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Volume 13 (4); July 27, 2023

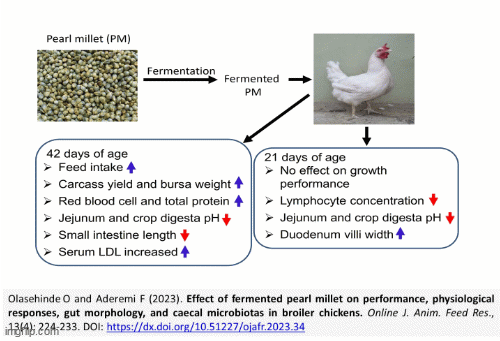
Research Paper

Effect of fermented pearl millet on performance, physiological responses, gut morphology, and caecal microbiotas in broiler chickens

Olasehinde O and Aderemi F.

Online J. Anim. Feed Res., 13(4): 224-233, 2023; pii: S222877012300034-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.34



Olasehinde O and Aderemi F (2023). **Effect of fermented pearl millet on performance, physiological responses, gut morphology, and caecal microbiotas in broiler chickens.** *Online J. Anim. Feed Res.*, 13(4): 224-233. DOI: https://dx.doi.org/10.51227/ojafr.2023.34

Abstract: This study evaluated the contribution of fermented pearl millet [*Pennisetum glaucum* (L) R. Br.,] on growth performance, physiological responses, gut morphology, and microbial composition in the caeca. One hundred and eighty day-old Arbor Acre broiler chicks were assigned into five groups and were fed starter (d 0 to 21) and finisher (d 22 to 42) diets. Test diets included a control composed of maize-soybean meal (0%); a similar diet with maize replaced with fermented pearl millet (FPM) at 25, 50, 75, and 100%. Results showed that there was no significant improvement in weight gain and feed conversion ratio although more feed was consumed (P = 0.035) as FPM increased in the diet. Carcass yield increased linearly (P = 0.05) at d 42. Bursa of Fabricius quadratically increased (P = 0.02) in weight particularly at 25% and 50% FPM levels at d 21. Concentrations of total protein (P = 0.026) and low-density lipoprotein (P = 0.037) increased linearly as FPM increased in the diets. Proventriculus weight, lymphocyte concentration in the blood, and size of gut segments linearly reduced (P < 0.05). Proventriculus and crop pH improved linearly (P = 0.05) while digesta pH in jejunum reduced linearly (P = 0.005) at d 21. Duodenal villus width increased quadratically (P = 0.008), and the highest width occurred in the 50% FPM group. Furthermore, dietary FPM did not influence caeca *Salmonella* and *Lactobacillus*. In conclusion, replacement of maize with FPM had no adverse effect on performance, physiological status, gut morphology and microbial composition of broiler chickens. Our results suggest that FPM represents a potential alternative in diets of broiler chickens without sacrificing the nutritional quality of the diet.
Keywords: Broiler chickens, Fermented pearl millet, Gut morphology, Gut microbiota, Nutritional quality.

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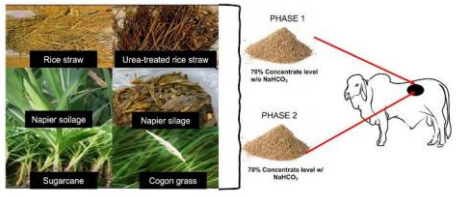
Research Paper

Nutrient digestibility of fibrous feedstuffs in high-concentrate diet with sodium-bicarbonate (NaHCO3) addition in rumen-fistulated Brahman bull

Niepes RA and Bestil LC.

Online J. Anim. Feed Res., 13(4): 234-241, 2023; pii: S222877012300035-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.35



Niepes RA and Bestil LC (2023). **Nutrient digestibility of fibrous feedstuffs in high-concentrate diet with sodium-bicarbonate ($NaHCO_3$) addition in rumen-fistulated Brahman bull.** *Online J. Anim. Feed Res.*, 13(4): 234-241. DOI: https://dx.doi.org/10.51227/ojafr.2023.35

Abstract: Ruminants are given substantial quantities of concentrate diets full of quickly fermentable carbohydrates to increase output performance; however, it can also lead to digestive disorders. This study aimed to investigate the effect of adding $NaCHO_3$ to a high-concentrate diet on the nutrient digestibility of locally available fibrous feedstuffs in the Philippines. The experiment utilized a rumen-fistulated Brahman bull. The treatment diets were the following: Treatment 1 (T1): Untreated rice (*Oryza sativa* L.) straw; T2: Urea-treated rice straw; T3: Napier grass (*Pennisetum purpureum* Schumach); T4: Napier silage; T5: Sugar cane (*Saccharum officinarum* L.) tops; and T6: Cogon grass (*Imperata cylindrica* L.). The nylon bags containing the treatment diets were incubated in the rumen at two periods: first at a high-concentrate (70% level), and second at a high concentrate added with NaCHO3). The results showed that the nutrient digestibility of locally-available feedstuffs varies significantly ($p<0.05$) both with and without $NaHCO_3$. The addition of $NaHCO_3$ in a high-fiber diet improves the digestibility of locally available fibrous feedstuffs in terms of dry matter (DM), organic matter (OM), and neutral detergent fiber (NDF). Therefore, the addition of NaHCO3 to a high-concentrate diet has the potential to positively stabilize rumen pH and enhance the nutrient digestibility of locally available fibrous feedstuffs.
Keywords: Fibrous feedstuffs, *in situ* digestibility, Nutrient digestibility, Rumen, Sodium bicarbonate.

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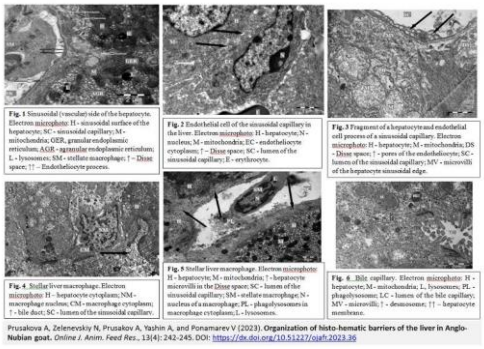
Short Communication

Organization of histo-hematic barriers of the liver in Anglo-Nubian goat

Prusakova A, Zelenevskiy N, Prusakov A, Yashin A, and Ponamarev V.

Online J. Anim. Feed Res., 13(4): 242-245, 2023; pii: S222877012300036-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.36



Abstract: The aim of this research was to establish features of the liver histo-hematic barriers ultrastructural organization of the Anglo-Nubian goat. The liver of an adult Anglo-Nubian goat was used as the material. The work was carried out using the electron microscopic method. Liver parenchymal tissue fragments were selected. These samples were fixed in a 2.0% glutaraldehyde solution on a cacodylate buffer for two hours. They were then washed in three portions of the same buffer and post-fixed in a 1.0% solution of osmium tetrachloride for one hour. The samples were then dehydrated in alcohols of ascending concentration and absolute acetone. The subsequent filling of the fragments was carried out in Epon-812. Ultrathin sections were obtained on an ultramicrotome, contrasted with a 2.0% aqueous solution of uranyl acetate and a solution of lead citrate. The ultrathin sections were photographed with a Jem-1011 electron microscope at magnifications of 2500-3000. Two histo-hematic barriers are detected in the liver of the studied animals hemato-hepatic and hepatobiliary. The hemato-hepatic barrier is formed by the plasmalemma of the apical end of the hepatocyte, covered by the glycocalyx, the perisinusoidal space of the Disse, the endotheliocyte of the sinusoid capillary, as well as Kupfer cells located in the lumen of the latter. The hepatobiliary includes all of the above structures, with the exception of Kupfer cells, as well as the plasmalemma of the basal end of the hepatocyte. All of the above structures in their organization have characteristic species features for Anglo-Nubian goats.

Keywords: Anglo-Nubian goat, Digestive organs, Hepatobiliary barrier, Hemato-hepatic barrier, Liver.

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Research Paper

Impact of climate change on dairy milk production in Nigeria

Osuji E, Igberi Ch, Enyia Ch, Nwachukwu E, Nwose R, Adeolu A, Akunna T-A, Nkwocha G, Eleazar A, and Gabrie ID.

Online J. Anim. Feed Res., 13(4): 246-252, 2023; pii: S222877012300037-13
DOI: https://dx.doi.org/10.51227/ojafr.2023.37

Abstract: This study explores the impacts of climate change on milk production in Nigeria. Climate variables such as temperature, rainfall, sunshine, relative humidity and wind speed were considered as covariates in the analysis. Time-series data spanning a period of forty years obtained from the Central Bank of Nigeria and FAOSTAT database was used. The autoregressive distributed lag model was used to analyze both the short run and long run impacts of climate change on milk production. As expected, not all the variables were stationary at levels, but they were all significant at the difference suggesting the presence of cointegration. The result showed that the Bound's test F-ratio was statistically significant implies the existence of long run and short run relationships among the variables studied. Present findings revealed that temperature, rainfall and relative humidity had a negative impact on milk production, while sunlight recorded a positive impact on milk production both in the short run and long run estimates. The study concludes that milk production in Nigeria dropped as a result of climate change particularly rising temperature and prolonged rainfall. Agricultural climate smart practices were recommended to mitigate impact of climate change on milk production.

Keywords: Climate Change, Dairy Products, Production, Rainfall, ARDL model.

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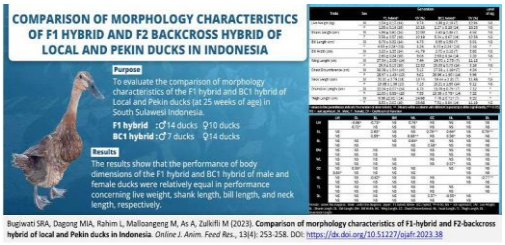
Research Paper

Comparison of morphology characteristics of F1-hybrid and F2-backcross hybrid of local and Pekin ducks in Indonesia

Bugiwati SRA, Dagong MIA, Rahim L, Malloangeng M, As A, Zulkifli M.

Online J. Anim. Feed Res., 13(4): 253-258, 2023; pii: S222877012300038-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.38



Abstract: The purpose of this study was to evaluate the comparison of morphology characteristics of two groups (the F1 and F2 backcross of Local and Pekin ducks at 25 weeks of age) in South Sulawesi Indonesia. The research material used 14 ducks males and 10 ducks females of F1 and 7 ducks males and 14 ducks females of F2-backcross. The data were measured on live weight, shank length, bill length, bill width, wing length, chest circumference, neck length, drumstick length, and thigh length. All mean differences of quantitative data from those two groups were analyzed using Independent T-test. The results showed that the performance of body dimensions of the F1 and F2-backcross of male and female ducks were relatively equal in performance concerning live weight, shank length, bill length, and neck length, respectively. The highest and positive correlation exists between shank length with chest circumference and drumstick (r=0.78) of F1 backcross female duck, between live weight with shank length (r=0.72) of F2-backcross female duck, between shank length with chest circumference (r=0.59) of F1 male duck and between live weight with chest circumference (r=0.84) of F2-backcross male duck, respectively. All measured variables had a coefficient of variation on both generations were less than 15%, except the bill width of the F1 male duck (41.79%) and both sex of thigh length of the F1 duck (24.68% and 23.68%, respectively).

Keywords: Breeding, Genetics, Morphology characteristic, Local duck, Pekin duck.

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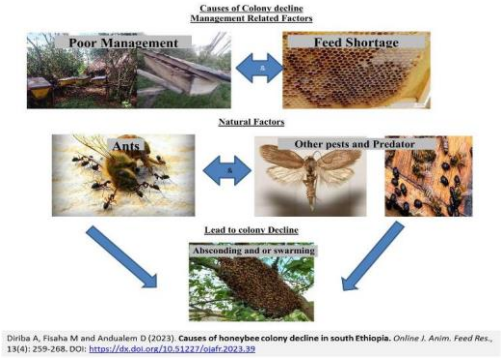
Research Paper

Causes of honeybee colony decline in south Ethiopia

Diriba A, Fisaha M and Andualem D.

Online J. Anim. Feed Res., 13(4): 259-268, 2023; pii: S222877012300039-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.39



Abstract: The purpose of this study was to identify the major causes of colony decline in the Gedeo Zone, South Ethiopia. Three districts, namely, Yirga Cheffe, Wonago, and Dilla Zuria, were purposefully selected based on beekeeping potential. A cross-sectional survey was conducted to collect data from 135 beekeepers and 15 key informants using a semi-structured questionnaire, focus group discussion, and personal observation of apiary sites. The results revealed two main causes of colony declines in the Gedeo zone: colony management-related factors and natural factors. Seventy percent of beekeepers lack the practical skills to perform hive inspection; 47% do not feed their colonies; 45% spray pesticides and insecticides near their apiaries; and 82% fail to control swarming. As a result, 87% of sampled beekeepers have experienced frequent colony absconding. The trends of colony decline showed an increase from 2008 to 2020 in the highlands and from 2008 to 2017 in the midlands and lowlands, respectively. The number of households facing colony declines increased in all agro-ecologies from 2008 to 2020. Pests and predators, like wax moths, and small hive beetles were take the first rank followed by ants, the inherent behavior of honeybees, a shortage of flora, and the presence of poisonous plants were the top five challenges among natural factors, respectively. Therefore, we strongly recommend educating beekeepers on scientific methods of colony management and planting bee flora. Laboratory diagnostics are required to identify bee diseases.

Keywords: Apiary management, Apiculture, Bee diseases, Colony decline, Swarm.

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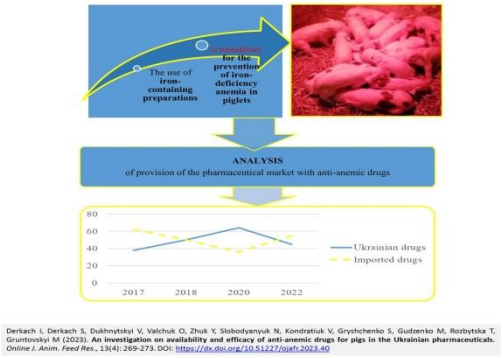
Research Paper

An investigation on availability and efficacy of anti-anemic drugs for pigs in the Ukrainian pharmaceuticals

Derkach I, Derkach S, Dukhnytskyi V, Valchuk O, Zhuk Y, Slobodyanyuk N, Kondratiuk V, Gryshchenko S, Gudzenko M, Rozbytska T, Gruntovskyi M.

Online J. Anim. Feed Res., 13(4): 269-273, 2023; pii: S222877012300040-13
DOI: https://dx.doi.org/10.51227/ojafr.2023.40



Derkach I, Derkach S, Dukhnytskyi V, Valchuk O, Zhuk Y, Slobodyanyuk N, Kondratiuk V, Gryshchenko S, Gudzenko M, Rozbytska T, Gruntovskyi M (2023). **An investigation on availability and efficacy of anti-anemic drugs for pigs in the Ukrainian pharmaceuticals.** *Online J. Anim. Feed Res.*, 13(4): 269-273. DOI: https://dx.doi.org/10.51227/ojafr.2023.40

Abstract: Antianemic drugs are used to prevent anemia, majorly iron deficiency anemia. Drugs with such pharmacological action are especially relevant for piglets, as animals of this species at this age are particularly sensitive to iron deficiency. The present investigation aimed at studying the pharmaceutical market of antianemic drugs registered in Ukraine during 2017-2022. It should be noted that if the drug is registered, it has been checked for safety according to the food industry standards of Ukraine. In 2017, the national market of veterinary iron-containing drugs was represented by 13 drugs from the group QB03A "Antianemic drugs. Drugs of iron", according to the ATC-vet classification. The range of these drugs by 38 % was provided by pharmaceutical products of Ukrainian manufacturers: "O.L.KAR-AgroZooVet-Service", "Pharmaton", "Brovapharma", "Experimental production of the Institute of Epizootology", "Biopharm", and "Vetsintez". Imported products (62 %) were represented by Pharmacosmos, Merial, Koofavet , "Vugen B&G" , "Biovet Pulawy", "Interchem Verken De Adelaar", and "Bioveta". In general, the modern pharmaceutical market of veterinary drugs in Ukraine during 2017–2022 was sufficiently provided with antianemic drugs for pigs and mostly imported drugs. The percentage of antianemic drugs of Ukrainian production prevailed in 2020, however the imported drugs of this pharmacological group's was higher in 2022. It can be concluded that the drugs of non-Ukrainian production predominated among antianemic drugs in Ukraine during 2017-2022. Based on the obtained results, we can state that in Ukraine there is a need for the development and/or production of domestic anti-anemic drugs. They must be effective and ensure a reduction in the dependence of the national pharmaceutical market of drugs of this group on foreign manufacturers.

Keywords: Anemia, Availability, Iron, Pigs, Veterinary drugs.

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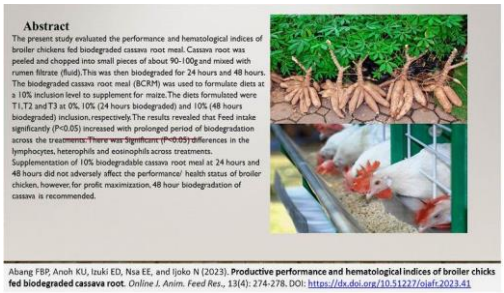
Research Paper

Productive performance and hematological indices of broiler chicks fed biodegraded cassava root

Abang FBP, Anoh KU, Izuki ED, Nsa EE, and Ijoko N.

Online J. Anim. Feed Res., 13(4): 274-278, 2023; pii: S222877012300041-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.41



Abang FBP, Anoh KU, Izuki ED, Nsa EE, and Ijoko N (2023). **Productive performance and hematological indices of broiler chicks fed biodegraded cassava root.** *Online J. Anim. Feed Res.*, 13(4): 274-278. DOI: https://dx.doi.org/10.51227/ojafr.2023.41

Abstract: To evaluate the performance and hematological indices of broiler chickens fed biodegraded cassava root meal an experiment was conducted in the Poultry Unit of the Livestock Teaching and Research Farm, Joseph Sarwuan Tarka University, Makurdi, Benue State. A total of one hundred and fifty five week-old (Ross 308) finishing broilers was used for the experiment. The birds were assigned randomly into three treatments and each treatment was replicated five times with ten birds per replicate. Cassava root was peeled and chopped into small pieces of about 90-100g and mixed with rumen filtrate (fluid). This was then biodegraded for 24 hours and 48 hours. The biodegraded cassava root meal (BCRM) was used to formulate broiler's diets at a 10% inclusion level to supplement for maize. The diets formulated were T1, T2 and T3 at 0%, 10% (24 hours biodegraded) and 10% (48 hours biodegraded) inclusion, respectively. The birds in each replicate were housed in separate cages in a completely randomized design (CRD). All routine management practices, including recommended vaccinations were strictly observed, feed and water were served ad libitum throughout the period of the study which lasted for 28 days. Performance indices such as body weight, body weight gain, feed intake and feed conversion ratio were measured. Hematological parameters were also taken; pack cell volume (PCV), red blood cell (RBC), hemoglobin (HB), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell (WBC) differential. Results revealed that there was no significant (P>0.05) differences in all the performance indices measured except in feed intake. Feed intake increased with prolonged period of biodegradation across treatments. There was significant (P<0.05) differences in the lymphocytes, heterophils and eosinophils across treatments. The study concluded that the dietary supplementation of

10% biodegradable cassava root meal at 24 and 48 hours did not adversely affect the performance/ health status of broiler chicken, however, for profit maximization, 48 hour biodegradation of cassava is recommended.

Keywords: Biodegradation, Broiler Chicken, Cassava Root, Maize, Productive performance.

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Research Paper

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Effect of combination of *Indigofera zollingeriana*, black soldier fly larvae, and turmeric on performance and histomorphological characterizes of native chicken at starter phase

Muhammad LN, Purwanti S, Pakiding W, Marhamah, Nurhayu, Prahesti KI, Sirajuddin SN and Mushawwir A.

Online J. Anim. Feed Res., 13(4): 279-285, 2023; pii: S222877012300042-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.42

Abstract: Sources of high protein feed ingredients can come from plants and animals (insects), namely *Indigofera zollingeriana syn.* and black soldier fly larvae. The addition of natural feed additives to feed can be obtained from turmeric phytobiotics which have many biological activities, such as anticancer, anti-inflammatory, antimicrobial and antioxidant. This study aimed to determine the effect of the combination of *Indigofera zollingeriana syn.*, black soldier fly (BSF) larvae and turmeric on the performance and histomorphology of the bursa Fabricius in the native chicken starter phase. The research design was completely randomized with 3 treatments and 5 replications with 6 chickens per unit. The combination treatments were P0 (Commercial feed as control); P1 (5% Indigofera flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% Indigofera flour + 20% BSF larvae flour + 2.5% turmeric flour). Parameters measured in this study were performance (body weight gain, feed consumption, FCR) and bursa of fabricius histomorphology in native chickens. The results of the analysis of variance showed that the combination of *Indigofera zollingeriana syn*., BSF larvae and turmeric had a significant effect on the performance of native chickens but could not match the performance of P0 (control feed). While the histomorphology of bursa Fabricius showed that the combination of *Indigofera zolliengeriana* flour up to 10% and 25% black soldier fly larvae flour in the feed identified the medulla width, cortex thickness and follicle width can increase lymphocyte cells to produce antibodies for native chickens at starter phase.

Keywords: Feed additives, *Indigofera zoillingeriana*, Insect, Native chicken, Larvae

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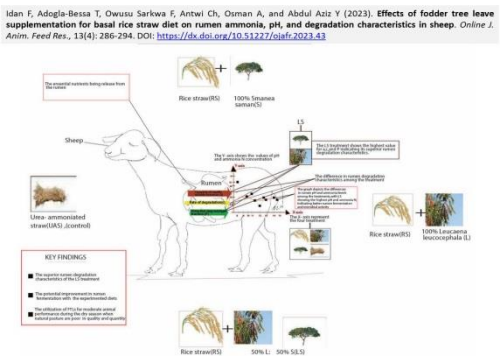
Research Paper

Effects of fodder tree leave supplementation for basal rice straw diet on rumen ammonia, pH, and degradation characteristics in sheep

Idan F, Adogla-Bessa T, Owusu Sarkwa F, Antwi Ch, Osman A, and Abdul Aziz Y.



Online J. Anim. Feed Res., 13(4): 286-294, 2023; pii: S222877012300043-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.43

Abstract:Fodder tree leaves (FTLs) contain high levels of protein, vitamins, and minerals that play a major role in enhancing roughage intake by ruminants, thus improving low-quality roughage utilization. The study sought to measure the rumen degradation characteristics, pH, and ammonia N concentration of sheep fed rice straw (RS) and supplemented with FTLs. Four forest-type rumen-fistulated rams of an average weight of 19.0±1.2 kg were randomly assigned to one of four treatments in a 4 × 4 Latin Square design. Treatment diets consisted of urea-ammoniated straw (UAS; control), RS+100% *Leucaena leucocephala* (L), RS+100% *Samanea saman* (S), and RS+50% L+50% S (LS). Data obtained were subjected to the Glimmix procedure of SAS (2016) and significant means were separated using Tukey's test at (P<0.05). Treatments

differed significantly (P<0.001) in the quantity of readily soluble materials (a), rate of degradation (c), and a potentially degradable fraction (P) with LS recording the highest a, c, and P among the treatments. Ruminal pH and ammonia concentrations differed significantly (P<0.0001) among the treatments. Overall mean rumen pH values obtained ranged from 6.44 in UAS to 6.72 in the S-supplemented diet whereas mean rumen ammonia values ranged from 4.59 mg/100 ml in sheep fed UAS diet to 9.15 mg/L in sheep fed L diet. The pH values obtained imply that the experimental diets could improve rumen fermentation and, hence, serve as good sources of feed for ruminants. The rumen DM degradation values indicated that sufficient amounts of DM would be degraded over a period of time, thus releasing substantial quantities into the small intestines for digestion to provide essential nutrients needed for better animal performance. The rumen ammonia values obtained were higher than the minimum values recommended for optimal microbial activity for animals fed lignocellulosic materials. This indicated that such FTLs could be utilized for moderate animal performance, especially during the dry seasons when natural pastures are qualitatively and quantitatively poor.

Keywords: Ammonia concentration, Degradation, *Leucaena leucocephala*, *Samanea saman*, Sheep.

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Research Paper

Effect of dietary crude protein levels on feed intake and nutrient digestibility of Wagyu crossbred cattle

Binh Truong N and Thanh Trung T.

Online J. Anim. Feed Res., 13(4): 295-301, 2023; pii: S222877012300044-13
DOI: https://dx.doi.org/10.51227/ojafr.2023.44

Binh Truong N and Thanh Trung T (2023). **Effect of dietary crude protein levels on feed intake and nutrient digestibility of Wagyu crossbred cattle.** *Online J. Anim. Feed Res.*, 13(4): 295-301. DOI: https://dx.doi.org/10.51227/ojafr.2023.44

Abstract: The objective of the experiment was to determine the suitable crude protein level on feed intake and digestible nutrient value of Wagyu crossbred cattle from 13 to 20 months of age. The research included 2 experiments. Experiment 1: five male Wagyu x Zebu crossbred cattle (12.2±0.56 months of age and average live weight of 179±24.2 kg, Mean±SD). Experiment 2: five male Wagyu x Zebu crossbred cattle (16.7±1.05 month's old and live weight of 276±22.0 kg). Two experiments were Latin square design (5x5) with 5 treatments and 5 periods (21 days per period). The treatments were different crude protein levels at 210, 245, 280, 315, and 350 g per 100 kg live weight (LW) corresponding to CP210, CP245, CP280, CP315, and CP350 treatments, respectively. The basal diet was commercial concentrate (1.2 kg/day), fresh Elephant grass (5.0 kg/day) and *ad libitum* rice straw. While soybean meal was used to adjust the dietary CP level per 100 kg LW in diets. The result showed that increasing nutrient consumption and metabolism energy (P<0.05) but reduced fiber intakes (P>0.05) by increasing crude protein levels. Experiment 1: the CP digestibility was highest (P<0.05) of CP350 (72.8%) treatment compare to CP210 (58.8%) treatments, while the CP280 (67.2%) treatment was not significant (P>0.05) with CP245 (62.9%) and CP315 (71.7%) treatments. Experiment 2: the highest CP digestibility (P<0.05) of CP350 treatment as compared to CP315, CP280, CP245, and CP210 treatments (80.2, 77.4, 73.1, 70.5, and 65.0%, respectively). As a result, increasing CP levels per 100 kg BW could rise nutrients digestibility and digestible value for Wagyu crossbred cattle. The level of 245 g CP per 100kg live weight in Wagyu crossbred cattle diet from 13 to 20 months of age could be recommended for application.

Keywords: Beef production, Crude protein, Digestion, Rumen escape protein, Ruminants.

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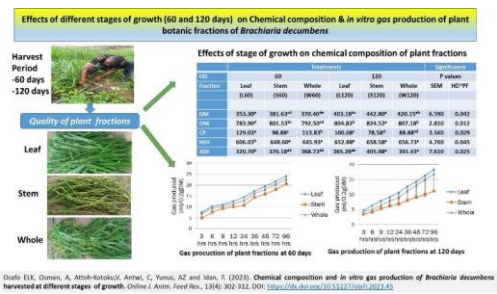
Research Paper

Chemical composition and in vitro gas production of *Brachiaria decumbens* harvested at different stages of growth in the hot humid region

Osafo ELK, Osman A, Attoh-Kotoku V, Antwi C, Abdul Aziz Y and Idan F.

Online J. Anim. Feed Res., 13(4): 302-312, 2023; pii: S222877012300045-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.45



Osafo ELK, Osman, A, Attoh-KotokuV, Antwi, C, Yunus, AZ and Idan, F. (2023). **Chemical composition and *in vitro* gas production *of Brachiaria decumbens* harvested at different stages of growth.** *Online J. Anim. Feed Res.*, 13(4): 302-312. DOI: https://dx.doi.org/10.51227/ojafr.2023.45

Abstract: The study evaluated the effect of harvesting date on the chemical composition and in vitro gas production of the botanic fractions of Brachiaria decumbens grass. The botanic fractions (leaf, stem and whole plant) of the grass at two maturities (60 and 120 days) in a Completely Randomised Design with factorial arrangement. Samples of botanic fractions at the different harvest dates were chemically analyzed

for dry matter (DM), crude protein (CP), fibre concentrations and in vitro gas production (IVGP) was measured at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h to estimate the volume and rate of gas production. Short-chain volatile fatty acids, microbial protein production, in vitro organic matter digestibility, and metabolizable energy were estimated from established models. Organic matter, crude fibre, NDF, ADF and ADL increased ($P<0.05$) with increasing maturity whereas the reverse was so for CP and ash contents ($P<0.05$). Significant interactions ($P<0.05$) between harvest date and plant fraction were present for both 'b' and 'c' attributable to treatment effects. Potential gas production 'b' elicited a negative response for all plant fractions across the two harvest dates as the values decreased linearly. The rate at which the gases were produced 'c' also induced a negative response for the leaf and whole fraction but a positive one for the stem fraction. The nutrient composition and gas production characteristics of grasses harvested at day 60 offer a better potential as high quality forage for improved intake and digestibility. The leaf fractions performed relatively better based on the afore-mentioned methods of quality assessment at both maturity periods.

Keywords: Botanic fraction, Brachiaria decumbens, Chemical composition, Feedstuff, In vitro gas production.

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Research Paper

A new reading of the animal production subsidy program for the Saharan regions: Opportunities and constraints

Bensaha A, Bensaha H and Bensaha L.

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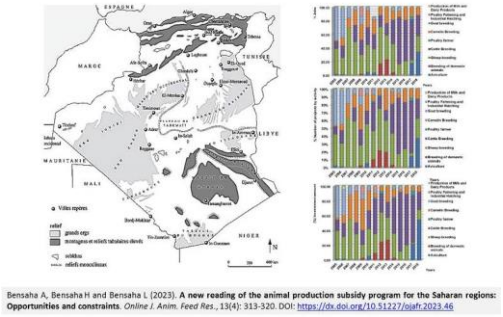

Bensaha A, Bensaha H and Bensaha L (2023). **A new reading of the animal production subsidy program for the Saharan regions: Opportunities and constraints**. *Online J. Anim. Feed Res.*, 13(4): 313-320. DOI: https://dx.doi.org/10.51227/ojafr.2023.46

Abstract: Like in other Saharan regions, the Animal Production Subsidy Program has increased the herd in Ghardaia. The creation of breeding farms in such areas has generated specific dynamics through a set of measures that have had different impacts on the components of this sector. The data from the guides addressed to the various stakeholders illustrates that the management of the breeding farms presents shortcomings at different levels and even compromises its sustainability. In our model, around 49 % of farmers are renting their farmland, while 51 % are managing their own land. A significant portion of breeders (approximately 75.88 %) expresses the view that the main obstacle hindering the progress of these treatments is the insufficient availability of outreach programs. Applying this management results in young breeders abandoning the farms and using them for other activities. As a solution, the government must revise its agricultural programs and investments in order to achieve the long-term development goals that have been set. The measures to be taken are discussed to preserve the sector and explain the substantial investments made by the public authorities.

Keywords: Agricultural policies, Algeria, Animal production, Breeding farms, Saharan region, Subsidy program.

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EFFECT OF FERMENTED PEARL MILLET ON PERFORMANCE, PHYSIOLOGICAL RESPONSES, GUT MORPHOLOGY, AND CAECAL MICROBIOTAS IN BROILER CHICKENS

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ABSTRACT: This study evaluated the contribution of fermented pearl millet [*Pennisetum glaucum* (L) R. Br.,] on growth performance, physiological responses, gut morphology, and microbial composition in the caeca. One hundred and eighty day-old Arbor Acre broiler chicks were assigned into five groups and were fed starter (d 0 to 21) and finisher (d 22 to 42) diets. Test diets included a control composed of maize-soybean meal (0%); a similar diet with maize replaced with fermented pearl millet (FPM) at 25, 50, 75, and 100%. Results showed that there was no significant improvement in weight gain and feed conversion ratio although more feed was consumed (P = 0.035) as FPM increased in the diet. Carcass yield increased linearly (P = 0.05) at d 42. Bursa of Fabricius quadratically increased (P = 0.02) in weight particularly at 25% and 50% FPM levels at d 21. Concentrations of total protein (P = 0.026) and low-density lipoprotein (P = 0.037) increased linearly as FPM increased in the diets. Proventriculus weight, lymphocyte concentration in the blood, and size of gut segments linearly reduced (P < 0.05). Proventriculus and crop pH improved linearly (P = 0.05) while digesta pH in jejunum reduced linearly (P = 0.005) at d 21. Duodenal villus width increased quadratically (P = 0.008), and the highest width occurred in the 50% FPM group. Furthermore, dietary FPM did not influence caeca *Salmonella and Lactobacillus*. In conclusion, replacement of maize with FPM had no adverse effect on performance, physiological status, gut morphology and microbial composition of broiler chickens. Our results suggest that FPM represents a potential alternative in diets of broiler chickens without sacrificing the nutritional quality of the diet.

Keywords: Broiler chickens, Fermented pearl millet, Gut morphology, Gut microbiota, Nutritional quality.

INTRODUCTION

The poultry industry is challenged by scarcity and increasing cost of maize which has enthused the use of alternative source of energy (Owen et al., 2012; Ravindran and Blair, 2009). Cereal crops including wheat, sorghum, and barley have been investigated in partial or total replacement of maize (Silva et al., 2015; Viliene et al., 2022; Biesek et al., 2022). However, cereal grains contain considerable amount of anti-nutrients which inhibits nutrient utilization and growth of broiler chickens (Mathlouthi et al., 2002).

Grain fermentation, an important affordable processing method in poor tropical countries, has been reported to boost the nutritive value of cereals by enriching crude protein and fat content, and reduce anti-nutrient thereby improving nutrient digestibility. It also enhances micronutrient bioavailability and density accompanied by reduced crude fibre content (Feng et al., 2007; Wang et al., 2010; Kasprowicz-Potocka, 2015; Sugiharto et al., 2015; Zaworska et al., 2016). The fermentation process can be carried out naturally or with the use of starter culture. Natural fermentation process is a traditional method that is relatively simple, common, inexpensive, and effective method available to small scale poultry farmers in developing countries like Nigeria.

The use of fermented feed products in poultry production has been reported extensively. Fermented feed enhanced growth performance, antioxidant system, size of the immune organs, egg weight, and strength of the egg shell of laying hens (Engberg et al., 2009; Zhu et al., 2020). Fermented feed tends to produce beneficial bacteria which could improve the structure and function of the gut and stimulate establishment of beneficial bacteria population (Gao et al., 2009; Li et al., 2020). Drażbo et al. (2019) showed that inclusion of 15% fermented rapeseed cake improved body weight in turkeys without effect on carcass quality. Previous studies revealed that fermented feed modulated intestinal microflora, subsequently contributing to improved growth, intestine structure, and immunity when broiler chickens were fed fermented rice bran (Kang et al., 2015), and cottonseed meal (Ranjitkar et al., 2016; Jazi et al., 2017). Feeding broiler chickens *B. licheniformis* fermented products improved the body weight of coccidia infected broilers and regulated caeca microbial composition (Cheng et al., 2021). However, information is limited on the impact of fermented pearl millet in broiler chickens nutrition. Pearl millet (*Pennisetum glaucum,* PM) contains a high amount of nutrients comparable to maize, rich in antioxidants and fiber (Boncompagni et al., 2018; Punia et al., 2021). Pearl millet contains fewer, anti-nutrient compounds including phytate, tannins, polyphenols, and enzyme inhibitors which could influence nutrient availability and digestion (Osman, 2011; Boncompagni et al., 2018).

Citation: Olasehinde O and Aderemi F (2023). Effect of fermented pearl millet on performance, physiological responses, gut morphology, and caecal microbiotas in broiler chickens. *Online J. Anim. Feed Res.*, 13(4): 224-233. DOI: https://dx.doi.org/10.51227/ojafr.2023.34

To our knowledge, in literature, there is limited information on the effect of fermented pearl millet in broiler chickens. Therefore, this study aimed to evaluate whether fermented pearl millet affects growth performance, physiological responses, gut morphology, and microbial composition in the caeca of broiler chickens.

MATERIALS AND METHODS

Ethical approval

Experimental procedures adopted in this study were approved by the Bowen University research and ethical committee in conformation with AARIVE 2.0 guidelines (Du Sert et al., 2020).

Experimental units

The experiment was conducted at the Teaching and Research Farm of Bowen University. One hundred and eighty, 180, broiler chickens arrived at the facility, immediately weighed, and randomly distributed into 5 treatment groups. Each treatment was allotted to 3 replicates with 12 broiler chickens per replicate in a completely randomized design. Broiler chickens in each treatment group had ad libitum access to feed and water throughout the experimental period. The pens were maintained in identical environmental conditions. The experiment lasted for 42 days, starter (0 - 21 d) and finisher (22 - 42 d). The light regime was 1 h darkness (0 - 7 d) and 4 h darkness (8 - 42 d) respectively.

Experimental diets

The pearl millet grains used in this study were fermented naturally in water. The PM grains were fermented following modified procedure of Osman (2011). The PM grains were sterilized in brine solution for 30 min. After this time, the solution was drained, and the grains thoroughly rinsed. The grains were soaked and fermented in distilled water for 24 h in darkness at ± 30^{o}C. The solution was drained, grains thoroughly rinsed, dried for 2 days and stored. Table 1 presents the chemical composition of fermented PM according to previous procedure of AOAC (2005).

The present study formulated five treatment diets fed as mash. A maize-soybean meal was formulated at a standard 22.5% CP as the basal diet to meet or exceed NRC (1994) nutritional requirements for broiler chickens. The five treatments adopted include a control diet which contains 100% maize and four experimental diets with 25%, 50%, 75%, and 100% of maize replaced by fermented PM. The ingredients and nutrient composition of treatment diets were presented in Table 2. The broiler chickens were fed at two phase feeding. The starter diet was fed for the first 21 days and then finisher diets fed until the end of the experiment at d 42 of age.

Table 1 - Nutrient composition of fermented pearl millet.

Composition	Fermented pearl millet
Metabolizable energy (Kcal/kg)	3,441.60
Dry matter (%)	88.6
Protein (%)	10.9
Crude fibre (%)	1.9
Ash (%)	1.3
Crude fat (%)	6.79
Phosphorus, Available (%)	0.11
Phosphorus, Phytate (%)	0.19

Table 2 - Basic ingredients and composition of experimental diets.

Ingredients	FPM (d 0 to 21)					FPM (d 22 to 42)				
	0%	25%	50%	75%	100%	0%	25%	50%	75%	100%
Maize	52.09	39.28	27.09	15.74	-	59.70	45.77	31.35	16.69	-
Pearl millet	-	14.81	28.91	42.03	60.29	-	16.20	32.86	49.75	67.06
Soybean meal	40.90	39.52	38.23	37.02	35.31	34.10	32.50	30.98	29.45	28.54
Soybean oil	2.68	2.03	1.41	0.83	-	2.15	1.46	0.72	-	-
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.38
DCP	2.16	2.14	2.11	2.09	2.05	1.95	1.92	1.89	1.87	1.85
Limestone	1.49	1.52	1.54	1.56	1.6	1.39	1.42	1.45	1.47	1.51
Vit-Min Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.38
Lysine	-	0.03	0.05	0.07	0.10	0.05	0.08	0.11	0.13	0.16
Methionine	0.18	0.17	0.16	0.16	0.15	0.16	0.15	0.14	0.14	0.12

Day 0 to 21 [crude protein: 22.5%; methionine: 0.50%; lysine: 1.21%; calcium: 1.10%; phosphorus: 0.50%; metabolizable energy: 2970 (kcal/kg)]; day 22 to 42 [crude protein: 20.00%; methionine: 0.45%; lysine: 1.10%; calcium: 1.00%; phosphorus: 0.45%; metabolizable energy: 3004 (kcal/kg)]; FPM = fermented pearl millet; DCP = dicalcium phosphate; vitamin-mineral premix supplied per kilogram of diet: vitamin A: 30,000 IU; vitamin D3: 6,250 IU; vitamin K: 5 mg; vitamin E: 75 mg; vitamin B1: 5.63 mg; vitamin B2: 15 mg; vitamin B6: 11.25 mg; vitamin B12; 0.0375 mcg; niacin; 100 mg; pantothenic acid: 37.5 mg; folic acid; 3.75 mg; choline chloride; 750 mg; manganese: 200mg; biotin: 0.125 mcg; zinc: 125 mg; iodine: 2.5 mg; copper: 12.5 mg; selenium; 0.5 mg; cobalt; 1.25 mg; iron; 50 mg; antioxidant; 312.5 mg.

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Performance, carcass and organ measurements

The body weight and feed intake were measured weekly. The FI, BWG and FCR were estimated for d 21 and d 42. Thirty broiler chickens (n = 30, 6 broiler chickens per treatment) were selected, fasted overnight, and weighed before slaughter. The resulting carcass were eviscerated, cut into different cuts and weighed. The carcass and internal organ weights were expressed as percentage live weight of each broiler chickens.

Hematological and biochemical determination

Blood samples were drawn from thirty broiler chickens (n = 30, 6 broiler chickens per treatment) at d 21 into vacutainer tubes for serum collection. Blood samples were centrifuged at 3000 rpm for 5 min at 4°C for serum collection. Serum samples were analyzed for cholesterol (CHL), low density lipoprotein (LDL), and high-density lipoprotein (HDL) using commercially available kits (AGAPPE Diagnostics Switzerland GmbH), albumin estimated by Bromocresol Green method (Wells et al., 1985), and total protein quantified by Biuret method (Kohn and Allen, 1995). The anti-coagulated blood samples were obtained for hematological analysis including white blood cell (WBC), lymphocytes (LYM), monocyte (Mono), packed cell volume (PCV), and red blood cell (RBC) were estimated using commercially available kits (RANDOX Laboratory Ltd, UK). Mean corpuscular hemoglobin concentration (MCHC) was determined using the equation described by Jain (1986).

Gut digesta pH

Broiler chickens (n = 30) were sampled at d 21 and d 42 to determine gut digesta pH. The entire intestine was separated into each segment. The pH in each segment from the crop to the caecum were measured by pH meter (Smart Spear pH tester, PH60S-Z Apera Instruments, USA). Stable readings were recorded for individual segments for each broiler chicken.

Gut morphology and caeca microbial composition

Gut morphology and caeca microbial composition of thirty broiler chickens (n = 30, 6 broiler chickens per treatment) were determined at d 21. The gut was removed and washed gently with Phosphate-buffered saline (PBS) solution. The gut segments including duodenum, jejunum, and ileum were separated, their individual length and weight subsequently measured and estimated as percentage of broiler chicken's live weight. For further morphological analysis, a 3-cm tissue sample of midpoint of each segment were placed in 10% formalin, dehydrated, cleared, and then embedded in paraffin (Suvarna et al., 2018). Cross sections of 5 µm for each sample were cut, mounted on glass slides, and hematoxylin-eosin stained. All morphometric images were taken and measured under a light microscope (VJ – 2005 DN Bio-microscope) and TX View CX Image® (Miotic Image 200, China). The contents of the caeca were collected, homogenized, and serially diluted 10-fold before plating. Samples were cultured on the Lactobacillus selective media, de Man, Rogosa and Sharpe agar (MERCK) and incubated for 48 h at 37°C before quantification. To enumerate Salmonella concentration, samples were incubated on Bismuth Sulfate agar for 24 h at 37°C. Bacterial colonies counted were reported as $\log_{10}$CFU for each gram of sample.

Statistical Analysis

A completely randomized design was adopted to analyse all data with analysis of variance (ANOVA) (Genstat Version 21.0, 2022). Bacteriological data was normalized using logarithmic transformation. Treatment means were separated at $P \leq 0.05$ by the Tukey least significant difference, post hoc test of ANOVA. Orthogonal polynomials were used to determine linear and quadratic effects of increasing levels of fermented pearl millet.

RESULTS

Growth performance of broiler chickens is shown in Table 3. During the experimental period, FPM did not affect BWG and FCR. On d 0 to 21 and d 0 to 42, there were no obvious effects of dietary FPM on FI. However, FI increased quadratically ($P = 0.035$) on d 22 to 42, with the highest FI obtained at the 25% and 100% FPM levels.

As presented in Table 4, on d 21, FPM in the diet had no significant effect on mean carcass yield. There was no impact of treatment diets on breast, thigh, and abdominal fat. However, drumstick at d 21 decreased quadratically ($P = 0.036$), with the lowest drumstick obtained at the 50% FPM level. Linear improvement in carcass yield was observed on d 42, although there was no significant effect on the breast, drumstick, thigh, and abdominal fat (Table 4).

The effects of FPM on organ weights of broiler chickens are shown in Table 5. By d 21, proventriculus weight reduced linearly ($P = 0.006$) as FPM increased in the diet while weight of bursa of Fabricius increased quadratically ($P = 0.02$), and highest weight was obtained in broiler chickens on 25% dietary FPM. Furthermore, FPM had no significant effect on weight of pancreas, gizzard, liver, and thymus. Similarly, by d 42, FPM did not influence organ weights.

Hematology and serum biochemistry of broiler chickens at d 21 are shown in Table 6. Increase in FPM up to 50% in the diets linearly decreased lymphocyte concentration in the blood ($P = 0.041$). Red blood cell concentration quadratically increased ($P = 0.048$) with the highest concentration obtained at 50% FPM level. The concentrations of Mono, WBC, PCV and MCHC were not altered. Similarly, FPM did not affect Albumin, CHL and HDL levels in the serum. Serum LDL

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concentration linearly increased {(P = 0.026) as FPM increased. Furthermore, TP level improved (P = 0.037) with up to 75% increase in FPM (Table 6).

The effects of FPM on both the length and weight of different segments of the intestine are shown in Table 7. On d 21, both ileum and cecum reduced in length linearly [(P = 0.003) and (P = 0.012), respectively] as FPM levels increased. In addition, both duodenum and jejunum lengths showed a decreasing trend. On d 42, complete replacement of maize with FPM reduced length of duodenum, jejunum and ileum at d 42. FPM also linearly reduced duodenum and ileum length [(P = 0.027) and (P = 0.015), respectively]. The least length was observed FPM had no effect on the length of the caecum (Table 7).

On d 21, FPM inclusion in the diets had no effect on empty weights of duodenum, jejunum, and caecum. On the contrary, empty weight of ileum (P = 0.034) decreased linearly as dietary FPM increased at d 21 while jejunum empty weight showed a similar decreasing trend at d 42 (Table 7).

The digesta pH in proventriculus and crop on d 21 increased linearly (P = 0.05) and jejunal pH decreased linearly (P = 0.005). However, FPM did not influence the pH of intestinal digesta from the gizzard, duodenum, ileum, and caecum. On d 42, FPM had no significant effect on the pH of the intestine. In contrast, crop digesta pH decreased quadratically (P = 0.048) with the addition of FPM to the diets (Table 8).

The dietary inclusion of FPM did not alter villus height and crypt depth of duodenum, however, duodenum villus height was quadratically increased (P = 0.008), and the highest observation was found in the 50% FPM group (Table 9). There was no effect of FPM on villus height, villus width and crypt depth in the jejunum (P > 0.05) at d 21 (Table 9). Furthermore, FPM did not influence (P > 0.05) *Lactobacillus* and *Salmonella* concentration in caeca of broiler chicken (Table 9).

Table 3 - Effect of fermented pearl millet on the performance parameters of broiler chickens during the rearing period

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
BWG								
d 0 to 21	599	602	609	635	633	8.070	0.483	0.794
d 22 to 42	1154	1255	1116	1097	1164	25.200	0.411	0.226
d 0 to 42	1753	1858	1725	1732	1797	28.200	0.631	0.350
FI								
d 0 to 21	865	890	909	919	949	10.400	0.307	0.374
d 22 to 42	2039^{a}	2140^{ab}	2098^{ab}	2095^{ab}	2172^{b}	18.200	0.694	0.035
d 0 to 42	2904	3030	3007	3014	3121	26.900	0.488	0.076
FCR								
d 0 to 21	1.45	1.48	1.50	1.45	1.50	0.012	0.986	0.204
d 22 to 42	1.78	1.71	1.88	1.91	1.87	0.032	0.224	0.476
d 0 to 42	1.66	1.63	1.75	1.74	1.74	0.022	0.316	0.792

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet; BWG = body weight gain; FCR = feed conversion ratio; FI = feed intake.

Table 4 - Effect of fermented pearl millet on carcass traits of broiler chickens.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
d 21								
Carcass yield, %	52.10	50.40	50.40	52.70	52.80	0.550	0.717	0.148
Breast, %	19.40	18.30	18.70	20.50	20.00	0.480	0.754	0.276
Drumstick, %	9.09^{a}	8.45^{ab}	8.32^{b}	8.92^{ab}	9.05^{a}	0.140	0.106	0.036
Thigh, %	9.52^{ab}	8.93^{a}	9.78^{b}	9.80^{b}	10.08^{b}	0.170	0.718	0.301
AF, %	1.60^{ab}	2.24^{b}	0.89^{a}	0.95^{a}	0.95^{a}	0.180	0.122	0.230
d 42								
Carcass yield, %	58.50^{ab}	55.00^{a}	59.70^{ab}	58.60^{ab}	61.70^{b}	0.920	0.050	0.193
Breast, %	23.30	22.30	24.20	23.30	24.60	0.680	0.339	0.680
Drumstick, %	9.58	9.68	9.77	9.74	10.32	0.120	0.114	0.437
Thigh, %	11.10	11.70	11.30	11.10	12.20	0.160	0.114	0.211
AF, %	0.92	1.16	1.45	1.17	0.86	0.110	0.897	0.115

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet; AF = Abdominal fat.

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Table 5 - Effect of fermented pearl millet on digestive organ weights of broiler chickens.

Parameters		FPM					SEM	P-value	
		0%	25%	50%	75%	100%		L	Q
d 21	Pancreas, %	0.29	0.28	0.21	0.26	0.29	0.013	0.836	0.068
	Gizzard, %	1.82	2.00	1.91	1.89	1.90	0.039	0.853	0.462
	Proventriculus, %	0.58^{a}	0.55^{ab}	0.51^{ab}	0.49^{ab}	0.46^{b}	0.016	0.006	0.820
	Liver, %	3.03	3.17	2.68	2.80	2.67	0.097	0.135	0.934
	Bursa, %	0.13^{a}	0.21^{b}	0.19^{bc}	0.15^{ac}	0.16^{abc}	0.008	0.923	0.020
	Thymus, %	0.37	0.39	0.40	0.37	0.38	0.024	0.990	0.754
d 42	Pancreas, %	0.20	0.22	0.22	0.22	0.18	0.010	0.390	0.120
	Gizzard, %	1.53	1.54	1.54	1.78	1.51	0.050	0.560	0.460
	Proventriculus, %	0.43	0.31	0.33	0.31	0.30	0.020	0.090	0.380
	Liver, %	1.95	1.86	1.70	1.84	1.91	0.060	0.800	0.240
	Bursa, %	0.08	0.08	0.12	0.12	0.09	0.010	0.600	0.330
	Thymus, %	0.27	0.31	0.33	0.24	0.25	0.020	0.220	0.140

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet.

Table 6 - Effect of fermented pearl millet on haematology and serum biochemistry of broiler chickens fed fermented whole PM-based diet on day 21.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
LYM, %	66.70^{a}	63.60^{ab}	59.40^{b}	62.70^{ab}	66.20^{ab}	1.060	0.041	0.591
Mono, %	3.00	3.80	3.20	3.17	2.83	0.180	0.579	0.363
WBC, x 10^{3}/µL	18,242	18,300	20,270	18,067	18,617	415	0.616	0.365
PCV, %	29.50	31.20	32.80	29.70	30.30	0.540	0.410	0.079
MCHC, g/dl	33.10	33.40	33.10	33.50	33.90	0.260	0.870	0.977
RBC, x10^{6} /µL	3.34^{ab}	3.47^{ab}	3.61^{a}	3.32^{b}	3.46^{ab}	0.048	0.750	0.048
Albumin, g/dl	1.36	1.42	1.37	1.56	1.46	0.034	0.162	0.524
CHL, mg/dl	175	163	170	184	189	4.540	0.994	0.318
HDL, mg/dl	103	111	93	120	124	4.000	0.851	0.852
LDL, mg/dl	35.50^{a}	46.10^{ab}	46.60^{ab}	51.00^{b}	51.80^{b}	1.800	0.037	0.208
TP, g/dl	2.22^{a}	2.12^{ab}	2.70^{b}	2.62^{b}	2.30^{ab}	0.077	0.026	0.452

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet; LYM = lymphocytes; Mono = monocytes; MCHC = mean corpuscular hemoglobin concentration; PCV = packed cell volume; WBC = white blood cell; RBC = red blood cell; CHL = cholesterol; TP = Total protein; HDL = high-density lipoprotein; LDL = Low-density lipoprotein; µL = microlitre; mg = milligram; dl = decilitre; g = gram.

Table 7 - Effect of fermented pearl millet on the relative length and weight of intestine segments of broiler chickens.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
d 21 - Relative length (cm/g) of BW								
Duodenum	3.75	3.80	3.59	3.57	3.29	0.096	0.074	0.511
Jejunum	8.14	8.08	7.47	7.65	7.17	0.180	0.087	0.977
Ileum	8.58^{a}	8.20^{ab}	7.63^{b}	7.60^{b}	7.30^{b}	0.150	0.003	0.531
Caecum	1.88^{ab}	1.89^{a}	1.84^{ab}	1.69^{ab}	1.61^{b}	0.039	0.012	0.367
Relative weights (g/g) of BW								
Duodenum	0.42	0.49	0.40	0.44	0.34	0.023	0.118	0.195
Jejunum	0.70	0.72	0.66	0.66	0.62	0.023	0.190	0.761
Ileum	0.58^{ab}	0.64^{a}	0.52^{ab}	0.47^{b}	0.47^{b}	0.027	0.034	0.702
Caecum	0.25	0.32	0.25	0.25	0.21	0.016	0.160	0.207
d 42 - Relative length (cm/g) of BW								
Duodenum	2.13^{a}	1.98^{ab}	1.84^{ab}	2.04^{b}	1.64^{b}	0.066	0.027	0.690
Jejunum	3.96	3.83	3.70	4.23	3.29	0.120	0.135	0.193
Ileum	4.61^{a}	4.22^{a}	4.28^{a}	4.55^{a}	3.57^{b}	0.120	0.015	0.183
Caecum	1.04	1.00	1.03	1.11	0.96	0.022	0.803	0.369
Relative weights (g/g) of BW								
Duodenum	0.37	0.36	0.36	0.34	0.30	0.015	0.243	0.624
Jejunum	0.60	0.59	0.56	0.52	0.48	0.024	0.052	0.690
Ileum	0.49	0.55	0.56	0.46	0.47	0.027	0.327	0.206
Caecum	0.21	0.22	0.25	0.24	0.18	0.012	0.739	0.127

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet.

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Table 8 - Effect of fermented pearl millet on gut pH of broiler chickens.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
d 21								
Crop	4.60^{ab}	4.43^{a}	4.68^{ab}	4.63^{ab}	4.85^{b}	0.320	0.050	0.244
Proventriculus	1.83^{a}	2.60^{ab}	2.48^{ab}	2.27^{ab}	3.30^{b}	1.120	0.050	0.753
Gizzard	2.38	2.57	2.13	2.82	2.95	0.630	0.060	0.203
Duodenum	5.83	5.95	6.03	5.92	5.90	0.390	0.839	0.354
Jejunum	6.00^{a}	5.98^{a}	5.97^{ab}	5.88^{ab}	5.78^{b}	0.190	0.005	0.532
Ileum	6.42	6.33	6.33	6.68	6.60	0.710	0.495	0.774
Caecum	5.88	6.15	5.58	5.57	6.20	0.710	0.938	0.245
d 42								
Crop	4.87^{a}	4.62^{ab}	4.36^{b}	4.47^{ab}	4.62^{ab}	0.075	0.166	0.048
Proventriculus	3.52	3.83	2.15	3.12	3.02	0.058	0.298	0.351
Gizzard	2.53	2.50	2.79	2.46	2.77	0.095	0.595	0.951
Duodenum	5.69	5.72	5.98	5.50	5.90	0.100	0.809	0.989
Jejunum	5.95	6.19	6.19	6.00	6.13	0.089	0.573	0.275
Ileum	6.59	6.87	7.07	6.35	6.69	0.095	0.573	0.237
Caecum	6.40	6.62	6.06	6.45	6.10	0.120	0.450	0.888

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet.

Table 9 - Effect of fermented pearl millet on gut morphology and caecal microbial composition of broiler chickens on day 21.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
Duodenum, µm								
Villus height	786	740	645	646	764	45.000	0.706	0.340
Villus width	141^{a}	168^{ab}	229^{b}	191^{ab}	149^{a}	11.600	0.525	0.008
Crypt depth	86.50	83.60	81.20	71.20	75.60	2.710	0.122	0.774
Jejunum, µm								
Villus height	548	538	741	655	667	38.800	0.227	0.470
Villus width	176	150	174	217	174	12.200	0.524	0.883
Crypt depth	78.30	77.40	96.80	84.90	85.50	5.580	0.612	0.584
Lactobacillus, log 10 cfu/g	5.31	5.07	5.04	5.33	5.04	0.076	0.479	0.628
Salmonella, log 10 cfu/g	4.72	4.94	4.99	4.85	5.13	0.069	0.208	0.880

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet.

DISCUSSION

In the present study, dietary inclusion of FPM had no obvious effect on the performance of broiler chickens. Our results are consistent with Guo et al. (2020), who showed that fermented soybean meal had no influence on broiler chicken's growth performance. In contrast, other studies reported improved weight gain and FCR of broiler chickens feeding on fermented cotton seed meal of Sun et al. (2013) and Wang et al. (2017). The difference in findings observed by above researchers might be due to changes in the environment and nature of feed ingredients including size of and feed particles. The small size of pearl millet, (3-4 µm) when fed to broiler chickens ad libitum coupled with its volume and resident time of feed in the crop may have contributed to increased FI (Picard et al., 2000; Classen et al., 2016).

In the present study, FPM had no effect on carcass traits except for drumstick. This observation is similar to the report of Khempaka et al. (2018) that cassava pulp fermented with *Aspergilus oryzae* did not affect carcass composition of broiler chickens. On the other hand, Yeh et al. (2018) showed that improved drumstick and carcass yield of broilers may be attributed to higher digestible amino acids of fermented feed accompanied by enhanced growth performance. The proportion of abdominal fat has direct influence on carcass yield and economic value (Wen et al., 2018), since increased deposition of excess abdominal fat is usually considered a waste of dietary energy resulting in economic losses, decreasing feed efficiency, reduced carcass yield and carcass quality of broiler chicken (Fouad and El-Senousey, 2014).

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Other authors reported that feeding fermented mulberry leaf powder significantly improved muscle yield and reduced abdominal fat in broilers (Ding et al., 2021). In another study, the proportion of carcass yield and abdominal fat in broiler chickens were increased by feeding fermented cotton seed meal (Nie et al., 2015). In this study, however, dietary FPM had no effect on abdominal fat at the different feeding phases but increased carcass yield at the end of d 42 suggest that dietary FPM did not have negative effect on economic value of broiler chickens' carcass traits.

Changes in the weight of the immune organs including the bursa of Fabricius is an indicator of the condition of the immune system of chickens (Heckert et al., 2002; Tong et al., 2012), since they may partly redirect absorbed nutrients from growth towards development of the immune system. In our study, replacement of maize with FPM in diets resulted in increased weight of the bursa of Fabricius. Our study agrees with Sugiharto et al. (2020) and Ao et al. (2011), who found that fermented commercial feed improved immune responses including increase in weight of the immune organ in broilers. Furthermore, bursa of Fabricius increased in weight in broiler chickens fed fermented sour cherry kernel (Gungor and Erener, 2020).

Blood indices are important measures to determine the state of health in animals (Johnstone et al., 2017). Red blood cells function in transporting oxygen and supporting increased metabolism. In this study, the level of RBC was influenced by dietary FPM and was within the standard limit reported for chickens (Bounous and Stedman, 2000).

Lymphocytes are immune cells that generally increase in concentration in response to infection (Akhtar et al., 2015). In this study, lymphocyte concentration decreased in broiler chickens on FPM diets, comparable to the findings of Sugiharto et al. (2020), that fermented cassava pulp and *Moringa olifera* leaf meal reduced lymphocyte count in broiler chickens. Previous research reported that lactic acid bacteria present in fermented feed produce organic acids which reduces pH in the intestine, and hinder the establishment of pathogenic bacteria (Sugiharto and Ranjitkar, 2019). Furthermore, our results suggest that broiler chickens on the FPM diets were healthy, and their physiological state were not negatively affected. They had comparable nutrient utilization for blood production and other hematological indices, although LDL concentration increased. Fermentation reduced fiber content but increased protein contents in grains (Sugiharto et al., 2015), while serum cholesterol level may be lowered by increasing dietary crude fibre level in broiler diets (Delaney et al., 2003). Therefore, it could be stated that reduction in fiber content of PM through fermentation may have contributed to high LDL concentration.

The development of the intestine indicated by the relative weight and length reflects the physiological status and function of broiler chickens (Zhong et al., 2019). The size of broiler chicken's intestine including its length and weight have been used as indicators of digestive and absorptive capacities (Gao et al., 2010; Li et al, 2018). Furthermore, increased intestine length has been suggested to improve absorption of nutrients (Wang et al., 2015). In this study, intestinal relative length and weight decreased as levels of dietary FPM increased. This finding contrast with Naji et al. (2016), who showed that the length and weights of the digestive tract increased in broiler chickens fed fermented corn-soybean feeds. Furthermore, one factor that may have contributed to the small intestine length and weight is the fiber content (Jørgensen et al., 1996; Al-Marzooki et al., 2000). Recent studies have shown that high fibre content in diet of broiler chickens may promote the absorption of nutrients by the intestinal segments, which ultimately stimulate the intestine (Alyileili et al., 2020; Zhang et al., 2023). However, natural fermentation process reduces the crude fiber of grains (Akinola et al., 2017). In this study, the decreased intestinal length may be an adaptive response associated with the fiber content or small size of millet grain in the diet. However, reduction in length and weight of small intestinal segments as dietary FPM increased, did not negatively impact growth performance, carcass characteristics and organ weights, which suggest that nutrient absorption and availability were not impaired in this study.

Intestinal morphology greatly influences growth performance, nutrient digestion and utilization of broiler chickens (Ravindran and Abdollahi, 2021). In the present study, duodenum villus height, jejunum villus height, and jejunum crypt depth were not affected by dietary FPM. However, FPM significantly increased the duodenum villus width. Feng et al. (2007) showed that fermented commercial feeds improved the structure of intestinal morphology of broiler chickens. In addition, Naji et al. (2016) reported that villus height, crypt depth and their ratio increased for broiler chickens on fermented commercial feed of maize-soybean. Previous study reported that fermented cottonseed meal improved villi structure in duodenum and jejunum of broiler chickens (Jazi et al., 2017). The contrast in our study compared to other reports may be due to grain characteristics or the method of fermentation process.

Low intestinal pH is regarded as an important barrier to prevent significant colonization of pathogenic microorganisms in the intestine (Abouloifa et al., 2020). Reduction in digestive pH attributed to acidifying effect of fermented feeds provide favorable environment to increase colonization of lactic acid producing bacteria in broilers (Wyszyńska and Godlewska, 2021; Peng et al., 2022). Yaşar et al. (2016) showed that fermented feed encouraged establishment of barriers against pathogens through reduction of pH in the upper intestinal tract. Lin and Lee (2020) reported that wheat bran fermented with *Lactoporus sulphureus* reduced digesta pH in ileum and cecum of broiler chickens. In the present study, FPM in diets reduced digesta pH in the jejunum. The jejunum, the longest segment of the intestine is considered highly efficient in absorbing nutrients (Liu et al., 2021). Vicentini et al. (2021) showed that regulation of structure and function of the intestinal tract is controlled by the intestinal microbiota. FPM had no impact on concentration of caeca bacteria including *Lactobacillus and Salmonella*. Lv et al. (2022) similarly showed that the predominant bacterial species was not impacted in laying hens fed fermented diet. The activity of microbiota within the intestine played a critical role in growth performance and health of broilers through modulating the growth of pathogenic

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bacteria (Niba et al., 2009). Hence, the fact that FPM did not affect caeca microbial count may partly explain the lack of compromise in growth and FCR of broiler chickens.

CONCLUSION

In conclusion, BWG and FCR were not affected by replacement of maize with FPM although more feed was consumed. Weight of bursa of Fabricius and duodenum villus width improved on treatment diets. In addition, FPM reduced length and weight of small intestine but increased LDL without undermining growth performance, carcass traits, gut morphology, and beneficial microbiota. Based on the results on growth performance, physiological responses, and intestinal morphology, it can be recommended that, partial replacement of maize with FPM can be used as alternative to maize in the diets without compromising the overall performance of broiler chickens.

DECLARATIONS

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Consent to publish

Not applicable.

Author's contribution

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

Competing interest

The authors declare that there is no competing interest to this research and publication.

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NUTRIENT DIGESTIBILITY OF FIBROUS FEEDSTUFFS IN HIGH-CONCENTRATE DIET WITH SODIUM-BICARBONATE ($NaHCO_3$) ADDITION IN RUMEN-FISTULATED BRAHMAN BULL

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ABSTRACT: Ruminants are given substantial quantities of concentrate diets full of quickly fermentable carbohydrates to increase output performance; however, it can also lead to digestive disorders. This study aimed to investigate the effect of adding $NaCHO_3$ to a high-concentrate diet on the nutrient digestibility of locally available fibrous feedstuffs in the Philippines. The experiment utilized a rumen-fistulated Brahman bull. The treatment diets were the following: Treatment 1 (T1): Untreated rice (*Oryza sativa* L.) straw; T2: Urea-treated rice straw; T3: Napier grass (*Pennisetum purpureum* Schumach); T4: Napier silage; T5: Sugar cane (*Saccharum officinarum* L.) tops; and T6: Cogon grass (*Imperata cylindrica* L.). The nylon bags containing the treatment diets were incubated in the rumen at two periods: first at a high-concentrate (70% level), and second at a high concentrate added with NaCHO3). The results showed that the nutrient digestibility of locally-available feedstuffs varies significantly ($p<0.05$) both with and without $NaHCO_3$. The addition of $NaHCO_3$ in a high-fiber diet improves the digestibility of locally available fibrous feedstuffs in terms of dry matter (DM), organic matter (OM), and neutral detergent fiber (NDF). Therefore, the addition of NaHCO3 to a high-concentrate diet has the potential to positively stabilize rumen pH and enhance the nutrient digestibility of locally available fibrous feedstuffs.

Keywords: Fibrous feedstuffs, *in situ* digestibility, Nutrient digestibility, Rumen, Sodium bicarbonate.

INTRODUCTION

The Philippines provides inexpensive sources of ruminant feeds based on its favorable climate for fodder and other fibrous feedstuffs (Van Hung et al., 2017). One agriculture by-product that can be utilized as feed is the rice straw (Van Hung et al., 2017; Zaghloul et al., 2018). Rice straw is known to have a low nutritional value (Vadiveloo, 2000), but when properly managed and supplemented, it can contribute to the diet of ruminants (Aquino, 2020). According to recent studies, rice straw contains on average 7-10% crude protein, 30-40% of digestible fiber, and provides about 6.7 MJ/kg of metabolizable energy (Devendra and Sevilla, 2002). The rice plant's (*Oryza sativa*) vegetative portion, cut during or after grain harvest, is known as rice straw. It can be incinerated and left on the rice field's surface before plowing, tilled into the ground to enhance the soil, or used as livestock feed (Kadam et al., 2000). In regions where rice is grown, rice chaff is a common forage. Rice straw's nutritional worth can be increased by treating it. These therapies aim to improve feed consumption and digestibility. Treatments involving mechanical, chemical, heat, and pressure can improve solubility. Napier grass, also known as Elephant grass (*Pennisetum purpureum*), is a prominent forage crop in the Philippines due to its high yield and resilience to various climatic conditions. Elephant grass, noted for its high fiber content, exhibits significant variations in neutral detergent fiber (NDF) concentrations ranging from 55% to 75% on a dry matter (DM) basis contingent on the stage of maturity. Due to its remarkable productivity, Napier grass is a crucial forage in the tropics. It is exceptionally well adapted for feeding buffalo and cattle. Napier grass is typically fed in stalls, made to silage or hay, or used in cut-and-carry methods (also known as "zero grazing"). A potential feedstuff for ruminants, especially areas with vast sugarcane plantations, is the sugarcane (*Saccharum officinarum* tops). A hectare of sugarcane can yield 11 to 21 tonnes of leaves and stalks, which are farm wastes (DA-Philmech). However, the majority of the crop residue from sugarcane fields is either burned or is allowed to decompose in the field, which may increase global warming.

Sugarcane tops can be used raw, dried, or ensiled for feeding animals. They have a wide range of nutritional value, which is affected by pre-harvesting methods, the stage at which the stem is removed, plant maturation, and the volume of dry leaves (McKenzie et al., 2007). Sugarcane forage, characterized by its high energy content, tends to offer optimal nutritional value during dry periods (Archimède et al., 2011). However, its nutritional profile is marked by low protein, mineral, and vitamin contents, making it a fibrous material of considerable bulkiness. Cogon grass (Imperata cylindrica) is a widespread weed that thrives throughout the Philippines. Cogon grass (*Imperata cylindrica*) can support an effective extensive livestock production system if they are given sources for energy, urea, and minerals (Falvey, 1981). Subsistence farmers keep the majority of the ruminant stock in the Philippines. So, feeding is mainly dependent on fibrous crop

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residues that are readily accessible or on any grasses on the farm, as well as grazing on unused land or shared pastures. Because concentrate is infrequently used in the country, animal productivity could be much higher. Increasing the number of concentrates in ruminant diets might improve production efficiency while utilizing the locally and economically available for ages.

Ruminants such as beef and dairy cattle are frequently given substantial quantities of concentrate diets full of quickly fermentable carbohydrates to increase output performance and financial returns (Klevenhusen and Zebel, 2021; Wang et al., 2023). In contrast to forages, concentrates feature low-fiber, high-energy diets with variable protein levels (Gallo et al., 2019). They are typically given to dairy cattle to increase the feed energy level and compensate for any deficiencies not covered by the forage part of the diet. While concentrated feed can significantly increase overall production, it also has adverse health effects. If the proper measures are not taken, a high-concentrate diet can cause digestive disorders like subacute ruminal acidosis (SARA) in cattle. Due to the buildup of the VFA and lactic acids in the rumen, a large amount of concentrate typically results in a temporary drop in the ruminal pH after feeding (Nocek and Rusell, 1998).

Sodium bicarbonate, represented by the chemical symbol NaHCO3, serves as a standard exogenous buffer. It plays a crucial role in normalizing the ruminal pH in cattle that might be susceptible to ruminal acidosis. In feedlots, the inclusion of sodium bicarbonate in diets is a response to the drastic dietary shift from highly fibrous feed to high-grain (carbohydrate) diets, which is associated with a decline in rumen pH, leading to rumen acidosis. However, sodium bicarbonate's effect on nutrient digestibility and buffer in the rumen environment varies. Additionally, it was not recorded how $NaHCO_3$ affected the intestinal digestibility of locally available feedstuffs in high-concentrate feeding. Therefore, the objective of this study was to investigate the effect of the addition of $NaCHO_3$ to a high-concentrate diet on the digestibility of dry matter (DM), organic matter (OM), and neutral detergent fiber (NFD) of locally available fibrous feedstuffs.

MATERIALS AND METHODS

Ethical consideration

All the procedures in caring for animals in this study followed the Animal Welfare Act 8485 of the Philippines. The methods of this study were approved by the Institutional Animal Care and Use Committee (IACUC) of the Visayas State University.

Experimental animal

All the procedures in caring for animals in this study followed the Animal Welfare Act 8485 of the Philippines. A rumen-fistulated Brahman Bull was dewormed using Ivermectin at a dose of 1ml/ 33kg LW prior to the conduct of the study. The bull was fed with the test diets for ten days as the adjustment period to allow the build-up of specific rumen microorganisms.

Treatments and experimental design

The experiment comprised six locally feedstuffs selected based on their fiber contents. The treatment diets were as follows: T1: Untreated rice (*Oryza sativa* L.) straw; T2: Urea-treated rice straw; T3: Napier grass (*Pennisetum purpureum* Schumach); T4: Napier silage; T5: Sugar cane (*Saccharum officinarum* L.) tops; and T6: Cogon grass (*Imperata cylindrica* L.).

The paddy rice straw (*Oryza sativa* L.) or the untreated straw for T1 was collected at Barangay Pangasugan, Baybay City, Leyte Rice Field. The collected feedstuff has undergone chopping using a mechanical chopper at approximately 3-4cm in length. For T2, the urea-treated rice straw has undergone (46%N) treatment. The straw was cut into 4cm lengths. The straw was moistened with a 3% urea solution. Forty to fifty grams of urea were dissolved in one liter of water and sprayed unto the rice straw for each kilogram. The treated rice straw was incubated for three days and dried for four to six hours for extended storage (Wanapat et al., 2009). The Napier grass (*Pennisetum purpureum* Schumach) for T3 was harvested at the Department of Animal Science at Visayas State University Forage Area. They were harvested at 45 days maturity and were chopped at approximately 3-4 cm length. Three weeks prior to rumen incubation is the preparation of Napier silage for T4. Napier grass was harvested after 45 days of re-growth and underwent chopping into 3-4 cm lengths. The small-scale silage was prepared by adding 4% molasses per fresh weight basis (Yokota et al., 1991) and was stored in a polyethylene bag. The silage was harvested at least three weeks (21 days) after the ensiling. The sugarcane (*Saccharum officinarum* L.) tops for T5 were collected at a plantation site at Ormoc City, Leyte. For animal consumption, the sugar cane tops were roughly chopped to a size of 3–4 centimeters, dried in the shade on a sheet of plastic, and then placed in a jute bag. For T6, Cogon (*Imperata cylindrica* L.) grass was harvested at the heading stage and has undergone drying to reduce moisture content, the chopped at 2-3 cm sizes.

The experiment was set up where the Nylon bags containing the test diets in six replicates each was incubated in a single rumen environment of a Brahman bull. This study was conducted using the Completely Randomized Design (CRD). The study was divided into two phases, phase 1 was the feeding of a high-concentrate diet (without $NaHCO_3$), and phase 2 was with the addition of $NaHCO_3$. The Brahman steer was fed ad libitum (5% feed allowance). The animal was fed a diet consisting of seventy percent (70%) commercial concentrate supplementation and thirty percent (30%) test diets at a dry

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matter DM basis. The test diets constituting 30% of the total ration comprised 5% Napier silage, 5% Napier grass, 5% urea-treated rice straw, 5 % untreated rice straw, 5% Sugar cane tops, and 5% Cogon grass.

Addition of $NaHCO_3$ to the diet

For the second phase of the study (Phase 2), 0.75% feed-grade sodium bicarbonate (NaCHO3) was added to the diet on a DM basis. The 0.75% addition on a DM basis was based on the recommendation of the study of Zinn, 1991.

Nylon-bag/In-situ/In-sacco experiment

The digestibility and degradability through in situ technique are widely used in several types of research to measure digestibility and evaluate feedstuffs (Hoffman et al., 2001). This method involves using small dacron/nylon bags and then inserted into the fistulated rumen of the experimental animal. The difference in the number of nutrients before and after incubation in the rumen is compared to the amount digested. The nylon bag experiment followed the schedule below:

Table 1- The *in-situ* digestibility schedule

Concentrate level	Day	Activity
Phase I (w/o $NaHCO_3$)	1-9	Adjustment period. Animal given basal diet, Napier grass soilage at *ad libitum* (giving 5% allowance for that day based on previous days voluntary intake), together with 70% commercial concentrate mixed with the test diets (constituting 30%).
	8-10	Daily monitoring of rumen pH at 2 hours after the morning feeding.
	9	Reduction of the total amount of feed given to 50-60% to accommodate the nylon bags containing the test diets.
	10	Incubation period. After being placed inside a lingerie bag with weights, the nylon bags containing the test diets were placed in the rumen for twenty-four (24) hours.
	11	Nylon bags were recovery from the rumen
Phase 2 (w/$NaHCO_3$)	11-17	Adjustment period. The animals were given the same type of diet, but with 0.75% $NaHCO_3$ in the diet.
	16-18	Rumen pH is measured every day two hours after the morning feeding
	18	Reduction of the total amount of feed given to 50-60% to accommodate the nylon bags containing the test diets.
	19	Incubation period. After being placed inside a lingerie bag with weights, the nylon bags containing the test diets were incubated in the rumen for 24 hours.
	20	Nylon bags were recovered from the rumen

To determine the initial DM and OM, the feed samples were oven-dried at 105℃ at 16hr and then weighed to determine the DM. The oven-dried sample was placed in a muffle furnace to determine the ash. The ash was subtracted from the DM content to obtain the OM. The feeds were ground into 2mm approximate sizes using the Wiley Mill by Thomas Scientific at the Department of Animal Science-Animal Nutrition Laboratory, VSU. The ground feedstuff samples were distributed into three replicates, weighing 5g each. The samples were placed in a nylon bag with a 5 × 10 cm dimension and pore size of + 53μ (Bar Diamond Lane, Parma, ID, USA). The nylon bags were incubated in the rumen of a cannulated Brahman steer with a body weight of 300kg for digestibility determination. After incubation, the nylon bags undergo 30-min washing in the running water and manual scrubbing until the water becomes clear. The washed nylon bags will be oven-dried under 65℃ for 48 hours and will be weighed to obtain the DM content.

Rumen pH

The rumen pH was determined by collecting a rumen fluid sample from the cannulated rumen and reading the pH value through a digital pH meter.

In situ nutrient degradation, %

The *in-situ* degradability of nutrients such as DM, OM, NDF from the samples incubated was calculated using the following formula:

$$\%\text{Nutrient Degradation} = \frac{\text{Amount before incubation} - \text{Amount after incubation}}{\text{Amount before incubation}} \times 100 \quad (1)$$

Where: Amount of nutrient before incubation = dry weight inside nylon bag after incubation x % nutrient of residue

Statistical Analysis

The collected data were analyzed by one-way analysis of variance (ANOVA), and treatment means were compared by Tukey's HSD test using the Statistical Package for Social Sciences (SPSS) version 26 software. A paired sample T-test was used to determine differences in treatment means between with and without $NaHCO_3$ addition.

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RESULTS AND DISCUSSION

Changes in the Rumen pH

The rumen pH from both treatments with and without $NaHCO_3$ gradually increases along with the sampling day (Table 2). It was clear that adding $NaHCO_3$ to a high-concentrate feed in a Brahman bull mitigates its effects. The diet without adding $NaHCO_3$ yields a pH of 5.75 which inclines to the acidity level, while the diet with $NaHCO_3$ has a mean of 6.49. Generally, the rumen pH under a high concentrate diet w/out the $NaHCO_3$ addition was 5.75, which falls below the optimum pH level for fiber digestion between 6.0-6.4 (Antanaitis et al., 2020). In a 2016 study by Sato, feeding Holstein cattle a high-concentrate diet reduced rumen pH and increased VFA concentration, indicating a negative correlation among rumen pH and VFA content. Rapidly increasing the proportion of concentrate in the feed (from 40% to 90% on a dry matter basis) led to a low pH level (Bevans et al., 2005). In this study, adding $NaHCO_3$ resulted in a pH level of 6.24, which falls to the optimal level for fiber digestion. At this pH level, cellulolytic microbes can digest fibre effectively. They are, however, inhibited at pH levels below 6.0 (AlZahal et al., 2009). However, the result in this study was contrary to the study of Rogers et al., 1985 by which the NaCHO3 addition has the lowest ruminal pH. The result was due to a higher feed intake and higher concentration of volatile fatty acids (VFA). These reactions might cause the rumen pH to decrease.

Table 2 - Changes in Rumen pH under a high-concentrate diet with and without $NaHCO_3$ addition

Day of sampling	w/o $NaHCO_3$	w/ $NaHCO_3$	Difference[1] (p-value)
1	5.90	6.12	0.427
2	5.73	6.25	0.385
3	5.62	6.36	0.049
Mean	5.75	6.24	0.259

[1]Paired-samples T-test; p<0.05=significant, p>0.05=not significant

Dry matter digestibility

The percentage of dry matter in the feed that an animal digest is known as dry matter digestibility. Table 3 presents the dry matter digestibility in the rumen under a high-concentrate diet with and without $NaHCO_3$ addition. It can be observed that Napier grass (T3) has the highest DMD of 43.67 under a higher concentrate diet alone. The urea-treated rice straw followed it with a DMD of 26.30, sugarcane tops at 22.87%, Napier silage at 21.53%, rice straw at 21.42%, and the least digested fiber was the cogon grass at 19.20%. On the other hand, the urea-treated rice straw has the highest DMD under the addition of $NaHCO_3$ in a high-concentrate diet. The addition of $NaHCO_3$ has generally increased the DMD across all fiber treatments. This means that adding $NaHCO_3$ improved DMD digestibility for locally able fiber feedstuffs. Moreover, significant differences (p<0.05) were observed in urea-treated rice straw (T2) and Napier silage (T3).

Table 3 - DMD (%) in rumen under a high-concentrate diet with and without $NaHCO_3$ addition

Treatment	$NaHCO_3$		Difference[2] (p-value)
	-	+	
T1 Rice Straw	21.42 ± 1.12^{b}	28.01 ± 1.25^{c}	0.571
T2 Urea-treated Rice Straw	26.30 ± 2.02^{ab}	62.81 ± 0.25^{a}	<0.001
T3 Napier grass	43.67 ± 0.56^{a}	38.53 ± 0.37^{ab}	0.400
T4 Napier Silage	21.53 ± 0.17^{b}	62.70 ± 1.85^{a}	<0.001
T5 Sugarcane Tops	22.87 ± 2.78^{b}	47.57 ± 0.34^{ab}	0.093
T6 Cogon grass	19.20 ± 0.65^{b}	31.30 ± 2.01^{c}	0.244
p-value[1]	0.16	0.003	

[1]One-way Analysis of Variance; p<0.05=significant, p>0.05=not significant; [2]Paired-samples T-test; p<0.05=significant, p>0.05=not significant

Although the Napier grass has the highest DMD among all fibrous feedstuffs at a high-concentrate level experiment (w/o $NaHCO_3$), it still appears to be lower than the DMD value of Napier which is 66% (Benedetti et al., 2008). Surprisingly, the Napier grass is higher than the Napier silage in terms of DMD, which contrasts with the study of Bureenok et al. (2012). The inclusion at an amount of 4% of molasses for silage making was insufficient to have an impact on digestion. Interestingly, when NaHCO3 was added in the high-concentrate diet, the Napier grass digestibility has reduced. Cogon grass (*Imperata cylindrica*) is a widespread weed that thrives throughout the Philippines. The inclusion of $NaHCO_3$ can help stabilize rumen pH, but its impact on DMD may be minimal, especially when it comes to high-quality forages like Napier grass. The inherent digestibility of Napier grass might not be significantly enhanced by the addition of $NaHCO_3$ or rumen pH alteration (Van Soest, 1994).

It can be observed that the urea-treated rice straw has a higher dry matter digestibility than rice straw alone for both treatments with or without $NaHCO_3$. The same results were also observed in the study of Asoy and Aban (2022), by which urea treatment on rice straw improves dry matter digestibility in goats. Rice straw's hemicelluloses-lignin complex swells

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due to ammonium hydroxide (NH4 OH) produced in UTRS (Mapato et al., 2010). These outcomes could explain how urea treatment improved rice straw rumen microbial degradation by increasing the accessibility of both cellulose and hemicellulose to the rumen microbes (Shen et al., 1999). Meanwhile, cogon grass had the lowest DMD due to its low DM content. *Imperata cylindrica*'s nylon bag digestibility was found to be 2/3 poorer than that of elephant grass (Pennisetum purpureum), buffel hay (*Cenchrus ciliaris*), and *Setaria sphacelata* (Holmes et al., 1980). Patiga et al. (2020) emphasized that to maximize utilization of cogon grass, it must be harvested early.

The improved dry matter digestibility (DMD) observed across various feedstuffs when high-concentrate diets are supplemented with sodium bicarbonate ($NaHCO_3$) can be attributed to the buffering capacity of $NaHCO_3$, which helps maintain optimal rumen pH levels. Rumen pH plays a crucial role in the activity and growth of different microbial populations involved in fiber and carbohydrate breakdown.

Fiber-digesting microbes, such as cellulolytic bacteria and protozoa, thrive optimally in a ruminal pH range of 6.0-6.4 (Antanaitis et al., 2020). These microbes are responsible for breaking down complex fiber components like cellulose and hemicellulose into simpler compounds that can be further fermented and utilized by the animal. By buffering the rumen pH, $NaHCO_3$ helps create an environment that is favorable for the growth and activity of these fiber-digesting microbes, thus enhancing the breakdown and utilization of fibrous feedstuffs (Nagaraja and Titgemeyer, 2007). By ensuring that the rumen pH remains within the appropriate ranges for both fiber and carbohydrate breakdown, $NaHCO_3$ supplementation promotes efficient fermentation and digestion of feedstuffs. This, in turn, leads to improved dry matter digestibility across various feedstuffs in high-concentrate diets.

Organic Matter Digestibility

The percentage of organic material in the feed that appears to have been digested in the entire ruminant digestive system is known as organic matter digestibility. Measurements of the amount of energy available and an estimation of the microbial protein production in the rumen can be made using the digestibility of organic matter. In table 4, the addition of $NaHCO_3$ improved the OMD of all fibrous feedstuffs and resulted in significant differences ($p<0.05$) to the urea-treated rice straw (T2) and Napier silage (T4).

The high-concentrate diet without $NaHCO_3$ addition resulted in a significant difference ($p<0.05$) among all feedstuffs. The Napier silage (T4) had the highest OMD (71.40%), which is slightly higher than that Napier grass (T3) at 70.80% OMD. The urea-treated rice straw has an OMD of 60.02%, higher than the untreated rice straw (T1) of 54.38%. Moreover, almost the same results were observed when $NaHCO_3$ was added. Significant differences were also observed ($p<0.05$).

It is possible that the addition of $NaHCO_3$ to a high-concentrate feed improved the digestion of OM because more cellulolytic bacteria and ciliate protozoa were present overall (Santra et al., 2003). This would have improved the rumen's ability to process fibrous feedstuffs. Santra et al. (2003) found that groups given feed with high levels of $NaHCO_3$ (2.25 and 1.5%) exhibited higher levels of total protozoa in the rumen (P0.01) than those fed diets with a lower level (0.75%) of $NaHCO_3$. These findings are in line with the understanding that cellulolytic bacteria and protozoa play a significant role in fiber degradation and utilization in the rumen. By increasing their populations through the addition of NaHCO3, the breakdown and digestion of complex carbohydrates, such as cellulose and hemicellulose, are enhanced. This, in turn, improves the overall digestibility of organic matter in the rumen. The result of this study was in contrast with Philip (1983), who reported that treatment with $NaHCO_3$ at a dosage of 3% of the diet was unaffected by DM. However, the bioavailability of nitrogen did show some improvement.

Table 4 - OMD (%) in rumen under a high-concentrate diet with and without NaHCO3 addition

Treatment	$NaHCO_3$		Difference[2] (p-value)
	-	+	
T1 Rice Straw	54.38±2.38^{cd}	63.40±1.67^{c}	0.367
T2 Urea-treated Rice Straw	60.02±3.12^{bc}	72.13±4.12^{abc}	0.041
T3 Napier grass	70.80±2.67^{a}	80.90±2.57^{ab}	0.359
T4 Napier Silage	71.40±3.94^{ab}	83.47±5.34^{a}	0.005
T5 Sugarcane Tops	58.12±2.01^{bc}	67.12±2.12^{bc}	0.246
T6 Cogon grass	40.12±1.02^{d}	58.03±2.42^{c}	0.052
p-value[1]	<0.001	0.001	

[1]One-way Analysis of Variance; $p<0.05$=significant, $p>0.05$=not significant; [2]Paired-samples T-test; $p<0.05$=significant, $p>0.05$=not significant

Neutral Detergent Fiber Digestibility

Neutral detergent fiber digestibility (NDFD) accurately measures rumen microbes' capacity to ferment fiber. NDF digestibility more accurately predicts feed intake capability, net energy from metabolism (NE), and total digestible nutrients (TDN). Greater NDF digestibility will generally lead to higher forage and digestible calorie intakes. Table 2 shows that $NaHCO_3$ had increased NDFD of the fibrous feedstuffs than a higher-concentrate diet alone. The data showed significant differences ($p<0.05$) of NDFD with and without $NaHCO_3$ addition for rice straw (T1), Napier silage (T4), and Cogon grass.

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Significant differences ($p<0.05$) among fibrous feedstuffs were observed for both experiments with and without $NaHCO_3$ addition. The urea-treated rice straw (T2) was the highest on a high-concentrate diet, with an NDFD of 25%. At a high-concentrate level with $NaHCO_3$ addition, the Napier silage has the highest NDFD of 60.53%. Since the rumen pH was altered by $NaHCO_3$ addition, it directly affects NDFD. Because ruminal pH significantly affects microbial populations, it is believed to be a crucial element in the rumen's regular and stable operation.

Table 5. NDFD (%) in rumen under a high-concentrate diet with and without NaHCO3 addition

Treatment	$NaHCO_3$		Difference[2] (p-value)
	-	+	
T1 Rice Straw	9.12±0.70[a]	33.35±2.76[c]	<0.001
T2 Urea-treated Rice Straw	25.13±1.45[c]	50.94±3.78[b]	0.024
T3 Napier grass	20.50±0.63[c]	31.69±2.45[c]	0.417
T4 Napier Silage	22.20±1.67[c]	60.53±2.46[a]	0.002
T5 Sugarcane Tops	14.12±0.98[b]	25.21±1.03[cd]	0.036
T6 Cogon grass	9.03±0.05[a]	24.37±0.12[cd]	0.001
p-value	0.002	.007	

[1] One-way Analysis of Variance; $p<0.05$=significant, $p>0.05$=not significant; [2]Paired-sample T-test; $p<0.05$=significant, $p>0.05$=not significant

Dietary fiber digestibility in ruminants is influenced by various factors, including ruminal pH, the amount of concentrate in the diet, and the addition of sodium bicarbonate. When high concentrate diets are fed without sodium bicarbonate supplementation, lower digestibility rates have generally been observed. This finding is consistent with a recent study conducted by Niepes et al. (2023), which showed that increasing the amount of concentrate in the diet from 20% to 40% resulted in a decrease in ruminal pH, making it more challenging for neutral detergent fiber (NDF) to be effectively digested.

On the other hand, improvements in dietary fiber digestibility have been occasionally noted and have been attributed to the maintenance of a pH level in the rumen that is more favorable for cellulolytic microbes. Zebeli et al. (2008) suggested that variables influencing ruminant conditions, such as ruminal pH and the amount of digesta exiting the reticulorumen, can impact the extent of fiber degradation. A study by Yang et al. (2002) demonstrated that reducing the mean ruminal pH level to a range of 6.18 to 5.78, along with an increase in the rate of nutrient disappearance through the reticulorumen, led to a reduction in NDF breakdown in the intestines and overall digestive tract. Specifically, the breakdown of NDF decreased from 40% to 37% and from 52% to 41%, respectively.

However, there are contrasting results in the literature regarding the effect of sodium bicarbonate on fiber digestibility. Marden et al. (2008) found that the addition of NaHCO3 to the diet resulted in a decrease in fiber digestibility. This finding aligns with the claims made by Mould and Orksov (1983), suggesting that in animals fed a high-concentrate diet, pH buffering with bicarbonate only partially restored ruminal cellulolytic activity.

CONCLUSION

This study investigated the effect of adding sodium bicarbonate ($NaHCO_3$) to a high-concentrate diet on the digestibility of locally available fibrous feedstuffs in ruminant animals. The results showed that NaHCO3 supplementation had a positive impact on rumen pH, dry matter digestibility (DMD), organic matter digestibility (OMD), and neutral detergent fiber digestibility (NDFD) of the tested feedstuffs. The addition of $NaHCO_3$ helped stabilize rumen pH, which is essential for maintaining optimal microbial activity in the rumen. The improved rumen pH led to increased DMD, OMD, and NDFD, indicating enhanced nutrient utilization and digestibility of the fibrous feedstuffs. This finding suggests that $NaHCO_3$ supplementation can effectively improve the utilization of locally available feed resources, which is particularly beneficial in areas where fibrous crop residues and grasses are the primary feed sources for ruminant animals. Among the tested feedstuffs, urea-treated rice straw and Napier silage showed the highest improvements in DMD, OMD, and NDFD when $NaHCO_3$ was added to the high-concentrate diet. These results highlight the potential of these feedstuffs as valuable sources of nutrients for ruminant animals when supplemented with $NaHCO_3$.

The findings of this study suggest that $NaHCO_3$ supplementation in high-concentrate diets can play a significant role in improving the digestibility and utilization of fibrous feedstuffs in ruminant animals

DECLARATIONS

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Authors' contribution

Richelle A. Niepes performed the experimental trial, analysis, and writing of the manuscript while supervised by Lolito C. Bestil.

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Conflict of Interests
The authors have not declared any conflict of interests.

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ORGANIZATION OF HISTO-HEMATIC BARRIERS OF THE LIVER IN ANGLO-NUBIAN GOAT

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Supporting Information

ABSTRACT: The aim of this research was to establish features of the liver histo-hematic barriers ultrastructural organization of the Anglo-Nubian goat. The liver of an adult Anglo-Nubian goat was used as the material. The work was carried out using the electron microscopic method. Liver parenchymal tissue fragments were selected. These samples were fixed in a 2.0% glutaraldehyde solution on a cacodylate buffer for two hours. They were then washed in three portions of the same buffer and post-fixed in a 1.0% solution of osmium tetrachloride for one hour. The samples were then dehydrated in alcohols of ascending concentration and absolute acetone. The subsequent filling of the fragments was carried out in Epon-812. Ultrathin sections were obtained on an ultramicrotome, contrasted with a 2.0% aqueous solution of uranyl acetate and a solution of lead citrate. The ultrathin sections were photographed with a Jem-1011 electron microscope at magnifications of 2500-3000. Two histo-hematic barriers are detected in the liver of the studied animals hemato-hepatic and hepatobiliary. The hemato-hepatic barrier is formed by the plasmalemma of the apical end of the hepatocyte, covered by the glycocalyx, the perisinusoidal space of the Disse, the endotheliocyte of the sinusoid capillary, as well as Kupfer cells located in the lumen of the latter. The hepatobiliary includes all of the above structures, with the exception of Kupfer cells, as well as the plasmalemma of the basal end of the hepatocyte. All of the above structures in their organization have characteristic species features for Anglo-Nubian goats.

Keywords: Anglo-Nubian goat, Digestive organs, Hepatobiliary barrier, Hemato-hepatic barrier, Liver.

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INTRODUCTION

Mammals have numerous histo-hematic barriers (Wong et al., 2013; Mruk and Cheng, 2015). Due to their presence, the regulation of metabolic processes between blood and tissues is regulated, which ensures the constancy of the composition, as well as the physicochemical and biological properties of the tissue fluid (Alekhin et al., 2023). At the same time, histo-hematic barriers ensure the timely excretion of cellular metabolic products and also block the release of certain substances into the bloodstream (Baryshev et al., 2022; Drozdova, 2004; Drozdova and Kundryukova, 2010). The structure of histo-hematic barriers is distinguished by its specific features and is mainly determined by the structure of the organ (Akmalova et al., 2018; Ponamarev et al., 2022). It is known that the main element that is part of any histo-hematic barrier is the hemocapillary, whose endotheliocytes in different organs and tissues have characteristic morphological features. The study of structural features of histo-hematic barriers is extremely important for both theoretical and practical veterinary medicine (Prusakov et al., 2019; Kuznetsov et al., 2022). This is due to the fact that the most pathological processes basis, both at the organ and tissue levels, are structural changes in these systems (Yashin et al., 2021). Considering the foregoing, we set ourselves the task of determining the features of the histo-hematic barriers ultrastructural organization of the Anglo-Nubian goat liver.

MATERIALS AND METHODS

The material for the study was the liver of an adult Anglo-Nubian goat. Liver parenchyma tissue fragments, no larger than 2.0 mm³, were selected for electron microscopic examination. The selected samples` fixation was carried out in a solution of 2.0% glutaraldehyde in cacodylate buffer (pH 7.2-7.4) for two hours. Then they were washed out three times in the same buffer and post-fixed in 1.0% osmium tetroxide solution (prepared in cacodylate buffer, pH 7.2-7.4) for one hour. Then the samples were dehydrated in ascending alcohols and absolute acetone. The subsequent filling of the selected tissue fragments was carried out in Epon-812 according to the generally accepted method (Weekly, 1975). Ultrathin sections were obtained on an ultramicrotome (LKB-III - Sweden), contrasted with a 2.0% aqueous solution of uranyl acetate and a solution of lead citrate (Reynolds, 1963). The resulting ultrathin sections were photographed using a Jem-1011 electron microscope (JEOL, Japan) at magnifications of 2500–3000. The terminology used is in accordance with the International Histological Nomenclature (Semchenko et al., 1999).

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Ethical regulation of study

The studies were carried out in accordance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, the rules of Good Laboratory and Clinical Practice (GLP and GCP), as well as the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union dated 22 September 2010 for the Protection of Animals Used for Scientific Purposes. The study design was approved by the Bioethics Committee of the St. Petersburg State University of Veterinary Medicine.

RESULTS AND DISCUSSION

In the liver of the studied animal, two histo-hematic barriers are revealed – hemato-hepatic and hepatobiliary. It has been established that the hemato-hepatic barrier is formed by the plasmalemma of the hepatocyte apical end, covered with glycocalyx, as well as the endothelial cell of the sinusoidal capillary, and Kupffer cells (stellate macrophages) located in the lumen of the perisinusoidal space of Disse. The composition of the hepatobiliary barrier of the Anglo-Nubian goat includes all of the above structures, with the exception of Kupffer cells, and the plasmalemma of the hepatocyte basal end.

The cytoplasmic membrane of hepatocytes in the studied animals consists of two clearly defined layers - outer and inner. Between the layers, a light osmiophobic layer is clearly visible, the thickness of which varies within 2.0-3.0 nm.

The apical (vascular, sinusoidal) surface of the hepatocyte (Figure 1) faces the sinusoidal capillary (sinusoid). Between them, the perisinusoidal Disse space is determined. The cytoplasmic membrane of the cell apical part forms a multitude of short and long microvilli covered with a thin layer of glycocalyx. Short microvilli face the perisinusoidal space. Long microvilli penetrate this space and through the pores of endotheliocytes, forming the sinusoid capillary, follow into its lumen, where they are in direct contact with the blood.

The endothelial cells of the liver sinusoid capillaries have flattened nuclei (Figure 2). Their cytoplasm contains well-developed organelles (short channels of the granular endoplasmic reticulum, many ribosomes and polyribosomes, slightly rounded mitochondria, lysosomes, microvesicles), and microfilaments.

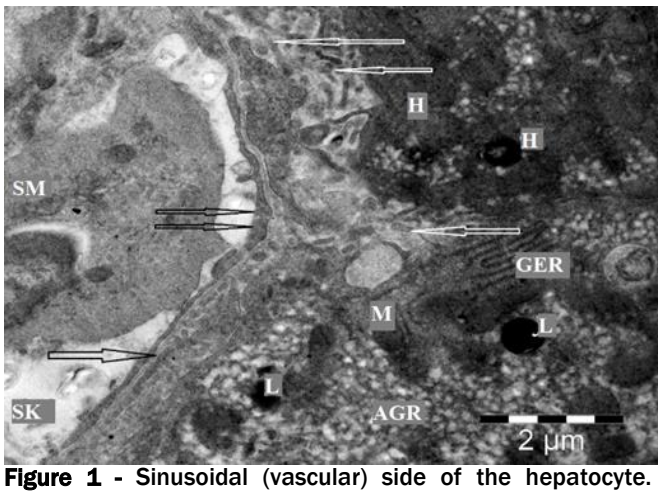

Figure 1 - Sinusoidal (vascular) side of the hepatocyte. Electron microphoto: H - sinusoidal surface of the hepatocyte; SC - sinusoidal capillary; M - mitochondria; GER, granular endoplasmic reticulum; AGR - agranular endoplasmic reticulum; L - lysosomes; SM - stellate macrophage; ↑ – Disse space; ↑↑ – Endotheliocyte process.

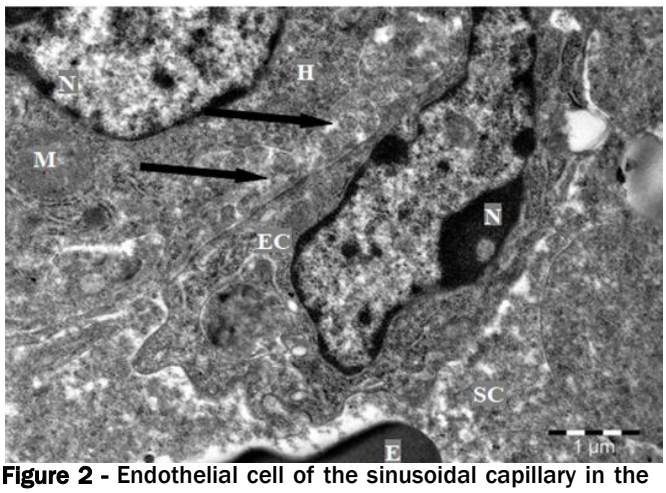

Figure 2 - Endothelial cell of the sinusoidal capillary in the liver. Electron microphoto: H - hepatocyte; N - nucleus; M - mitochondria; EC - endotheliocyte cytoplasm; ↑ – Disse space; SC - lumen of the sinusoidal capillary; E - erythrocyte.

Long processes of endothelial cells stretched along the hepatic plates are characterized by the presence of numerous pores - fenestra, through which there is a free exchange between blood and intercellular fluid (Figure 3). It should be noted that there is no basement membrane in the sinusoidal capillaries of the liver. In the lumen of sinusoidal capillaries in many parts of the liver, there are stellate macrophages (Kupffer cells), which are large process cells with high phagocytic activity (Figure 4). Their nuclei are large, sometimes elongated oval, sometimes with strongly indented edges. In the nuclei, a large amount of heterochromatin is determined, which lies on the inner karyolemma or is located throughout the nucleus in large, coarse lumps. At one end, stellate macrophages are attached in bifurcations between hepatocytes, and the cell body with processes most often lies freely in the sinusoid lumen (Figure 5).

Macrophages "purify" the blood brought from the portal vein from toxins, antigens, microorganisms, etc., therefore their cytoplasm contains many small dark lysosomes and heterogeneous residual bodies. The cytoplasm of hepatic macrophages appears optically dark due to the presence of a large number of ribosomes, polyribosomes, small granules, and various microvesicles. In addition, many rounded small mitochondria, a well-developed Golgi complex, and short cisterns of the granular endoplasmic reticulum are found in their cytoplasm. Sometimes there are fragments of obsolete erythrocytes and inclusions of hemosiderin.

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The opposite apical, biliary surface of the hepatocyte faces the bile capillary (Figure 6). In the area of the capillary mouth, the cell membranes of hepatocytes are firmly bound by dense osmiophilic compounds - desmosomes, which provide stable adhesion between cells. The sections of the cytolemma forming the bile capillary wall create pronounced intussusceptions and microvilli facing the lumen.

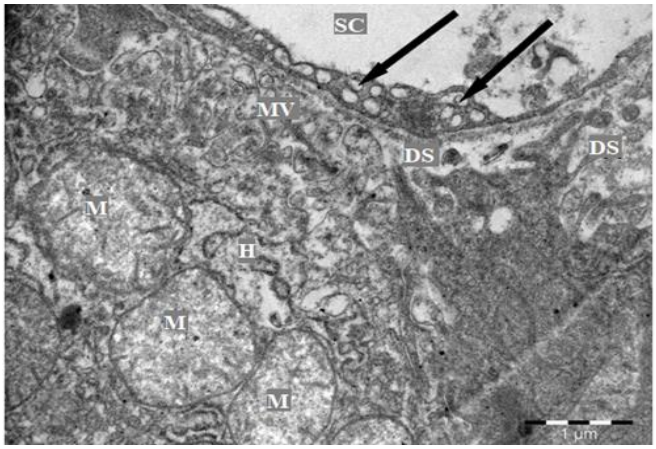

Figure 3 - Fragment of a hepatocyte and endothelial cell process of a sinusoidal capillary. Electron microphoto: H - hepatocyte; M - mitochondria; DS - Disse space; ↑ - pores of the endotheliocyte; SC - lumen of the sinusoidal capillary; MV - microvilli of the hepatocyte sinusoidal edge.

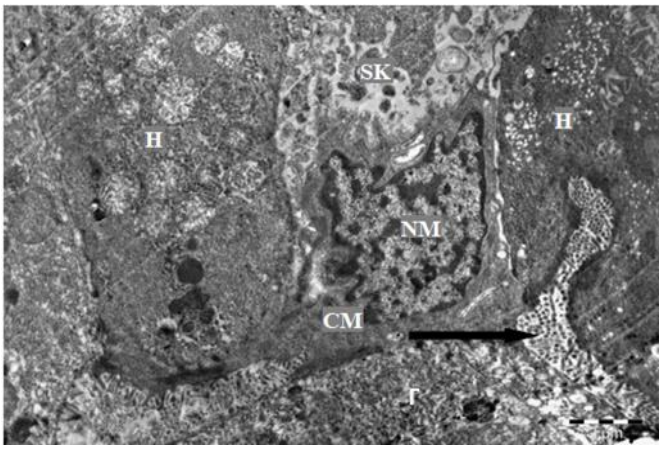

Figure 4 - Stellar liver macrophage. Electron microphoto: H – hepatocyte cytoplasm; NM - macrophage nucleus; CM - macrophage cytoplasm; ↑ - bile duct; SC - lumen of the sinusoidal capillary.

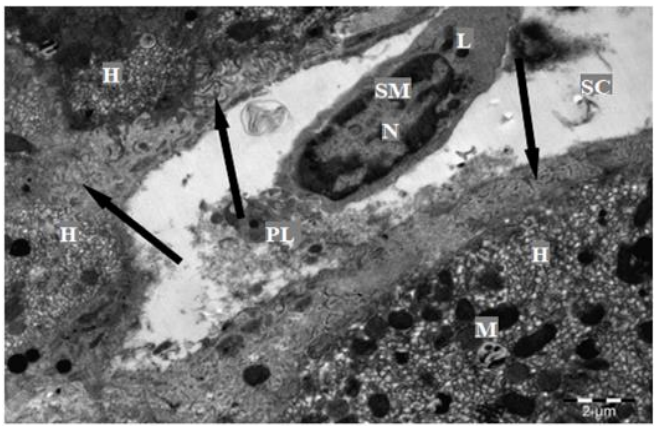

Figure 5 - Stellar liver macrophage. Electron micro-photo: H - hepatocyte; M - mitochondria; ↑ - hepatocyte microvilli in the Disse space; SC - lumen of the sinusoidal capillary; SM - stellate macrophage; N – a nucleus of a macrophage; PL - phagolysosomes in macrophage cytoplasm; L - lysosomes.

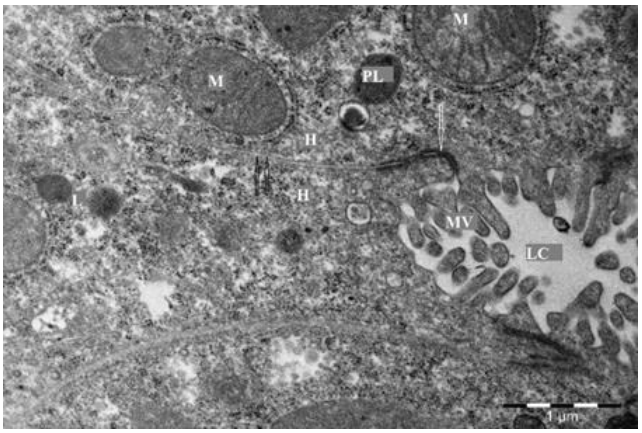

Figure 6 - Bile capillary. Electron microphoto: H - hepatocyte; M - mitochondria; L, lysosomes; PL - phagolysosome; LC - lumen of the bile capillary; MV - microvilli; ↑ - desmosome; ↑↑ – hepatocyte membrane.

CONCLUSION

In the liver of the studied animal, two histo-hematic barriers are revealed – hemato-hepatic and hepatobiliary. The hemato-hepatic barrier is formed by the plasmalemma of the apical end of hepatocytes covered with glycocalyx, as well as the endothelial cell of the sinusoidal capillary, and Kupffer cells (stellate macrophages) located in the lumen of the perisinusoidal space of Disse. The composition of the hepatobiliary barrier of the Anglo-Nubian goat includes all of the above structures, with the exception of Kupffer cells, and the plasmalemma of the basal end of hepatocytes. All of the above structures in their organization have characteristic species features characteristic for the Anglo-Nubian goat.

DECLARATIONS

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Authors' contributions
Zelenevskiy V. Nikolay, Yashin V. Anatoly: modelling and supervising all work, literature overview.
Prusakova V. Anna, Prusakov V. Aleksey: performing research, taking and preparing samples, article writing.
Ponamarev S. Vladimir: analyzing results, translating, editing.

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Competing interests
The authors declare that they have no competing interests.

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IMPACT OF CLIMATE CHANGE ON DAIRY MILK PRODUCTION IN NIGERIA

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study explores the impacts of climate change on milk production in Nigeria. Climate variables such as temperature, rainfall, sunshine, relative humidity and wind speed were considered as covariates in the analysis. Time-series data spanning a period of forty years obtained from the Central Bank of Nigeria and FAOSTAT database was used. The autoregressive distributed lag model was used to analyze both the short run and long run impacts of climate change on milk production. As expected, not all the variables were stationary at levels, but they were all significant at the difference suggesting the presence of cointegration. The result showed that the Bound's test F-ratio was statistically significant implies the existence of long run and short run relationships among the variables studied. Present findings revealed that temperature, rainfall and relative humidity had a negative impact on milk production, while sunlight recorded a positive impact on milk production both in the short run and long run estimates. The study concludes that milk production in Nigeria dropped as a result of climate change particularly rising temperature and prolonged rainfall. Agricultural climate smart practices were recommended to mitigate impact of climate change on milk production.

Keywords: Climate Change, Dairy Products, Production, Rainfall, ARDL model.

INTRODUCTION

On a global scale, milk production is done by over 150 million dairy homes. Smallholder dairy farmers produce milk in the majority of developing nations, and milk production has become a source of livelihood for most families around the world (Thitiya et al., 2020). From 530 million tonnes in 1988 to 843 million tonnes in 2018, the worldwide milk output has climbed by more than 59 percent (FAO, 2023). The United States of America, China, Pakistan, and Brazil are the next five countries in order of milk production output, each producing 22% of the world's total. South Asia has been the primary driver of milk production increase in the developing world since the 1970s, where production has increased rapidly (FAO, 2023). Germany, France, Australia, Ireland, New Zealand, and the United States of America have the greatest milk surpluses (OCED and FAO, 2020) while the nations with the greatest milk shortfalls are China, Italy, Russia, Mexico, Algeria, and Indonesia (Jeffrey, 2022). In many developing nations, milk productions has been limited by various factors such as health of dairy farmers, capital, and changing weather conditions combined with low genetic potential of dairy animals (Duguma, 2022). Contrary to developed nations, a large number of developing nations have hot and variant weather conditions that are unfavorable for milk production. Sudan, South Africa, Kenya, and Ethiopia are the nations in Africa that produce the most milk (Mebrate et al., 2019). Though, due to poverty and difficult climatic circumstances in Africa, milk production is experiencing a slow downturn. Nigeria produces just between 560,000 and 570,000 tonnes of fresh milk annually, against the expected industry and domestic consumption and market demand of 1.7 million tonnes (Elekwachi et al., 2021).

According to literature, In Nigeria milk production is synonymous with the northern nomads/herdsmen. The decrease in its production in Nigeria is attributed mostly to climate change and other internal and external factors (Elekwachi et al., 2021, FAO, 2023). Milk production in dairy cows is reduced as a result of heat stress brought on by excessive heat and humidity (Abbas et al., 2019, Hossain et al., 2023). The discomfort and physiological changes that result from temperatures rising above a cow's thermo neutral zone causes the animals to produce less milk (Bhimte et al., 2021, Chawicha and Mummed, 2022). Extreme heat and higher temperatures are difficult for dairy cows to tolerate, and as a result, their milk production is frequently reduced and they become more susceptible to diseases and other health issues (Das et al., 2016). Excessive rainfall subjects the dairy farm animals to extreme cold causing abnormal body functioning and inhibits fodder availability leading to a fatal drop in animal body weight and milk production (Hoffmann, 2013; hang-Fung-Martel et al., 2017). This in turn truncates the profits of dairy farmers who depend on it for economic

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livelihoods and human sustenance. It is in the light of these circumstances that Nigeria spends over 28 billion in milk importation in 2022 (NBS, 2023). These events created the gap in knowledge and led to the conceptualization of the study to ascertain the true impacts of climate change on dairy milk production in Nigeria.

MATERIALS AND METHODS

This study employed time-series data (1981–2021) from two databases, FAOSTAT and the Statistical Bulletin of the Central Bank of Nigeria. The choice of the dependent and independent variables was influenced by the data that were available. The Central Bank of Nigeria Statistics Bulletin was specifically used to acquire the quantity of dairy milk produced, and the FAOSTAT database was used to obtain the climatic parameters. Table 1 displays specifics about the information and its sources. The effect of climate change on dairy milk production in Nigeria was examined using an autoregressive distributed lag (ARDL) model. The ARDL can simulate the effects of the dependent variable and independent variables over the short and long terms. It can also use the F-statistic to test for cointegration between the variables at the first and second levels. The ARDL Bound's test is typically used for cointegration tests. it's crucial to run unit root tests on time-series data to find out whether the dataset in question is stationary or has a unit root. In this study, the unit root tests were performed using the Augmented Dickey Fuller (ADF) and Phillips-Perron tests. Our model was further put to the test for missing variables, autocorrelation, homoscedasticity, heteroskedasticity, multicollinearity, and parameter stability using the LM test, F test, DW test, Ramsey reset test, White test, ARCH test, Variance Inflation Factor, and Cusum test. The amount of dairy milk produced for the research is the dependent variable, while the independent variables are mean temperature, total rainfall, sunshine, relative humidity, and wind speed. The implicit model of our autoregressive distributed lag framework is stated as follows:

$Y = f(X_1, X_2, X_3, X4, X_5, e_t)$ — eqn. 1

Where, Y = Quantity of dairy milk produced (tons); X_1 = Mean temperature (^{0}C); X_2 =Total rainfall (mm); X_3 = Sunlight (hours); X_4 = Relative humidity (%); X_5 = wind speed (km/h); e_t = error term

Transforming eqn.1 into natural logarithms, thus:

$LnY = LnX_1 + LnX_2 + LnX_3 + LnX_4 + LnX_5 + et$ eqn. 2

$LnY = LnQDMP = \beta_0 + \beta_1 LnX_1 + \beta_2 LnX_2 + \beta_3 LnX_3 + \beta_4 LnX_4 + \beta_5 LnX_5 + e_t$ eqn. 3

The ARDL model specification of equation (3) is expressed as unrestricted error correction model (UECM) to test for cointegration between the variables under study, this is specified as follows:

$\Delta LnQDMP_t = \phi_o + \sum_{i=0}^{t} \phi_1 \Delta \mathrm{LnQDMP}t - 1 + \sum_{i=0}^{t} \phi_2 \Delta \mathrm{LnX}_1 t - 1 + \sum_{i=0}^{t} \phi_3 \Delta \mathrm{LnX}_2 t - 1 + \sum_{i=0}^{t} \phi_4 \Delta \mathrm{LnX}_3 t - 1 + \sum_{i=0}^{t} \phi_5 \Delta \mathrm{LnX}_4 t - 1 + \sum_{i=0}^{t} \phi_6 \Delta \mathrm{LnX}_5 t - 1 + \beta_1 LnX_{1t-1} + \beta_2 LnX_{2t-1} + \beta_3 LnX_{3t-1} + \beta_4 LnX_{4t-1} + \beta_5 LnX_{5t-1} + e_t$ eqn. 4

Once cointegration is established, the long run relationship is estimated using the conditional ARDL model specified as:

$LnQDMP_t = \phi_o + \beta_1 QDMP_{t-1} + \beta_1 LnX_{1t-1} + \beta_2 LnX_{2t-1} + \beta_3 LnX_{3t-1} + \beta_4 LnX_{4t-1} + \beta_5 LnX_{5t-1} + e_t$ eqn. 5

The short run dynamic relationship is estimated using an Error Correction Model (ECM) specified as:

$\Delta LnQDMP_t = \phi_o + \sum_{i=0}^{t} \phi_1 \Delta \mathrm{LnQDMP}t - 1 + \sum_{i=0}^{t} \phi_2 \Delta \mathrm{LnX}_1 t - 1 + \sum_{i=0}^{t} \phi_3 \Delta \mathrm{LnX}_2 t - 1 + \sum_{i=0}^{t} \phi_4 \Delta \mathrm{LnX}_3 t - 1 + \sum_{i=0}^{t} \phi_5 \Delta \mathrm{LnX}_4 t - 1 + \sum_{i=0}^{t} \phi_6 \Delta \mathrm{LnX}_5 t - 1 + \Im ECMt - 1 + e_t$ eqn. 6

Where, Δ = First difference operator; Ln = Natural logarithm; φo = Constant term; φ1—φ5 = Short run elasticities (coefficients of the first-differenced explanatory variables); β1—β5 = Long run elasticities (coefficients of the explanatory variables); ECMt -1 = Error correction term lagged for one period; ʒ = Speed of adjustment; p = Lag length

Table 1 - Type of variable and data source

Variable	Source
Diary milk quantity	CBN
Temperature	FAOSTAT
Rainfall	FAOSTAT
Sunlight	FAOSTAT
Relative humidity	FAOSTAT
Wind speed	FAOSTAT

RESULTS AND DISCUSSION

Descriptive statistics of variables used

Table 2 presents the descriptive statistics of the variables used which comprises of the dependent and independent variables. From the Table, the mean diary of milk produced in Nigeria during the study period was 478.93 metric tons which is far less of 1.7 million metric tons expected per annum (Elekwachi et al., 2021). The minimum and maximum range further showed that Nigeria is producing less of its expected outcome. The mean temperature had a value of

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25.09^{0}C with a high standard deviation of 7.09 indicating that temperature was not normally distributed during the study period. Rainfall mean had a value of 999.03mm, which possibly indicates high showers of rainfall during the study period. Its standard deviation was 0.54 showing moderately distributed. Sunlight had a minimum and maximum value of 101.91 and 487.23 with a mean of 371.03, its maximum value reflects high intense of sunrays. The mean relative humidity was 71.03; this value indicates a relatively unfair distribution of relative humidity with its skewness and kurtosis values being negatively related. Wind speed average was 56.03 and ranges between 40.00 and 110.03 respectively.

Table 2 - Descriptive statistics of variables

Variable	N	Minimum	Maximum	Mean	Standard Deviation	Skewness	Kurtosis
Diary milk quantity	40	101.28	765.93	478.93	4.90	-0.57	-0.29
Temperature	40	22.09	28.05	25.09	7.09	-0.91	-0.45
Rainfall	40	671.01	1457.61	999.03	0.54	-0.48	0.12
Sunlight	40	101.91	487.23	371.03	2.94	0.45	0.31
Relative humidity	40	55.00	99.61	71.03	0.39	-0.25	-1.01
Wind speed	40	40.00	110.03	56.03	0.69	-0.81	-0.41

Unit Root Test using Augmented Dickey-Fuller and Phillips–Perron Test

The unit root test utilizing the Augmented Dickey-Fuller and Phillips-Perron tests are shown in Table 3. They were used to establish the stationary nature of the dependent and independent variables. The amount of dairy milk produced and temperature were not steady under the Augmented Dickey-Fuller test when integrated with order zero [I (0)]; this suggests the presence of the unit root that characterizes the null hypothesis. Yet, all of the variables became stationary at the first difference [I (1)], indicating that they were all integrated at order one. This supports the existence of the alternative hypothesis, which characterizes time series as stationary, and further refutes the null hypothesis. The amount of dairy milk produced, the temperature, and the amount of rainfall were also not stationary under the Phillips-Perron Test when integrated at order zero [I (0)], but became stationary at the first difference [I (1)]. The test for co-integration is justified since this suggests that the variables under consideration were not integrated in the same order under the Augmented Dickey-Fuller and the Phillips-Perron tests (Onyeneke et al., 2022).

Table 3 - Unit root test using Augmented Dickey-Fuller and Phillips–Perron test

Variable	At level I (0) *t*-statistic	Remark	At first difference I (1) *t*-statistic	Remark	Decision: *H0*	Order of integration
Augmented Dickey – Fuller test						
Y	-1.267	Non-Stationary	-2.679**	Stationary	Reject	I (1) at 5%
X_1	-1.309	Non-Stationary	-2.821**	Stationary	Reject	I (1) at 5%
X_2	-2.403**	Stationary	-3.853***	Stationary	Reject	I (0) at 1%
X_3	-3.462***	Stationary	-4.143***	Stationary	Reject	I (0) at 1%
X_4	-3.852***	Stationary	-3.426***	Stationary	Reject	I (0) at 1%
X_5	-4.934***	Stationary	-4.521***	Stationary	Reject	I (0) at 1%
Phillips – Perron test						
Y	-1.409	Not stationary	3.734***	Stationary	Reject	I (1) at 1%
X_1	-1.056	Not stationary	3.982***	Stationary	Reject	I (1) at 1%
X_2	-1.034	Not stationary	2.582**	Stationary	Reject	I (1) at 5%
X_3	-3.983***	Stationary	4.532***	Stationary	Reject	I (0) at 1%
X_4	-2.321**	Stationary	5.821***	Stationary	Reject	I (0) at 1%
X_5	-4.733***	Stationary	4.624***	Stationary	Reject	I (0) at 1%

** and *** indicate significance at 5% and 1% levels, respectively; *H0* = series have a unit root

ARDL-Bounds Test for Cointegration

Table 4 presents the ARDL-bounds test for cointegration. Cointegration is a vital method for analyzing time series data' long-term connections. Co-integration is used to look at non-stationary variables' correlations and the long-term effects of explanatory factors on dependent variables. The table demonstrates that the F-statistics for the dependent and independent variables were significant at the 1% and 5% level of probability for both the lower and higher boundaries. As a result, the alternative hypothesis, which indicates the presence of cointegration, was accepted and the null hypothesis of no cointegration was rejected. This further supports the existence of both short- and long-term relationships among the dependent and independent variables under investigation. According to the findings of (Gershon and Mbajekwe, 2020 and Emenekwe et al. 2022), even though these factors may diverge in the near term, their correlations are predictable over

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the long term. Thus, the estimation of an autoregressive distributed lag ECM model is justified by the presence of cointegration among the variables.

Table 4 - ARDL-bounds test for co-integration

Critical value	F-Statistic lower bound	F-Statistic upper bound	Remark
1%	3.06***	4.72***	Reject H0
5%	2.43**	2.08**	

Long and short run ARDL estimates on impact of climate change on diary milk production

Table 5 presents the ARDL estimates on impact of climate change on diary milk production. Temperature had a long-term negative correlation with milk production, according to the coefficient of temperature, which was negative and significant at the 1% level. Thus, a 1% rise in the average temperature will result in a 96% reduction in milk output. Cows that produce a lot of milk are sensitive to heat and tend to produce less milk as the temperature rises (Yano et al., 2014). Once more, dairy cows under heat stress have physiological body changes and decreased dry matter intake, which lower milk output. Rainfall had a negative coefficient that was statistically significant at the 1% level, suggesting that rainfall has a long-term negative impact on milk output. According to this, a change in the pattern of rainfall will result in a proportional decline in the output of dairy milk. Milk output is predicted to plummet by 85% as precipitation rises. Prolonged droughts are brought on by seasonal variations in rainfall patterns, which have a negative effect on the production of dairy milk. Increased infestations of cow diseases brought on by excessive rain damage the animals' health and result in a sharp decline in milk output (Somoza et al., 2018). Due to the animals' low health, this equally causes poor reproduction and abortion. Lack of rain hinders the optimal growth of dry matter feed or dairy fodder, which results in a shortage of feed and thus increases the mortality of calves and cows. These aberrations inevitably cause dairy cow animals to produce less milk. The correlation between sunshine and milk production is positive and significant at the 1% level, this suggests that a 1% increase in sunshine would result in a 76% rise in milk output. Cows that are exposed to sunshine react favorably to the physiological processes of the environment, increasing milk output. Sunlight aids dairy farm animals' physical development, which increases milk quality and output (Lim et al., 2021). Moreover, sunlight enhances dairy cows' consumption of dry matter and water, which improves the quality and amount of milk produced. The coefficient for relative humidity was negative and statistically significant at the 5% level, suggesting that relative humidity has a long-term detrimental impact on milk production. This implies that any rise in relative humidity will immediately result in a drop in milk output. Relative humidity increases the amount of microbial activity in dairy cows, which lowers milk quality and output. This is in conflict with Abbaya et al. (2022) and supports Bohmanova et al. (2007) and Xiaoyan et al. (2020). The results of the short-run ARDL estimates, however, were consistent with those of the long-run ARDL estimates, with the exception that the coefficient of wind speed turned positive and significant at the 5% level, which indicates that a 1% increase in wind speed will result in a corresponding increase in milk production of 71%. Wind speed reduces dairy cows' heat stress, keeping their ability to operate normally and maintaining a healthy balance, which results in the production of high-quality milk (Hill and Wall, 2015). The calculated error correction coefficient, which is -0.899, has the predicted sign, highly significant at the 1% level, and it suggests that equilibrium returns reasonably quickly following a shock. 90% of the disequilibria caused by the shock of the previous year converge to the long-term equilibrium in the current year. This further suggests that there was a considerable and steady adjustment process for the examined variables during the course of the relationship.

Table 5 - ARDL estimates on impact of climate change on diary milk production

Variables	Coefficient	T-values	Std. Error
Long-run Estimates			
LnX_1	-0.956	-3.939***	0.243
LnX_2	-0.848	-3.710***	0.228
LnX_3	0.758	4.081***	0.186
LnX_4	-0.934	-2.023**	0.462
LnX_5	-0.352	-1.028	0.342
Short-run Estimates			
ECM (-1)	-0.899	-4.921***	0.183
ΔLnX_1	-0.931	-3.201***	0.291
ΔLnX_2	-0.845	-3.011***	0.281
ΔLnX_3	0.671	3.610***	0.186
ΔLnX_4	-0.801	-2.220**	0.189
ΔLnX_5	0.710	2.205**	0.322
Constant	0.566	3.670***	0.154

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Diagnostic Statistical Test

Table 6 presents the diagnostic statistical test. The result of variance inflation factor (VIF) was less than 5; this indicates the absence of multicolinearity among the independent variables. LM test was not significant showing that no serial correlation exists among the explanatory variables. ARCH test and the white test values was not significant indicating the absence of homoscedasticity and heteroskedasticity among the variables. Ramsey RESET test shows that the model is free from omitted variables. R^2 value of 0.8902 indicates that 89.02% of the total variation in diary milk production was explained by the climatic variables investigated. F-statistic was highly significant at 1% level showing the overall fitness of the model. DW-Statistic value of 1.8063 was higher than the value of R^2 indicating that the result is not spurious and absence of absence of autocorrelation among the variables. Cusum test further confirmed the goodness of fit of the model used and establish that the econometric model is structurally stable (Onyeneke et al., 2022).

Diary milk production in Nigeria (1981 to 2021 in metric tons)

Figure 1 presents the trend analysis showing the trend pattern of milk produced in Nigeria during the period covered. It could be seen that milk production in Nigeria is unstable and fluctuates arbitrarily per year.

Diary milk consumption in the six geo-political zones in Nigeria

Table 7 presents the diary milk consumption in the six geo-political zones in Nigeria. The Table shows that a total of 266 litres of milk was consumed in the north with the north central having the highest consumption rate. While the south had a total of 302 litres with outstanding consumption in the south east. The total litres of milk consumed (568) in both the north and south regions implies that milk consumption in Nigeria is generally low probably due to climate change impacts on dairy cows (FAO, 2020).

Table 6 - Diagnostic Statistical Test

Diagnostic statistical test	Statistical values
VIF Test	2.903
LM Test	0.867
ARCH Test	0.762
White Test	1.045
Ramsey RESET test	1.221
R^2	0.8902
F-statistic	4.934
DW-Statistic	1.8063
Cusum Test	Stable

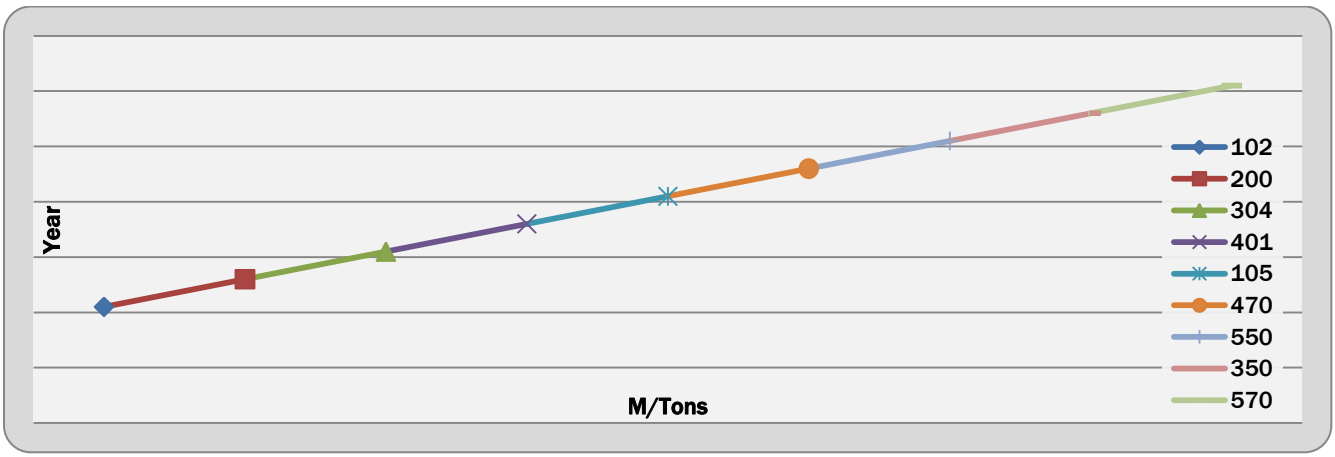

Figure 1 -Diary milk production in Nigeria (1981 to 2021 in metric tons)

Table 7 - Diary Milk Consumption in the Six Geo-political Zones in Nigeria

Zones	Diary milk consumption
North East	78
North Central	101
North West	87
South East	116
South South	104
South West	82

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CONCLUSION

The average amount of milk produced in Nigeria during the course of the study was 478.93 metric tons, far less than the 1.7 million metric tons anticipated annually. Climatic variables including temperature, rainfall, and relative humidity negatively impacted milk production both in the long and short run. Heat stress brought on by a rise in temperature causes dairy cows to consume less dry matter, which lowers milk quality and output. Prolonged droughts have a negative effect on the production of dairy milk. Increased infestations of cow diseases brought on by excessive rain damage the animals' health and result in a sharp decline in milk output. Low milk quality and quantity are caused by dairy cows' increased microbial activity when relative humidity is high. Cows exposed to sunlight retain healthy physiological attributes which improves dairy cows' milk output. The ECM value indicated the rapid response of milk production to climate change shocks. Increasing milk production in Nigeria to meet the market demand requires prompt and engaging actions in checkmating climate change.

DECLARATIONS

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Authors' contribution
E. Osuji = Conceptualization, model design, data analysis, section writing and proof-read.
C. Igberi = Data analysis, results editing, proof-reading and grammar checking.
C. Enyia = Conceptualization, result design, section writing, reference sorting and editing.
E. Nwachukwu = Questionnaire design, methodology design, section writing, editing and proof-read.
R. Nwose, A. Adeolu, A. Tim-Ashama, G. Nkwocha, A. Eleazar and D. Gabriel = Data collection, data processing, data curation, data sorting, and data coding.

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Conflict of interest
The authors declare that no conflict of interest exists.

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MORPHOLOGY CHARACTERISTICS COMPARISON OF F1 AND F2-BACKCROSS OF LOCAL AND PEKIN DUCKS IN INDONESIA

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The purpose of this study was to evaluate the comparison of morphology characteristics of two groups (the F1 and F2 backcross of Local and Pekin ducks at 25 weeks of age) in South Sulawesi Indonesia. The research material used 14 ducks males and 10 ducks females of F1 and 7 ducks males and 14 ducks females of F2-backcross. The data were measured on live weight, shank length, bill length, bill width, wing length, chest circumference, neck length, drumstick length, and thigh length. All mean differences of quantitative data from those two groups were analyzed using Independent T-test. The results showed that the performance of body dimensions of the F1 and F2-backcross of male and female ducks were relatively equal in performance concerning live weight, shank length, bill length, and neck length, respectively. The highest and positive correlation exists between shank length with chest circumference and drumstick (r=0.78) of F1 backcross female duck, between live weight with shank length (r=0.72) of F2-backcross female duck, between shank length with chest circumference (r=0.59) of F1 male duck and between live weight with chest circumference (r=0.84) of F2-backcross male duck, respectively. All measured variables had a coefficient of variation on both generations were less than 15%, except the bill width of the F1 male duck (41.79%) and both sex of thigh length of the F1 duck (24.68% and 23.68%, respectively).

Keywords: Breeding, Genetics, Morphology characteristic, Local duck, Pekin duck.

INTRODUCTION

Local ducks in Indonesia are one ancestor but there are several types of local ducks scattered throughout the archipelago with various names according to their respective regions or locations (Su, 2022). Several local duck breeds in Indonesia, namely the Alabio duck, Bayang duck, Magelang duck, Mojosari duck, Pegagan duck, Pitalah duck, Rambon duck, Tegal duck, and Turi duck (Hariyono et al., 2019). Those were included as important assets by the Indonesian Ministry of Agriculture and play an important role in a socio-economic aspect as they provide a livelihood to smallholders as well as food for humans. Each type of local duck has a very diverse variation. Phenotypic variation can be caused by uncontrolled crossbreeding, even though the parental generation is used to be one family (Besbes, 2009).

Many indigenous animal breeds, including local ducks from South Sulawesi Province Indonesia, still require scientific documentation and characterization to be conserved. As genetic resources, local ducks of South Sulawesi were kept traditionally and cultivated as dual-purpose ducks (as egg producer/laying type) and meat producers/meat type). The weakness of the local South Sulawesi duck is its relatively slow growth character, low live weight, and a large variety of performance compared with meat-type duck (Mahsyar, 2016). Genetic and morphometric diversity are important for breeding management and increasing the productivity of Local ducks of South Sulawesi. Unfortunately, there was limited information regarding the quantitative traits of Local ducks in South Sulawesi province.

There is traditional cuisine (*Nasu Palekko*) from South Sulawesi that uses the meat from rejected female or male of local duck as raw material. Therefore, the meat quality and quantity of Local ducks tend to be lean, tough, and less than optimal. On the other hand, *Nasu palekko*'s consumers love the distinctive smell and toughness of local duck meat (Bugiwati et al., 2021). The specific meat-type of local ducks from South Sulawesi are needed to meet the demand for meat in traditional culinary delights. There are some broiler ducks in Indonesia, such as the Pekin duck from China which is commonly known as a good meat-type duck and has the advantages of several characteristics (large size and body weight, fast growth, high carcass weight, and good carcass quality).

Unfortunately, the meat characteristics of Pekin duck are less favored by expert consumers (Bugiwati et al., 2021; Baéza et al., 2022). Therefore, it is necessary to have a new line of specific meat types of Local duck from South Sulawesi which has better meat quantity and quality, and good adaptability to tropical climatic conditions but still has the specific characteristics smell, and flavors like Local duck meat. It means that the genetic potential of local ducks in South Sulawesi needs to improve to have better meat quality and quantity that is more oriented towards meat-type ducks.

Citation: Bugiwati SRA, Dagong MIA, Rahim L, Malloangeng M, As A, Zulkifli M (2023). Comparison of morphology characteristics of F1-hybrid and F2-backcross hybrid of local and Pekin ducks in Indonesia. *Online J. Anim. Feed Res.*, 13(4): 253-258. DOI: https://dx.doi.org/10.51227/ojafr.2023.38

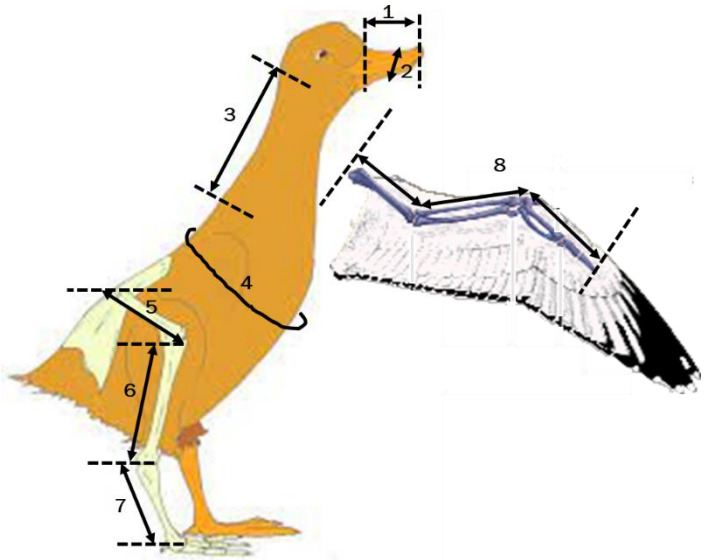
The first step that can be taken to develop the genetics of the Local duck of South Sulawesi is through exploration of the genetic resources of the Local duck. Crossbreeding and back-cross methods will be tried to improve the quality and quantity of Local ducks in South Sulawesi. A backcross hybrid is one method to improve growth and production potentials through the exploitation of heterosis besides forming a new composite meat-type duck line in South Sulawesi. A backcross hybrid is a progeny derived from F1-hybrid mating with a member of one of its parental species and forming an F2-backcross hybrid. Morphometric measurements of different generations can be used as study data for local ducks of South Sulawesi to know genetic characteristic differences inter-generational. The current trend in the improvement of Local ducks relies on the variation between and within breeds of certain traits (Maharani et al., 2019). The aim of this research was to investigate the morphological features of the F1-hybrid and F2-backcross hybrid of crossbreeding between Local with Pekin ducks under intensive management conditions. The study was also conducted to identify the level of phenotypic correlation that exists between the body weight and body measurement of two different generations (F1-hybrid and F2-backcross hybrid of crossbreeding between Local with Pekin ducks) at 25 weeks. The results of this study are expected to provide basic information for the possibility of developing new meat-type Local ducks of South Sulawesi.

MATERIALS AND METHODS

The data used for this study were progeny from Local and Pekin breeding ducks as a parental generation. The progenies of those parental generations (F1). Then the F1 mated back-cross with their parents (Peking ducks) and produced F2 offspring (F2-backcross). Finally, we used 24 ducks of F1 and 21 ducks of F2-backcross. Each generation was divided into two subgroups, with 14 ducks male and 10 ducks female of F1 and 7 ducks male and 14 ducks female of F2-backcross.

All ducks were reared under an intensive management system at Duck Research Center, Laboratory of Animal Breeding and Genetics, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia from July 2021 to September 2022. All ducks were kept in a colony cage and used separate fences between groups of ducks. The size cage is nine meters long, and six meters wide with 60 cm of insulated fence. All pens have a concrete floor which is covered by wood shaving and are provided with a feeding and watering space for each pen. Duck feed ration (for 100 kg) contains 33 kg pollard, 30 kg bran, 15 kg corn, 20 kg concentrate, and 2 kg mineral mix. The feed nutrition contents are crude protein min 16%, crude fat min 3%, crude fiber max 8%, calcium 4.25%, phosphor 1%, lysine min 0.70%, methionine min 0.30%, methionine + cysteine min 0,5% and tryptophan min 0.15%, respectively. The total feed base on the wet feed system was 200 g per bird/day and was provided two times a day. Throughout the study, ducks had unrestricted access to water.

The morphometric characteristics data were collected from the F1 and F2-backcross of Local and Pekin Duck. Live weight and all body measurements were always taken in the morning before supplying feed and water to make the homogeneity of data. The morphometric characteristic measured were shank length (cm), bill length (cm), bill width (cm), wing length (cm), chest circumference (cm), neck length (cm), drumstick length (cm), and thigh length (cm), respectively were measured on spot by using a standard measuring tape calibrated in centimeters with an accuracy of 1 mm. Ducks were individually weighed every two weeks with an electronic hook scale to within 5 g up to the 25th week of age. The anatomical reference points were shown in Figure 1.



1. Bill length
2. Bill width
3. Neck length
4. Chest circumference
5. Thigh length
6. Drumstick length
7. Shank length
8. Wing length

Figure 1 - Main measurement taken on duck

Statistical analysis

Arithmetic means and standard deviation of the mean (collectively for both groups) were calculated for each tested trait. In the first stage of the analysis, the Shapiro-Wilk test and Kruskal Wallis normality test were applied to the data set. The homogeneity test was analyzed with the Levene test. A coefficient of variation for the data on quantitative traits

Citation: Bugiwati SRA, Dagong MIA, Rahim L, Malloangeng M, As A, Zulkifli M (2023). Comparison of morphology characteristics of F1-hybrid and F2-backcross hybrid of local and Pekin ducks in Indonesia. *Online J. Anim. Feed Res.*, 13(4): 253-258. DOI: https://dx.doi.org/10.51227/ojafr.2023.38

at various duck populations was performed. The simple correlation (r) between live weight and body measurements was calculated. The differences between the mean values of all compared generations, as well as between males and females, were determined using Independent Sample T-test and be considered significant at $p<0.05$. SPSS package (version 25) was used for the statistical analysis. Animal care and maintenance were performed in accordance with the Protocol stated in the Republic of Indonesia's law (number 41-2014) regarding guidelines of Animal Welfare Standards for Research Treatment in Indonesia.

RESULTS AND DISCUSSION

Table 1 shows descriptive statistics for all morphometric characteristics of the male and female F1 and F2-backcross duck. F1 ducks were considerably significantly ($p<0.05$) and longer in bill length (female), wing length (male), chest circumference (male and female), drumstick length (male and female), and thigh length (male), respectively than F2-backcross duck. There were no marked differences in live weight, shank length, bill width, and neck length (both male and female) and for bill length (male), wing and thigh length (female), respectively of F1 and F2-backcross ducks.

Table 1 - Mean, standard of deviation, and coefficient of variation of morphometric traits of male and female F1 and F2-backcross duck (at 25 weeks of age).

Traits	Sex	Generation				Level of sig.
		F1[1]	CV (%)	F2-backcross[1]	CV (%)	
Live weight (kg)	M	1.54 ± 0.15 (14)	9.74	1.39 ± 0.18 (7)	12.94	NS
	F	1.38 ± 0.14 (10)	10.15	1.27 ± 0.13 (14)	10.23	NS
Shank length (cm)	M	5.98 ± 0.60 (14)	10.03	5.63 ± 0.48 (7)	8.53	NS
	F	5.59 ± 0.57 (10)	10.19	5.34 ± 0.67 (14)	12.56	NS
Bill length (cm)	M	6.76 ± 0.32 (14)	4.73	6.55 ± 0.59 (7)	9.01	NS
	F	6.55 ± 0.28 [a] (10)	4.28	6.25 ± 0.34 [b] (14)	5.44	*
Bill width (cm)	M	3.23 ± 1.35 (14)	41.79	2.70 ± 0.16 (7)	5.93	NS
	F	2.65 ± 0.24 (10)	9.06	2.63 ± 0.14 (14)	5.23	NS
Wing length (cm)	M	27.54 ± 2.05 [a] (14)	7.44	24.70 ± 2.75[b] (7)	11.13	*
	F	25.90 ± 3.19 (10)	12.32	25.03 ± 0.79 (14)	3.16	NS
Chest circumference (cm)	M	30.08 ± 1.54 [a] (14)	5.12	27.81 ± 1.18 [b] (7)	4.24	*
	F	28.57 ± 1.43 [a] (10)	5.01	26.96 ± 1.50 [b] (14)	5.56	*
Neck length (cm)	M	20.31 ± 2.79 (14)	13.74	19.44 ± 2.31 (7)	11.88	NS
	F	19.08 ± 1.36 (10)	7.13	18.21 ± 1.66 (14)	9.12	NS
Drumstick length (cm)	M	12.04 ± 0.57 [a] (14)	4.73	11.09 ± 0.79 [b] (7)	7.12	*
	F	11.60 ± 0.88 [a] (10)	7.59	10.39 ± 0.75 [b] (14)	7.22	*
Thigh length (cm)	M	9.36 ±2.31 [a] (14)	24.68	7.70 ± 0.71 [b] (7)	9.22	*
	F	8.53 ± 2.02 (10)	23.68	7.51 ± 0.84 (14)	11.19	NS

[1] values in the parentheses indicate the number of observations; [a,b]= Means within a column with different superscripts differ significantly (*p<0.05). NS= not significant; M: Male; F: Female; CV= Coefficient of Variation

Body dimensions will significantly determine the body size of the animal. Therefore, it can be used as a parameter in growth. Differences in body size parts in various breeds of ducks are influenced by the environment in which these ducks live and the genetic influence of each breed.

The average live weights were 1.54 kg of duck male F1, 1.39 kg of duck male F2-backcross, 1.38 kg of female duck of F1, and 1.27 kg of female duck F2-backcross, respectively. There are no significant differences between those generations. A similar result was reported on the Talang Benih female duck at 1.38 kg (Kususiyah and Desia, 2008). Hidayati and Desrita (2021) reported the results body weight of Sawang female duck (1.45 kg). El-Deghadi et al. (2022) reported a rather similar live weight (1.667 kg) of the Domyati duck breed at 20th weeks of age compared with male duck F1 at 25th weeks of age. The average live weight of F1 and F2-backcross ducks was lower than Morduzzaman et al. (2016) who reported average adult body weight of Nageswari duck was 1.66 kg in males and 1.51 kg in females.

In the present observation, the shank length of the F1 and F2-backcross was recorded as 5.98 cm Vs 5.63 cm (male duck) and 5.59 cm vs 5.34 cm (female duck), respectively. There are no significant differences between those generations. Shorter shank lengths were shown by a female duck (4.75 cm) of Sawang duck (4.38 cm; Hidayat and Desrita, 2021).

The average bill length was found to be 6.76 cm (male duck) and 6.55 cm (female duck) for F1 and 6.55 cm (male duck) and 6.25 cm (female duck) for F2-backcross. There are no significant differences between male duck grups. Ajit et al. (2009) reported relatively similar bill lengths in Chara duck (6.70 cm) and Chemballi ducks (6.80 cm). Matitaputty (2012) also found a long bill length of the Cihateup duck (6.79 cm). Ajit et al. (2009) showed that Chemballi female ducks have nearly similar bill lengths (6.30 cm) compared to the F2 backcross of female ducks. Matitaputty (2012) reported that Alabio male ducks (6.59 cm) have longer bill lengths than F1 (female) and F2-backcross (male and female). Shorter bill lengths were reported on the Nageswari male duck (5.87 cm) in Bangladesh (Morduzzaman et al, 2016). A

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similar finding of shorter bill length for Nageswari duck of Bangladesh (5.54 cm) (Morduzzaman et al., 2016), and Cirebon duck (5.55 cm) (Maharani et al., 2019), respectively.

Wing lengths of both F1 and F2-backcross ducks were shorter than those of the Cihateup duck (28.87 cm of males and 26.83 cm of females) (Matitaputty and Suryana, 2015), respectively. But the Cihateup female duck (21.6 cm) has a shorter wing length compared with F1 or F2-backcross. The chest circumference of the F1 duck is different from the F2-backcross duck (male of 30.08 cm vs. 27.81 cm) and female (28.57 cm vs. 26.96 cm). These results were lower compared to Magelang duck male (33.57 cm) (Rahayu et al., 2022)

In the present study, the average neck length (cm) recorded was no significant difference between the F1 and F2-backcross (male of 20.31 cm and 19.44 cm and female of 19.08 cm and 18.21 cm), respectively. Those results were shorter than the Cihateup duck male 24.36 cm and female 20.93 cm (Matitaputty, 2012), and the Cihateup duck male of 25.5 cm (Dudi, 2007), respectively. The overall mean value of neck length of female F1 and F2-backcross were longer than Alabio female duck (17.14 cm), Magelang female duck (14.83 cm), Rambon female duck (15.45 cm), Pegagan female duck (16.51 cm), and Pitalah female duck (16.40 cm), respectively (Maharani et al., 2019). The variation in bill length and neck length might be due to the differences in the breed. The neck length of the Indian local duck breed tended to be shorter than the Indonesian Local duck breed. Morduzzaman et al. (2016) found that the neck length of the Nageswari duck ranged between 23.46 cm (male) and 21.59 cm (female), which was longer than the current study. Matitaputty (2012) reported that Cihateup male ducks have longer drumsticks (12.32 cm) than those of the F1 and F2-backcross. However, both sexes of Cihateup duck show shorter thigh lengths (male of 6.45 cm and female of 7.26 cm) than those of the F1 and F2-backcross.

Our results revealed that generation and mating effects on some traits were significantly different (p<0.05). Many backcrosses are required to produce a new cultivar. The reciprocal crosses among breeds of ducks increased the morphometric measurements (Henrik et al., 2018). Ayorinde and Oke (1995) reported that variation in body weight within a flock can be attributed to genetic variation and environmental factors that influence an individual's performance. The differences in the value of morphometric characteristics might be due to the variation in the duck size, age, and conformation of the distinct variety of duck breeds, differences in genetic ability among those breeds include the effect of hybrid groups besides feeding and management practices. On the other, the difference in the body dimension of different varieties of indigenous local ducks might be attributed to the variation among indigenous germplasm and adaptability to the rearing environment.

All measured variables have a coefficient of variation is less than 15 % except bill width (male F1 of 41.79%) and thigh length (male and female F1 of 24.68% and 23.68%, respectively). These results show that the variance of traits is relative homogeny. This indicates that the size of all the variables is uniform. It is stated that it is recommended to do crossbreeding to improve the genetic quality and quantity of local ducks in South Sulawesi. The genetic diversity level will produce quantitative and qualitative phenotypes in ducks. This information can be used as one of the breeding decisions. Conventional duck breeding activities can be based on production performance related to certain phenotypic traits. The variation of a trait within a population could be the basis for the implementation of selection for the implementation of livestock breeding programs. Reciprocal crosses can trigger the emergence of phenotypic variations in the morphometric traits of ducks due to the heterosis results of reciprocal crosses (Henrik et al., 2018).

Correlations among live weight and some linear body measurements

The relationships existing among linear body measurements provide useful information on performance, productivity, and carcass characteristics. The phenotypic correlations between live weight and linear body measurement traits of F1 and F2-backcross of Local and Pekin ducks male and female at 25 weeks of age are presented in Table 2. The results of F1 male duck showed that chest circumference was significant (p<0.05) and positively correlated with shank length (0.59) and with drumstick length (0.57). Neck length was significantly (p<0.05) and negatively correlated with body length (-0.62) and drumstick length (-0.55). The results of the F1 female duck showed that shank length was high significantly (p<0.01) and positively correlated with chest circumference (0.78) and with drumstick length (0.78). Live weight was significant (p<0.05) and positively correlated with wing length (0.76), shank length with body length (0.65) and neck length (0.64), body length with wing length (0.64). The value obtains for the coefficient of correlation at 25 weeks of live weight and wing length agreed with literature values reported by Ologbose and Mbara (2020) using Muscovy duck at week 4 (0.71) and Mallard duck at 4 weeks (0.75), respectively. Significant (p<0.05) but negative correlation showed at live weight with shank length (-0.66) and highly significant (p<0.01) and negative correlation showed at neck length with drumstick (-0.77).

Positive and significant (p<0.05) correlation of F2-backcross female duck only existed between chest circumference with live weight (0.84). This result is in line with the report of Ologbose and Mbara (2020) who recorded positively high phenotypic correlation estimates in mallard ducks. Positive and significant (p<0.05) correlation of F2-backcross male showed at shank length with live weight (0.72), with body length (0.59), and with neck length (0.56). It was also observed that chest circumference was positively correlated with body length (0.58), and neck length with wing length (0.57). This positive and mostly significant phenotypic relationship between live weight and some body measurements indicates that an improvement in one trait could lead to an improvement in the other if they do demonstrate a positive association (Olanwumi et al., 2011).

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Table 2 - Phenotypic correlation among traits of F1 and F2-backcross of Local and Pekin duck at 25 weeks of age.

	LW	SL	BL	BW	WL	CC	NL	TL	DL
LW		- 0.66*	-0.73*	NS	0.76*	NS	NS	NS	NS
		0.72*	NS	NS	NS	NS	NS	NS	NS
SL	NS		0.65*	NS	NS	0.78**	0.64*	NS	0.78**
	NS		0.59*	NS	0.68**	NS	0.56*	NS	NS
BL	NS	NS		NS	0.64*	NS	NS	NS	NS
	NS	NS		NS	NS	0.58*	NS	NS	NS
BW	NS	NS	NS		NS	NS	NS	NS	NS
	NS	NS	NS		NS	NS	NS	NS	NS
WL	NS	NS	NS	NS		NS	NS	NS	NS
	NS	NS	NS	NS		NS	0.57*	NS	NS
CC	NS	0.59*	NS	NS	NS		NS	NS	NS
	0.84*	NS	NS	NS	NS			NS	NS
NL	NS	NS	-0.62*	NS	NS	NS		NS	-0.77**
	NS	NS	NS	NS	NS	NS		NS	NS
TL	NS	NS	NS	NS	NS	NS	NS		NS
	NS	NS	NS	NS	NS	NS	NS		NS
DL	NS	NS	NS	NS	NS	0.57*	-0.55*	NS	
	NS	NS	NS	NS	NS	NS	NS	NS	

Female: above the diagonal; Male: under the diagonal; Upper: F1 ; Below: F2-backcross; *P<0.05; NS = not significant; LW: Live Weight, SL: Shank Length, BL: Bill Length, BW: Bill Width, WL: Wing Length, CC: Chest Circumference, NL: Neck Length, TL: Thigh Length, DL: Drumstick Length

The coefficient of correlation from this study varied from strong to moderate, positive, and significant to most of the generations considered. Correlation coefficients indicate the strength of a linear relationship between traits and thus provide valuable information about the traits involved in breeding and improvement plan. The results show favorable relationships exist among traits with higher correlation coefficients. Generally, there are no significant differences between F1 and F2-backcross in their morphological characteristics. Those results revealed that the body dimensions of the F1 and F2-backcross are relatively equal in performance in relevancy to live weight, shank length, bill length, and neck length for each sexual. Pekin duck can also be used to upgrade local ducks of South Sulawesi for better performance in a systematic breeding program. Therefore, present information could aid the management, conservation, future selection, and breeding programs of local ducks from South Sulawesi.

CONCLUSION

The morphological characteristics of the F1 and F2-backcross of Local and Pekin ducks were relatively similar. Improving the genetic quality and quantity of local ducks in South Sulawesi should use the crossbreeding method. It is advisable to research the optimal growth age of duck (1-8 weeks of age)

DECLARATIONS

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Authors' contribution

SRA. Bugiwati led and fully managed the research project and was responsible for data collection, data analysis, the write-up of the manuscript, and the publication process; MIA. Dagong contributed to interpreting research data and the write-up of the manuscript; L. Rahim contributed to the provision of library resources and translation process; M. Malloangeng, A. As, and M. Zulkifli were responsible for data collection and data input.

Conflict of interests

The authors have declared no conflict of interest.

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CAUSES OF HONEYBEE COLONY DECLINE IN SOUTH ETHIOPIA

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ABSTRACT: The purpose of this study was to identify the major causes of colony decline in the Gedeo Zone, South Ethiopia. Three districts, namely, Yirga Cheffe, Wonago, and Dilla Zuria, were purposefully selected based on beekeeping potential. A cross-sectional survey was conducted to collect data from 135 beekeepers and 15 key informants using a semi-structured questionnaire, focus group discussion, and personal observation of apiary sites. The results revealed two main causes of colony declines in the Gedeo zone: colony management-related factors and natural factors. Seventy percent of beekeepers lack the practical skills to perform hive inspection; 47% do not feed their colonies; 45% spray pesticides and insecticides near their apiaries; and 82% fail to control swarming. As a result, 87% of sampled beekeepers have experienced frequent colony absconding. The trends of colony decline showed an increase from 2008 to 2020 in the highlands and from 2008 to 2017 in the midlands and lowlands, respectively. The number of households facing colony declines increased in all agro-ecologies from 2008 to 2020. Pests and predators, like wax moths, and small hive beetles were take the first rank followed by ants, the inherent behavior of honeybees, a shortage of flora, and the presence of poisonous plants were the top five challenges among natural factors, respectively. Therefore, we strongly recommend educating beekeepers on scientific methods of colony management and planting bee flora. Laboratory diagnostics are required to identify bee diseases.

Keywords: Apiary management, Apiculture, Bee diseases, Colony decline, Swarm.

INTRODUCTION

Apiculture is an important agricultural sub-sector that allows for the use of natural resources that would otherwise be wasted and contributes to national food production through pollination (Melaku et al., 2008). Due to their effectiveness and widespread distribution over the world (Durazzo et al., 2021) honey bees are regarded as one of the most significant pollination agents globally (Klein et al., 2007; López et al., 2017; El-Naggar et al., 2022). Furthermore, they are essential pollinators for agricultural crops (Aizen et al., 2009; Verde, 2014; Robinson et al., 2021). Honey bees play a vital function and have a positive impact on the environment by keeping the balance of ecosystems through enhanced pollination (Gidey and Kibrom, 2010; Famuyide et al.,2014). Recent studies (Aryal et al., 2020; Kline and Joshi, 2020; Patel et al., 2021) have also appreciated the pollination effort performed by wild pollinators. As a result, more seeds and plants (Gidey and Kibrom, 2010) provide food for wildlife (Bradbear, 2009) and aid in reducing soil erosion and degradation (Ahmad et al., 2003) through ensuring the preservation of floral diversity. Pollination services support biological diversity and ecological harmony (Toni and Djossa, 2015). Therefore, apiculture is the most important intervention area for countries like Ethiopia to increase crop yield and for sustainable development (Gibbon, 2001).

In Ethiopia, apiculture has been practiced for centuries throughout the country (Melaku et al., 2008). Despite long-standing beekeeping practices, more than 96% of apiculture is still conducted in a backyard and traditional system (CSA, 2019). This implies the need for modernization (Melaku et al., 2008). Ethiopia is home to an estimated 10 million honeybee colonies; 60 percent of which are managed in various hives (MoARD, 2007). Unfortunately, the MoARD report indicated a 50% decrease in colony population (4,993,815). However, the recent report of the CSA (2019) showed that there are 7,075,188 colonies in the country, with 96.1 percent, 2.8 percent, and 1.1 percent being kept in traditional, modern, and transitional hives (Kenyan Top bar hive), respectively. Beekeeping employs an estimated one million beekeepers, thousands of honey collectors and traders, and thousands of "Tej" (local beverage) makers in urban areas of Ethiopia (Beyene and David, 2007).

Despite Ethiopia's vast beekeeping potential, numerous studies have found that deforestation, agrochemical poisoning, pests and predators, and a lack of bee flora are major challenges in the apiculture sub-sector, resulting in colony declines and a decline in honey yield (Ejigu et al., 2009; Mengistu and Beyene, 2014; Beyene and Verschuur, 2014). The colony decline has been increasing because of unwise pesticide application to crop fields (Fikadu, 2020). Poor honey harvesting techniques that result in the complete destruction of brood comb, honeybee disease, and pests and predators such as ants, wax moths, and small hive beetles are major destructors of honeybees, leading to colony absconding (Kenesa, 2018). Except for a very few studies that identified general challenges in the beekeeping sub-sector, there is a lack of scientific information about the causes of colony decline in the Gedeo zone. Therefore, the primary purpose of this study was to identify the causes of colony decline and the status of declines in the Gedeo zone.

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MATERIALS AND METHODS

Description of study area

The study was conducted in Gedeo Zone, Southern Ethiopia, which is about 360 km from Addis Ababa and 90 km from Hawassa, the capital of Sidama Region, and the South Nations Nationality and People Region (SNNPR). The Gedeo zone lies approximately between 5^0 53'N and 60 27'N latitude and from 38^0 8' to 38^0 30' E longitude (Figure 1). The average monthly temperature is 21.5 degrees Celsius, with maximum and minimum monthly temperatures of 25 degrees Celsius and 18 degrees Celsius, respectively (CSA, 2006).

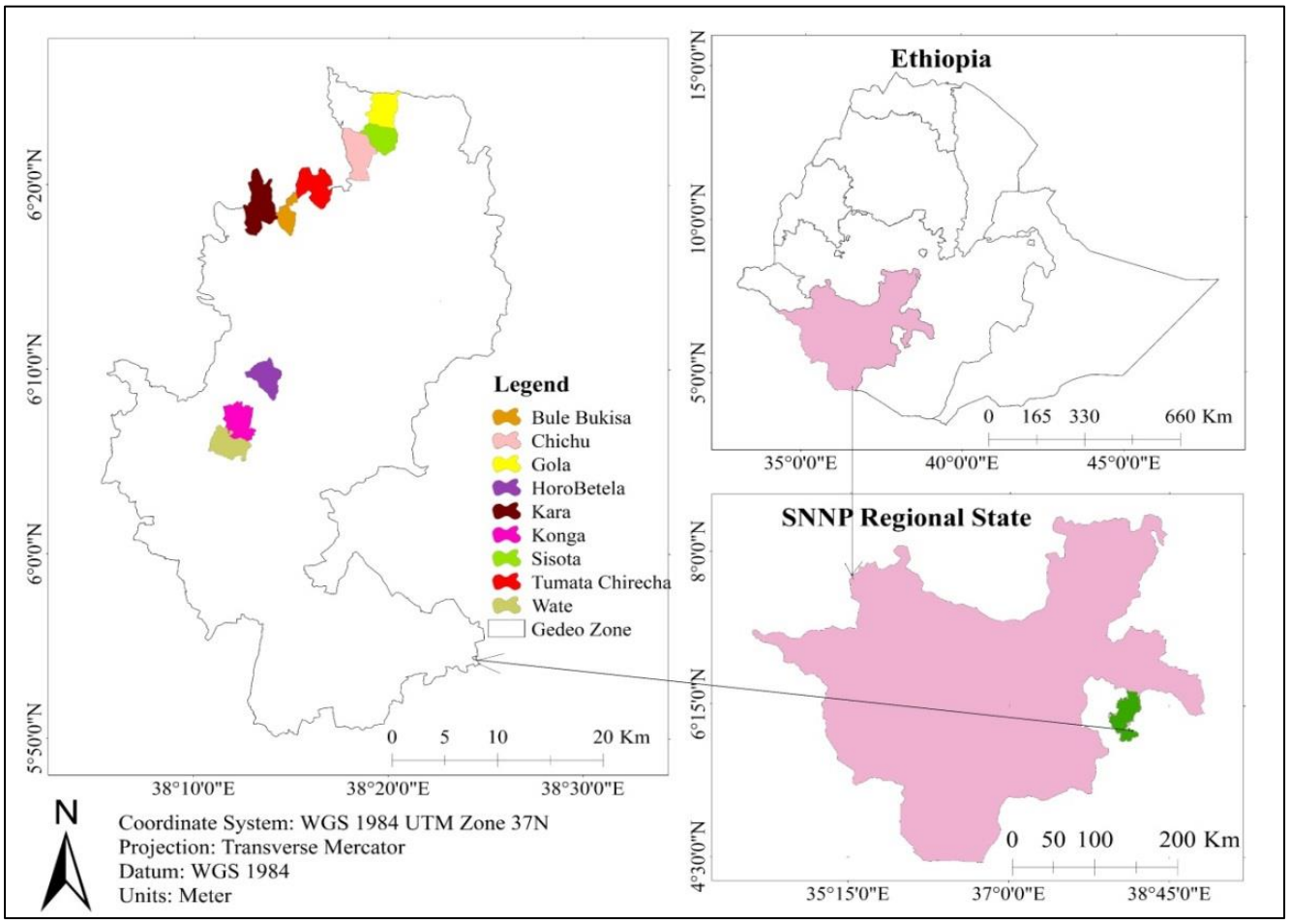

Figure 1 - Map of the study area

Sampling techniques and sample size

Three districts, namely Yirga Cheffe (highland), Wonago (midland), and Dilla Zuria (lowland), were purposefully chosen based on their beekeeping potential in the Gedeo zone. From each district, three Kebeles (the smallest administration unit in Ethiopia) were selected based on agro-ecology: highland, mid-altitude, and lowland. From each kebele, fifteen respondents were randomly selected, and the primary information regarding all beekeeping management and the history of colony decline was gathered from different agro-ecological regions. A total of 135 sample respondents and 15 key informants were involved in this study.

Data collection methods

Before the commencement of the survey questionnaire, the districts' livestock and fishery bureau officers were consulted regarding the accessibility of intended information from farmers and key informants, which include knowledgeable local leaders of the peasant association, district beekeeping experts, and development agents. Five key informants were contacted from each district. Finally, primary data from respondents was gathered using a semi-structured questionnaire and personal observations of the apiary sites.

Statistical analysis

The data generated through the survey questionnaire was analyzed using SPSS software, version 20. Then descriptive statistics such as mean, tables, percentages, and figures were used to analyze the data. The rate at which constraints contributed to colony decline in the study area was determined by the relative importance index (Tam and Le, 2006).

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$$RII = \frac{\Sigma w}{AN} = \frac{10n_{10}+9n_9+8n_8+7n_7+6n_6+5n_5+4n_4+3n_3+2n_2+1n_1}{10N} \quad (1)$$

Where RII is Relative Importance Index w is the weighting given to each factor by the respondent, ranging from 1 to 9. n_1 = number of respondents for little important, n10 = number of respondents for very important. A is the highest weight (i.e. 10 in the study area) and N is the total number of respondents (Tam and Le, 2006).

RESULT

Demographic characteristics of respondents

The mean age of the entire respondents was 46.6 years. Males were the dominant owners of bee colonies (91.1%) in the study area (Table 1). The majority (65.9%) of the respondents were attending from elementary school to college level and about 30.4% were able to read and write. Regarding beekeeping experiences, 72% of sampled farmers had more than ten years of experience in beekeeping while 27% of respondents had less than five years of experience. The mean colony holding per household was 10.5 (Table 1). A male (54.1%) house head mainly carried out colony management activities. However, about 36.3% of respondents reported that all family members equally participate in the colony's management, while only 5.2% of children and 4.4% of women reported that they manage their colony independently. As shown in the result, the majority (60.7%) of the respondents receive consultation services from extension workers during active (honey flow) and dearth seasons. Nonetheless, 39.3% of beekeepers reported receiving no assistance from extension workers (Table 1). Seventy-seven percent of the entire group of respondents reported that they obtained their colonies from swarm bees, and the remaining 22% of beekeepers obtained them from both swarms and parental gifts. The main purpose of beekeeping in the studied area was only for honey production (Table 1).

Management related factors contributed to colony decline in the study area

According to the results (Table 2), nearly half of the respondents (46.7%) did not provide supplementary feed to their colonies during the dearth season. In terms of hive inspection, 68.9% of respondents stated that they did not perform internal hive inspections during drought seasons. Besides, 45% of sampled respondents reported that pesticides were sprayed close to their apiary sites for cash and cereal crops. Furthermore, 82% and 87% of sampled beekeepers reported the occurrence of frequent swarming during honey flow season and absconding of their colony in all seasons, respectively (Table 2). On the other side, farmers ranked natural factors that had the main contribution to colony declines based on their relative importance (Table 3). According to the results, pests and predators, ants, the inherent behavior of honeybees, a shortage of flora, and the presence of poisonous plants were ranked as the top five challenges, respectively, in the study areas. The incidence of collective death of honeybees, external disturbances, indiscriminate agrochemical application, frequent hive inspection, and disease prevalence were ranked from six to ten causes for colony declines, respectively (Table 3).

Trends of colony decline in study area

The number of colonies lost from 2008 to 2012 was the lowest in all agro-climatic zones (Figure 2). However, between 2013 and 2017, the number of colonies lost increased fourfold in the highlands and mid-altitudes, while it increased eightfold in the lowlands (Table 4). In the highland area, the declines continued to increase until 2018–2020, but in the mid- and low-altitude zones, a slight decline was observed in the years 2018–2020 compared to the previous five years (Figure 2). Our results showed that the overall colony decline was highest in the highlands (439), followed by the midlands (325), and relatively lowest in the lowlands (201) in the study area (Figure 2).

The majority of entire respondents reported that they experienced colony declines in the years 2013–2017 (79.26%) and 2018–2020 (83.7%). One hundred percent of sampled households in the highlands and above 90% in the midlands reported that they lost their colonies between the years 2013 and 2020. Colony declines increased from 2008 to 2020 in the highlands and from 2008 to 2013 in the midlands and lowlands, with a slight decline between 2018 and 2020 in the mid and lowlands areas (Figure 3).

Types of hive colony declines most occurred in the study area

The types of hives and colony declines most frequently observed are presented in Table 5. As shown in the result, 54% of the sampled respondents reported that the colony decline most frequently occurred from traditional hives (Table 5). The highest colony declines in the traditional hives were reported in the midland and highlands, respectively, compared to lowland areas. The results further revealed that the lowest colony declines occurred in frame and transitional hives, respectively. However, 32% of beekeepers reported that the colony decline did not depend on the types of hives but happened in all types of hives.

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Table 1 - Demographic characteristics of respondents (n = 135)

Variable \ Topography	Highland	Mid land	low land	Overall
Mean Age (Range)	51(30-68)	44(19-66)	45(24-65)	46.6(19-68)
Gender	n(%)	n(%)	n(%)	n(%)
Male	42(93.3)	41(91.1)	40(88.9)	123(91.1)
Female	3(6.7)	4(8.9)	5(11.1)	12(8.9)
Education status	n(%)	n(%)	n(%)	n(%)
Illiterate	0(0)	3(6.7)	2(4.4)	5(3.7)
Read and write	12(26.7)	16(35.6)	13(28.9)	41(30.4)
Elementary school	21(46.7)	14(31.1)	11(24.4)	46(34.1)
Secondary school	12(26.7)	7(15.6)	18(40)	37(27.4)
College and above	0(0)	5(11.1)	1(2.2)	6(4.4)
Experience on beekeeping	n(%)	n(%)	n(%)	n(%)
0-5 year	9(20)	12(26.7)	16(35.6)	37(27.4)
5-15 year	8(17.8)	15(33.3)	14(31.1)	37(27.4)
15-25 year	15(33.3)	11(24.4)	9(20)	35(25.9)
25-40 year	13(28.9)	7(15.6)	6(4.4)	26(19.3)
Colony holding status /HH (Mean)	11.86	7.74	11.63	10.51
Family participation in colony management	n(%)	n(%)	n(%)	n(%)
Male	26 (57.8)	25(55.6)	22(55.6)	73(54.1)
Female	0(0)	3(6.7)	3(6.7)	6(4.4)
Children	3(6.7)	0(0)	4(8.9)	7(5.2)
All members equally	16(35.6)	17(37.8)	16(35.6)	49(36.3)
Source of colony	n(%)	n(%)	n(%)	n(%)
Parents gift	1(2.2)	1(2.2)	11(24.4)	13(9.6)
swarm and parent gift	1(2.2)	4(8.9)	12(26.7)	17(12.59)
Swarm	43(95.6)	40(88.9)	22(48.9)	105(77.8)
Consultation from extension workers	n(%)	n(%)	n(%)	n(%)
Yes	23(51.1)	27(60)	32(71.1)	82(60.7)
No	22(48.9)	18(40)	13(28.9)	53(39.3)
Purpose of beekeeping	n(%)	n(%)	n(%)	n(%)
Honey production	44(97.8)	45(100)	45(100)	134(99.3)
Wax production	1(2.2)	0(0)	0(0)	1(0.7)

Table 2 - Management factors led to colony decline in the study area (n = 135)

Factors \ Topography	Highland	Mid land	low land	Overall
Supplementary feeding	n(%)	n(%)	n(%)	n(%)
Yes	25(55.6)	26(57.8)	21(46.7)	72(53.3)
No	20(44.4)	19(42.2)	24(53.3)	63(46.7)
Hive inspection at active season				
Yes- external inspection	45(100)	33(73.3)	32(71.1)	110(81.5)
No- external inspection	0(0)	12(26.7)	13(28.9)	25(18.5)
Yes- internal inspection	29(64.4)	23(51.1)	17(37.8)	69(51.1)
No- internal inspection	16(35.6)	22(48.9)	28(62.2)	66(48.9)
Hive inspection at dearth season				
Yes- external inspection	42(93.3)	33(73.3)	23(51.1)	98(72.6)
No- external inspection	3(6.7)	12(26.7)	22(48.9)	37(27.4)
Yes- internal inspection	10(22.2)	23(51.1)	9(20)	42(31.1)
No- internal inspection	35(77.8)	22(48.9)	36(80)	93(68.9)
Pesticide sprayed close to apiary	n(%)	n(%)	n(%)	n(%)
Yes	23(51.1)	34(75.6)	4(8.9)	61(45.2)
No	22(48.9)	11(24.4)	41(91.1)	74(54.8)
Frequent swarming occurred	n(%)	n(%)	n(%)	n(%)
Yes	45(100)	38(84.4)	27(60)	110(81.5)
No	0(0)	7(15.6)	18(40)	25(18.5)
Absconding occurred	n(%)	n(%)	n(%)	n(%)
Yes	45(100)	42(93.3)	30(66.7)	117(86.7)
No	0(0)	3(6.7)	15(33.3)	18(13.3)

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Table 3 - Natural factors led to colony decline in the study area (n = 135)

Variable	Highland	Midland	Lowland	Overall average	Overall Rank
Shortage of flora	0.798	0.80	0.84	0.813	4
Ants	0.80	0.840	0.96	0.8667	2
External disturbance	0.56	0.53	0.77	0.62	7
Indiscriminate agrochemical application	0.56	0.61	0.61	0.593	8
Disease	0.48	0.38	0.52	0.46	10
Inherent behaviour of honey bees	0.91	0.76	0.78	0.817	3
Other pests and predators	0.93	0.93	0.84	0.9	1
Frequent inspection	0.58	0.48	0.71	0.59	9
Poisoning plants	0.81	0.51	0.65	0.657	5
Collective death of honey bee	0.62	0.62	0.65	0.63	6

Table 4 - Number of household facing colony declines in the study area

Topography / Household faces colony declines	Highland	Mid land	low land	Overall
	n(%)	n(%)	n(%)	n(%)
2008-2012	17(37.78)	11(24.4)	12(8.89)	40(29.63)
2013-2017	45(100)	41(91.11)	21(46.67)	107(79.26)
2018-2020	45(100)	40(88.89)	28(62.22)	113 (83.7)

Table 5 - Proportion of colony declines in the different types of hives in the study area

Topography / Variable	Highland	Mid land	low land	Overall
	n(%)	n(%)	n(%)	n(%)
Traditional hive	30(66.7)	34(75.6)	9(20)	73(54.1)
Traditional and Transitional hive	2(4.4)	1(2.2)	0(0)	3(2.2)
Transitional hive	1(2.2)	5(11.1)	8(17.8)	14(10.4)
Transitional and Frame hive	0(0)	0(0)	1(2.2)	1(0.7)
Frame hive	0(0)	1(2.2)	0(0)	1(0.7)
Do not depend on type of hive	12(26.7)	4(8.9)	27(60)	43(31.9)

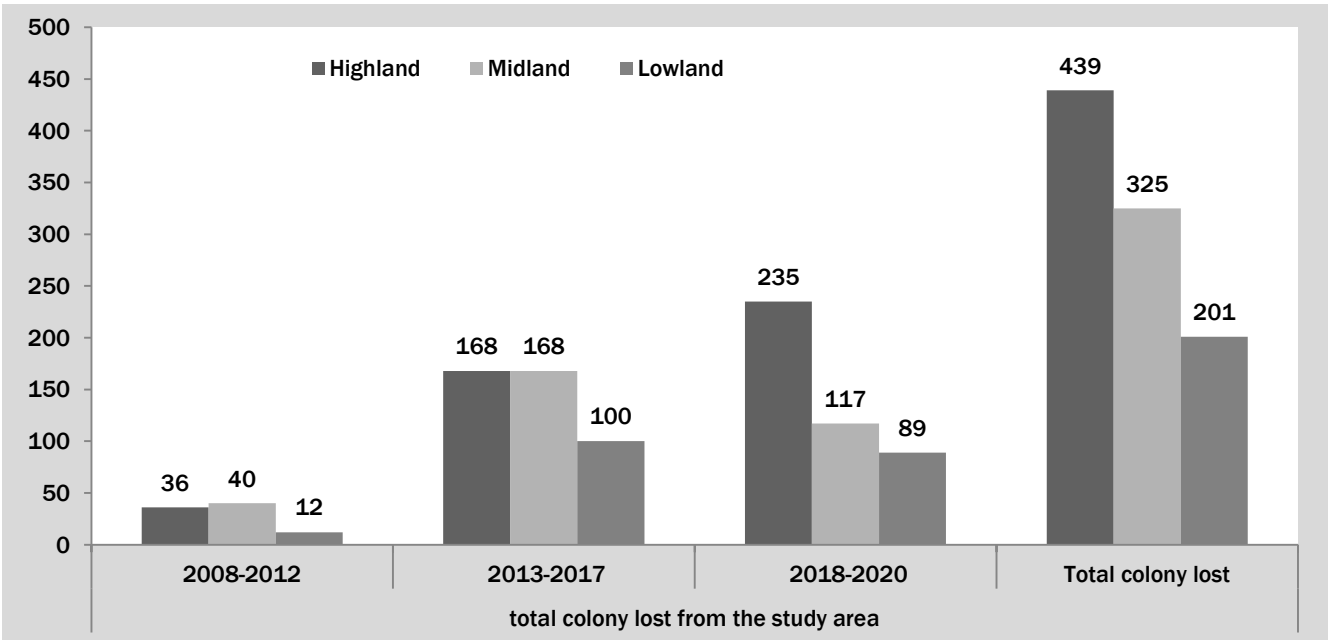

Figure 2 - Lost colonies during different years of production (2008 to 2020)

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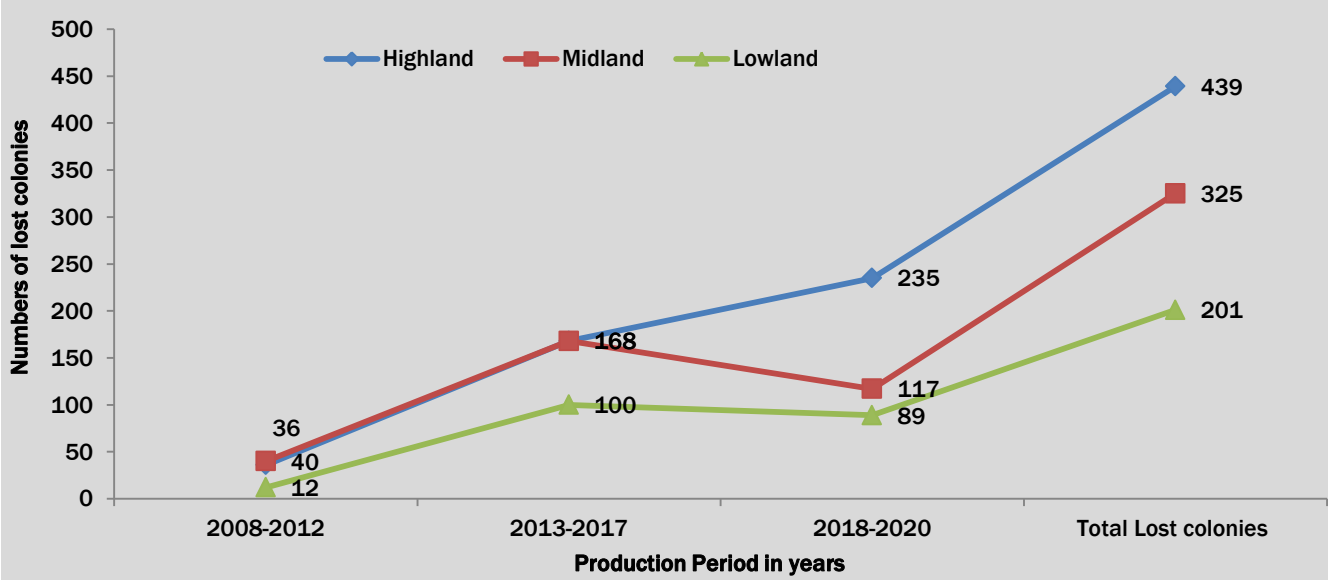

Figure 3 - Trends of colony in the study area

DISCUSSION

In Ethiopia, the Gedeo Zone is well known for its natural forest conservation heritage and agroforestry practices. The zone has a wide range of agricultural climates that are suitable for beekeeping practices. Beekeepers in the Gedeo zone can easily catch swarm bees by hanging bait hives over long tree branches and under their house roofs and rearing bees is the most common method. Catching swarms of bees to start beekeeping is a very common practice in the south and southwest parts of Ethiopia. For instance, beekeepers in Kaffa, Sheka, and Bench-Maji Zones attract honeybees to new and previously used hives by hanging bait hives on the trees (Awraris et al., 2015). Some farmers in the Gedeo Zone get started in the beekeeping business by receiving bees as a gift from their parents. Similarly, different authors noted that beekeeping is a long tradition and an indigenous practice that is passed down from parents to sons in Southwest Ethiopia (Hartmann, 2004) and in the Guji and Borana Zones of Oromia Region (Birhanu, 2016). Unlike in the north and central parts of the country, there is no colony market or queen multiplication center to start beekeeping in the Gedeo zone.

Despite having long beekeeping experiences and traditions, most beekeepers in the study area had been losing a huge number of colonies every year due to a lack of scientific and practical skills and because of many socio-economic reasons. In the last ten years, beekeepers and local government officials have repeatedly reported colony declines in the Gedeo zone. However, little was known about the causes and extent of colony declines. This study indicated major causes of colony decline, the status of the decline, and the types of hives from which most declines occurred in the different agro-climatic zones of the studied areas. Sampled farmers had different ages and educational backgrounds, varied beekeeping experiences, and accessed different levels of extension advice in the highland, midland, and lowland agro-ecologies. For example, sampled respondents in the Highlands were more educated, older in age, had more beekeeping experience, and had more colonies per household than beekeepers in other agro-ecologies. Nonetheless, the total number of colonies lost over time and the number of families affected were higher in the highland agro-ecology. This is clearly revealed by the differences in the extension services provided for beekeepers among various agro-climatic zones and their lack of scientific knowledge and practical skills to manage their colonies. Besides, the majority of cereal crops are grown in the highlands and mid-altitude regions of the zone. All farmers in the studied area practice a crop-livestock mixed farming system; in fact, they apply a varied level of pesticides, insecticides, and fungicides to their crops near their apiary sites. This could have resulted in colony declines, either entirely or partially. This result was in line with the findings of Steinhauer et al. (2018) and Dolezal et al. (2019) that hives bounded by more cultivated land result in higher colony declines. According to Tesfaye et al. (2017), pesticide and herbicide application is the leading cause of colony decline in the Bale Zone of Southeast Ethiopia. The colony declines in the lowlands were lower than those in the highlands and mid-altitudes. This is because of better extension service provision for beekeepers and less intensity of agrochemical application, hence better management of their colonies. This finding is consistent with Rasa (2020) and Markos and Samuel (2021), who stated that beekeepers who were more frequently visited by extension workers managed their colonies better and adopted improved hive technologies.

The study conducted elsewhere speculated whether a combination of the stressors, including mites, disease, and nutritional stress, are interacting to weaken bee colonies and allowing stress-related pathogens such as fungi, thus causing a final collapse (Kluser, 2007). Colony mortality can be caused by a number of interrelated factors, such as the unavailability of forage (Decourtye et al., 2010), pesticide exposure (Zhu et al., 2014), problems associated with the ectoparasitic mite, Varroa destructor (Neumann and Carreck, 2010), other pests, parasites, and diseases (Berthoud et al., 2010), as well as various socioeconomic factors (Gallai et al., 2009).

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The current study identified two main causes of colony declines in the Gedeo zone: colony management-related factors and natural factors. Management-related factors such as lack of seasonal colony inspection, feeding, swarm control, unwise use of agrochemicals, and poor honey harvesting techniques are the most responsible contributors for colony decline in the Gedeo zone. Nearly half of the sampled beekeepers, for example, did not perform hive inspections during active or dearth seasons, nor did they provide supplementary feed during dearth season. Consequently, the colonies weakened due to frequent swarming during the active season and pests and predators attacking in all seasons. Farmers are unable to control reproductive swarming by adding supers, removing old and black combs that are not used by worker bees, and controlling pests and enemies that compete for comb space, such as wax moths (*Galleria mellonella*). The common honey harvesting practice in the studied area was the complete destruction of brood and honeycombs in all types of hives. Finally, due to a lack of food reserves during the scarce periods, such poor management practices resulted in colony absconding. Pathogens, parasites, environmental pressures, and bee management stresses such as insufficient feeding were the focus of the earliest scientific investigation into the potential causes of colony collapse disorder (van Engelsdorp et al., 2008). The study conducted by Kumsa and Takele (2014) at Jimma Zone revealed that high absconding rates are characteristics of poor bee management practices (absence of supplementary feed during dearth periods, cleaning, and inspection).

Among natural factors identified as major causes of colony declines, beekeepers ranked the factors based on the relative index of importance from very worst to least worst. Pests and predators (such as wax moths, small hive beetles, large hive beetles, and termites) were ranked in the first position by causing the highest colony declines in the study area. Ants were the colony's second-largest destroyers, trailing only the combined damage of other pests (wax moths and small hive beetles) and predators (lizards). According to Gidey et al., (2012), honey bee pests and predators include ants, insects, spiders, monkeys, snakes, and lizards; the wax moth (*Galleria mellonella*); bee-eater birds; bee lice (*Braula coecal*); honey badgers (*Mellivora capensis);* and small hive beetles. Inherent behaviors of honeybees (absconding and swarming tendencies), shortage of bee flora, and the presence of poisonous plants were ranked third to fifth, respectively, in the study areas. Numerous studies (Workneh and Ranjithan 2011, Kinati et al., 2012, Assemu et al., 2013, and Kenesa, 2018) performed in different regions of Ethiopia noted that pest, predator, and disease incidence, shortage of flora, drought, and deforestation are major factors for colony absconding and migration. However, other factors that were ranked from six to ten had a relatively smaller contribution to colony declines in the study area. This finding is consistent with Teklu (2016), who stated that pests and predators are major challenges in the Sidama and Gedeo zones of the selected districts.

The colony declines showed increasing trends from 2008 to 2020 in the highlands and from 2008 to 2017 in the mid- and lowlands. The reason for this increase may be the increased use of farm inputs such as agrochemicals and clearing forestland for food crop production. Agrochemical applications have been dramatically increased in Ethiopia to improve crop productivity (Nigatu et al., 2016). However, they have a negative effect on the honeybees and other pollinators (Fikadu, 2020). On the other hand, even if there was a slight decline in the trends of colony declines between 2018 and 2020 in the mid- and low-land areas, the number of families reporting colony declines increased in all agro-ecologies from 2008 to 2020. In a similar way, several authors (Oldroyd, 2007; EFSA, 2008; Van Engelsdorp, 2008) have reported unexpected and alarming colony declines in various parts of the world over the last few years. Numerous variables, including beekeeping techniques, temperatures, human activities, the genetic makeup of honey bees, enemies, forage shortages, and others, can be implicated in the variations of colony declines among different regions (Le et al., 2010; Büchler et al., 2014; Goulson et al., 2015).

Traditional hives accounted for more than half of colony declines, especially in mid- and highland agro-ecologies. This is due to the smaller size of local hives, their design, and their inability to protect bees from the rain, sun, heat, and enemies. Internal hive inspection and other colony management practices are not common in traditional beekeeping (Gebremedhn, 2015). One of the factors driving colony declines in the current study is a lack of colony inspection. Traditional hives, which were suspended from long trees in the forest or in the backyard, were only visited when beekeepers anticipated honey harvesting during an active season. The studies conducted in the southwest parts of the country revealed that traditional hives were more affected by disease and pests (Solomon et al., 2021). The same authors concluded that Nosema *apis* disease prevalence was most pronounced in highland agro-ecology (Abi, 2017; Bizuayehu Ayele et al., 2020). On the other hand, some of the surveyed beekeepers believe that colony declines can happen in any type of hive due to many reasons (Stokstad, 2007; Stanimirović et al., 2019). Disorganization, depopulation, and mortality of honeybee colonies are caused by a variety of environmental variables, including nutritional imbalances and pathogen infections (Dolezal and Toth, 2018). Despite the fact that the declines were lowest in the frame and transitional (Kenyan to bar) hives compared to traditional hives in the Gedeo zone, this finding is also in agreement with Solomon et al., (2021).

CONCLUSION

This study identified two major sources of colony declines in the Gedeo zone: one resulting from poor colony management practices and the other from natural factors. High absconding and swarming rates, a lack of internal and external colony (hive) inspection during active and dearth seasons, a lack of supplementary feeding during the dearth period, and the indiscriminate application of agricultural chemicals were identified as major management-related factors causing colony

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decline in the study area. Among natural factors, pests and predators, like wax moths, and small hive beetles(together) were take the first rank followed by ants, the inherent behavior of honeybees (absconding), a shortage of bee flora, and the presence of poisoning plants were the top five challenges that are causing colony declines. Whereas disease was not an important issue in the study area, it was ranked last of all constraints leading the colonies to decline. The traditional hive is one of the most commonly reported hive types, and colony declines frequently occur. In general, colony declines and the number of beekeepers dealing with these issues is increasing in all agro-ecologies. Therefore, we strongly advise educating beekeepers about practical scientific methods of colony management. Even though the respondent ranks the disease as least constrained, conducting laboratory examinations to test the prevalence of diseases would bring additional information regarding colony losses. We also suggest local governments widely disseminate improved hive technologies to avoid the limitations of traditional hives associated with their dimensions and designs.

DECLARATIONS

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Authors' contributions
A. Diriba: design and acquisition of data, analysis and interpretation of data; and write up
M. Fisaha: acquisition of data, drafting the article, revising; and approval
D. Andualem: analysis and final approval of the version to be published

Competing interest
There is no potential conflict of interest between the authors.

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AN INVESTIGATION ON AVAILABILITY AND EFFICACY OF ANTI-ANEMIC DRUGS FOR PIGS IN THE UKRAINIAN PHARMACEUTICALS

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ABSTRACT: Antianemic drugs are used to prevent anemia, majorly iron deficiency anemia. Drugs with such pharmacological action are especially relevant for piglets, as animals of this species at this age are particularly sensitive to iron deficiency. The present investigation aimed at studying the pharmaceutical market of antianemic drugs registered in Ukraine during 2017-2022. It should be noted that if the drug is registered, it has been checked for safety according to the food industry standards of Ukraine. In 2017, the national market of veterinary iron-containing drugs was represented by 13 drugs from the group QB03A "Antianemic drugs. Drugs of iron", according to the ATC-vet classification. The range of these drugs by 38 % was provided by pharmaceutical products of Ukrainian manufacturers: "O.L.KAR-AgroZooVet-Service", "Pharmaton", "Brovapharma", "Experimental production of the Institute of Epizootology", "Biopharm", and "Vetsintez". Imported products (62 %) were represented by Pharmacosmos, Merial, Koofavet , "Vugen B&G" , "Biovet Pulawy", "Interchem Verken De Adelaar", and "Bioveta". In general, the modern pharmaceutical market of veterinary drugs in Ukraine during 2017–2022 was sufficiently provided with antianemic drugs for pigs and mostly imported drugs. The percentage of antianemic drugs of Ukrainian production prevailed in 2020, however the imported drugs of this pharmacological group's was higher in 2022. It can be concluded that the drugs of non-Ukrainian production predominated among antianemic drugs in Ukraine during 2017-2022. Based on the obtained results, we can state that in Ukraine there is a need for the development and/or production of domestic anti-anemic drugs. They must be effective and ensure a reduction in the dependence of the national pharmaceutical market of drugs of this group on foreign manufacturers.

Keywords: Anemia, Availability, Iron, Pigs, Veterinary drugs.

INTRODUCTION

Iron deficiency anemia is the most common anemic syndrome and accounts for approximately 80% of all anemias (Lopez et al., 2016). On the other hand, anemia is one of the most common diseases in newborn piglets (Szudzik et al., 2018). anemia is one of the most common diseases of newborn piglets, causing significant economic damage. Some reports describe that anemia rate in newborn piglets is 100%, and 20–30% of the mortality of young pigs in the first weeks of life is caused by iron deficiency. This is explained by the fact that the reserves of vital microelements deposited in the tissues are depleted within the first 5–7 days of piglets' life and this deficiency provokes the development of alimentary or iron deficiency anemia. This is one of the most important and unresolved issues in modern pig farming (Svoboda and Drabek, 2005; Levchenko et al., 2015; Perri et al., 2016).

In medicine and veterinary medicine of the twentieth century, internally used drugs were widely based on iron(II) sulfate, and parenteral used ones on iron(III) dextran (Svoboda et al., 2017; Szudzik et al., 2018). However, the use of iron dextran drugs in large doses is not always safe, because iron has prooxidant properties (Pamučar, 2018). Iron dextran drugs are still widely used in practical veterinary medicine. Consequently, the basis of treatment and prevention of anemia of pigs is the use of appropriate antianemic drugs. Moreover, their safe use in animals is important for the food industry's safety.

Considering that anemia causes great losses in pig farming worldwide (Godyń et al., 2016; Perri et al., 2016), many researchers are working on the pharmacological aspects of new anti-anemic drugs and methods of their administration. For instance, Maes et al. (2011) compared the effect of oral versus parenteral iron supplementation on the health and productivity of piglets; Meier et al. (2011) studied the physicochemical and toxicological characterization of a new generic iron sucrose preparation; Starzyński et al. (2013) analyzed correction of iron deficiency anemia without affecting plasma hepcidin levels; Streyl et al. (2015) described the field evaluation of the effectiveness of an oral toltrazuril and iron combination (Baycox® Iron) in maintaining weaning weight by preventing coccidiosis and anaemia in neonatal piglets;

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Antileo et al. (2016) characterized a novel encapsulated oral iron supplement to prevent iron deficiency anemia in neonatal piglets; Gan et al. (2017) described the effect of iron dextran on the transcriptome of pig hippocampus; Szudzik et al. (2018) studied iron supplementation in suckling piglets as an ostensibly easy therapy of neonatal iron deficiency anemia; Churio et al. (2018) described the use of encapsulation technology to improve the efficiency of an iron oral supplement to prevent anemia in suckling pigs; Knight et al. (2018) analyzed the longitudinal effects of iron deficiency anemia and subsequent repletion on blood parameters and the rate and composition of growth in pigs; and Szudzik et al. (2020) showed the long-term effect of split iron dextran/hemoglobin supplementation on erythrocyte and iron status, growth performance, carcass parameters, and meat quality of pigs.

Some studies reported the application of iron-containing drugs in pregnant sows in the last weeks of (Ulyzko and Todorov, 2014; Bhattarai et al., 2019). Dukhnytsky et al. (2021) and Derkach et al. (2021) found that the content of iron in the colostrum of sows, supplemented with solutions of iron (IV) clatrochelate and cyanocobalamin during gestation was about 52% higher than sows of the control group on the first day after farrowing. After 4 days, it was about 111 higher and after 7 days, about 175 % higher.

The present investigation aimed at analyzing the pharmaceutical market trends of the pharmaceutical market of antianemic drugs for pigs registered in Ukraine during 2017–2022. Official state websites with information on the registration of veterinary drugs were used in particular.

MATERIALS AND METHODS

Structure

The comparative analysis trends of the pharmaceutical market of antianemic drugs for pigs registered in Ukraine during 2017–2022 was conducted by analyzing literary information sources and and official government sources in this regard. Description of the Experiment: we analyzed official state data on veterinary drugs registered in Ukraine, which were initially available via a link http://vet.gov.ua/node/888 (no longer available), and in recent years, due to the reorganization of state structures, using another official source (https://dpss.gov.ua/bezpechnist-harchovih-produktiv-ta-veterinarna-medicina/reyestri). Number of repeated analyses: 6 (every year from 2017 to 2022).

Design of the experiment: our experiment aimed at establishing the main trends of development in the pharmaceutical market of anti-anemic drugs. Official public state data showing the registration of veterinary drugs were used for this purpose. The number of anti-anemic drugs and their manufacturers during the last 6 years was analyzed. Also, the composition of drugs presented by various Ukrainian and foreign pharmaceutical companies was compared. Thus, it was concluded that the national pharmaceutical market of veterinary drugs is provided with anti-anemic drugs for pigs since the anemia problem in pig farming does not lose its relevance. It should be noted that if the drug is registered in Ukraine, it has been tested by all necessary procedures, the results of which prove its safety for the food industry of Ukraine.

The analysis of research results with the use of descriptive statistics is carried out. Statistical analysis data were prepared using Microsoft Excel.

RESULTS AND DISCUSSION

The Ukrainian scientists are working on developing and studying the pharmacological action of new antianemic drugs to provide the national pharmaceutical market with high-quality drugs that can compete with imported ones and meet the demand for such drugs in Ukraine (Prokopenko and Martynov, 2012; Pristupa et al., 2013; Derkach et al., 2021; Dukhnytsky et al., 2021). Based on the fact that if dextran drugs contain only iron, the absorption of the drug after injection is 60–70%, besides the possible polyetiology of anemia, the researchers tried to combine iron dextrans with other trace elements (zinc, copper, and cobalt) and vitamins (B_2, B_3, B_6, B_{12}, biotin, and ascorbic acid), or with protein drugs to stimulate hematopoiesis. Furthermore, because the complex drugs (microanemin, suiferovit) have the best results, their developers are trying to minimize the dose of iron for piglets. Todoriuk (2011, 2012) proved the effectiveness of the microelement-containing composition of the drug minbevit in increasing the level of hemoglobin in the blood of animals. Levchenko et al. (2015) investigated the effect of the national drug ferrolife on hemocytopoiesis in young animals. Tokarchuk et al. (2016, 2017) studied the effect of vitamin E, citrates of Zinc, Iron and Germanium on body weight and morphological parameters of piglets' blood.

Our research has established that in 2017, 13 iron-containing drugs (38 %) were registered on the Ukrainian pharmaceutical market. These drugs were provided provided by the national manufacturers: Ferolife ("O.L.KAR AgroZooVet-Service"), Feropharm ("Pharmaton"), Brovaferan-100 ("Brovapharma"), Ferodev ("Experimental production of the Institute of Epizootology", "Biopharm"), Ferrovet + B_{12} ("Vetsintez"). One drug was offered by the foreign manufacturers: Pharmacosmos (Kingdom of Denmark), Merial (France), Wugen (South Korea), by Biovet Pulawy (Poland). Two drugs was offered by the foreign manufacturers: Interchem Verken "De Adelaar" (Estonia; drugs Intrafer-200 B_{12}, Intrafer-100 B_{12}), Bioveta (Czech Republic; drugs Ferribion 10%, Gafervit). These drugs are in great demand as anti-anemic drugs abroad, in particular in the countries that produce them.

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These drugs include the dextran complex of iron (III) hydroxide, and 46% of such drugs contain its combinations with other substances. For example, 4 drugs, Intrafer-100 B_{12}, Intrafer-200 B_{12}, Ferovita 200, and Ferrovet+B_{12} contain cyanocobalamin. There are 2 more antianemic drugs, which are multi-component drugs. Thus, Gafervit (Bioveta, Czech Republic) includes iron (III) dextran complex, vitamin B_1, vitamin B_2, vitamin B_6, calcium pantothenate, copper chloride, cobalt chloride, and inactivated normal pig serum. Suiferovit (Biovet Pulawy, Poland) contains active substances: immunoglobulin of normal pig serum, iron dextran, thiamine chloride, riboflavin, pyridoxine hydrochloride, nicotinamide, calcium pantothenate, copper chloride, cobalt chloride.

According to the international ATC-vet classification, these iron-containing drugs belong to the pharmacological group with the code QB03A antianemic. Such drugs have an antianemic effect due to the presence of low-toxic and water-soluble iron dextran complex. They stimulate the functional state of the hematopoietic system and the synthesis of hemoglobin, which leads to an increase in the number of erythrocytes. The activation of metabolic processes in tissues stimulates animal growth, increasing their resistance to the effects of negative environmental factors. Salts of copper and cobalt, and vitamins of group B act synergistically, enhancing the effect of iron, regulating metabolism, and compensating for the lack of these elements in the food. After intramuscular injection of iron, dextran is rapidly absorbed through capillaries and lymphatic vessels. It is removed from blood plasma by cells of the reticuloendothelial system, then divided into iron and dextran. Iron binds to proteins to form hemosiderin, ferritin, and transferrin complexes. Vitamin B_{12} is required for DNA synthesis.

In 2018, the national market for iron-containing veterinary drugs was equally represented by national and foreign drugs. We also analyzed the market of food additives and premixes containing iron. Thus, ones for pigs were presented in dry dosage forms. In the list registered in 2018, the number of drugs in Ukraine was 55, 14 (26% of the total) of which were Ukrainian-made. There was a tendency for one manufacturer or several in cooperation to produce several drugs for animals of different ages and species, taking into account the body's physiological needs.

The analysis of the pharmaceutical market of iron-containing drugs registered in Ukraine in 2020 showed that the share of Ukrainian drugs in this group outweighed the share of imported ones and was 10 (64%) of the total. It is noteworthy that one of the trends in the development of this market of pharmaceutical products was that the drugs were produced in cooperation with several companies; for example, Ferroselenite was represented by manufacturers "Circle" and "Nova Plus".

In 2021, the pharmaceutical market of national antianemic drugs expanded. In particular, the drug Ferum+, was registered by Biotestlab.

According to the available information sources in the list of veterinary drugs for pigs registered in Ukraine as of 1.01.2022, 11 were identified, of which 5 (45%) are Ukrainian products (manufacturers "Biotestlab", "Brovapharma", "Vetsintez", and "Fortis-pharma"). 6 (55%) of such drugs are offered by foreign pharmaceutical companies, including companies from France, the Czech Republic, Estonia, and Denmark. manufacturers such as Bioveta a.s. and Interhemi Verken De Adelaar Esti AS offer two antianemic drugs on the Ukrainian pharmaceutical market of veterinary drugs. The results of the analysis show that the registration of drugs is valid during 2022–2026: for Ferofort – until 2022; for Ferro 2000 and Uniferon – until 2023; for Bioferon Forte, Ferrovet + B_{12}®, Ferribion 10%, Intrafer-200 B_{12} – until 2024; for Brovaferan-100, Gafervit, Intrafer-100 B12 – until 2025; for Iron+ – until 2024 (Table 1).

Table 1 - Iron-containing drugs registered in Ukraine as of 1.01.22

Name of drug	Date of registration	Registration is valid until	Procedure of registration	Manufacturer	Country of manufacture
Iron +	04.03.2021	03.03.2026	re-registration	Biotestlab	Ukraine
Brovaferan-100	24.12.2020	23.12.2025	re-registration	Brovapharma	
Ferrovet +B12®	26.11.2019	25.11.2024	re-registration	Vet-synthesis	
Ferofort	12.04.2017	11.04.2022	registration	Fortis-pharma	
Bioferon Forte	26.11.2019	25.11.2024	registration	Biopharm	
Ferro 2000	07.07.2020	16.12.2023	changes	Dopharma France	France
Gafervit	07.07.2020	06.07.2025	re-registration	Bioveta, a.s	Czech Republic
Ferribion 10 %	15.07.2019	14.07.2024	re-registration		
Intrafer-100 B12	07.07.2020	06.07.2025	re-registration	Interchem Verken "De Adelaar" Esti AS	Estonia
Intrafer-200 B12	04.03.2019	03.03.2024	re-registration		
Uniferon	28.02.2018	27.02.2023	re-registration	Pharmacosmos A/S	Denmark

As for the composition of antianemic drugs, their main active ingredient is a complex of iron (III) hydroxide with low molecular weight dextran. In addition to this combination, the following drugs contain Ferovet B_{12}, Intrafer-200 B_{12}, Intrafer-100 B_{12} – cyanocobalamin; Iron+ – cyanocobalamin, folic acid; Ferofort – vitamins B_6 and B_{12}; Bioferon forte – copper chloride, cobalt chloride, cyanocobalamin. The most diverse composition is in the drug Gafervit, which contains

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normal pig serum immunoglobulin, iron dextran, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, nicotinamide, calcium pantothenate, copper chloride, and cobalt anhydrous chloride.

Thus, according to the results of our research, the modern pharmaceutical market of veterinary drugs in Ukraine during 2017–2022 was sufficiently provided with antianemic drugs for pigs, and mostly imported drugs.

As seen from Table 1, the part of Ukrainian antianemic veterinary drugs for pigs registered was slightly higher in 2020. Still, in 2022 the ratio of imported drugs of this pharmacological group again prevailed.

CONCLUSION

The modern pharmaceutical market of veterinary drugs in Ukraine from 2017 to 2022 was analyzed. Analysis of the national market of veterinary iron-containing drugs in 2017, showed that it was represented by 13 drugs of group QB03A «Antianemic drugs. Drugs of iron», according to the ATC-vet classification. 38% of this market was provided with pharmaceutical products from Ukrainian manufacturers. According to the results of our research, the modern pharmaceutical market of veterinary drugs in Ukraine during 2017–2022 was sufficiently provided with antianemic drugs for pigs, but mostly imported drugs. This motivates Ukrainian scientists and manufacturers to work on creating and producing national antianemic drugs to reduce Ukraine's dependence on foreign manufacturers.

DECLARATIONS

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Authors' contribution
The authors participated equally in data analysis and writing the manuscript.

Conflict of interests
The authors have not declared any conflict of interests.

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PRODUCTIVE PERFORMANCE AND HEMATOLOGICAL INDICES OF BROILER CHICKS FED BIODEGRADED CASSAVA ROOT

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ABSTRACT: To evaluate the performance and hematological indices of broiler chickens fed biodegraded cassava root meal an experiment was conducted in the Poultry Unit of the Livestock Teaching and Research Farm, Joseph Sarwuan Tarka University, Makurdi, Benue State. A total of one hundred and fifty five week-old (Ross 308) finishing broilers was used for the experiment. The birds were assigned randomly into three treatments and each treatment was replicated five times with ten birds per replicate. Cassava root was peeled and chopped into small pieces of about 90-100g and mixed with rumen filtrate (fluid). This was then biodegraded for 24 hours and 48 hours. The biodegraded cassava root meal (BCRM) was used to formulate broiler's diets at a 10% inclusion level to supplement for maize. The diets formulated were T1, T2 and T3 at 0%, 10% (24 hours biodegraded) and 10% (48 hours biodegraded) inclusion, respectively. The birds in each replicate were housed in separate cages in a completely randomized design (CRD). All routine management practices, including recommended vaccinations were strictly observed, feed and water were served *ad libitum* throughout the period of the study which lasted for 28 days. Performance indices such as body weight, body weight gain, feed intake and feed conversion ratio were measured. Hematological parameters were also taken; pack cell volume (PCV), red blood cell (RBC), hemoglobin (HB), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell (WBC) differential. Results revealed that there was no significant ($P>0.05$) differences in all the performance indices measured except in feed intake. Feed intake increased with prolonged period of biodegradation across treatments. There was significant ($P<0.05$) differences in the lymphocytes, heterophils and eosinophils across treatments. The study concluded that the dietary supplementation of 10% biodegradable cassava root meal at 24 and 48 hours did not adversely affect the performance/ health status of broiler chicken, however, for profit maximization, 48 hour biodegradation of cassava is recommended.

Keywords: Biodegradation, Broiler Chicken, Cassava Root, Maize, Productive performance.

INTRODUCTION

Feeding farm animal especially the non- ruminant has been a serious problem to livestock farmers. Feed takes the much of production cost; about 70-75% of total cost of production (Abang et al., 2013). This is largely due to the hike in conventional feed stuffs like maize and cereal grains on the whole as a result of stiff competition between man, livestock and, industry (Anoh and Akpet 2013; Abang et al., 2023). The need to look inward in search of feed resources that are available all round, less competed for by animal farmers is being sort for. Cassava is grown in Nigeria all year round; it is always available (Enesi et al., 2022; Obayelu et al., 2022). The yield is impressive; Nigeria is among the countries with highest tons/ metrics of cassava (Enesi et al., 2022; Abang et al., 2023).

Early studies indicated statistically significant growth depression in chicks with increasing amounts of cassava, which led to a recommendation that no more than 10% cassava should be included in chick rations. However, there are several reports on the use of cassava meal in poultry diets in the past few decades with encouraging results (Akinfala et al., 2002; Aderemi et al., 2010): birds fed ensiled cassava peel meal diet had similar feed intake and body weight gain as the control group whereas the FCR of birds on sun-dried cassava peel meal was poor (Obikaonu and Udedibie, 2006). Aderemi et al. (2020) asserted that whole cassava root meal could replace 25% of corn in laying hen diets without negative effect on the performance. In a study by Oyebimpe et al. (2006), 200 g/kg cassava peel meal could replace maize in broiler diets with no reduction in growth performance. However, its' limitation is the presence of anti-nutritional factors especially; hydrogen cyanide, saponins, tannins, oxalate, phytates; processing methods like: fermentation/ soaking, boiling, toasting, sun drying auto cleaving etc. is able to reduce these anti nutrients to a tolerable level (Abang et al., 2018; Odunlade et al., 2020; Gyang et al., 2021). The presence of these anti nutritional factors informed the assay for hematology. Anti-nutritional factors chelates divalent ions (Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+}) also react with the charged groups of protein and polysaccharides thereby forming indigestible complexes while the toxic substances interfere with bio availability and utilization. Blood contains several metabolites which provide useful information of nutritional status and clinical investigation of an individual; WHO recommends blood parameters for medical and nutritional assessments (Abang et al., 2017).

The aim of this study is to evaluate the productive performance and hematological indices of broiler chicks fed biodegraded cassava root.

Citation: Abang FBP, Anoh KU, Izuki ED, Nsa EE, and Ijoko N (2023). Productive performance and hematological indices of broiler chicks fed biodegraded cassava root. *Online J. Anim. Feed Res.*, 13(4): 274-278. DOI: https://dx.doi.org/10.51227/ojafr.2023.41

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Experimental Poultry unit of the Teaching and Research Farm of Joseph Sarwuan Tarka University Makurdi, Benue State of Nigeria. Makurdi is the Capital of Benue State located on central Nigeria along the Benue River. It lies within the geographical coordinates of Latitude 7^{0}44` north, and Longitude 8$^0$20` east. The area is warm with a minimum temperature range of 22.71±3.43°C and a maximum range of 39.98±2.43°C (TAC, 2011). It is characterized by two seasons, the dry and wet. The wet season also known as rainy season starts from April to October with minimum break at July under normal basis. The total annual rainfall is estimated from 1371-1321 mm, characterized by warm climate with average temperature and relative humidity (TAC, 2004).

Experimental material

The experimental test ingredient was biodegraded cassava root meal. The cassava root was obtained from local markets around Markurdi metropolis. The rumen content was obtained from the abattoir in North Bank Makurdi in the early hours of the day. It was collected from three different cows and mixed thoroughly to obtain a homogeneous mixture.

Processing of test ingredient

The cassava roots were hand-peeled using kitchen knife and chopped into smaller pieces of about 90 - 100 grams. Rumen content was mixed with water at a ratio of 1:1 (1 kg of rumen content to 1 liter of water) to obtain a filtrate. The filtrate was mixed with the chopped cassava root and packed into air tight bags. The bags were kept under shade with their open ends tied tightly and labeled accordingly. After 24 hours of fermentation, the bags labeled "24" were poured on a concrete floor for sun-drying while this was repeated for the remaining bags at the end of the 48th hour. The biodegraded cassava was dried to a moisture level below 10 percent and was crushed into meal. A fraction was taken for proximate.

Preparation of diets

A total of three experimental diets were formulated. Milled biodegraded cassava root meal was included in the diets at 0%, 10% (24 hours biodegraded) and 10% (48 hours biodegraded) to give diets T1 (control) T2 and T3 respectively to replace maize.

Management of birds

A total of 150 (Ross-308) five week old finishing broiler chickens were obtained from the hatchery's area distributors in Makurdi, Benue State. The birds were assigned randomly into three treatments and each treatment was replicated five times with ten birds per replicate. The birds in each replicate were housed separately. The experimental design used was completely randomized design (CRD). All routine management practices, including recommended vaccinations and medications, were strictly observed throughout the period of the study. A known quantity of feed was served daily, left over was measured to ascertain feed intake. Quantity of feed was increased weekly to enable birds feed ad libitum. The weights of the birds were taken weekly using electronic scale. Feeders and drinkers were washed and disinfected when appropriate. Litter materials were changed when due and replaced accordingly. Other drugs that were given to the birds include: Panteryl (antibiotics) as prophylaxis and vitaminolyte (vitamins). Feed and water were served ad libitum throughout the experimental period. The experiment lasted for 4 weeks (5th-8th).

Table 1 - Composition of broiler finisher diet using biodegraded cassava root meal

Ingredients	Control	24 Hours	48 Hours
Maize	63.75	57.25	7.14
BCRM	-	5.73	6.00
FFSB	17. 69	18.17	4.86
Blood meal	6.63	6.81	5.71
Bone meal	4.42	4.54	6.29
Rice bran	1.00	1.00	1.00
Fish meal	5.00	5.00	5.00
Palm oil	1.00	1.00	1.00
Salt	0.25	0.25	0.25
Premix	0.25	0.25	0.25
Total	100.00	100.00	100.0
Analyzed nutrient			
ME (Kcal/kg)	3290.48	3217.33	3203.15
Crude protein%	20.54	20.13	20.14
Crude fiber%	3.47	3.47	3.48
Ether extract%	7.34	7.10	7.10
Calcium%	1.04	1.05	1.05
Phosphorus%	0.71	0.78	0.69
Lysine%	1.11	1.50	1.10
Methionine%	0.46	2.64	0.21
Ash%	3.36	2.64	1.91

Premix supplied per kilogram Vit A: 10000000IU; Vit D3: 2000001U; Vit K3: 2000mg; Vit B1: 3000mg; Vit B2: 5000mg; Niacin: 45000mg; Calcium panthothenate: 10000mg; Vit B6: 4000mg., Vit B12: 20mg; Choline chloride: 300000; Folic acid: 1000mg; Biotin: 50mg; Manganese: 300000mg; Iron: 120000mg; Zinc: 80000mg; Copper: 8500mg; Iodine: 1500mg; Cobalt: 300mg; Selenium: 120mg; Antioxidant: 120000mg. BCRM=Biodegraded cassava root meal; FFSB=Full fat Soybean; ME=Metabolizable energy; T1=Control diet; T2=24 hours BCRM; T3=48 hours BCRM.

Citation: Abang FBP, Anoh KU, Izuki ED, Nsa EE, and Ijoko N (2023). Productive performance and hematological indices of broiler chicks fed biodegraded cassava root. *Online J. Anim. Feed Res*., 13(4): 274-278. DOI: https://dx.doi.org/10.51227/ojafr.2023.41

Data collection

Performance indices

Productive performance indices were measured according to the methods of Abang et al. (2023).

Blood samples and preparation for hematological indices

This was carried out according to the methods of Abang et al. (2017).

Ethical approval

All authors hereby declared that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Statistical analysis

Data collected were subjected to one-way Analysis of Variance (ANOVA) using Special Package for Social Science (SPSS) version 22.0 statistical software. Significant means were also separated using Duncan's Multiple Range Test of the same package at 5% probability level.

RESULTS

Performance

The result of the growth performance of broiler finisher birds fed biodegraded cassava root meal-based diets is shown in Table 3. There were no significant (P>0.05) differences in all the growth parameters measured across treatment groups. This revealed that birds served BCRM competed favourably with birds fed sole maize grains. The impressive performance showed that the use of rumen fluid in biodegradation of cassava root meal results in effective utilization of the diets. This confirms the position of Adeyemi et al. (2008), whose reports showed that cassava root meal can completely replace maize in broiler diets without adverse effects on utilization, but, contradicts the results of Nsa et al. (2016), who observed a depressive growth in broilers fed cassava. The non-significant (P>0.05) difference observed in feed intake reveals the palatability of feed; this is a pointer to the fact that the anti-nutritional factors present in cassava were adequately handled by the microbes from rumen fluid. Akinmutimi (2004) and George and Sese (2012) had similar results of high feed intake when birds were served biodegradable cassava root meal. However, Aderemi (2010) and Ogbamgba and George (2015) had a contrary report; feed intake was observed to have decreased with increased levels of supplementation across treatments, probably because of the higher fiber and Hydrogen cyanide (HCN) contents of diets. More so, the dustiness of the feed associated with cassava peel/root meal and low palatability, could also be the reason for the reduction in feed consumption of birds fed diets with high levels of cassava meal.

The feed cost savings per gram meat (₦) was recorded to be (0.01) at 48 hours of biodegradation. This infers that, the inclusion of T3 in finisher diet resulted in saving 0.01 kobo for every gram of meat produced.

The result of profit ranged from ₦465.90 - ₦1071.40, RNI ranged from 0.39-0.91, with the highest profit margin and RNI recorded with birds served 48 hours BDCR. Birds serve 24 hours BDCR recorded the least values probably because of the highest amount of feed consumed as well as time of biodegradation; perhaps not sufficient enough to reduce HCN present in cassava root which subsequently affected their body weight. The highest BCR was recorded with birds served 48 hours BDCR, followed by birds fed control diet. It is worthy of note that a BCR greater than one signifies viability of an enterprise. However, all treatments recorded BCR greater than one implying that, no losses will be incurred when these treatments are used in finisher broiler production.

Table 2 - Productive performance of finisher broiler chickens fed diet containing biodegraded Cassava root meal.

Parameters	Control	24 Hours	48 Hours	SEM	P-value
Initial body weight (g)	726.00	660.00	664.00	15.57	0.153
Final body weight (g)	3000.89	2877 .05	2910.11	41.92	0.122
Total body weight gain (g)	2274.00	2217.05	2246.11	38.17	0.157
Average daily weight gain (g)	32.49	31.67	32.08	1.36	0.157
Total feed intake (g)	4333[a]	41 62[a]	3831[b]	10.73	0.112
Average daily feed intake (g)	61.90[a]	59.46[b]	54.73[b]	3.60	0.112
Feed conversion ratio	1.91	1.88	1.7 1	0.24	0.162
Cost of feed/weight gain (#/g)	0.26	0.26	0.25	-	-
Feed cost savings/g meat (#/g)	-	0	0.01	-	-
Profit: TR-TC	817.84	465.90	107.1.4	-	-
BCR: TR/TC	1.69	1.40	1.91	-	-
RNI: Profit/TC	0.69	0.39	0.91	-	-

ab= means with different superscript within same row are significantly different (p<0.05); SEM= Standard Error of Mean; BCR= Benefit cost ratio; RNI= Return to Naira invested; TR= Total Revenue; ,TC= Total Cost

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Hematology

The result of the hematological parameters of broiler chicken fed biodegraded cassava root meal is presented in Table 4. There was no significant (P>0.05) difference in PCV, RBC, WBC, Hb, MCV, MCH, MCHC and Monocytes across treatments. The value for pack cell volume (PCV) ranged from 31.20-32.00%, RBC (3.14-3.28) while WBC (21.02-21.92), Hb (10.38-10.80) MCH (32.02-32.86) for MCHC (33.26-33.75). These values fell within the normal reference ranges as reported by Bounous and Stedman (2000) and Talebi et al. (2005) indicating that the birds had no traces of anemia. Non-significant (P>0.05) difference in the values of PCV, RBC and WBC was also observed by Adeyemi et al. (2008) when cassava root was fermented with rumen filtrate. However, the results of leucocytes, heterophils and eosinophils were significantly (P<0.05) different across treatment groups. The value of lymphocytes was within the recommended reference value of 31.00-72.00% reported by Scholtz et al. (2009). It was observed that, the levels of heterophils and eosinophils decreased across treatments with birds fed diets containing BCRM having the least values, however, all the values were within the normal reference ranges. Since lymphocytes, heterophils and eosinophils are components of the white blood cell; the values obtained did not give an indication of any disease or stress condition resulting from the dietary treatments. Fafiolu et al. (2014) reported that the birds with higher leucocytes count could perform their phagocytic functions for optimal immunity levels and would cope successfully under stress conditions. Also, Talebi et al. (2005) reported that birds with high leukogram counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases. Lymphocytes offer a more versatile means of defense as they are capable of recognizing different foreign invaders thereby producing cytokines, lymphokines, and ultimately, antibodies with specificity for antigens.

Table 3 - Hematological indices of broiler chicken fed diet containing biodegraded cassava root meal.

Parameters	Control	24 Hours	48 Hours	SEM
PCV %	31.20	32.00	32.00	0.43^{NS}
RBC $\times 10^{12/l}$	3.14	3.22	3.28	0.06 ^{NS}
WBC $\times 10^{12/l}$	7.02	7.50	7.92	0.18 ^{NS}
HB/dl	10.38	10.68	10.80	0.14 ^{NS}
MCV fl	99.36	99.38	99.25	0.18 ^{NS}
MCH pg	32.86	33.22	33.02	0.38 ^{NS}
MCHC g/dl	33.26	33.37	33.75	0.12 ^{NS}
Lymphocytes %	42.20^{c}	44.60^{b}	47.20^{a}	0.68*
Heterophil %	53.60^{a}	51.00^{b}	50.20^{b}	0.70*
Eosinophil %	2.40^{a}	0.40^{b}	1.00^{b}	0.30*
Basophil %	0.00	0.00	0.00	0.00 ^{NS}
Monocyte %	1.40	1.00	1.60	0.34 ^{N}

^{abc} = means with different superscripts within same row are significantly different (P<0.05). NS= non significance, T1= Control diet, T2= Diet containing biodegraded level of cassava root meal at 24 hours of fermentation, T3= Diet containing biodegraded level of cassava root meal at 48 hours of Fermentation, SEM= Standard error of mean. PCV= pack cell volume; RBC= red blood cell; WBC= white blood cell; HB= hemoglobin; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration.

CONCLUSION

The study concluded that the dietary supplementation of 10% biodegradable cassava root meal at 24-hour and 48 hours did not adversely affect the performance/health status of broiler chicken, however, for profit maximization, 48-hour biodegradation of cassava is recommended.

DECLARATIONS

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Authors' contribution

F. B. P. Abang performed conceptualization, writing, original draft preparation, review and editing. K. U. Anoh performed conceptualization and evaluation of manuscript before submission. E. D. Izuki performed conceptualization and evaluation of manuscript before submission. E. E. Nsa performed conceptualization and evaluation of manuscript before submission. N. Ijoko performed conceptualization and evaluation of manuscript before submission.

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Conflict of Interests

The authors declare that there is no conflict of interest.

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EFFECT OF COMBINATION OF *Indigofera zollingeriana,* BLACK SOLDIER FLY LARVAE, AND TURMERIC ON PERFORMANCE AND HISTOMORPHOLOGICAL CHARACTERISTICS OF NATIVE CHICKEN AT STARTER PHASE

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Sources of high protein feed ingredients can come from plants and animals (insects), namely *Indigofera zollingeriana syn.* and black soldier fly larvae. The addition of natural feed additives to feed can be obtained from turmeric phytobiotics which have many biological activities, such as anticancer, anti-inflammatory, antimicrobial and antioxidant. This study aimed to determine the effect of the combination of *Indigofera zollingeriana syn.*, black soldier fly (BSF) larvae and turmeric on the performance and histomorphology of the bursa Fabricius in the native chicken starter phase. The research design was completely randomized with 3 treatments and 5 replications with 6 chickens per unit. The combination treatments were P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour). Parameters measured in this study were performance (body weight gain, feed consumption, FCR) and bursa of fabricius histomorphology in native chickens. The results of the analysis of variance showed that the combination of *Indigofera zollingeriana syn.*, BSF larvae and turmeric had a significant effect on the performance of native chickens but could not match the performance of P0 (control feed). While the histomorphology of bursa Fabricius showed that the combination of *Indigofera zolliengeriana* flour up to 10% and 25% black soldier fly larvae flour in the feed identified the medulla width, cortex thickness and follicle width can increase lymphocyte cells to produce antibodies for native chickens at starter phase.

Keywords: Feed additives, *Indigofera zoillingeriana*, Insect, Native chicken, Larvae

INTRODUCTION

In native farming, to achieve fast growth and high productivity, it is necessary to feed that contains the needed nutrients and alternative feedstuffs (Ogbuewu et al., 2017; Truong et al., 2019). According to Varianti et al. (2017) that the quality of poultry feed is mainly estimated from the protein content (the higher and complete the amino acid content).

The protein content of the feed is related to growth rate because protein is used to form new tissue, maintain existing tissue and replace damaged tissue (Post and Hocquette, 2017). The protein content of feed has an important role in increasing protein consumption in chickens, according to Saputro et al., (2016) explaining if low protein consumption causes antibodies to be formed slightly so that the physiological function of chickens is disturbed, they are susceptible to diseases that affect the development of the bursa of Fabricius organs in chickens.

One source of herbal feed ingredients derived from plants is *Indigofera zollingeriana syn.* which is rich in nutrients; especially crude protein of 27.9% and 27.68% (Abdullah, 2010). Muhammad et al. (2021) stated that the substitution of soybean meal with *Indigofera zollingeriana syn.* up to a level of 15% (Soybean meal substitution PK = 4.23%) + 2.5 turmeric was able to increase the commercial cut of carcass and the percentage of the weight of the digestive tract of native chicken in the grower phase.

Another source of alternative feed ingredients derived from insect species is black soldier fly (BSF) larvae (*Hermetia illucens*) with a protein content of 40 - 50% (Lu et al., 2022). BSF larvae have a high lauric acid content and can function as a natural antimicrobial agent (Kim and Rhee, 2016). BSF larvae extract or maggot has inhibitory activity against the Gram-negative bacteria group (Sundari et al., 2022). Antimicrobial activity is very influential on the health and development of digestive tract organs and lymphoid organs (Derthi, 2012). Increasing protein consumption in native chickens can be done by using a natural feed additive made from herbs, namely curcumin. Curcumin is an active ingredient that is derived from turmeric phytobiotics, which have a wide range of biological benefits, including antioxidant, anti-inflammatory, anti-cancer, and anti-inflammatory properties (Araújo and Leon, 2001). Histopathological examinations of lymphoid organs after injection of Salmonella pullorum and *E. coli* showed that the use of turmeric at a level of 2.5% was able to maintain performance, internal organs and lymphoid organs in good condition (Purwanti et al, 2019).

This study aimed to determine the effect of the combination of *Indigofera zollingeriana syn*, black soldier fly (BSF) larvae and turmeric on the performance (feed consumption, weight gain and feed conversion ratio) and histomorphology of the bursa Fabricius in the native chicken starter phase.

Citation: Muhammad LN, Purwanti S, Pakiding W, Marhamah, Nurhayu, Prahesti KI, Sirajuddin SN and Mushawwir A (2023). Effect of combination of *Indigofera zollingeriana,* black soldier fly larvae, and turmeric on performance and histomorphological characterizes of native chicken at starter phase. *Online J. Anim. Feed Res*., 13(4): 279-285. DOI: https://dx.doi.org/10.51227/ojafr.2023.42

MATERIALS AND METHODS

From June to August 2022, at the Poultry Production Laboratory, Faculty of Animal Science, Hasanuddin University, South Sulawesi, Indonesia, 90 native chickens with the same body weight were randomly divided into 15 units for three treatments and five replications. First of all, the maintenance of native chickens in this study was through a brooding period of 100 aged chicks 1 day for 7 days. Domestic chicken rearing from day 8 to day 28 is the starter phase and day 29 to day 56 is the grower phase. The treatments groups consisted of P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour). The research began with the preparation of *Indigofera zollingeriana* shoot flour, the manufacture of turmeric flour and the manufacture of BSF (*Hermetia illucens*) flour. During maintenance, feed and drinking water were provided *ad libitum* based on treatment. The composition of the feed nutrients used during this study is in Table 1.

The parameters measured in this study were the performance and histomorphology of the bursa of the Fabricius organ of native chicken. Native chicken performance consists of feed consumption obtained from weighing the remaining feed given minus the remaining feed, weight gain can be obtained from the difference in body weight of chickens and feed conversion is obtained from the amount of feed consumed at a certain time divided by weight gain. The bursa of the Fabricius organ was obtained by slaughtering and cutting the samples in the starter phase (4 weeks) in each treatment. Slaughtering of chickens is done at the base of the neck by severing the respiratory tract (trachea), the feeding tube (esophagus) and the two neck veins (blood vessels on the right and left of the neck) with a single incision without lifting the knife. The slaughter process is done from the front of the neck and does not break the neck bone. According to Masyitha and Budiman (2017) the histological description of the bursa Fabricius includes the cortex, medulla, number, and diameter of follicles as well as the epithelial zone.

Table 1 – Nutrient composition

Ingredients	ME (Kkal/Kg)	CP (%)	EE (%)	CF%)	Lysine (%)	Methionine (%)	P (%)	Ca (%)
Yellow Corn	3291.27	9.88	1.79	5.70	0.06	0.18	0.60	0.02
Rice Bran	2730.00	13.40	5.10	11.50	0.42	0.30	2.50	0.20
Coconut meal	1525.00	16.00	15.00	16.00	0	0	0.75	0.30
BSF larvae	3596.40	46.14	21.88	13.12	0	0.00	0.93	1.28
Indigofera zollingeriana	2617.41	36.18	4.74	11.11	2.05	0.67	0.58	0.13
Cassava Flour	3200.00	2.00	12.70	11.40	0.07	0.01	0.40	0.33
Rice polish	1451.85	10.60	13.66	27.80	0	0	1.48	0.05
Heap	3500.00	1.88	15.62	0.25	0	0	0.05	0.31
DCP	0	0	0	0	0	0	21.00	16.00
$CaCO_3$	0	0	0	0	0	0	0.04	39.00
Lysine	0	0	0	0	99	0	0	0
Methionine	0	0	0	0	0	99	0	0

Turmeric flour as much as 2.50% as a feed additive (Purwanti et al., 2014). The Result Laboratorium of Livestock Biotechnology Integrated Laboratory Test, 2022.

Table 2 - Feed ingredients composition and nutrients value in the starter phase of the feed

Ingredients	P0	P1	P2
Yellow corn	Commercial Feed as feed control	47	46
Rice Bran		12.4	14.9
Coconut meal		6	4.5
NaCl		0.5	0.5
Premix		1.5	1.5
BSF larvae		25	20
Indigofera		5	10
DCP		1	1
CaCO3*		1	1
L-Lysin		0.3	0.3
DL-Metionin		0.3	0.3
Nutrient Composition			
ME (Kkal/Kg)	-	3006.89	2970.4
Crude Protein (%)	20	20.97	20.47
Crude Fat (%)	5	8.08	7.11
Crude Fiber (%)	5	8.9	8.79
Lysin (%)	1.2	0.48	0.59
Metionin (%)	0.45	0.45	0.49
P (%)	0.5	1.11	1.14
Ca (%)	0.6	0.93	0.87

Calculated based on the results of data analysis and calculation Table 1. P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour).

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Ethical regulation

Maintenance management in this study refers to the Australian Animal Welfare Standards and Guidelines for Poultry and slaughtering native chickens in this study refers to the Indonesian National Standard (INS) number 99002 of 2016 concerning halal slaughtering of poultry.

Statistical analysis

The research design was carried out using a Completely Randomized Design (CRD) with a significance level of 5%. If the data is significantly different at the P<0.05 on the measured parameters, then the differences between treatments were analyzed by an orthogonal contrast test.

RESULTS AND DISCUSSION

The results of the study on the combination of *Indigofera zollingeriana syn,* BSF larvae and turmeric on the performance of native chickens can be seen in table 2. The data in table 2 shows a significant effect (P<0.05), then proceed with further contrast tests which can be presented in table 3.

Table 3– Performance of native chicken fed a combination of *Indigofera zollingeriana*, larvae BSF and turmeric in the ration

Parameter \ Treatment	P0	P1	P2
Weight gain (g/bird/day)	9.47 ± 0.20	4.27 ± 0.29	3.46 ± 0.37
Feed consumption (g/bird/day)	18.32 ± 0.36	13.11 ± 0.56	11.41 ± 1.19
FCR	1.94 ± 0.05	3.08 ± 0.10	3.30 ± 0.04

P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour).

Table 4– Performance of native chicken (orthogonal contrast test)

Parameter		Significant
Weight gain	P0 Vs P1 and P2	114.289*
	P1 Vs P2	18.698*
Feed consumption	P0 Vs P1 and P2	118.227*
	P1 Vs P2	11.485*
FCR	P0 Vs P1 and P2	1186.18*
	P1 Vs P2	0.001^{ns}

*significant. ^{ns} non significant

Feed consumption

Based on Table 1 on feed consumption during the study were obtained from the average consumption of native chickens during the rearing phase. Feed consumption in the starter phase of native chicken in treatment P0 which was 18.32 grams/bird/day was higher than that of treatment P1 and P2. Namely 13.11 and 11.41 grams/bird/day. Further tests showed feed consumption in the starter phase, namely P0 Vs P1 and P2 showed a significant effect and P1 Vs P2 also showed a significant effect. This was because the P1 and P2 feed contained *Indigofera zollingeriana syn.* and BSF mangosteen which had high crude fiber content compared to the P0 treatment (control feed). The higher the crude fiber content, the faster the digesta rate, the shorter the digestion process in the digestive tract so that a lot of nutrients are lost to faces or excreta. This is in line with the opinion of Supriyanto et al. (2021) who reported that high crude fibre feed prevents chicks from utilizing nutritional feed properly and undigested crude fibre will carry nutrients out with faeces or excreta. Feeds that contain high crude fibre cannot be digested completely and will cause a full fast cache which results in limited feed consumption. The high crude fibre content in the ration will make the chickens feel full quickly because the crude fibre is bulky and will expand when exposed to water Supriadi et al. (2021). BSF flour contains high fibre so it can cause clumping of feed. Therefore, birds become full quickly and reduce the level of feed consumption (Rahayu et al. 2021).

Weight gain

The increase in body weight gain is influenced by the type of feed given to the chickens. Table 2 shows that the body weight gain of treatment P0 (9.47 grams/bird/day) was much higher than that of treatment P1 (4.27 grams/head/day) and P2 (3.46 grams/head/day). This was due to the low level of feed consumption of P1 and P2 obtained during the maintenance of Native chickens in the starter phase. This is in line with the opinion of Nugraha et al. (2017) reported that body weight gain is closely related to feeding, in terms of quantity related to feeding consumption if feed consumption is disturbed it will interfere with growth.

Citation: Muhammad LN, Purwanti S, Pakiding W, Marhamah, Nurhayu, Prahesti KI, Sirajuddin SN and Mushawwir A (2023). Effect of combination of *Indigofera zollingeriana,* black soldier fly larvae, and turmeric on performance and histomorphological characterizes of native chicken at starter phase. *Online J. Anim. Feed Res*., 13(4): 279-285. DOI: https://dx.doi.org/10.51227/ojafr.2023.42

Further tests showed body weight gain in the starter phase, namely P0 Vs P1 and P2 showed a significant effect (P<0.05) and P1 Vs P2 also showed a significant effect (P<0.05). This is because the balance of energy metabolism and protein in the P0, P1 and P2 feed treatments is not much different, ranging from 143.39% - 145.10%. This is the opinion of Allama et al. (2012) who reported that the balance of food substances, especially protein and energy, is very important because it significantly affects the speed of body weight gain. The balance of energy and protein is intended to meet the minimum protein requirement because a lack of energy will convert protein into energy.

Chickens that received feeds with high energy and protein became efficient in changing feed to increase body weight gain, while chickens that received feeds with lower energy and protein were less efficient in using the feed to increase body weight gain (Eriko and Nur, 2015) According to Rahayu et al. (2021), protein plays an important role in chicken growth, because it contains essential and non-essential amino acids.

Feed conversion ratio

The relationship between feed consumption and body weight gain is determined by feed conversion. Low feed conversion values indicate better feed use efficiency. This means that the chickens are more efficient in consuming feed for meat production (Allama et al., 2022). Feed Conversion Ratio (FCR) in the starter phase showed that the mean of treatment P1 (3.08) and P2 (3.30) was higher than treatment P0 (1.94). Further tests showed that the FCR in the starter phase, namely P0 Vs P1 and P2, showed a significant effect (P<0.05) while P1 Vs P2 also showed a significantly different effect (P>0.05). The results of this study are different from Wahid et al. (2021) study which concluded that the substitution of fish meal with BSF maggot flour in native chickens was 25% FCR obtained by 4.62% while Supriadi et al. (2021) study reported that the use of 10% *Indigofera zollingeriana syn*. flour in chicken feed the village FCR obtained is 2.65. The difference in FCR in these three studies was due to the difference in feed consumption and body weight gain of each study, this was influenced by the different breeds of Native chickens used. This is in line with the opinion of Herlina et al. (2015) who said that the things that affect the consumption of rations are the breed of chickens, environmental temperature, production stage and energy in the ration. Feed conversion is closely related to feeding consumption and body weight gain.

Histomorphology of bursa Fabricius chicken native starter phase

The bursa of Fabricius is the main lymphoid organ in avian species. The bursa of Fabricius in birds has an important role as a central lymphoid organ for B lymphocyte differentiation. In addition, the bursa has B lymphocytes that have immune competence capable of producing local antibodies (Wu et al., 2013). The results of the histological study of the bursa of Fabricius of native chicken in the starter phase in each treatment can be seen in Figures 1, 2 and 3.

The bursa of Fabricius has follicles consisting of a medulla and cortex, according to Madej et al. (2013) saying that the development of the bursa of Fabricius occurs through the formation and colonization of the medulla during the embryonic stage, while the cortex develops after hatching. The figure below shows that the medulla in the P0 treatment was wider than in the P1 treatment but the medulla in the P2 treatment was wider than in the P0 treatment. This shows that there are more lymphocyte cells in P2 treatment compared to other treatments, this is in accordance with the opinion of Ebru et al. (2015) who reportede that the medulla only contains lymphocyte cells, these lymphocyte cells function to produce antibodies in chickens. The area of the medulla in the P2 treatment was influenced by the turmeric content in the ration, in the opinion of Nasrullah et al. (2020) saying that giving turmeric to chickens was thought to affect the increase in the number of lymphocytes. Curcumin has the ability to activate T lymphocyte cells and B lymphocyte cells which are part of lymphocytes.

The histological cortex of the bursa of Fabricius of native chicken in the starter phase showed that the P1 and P2 treatments were thicker than the control diet group. The width of the histological bursa follicles of native chickens in the starter phase showed that P1 and P2 were wider than those of the control diet. Ismiraj (2020) reported that most cell division occurs in the cortex, which the cortex consists of lymphocytes, plasma cells, and macrophages. In addition, Selim et al. (2021) reported that the width of the follicle which is higher than the bursa indicates an increase in the number of B lymphocytes and the formation of B lymphocytes for antibodies. The number of B lymphocytes in the treatment increased P1 and P2 due to the addition of immunomodulatory substances, namely *Indigofera zollingeriana syn*, black soldier fly larvae and turmeric. According to Sulistiyanto et al. (2019), an imunomodulator is a chemical substance, drug, or action of an immune system that can increase the body's immune system.

The results of the study on the number and diameter of the bursa of Fabricius follicles in the starter phase of native chickens can be seen in Table 4. The data in Table 4 shows a significant effect (P<0.05), then continued with the contrast test which can be seen in Table 5. The bursa of Fabricius has follicles consisting of a cortex and a medulla, each of which contains B lymphocytes. Aged B lymphocytes are released into the blood vessels to boost immunity. This release can be induced to produce optimum immunity. One way to generate immunity in chickens is to provide local/herbal feeds such as *Indigofera zollingeriana syn*, larvae BSF and turmeric. Table 3 shows the results of the calculation of the number of follicles in treatment P2 (41.87) which was higher than in treatment P0 (38.80) and P1 (31.40). The diameter of the follicles in the bursa can be seen in Table 3. It shows that the P2 treatment was higher than the P0 and P1 treatments. Further tests showed that the diameter of the follicles P0 Vs P1 and P2 showed a significant effect (P<0.05) and P1 Vs P2 also showed a significant effect (P<0.05). This is due to the content possessed by the combination of *Indigofera zollingeriana syn*, larvae BSF and turmeric which can help the growth of the bursa of Fabricius. *Indigofera zollingeriana*

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syn. contains anti-nutritional substances in the form of tannins which function as antibacterial (Azis et al., 2019). Larvae BSF has a high lauric acid content and can function as a natural antimicrobial agent (Kim and Rhee, 2016). Turmeric has many biological activities, such as anticancer, anti-inflammatory, antimicrobial and antioxidant (Araújo and Leon, 2001).

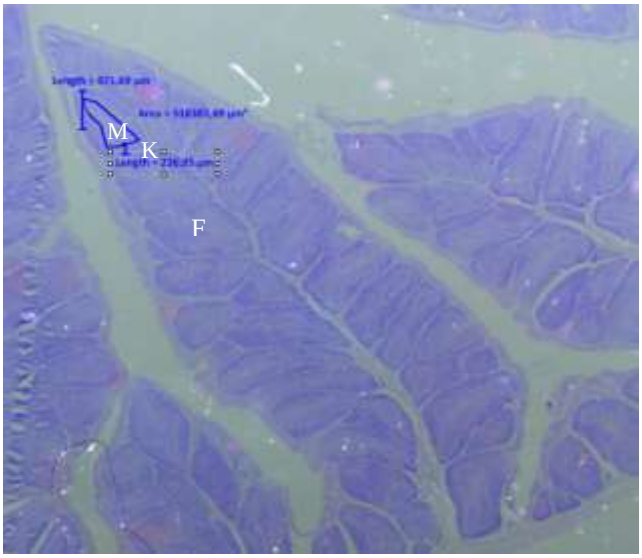

Figure 1 - Bursa Fabricius Feed Treatment P0. M = Medula; K = Corteks; F = Follicle

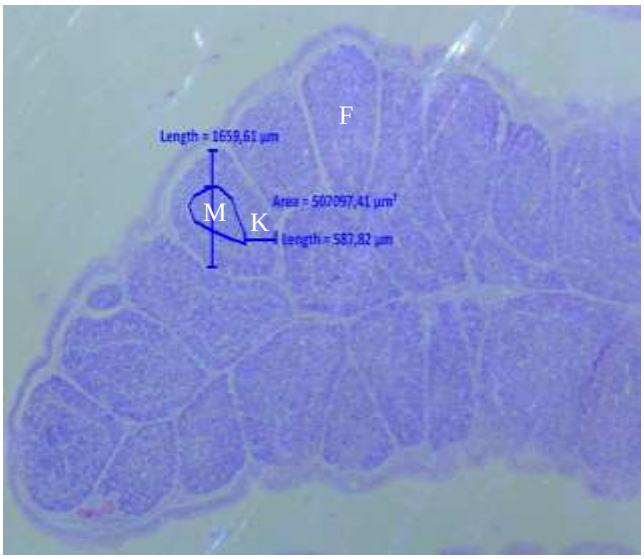

Figure 2 - Bursa Fabricius Feed Treatment P01. M = Medula; K = Corteks; F = Follicle

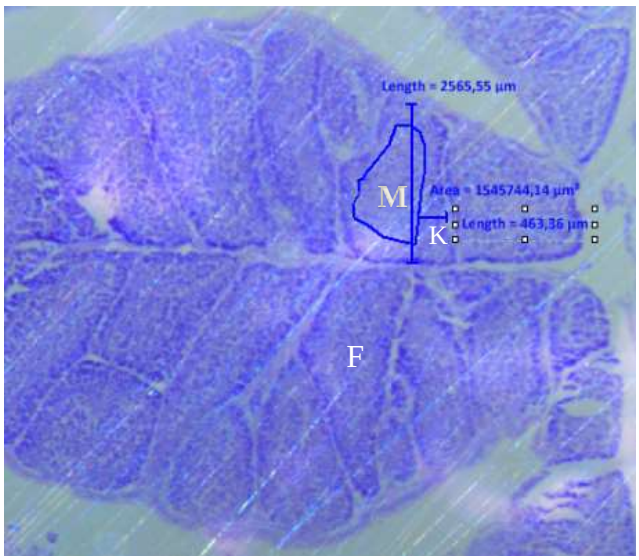

Figure 3 - Bursa Fabricius Feed Treatment P02. M = Medula; K = Corteks; F = Follicle

Table 5– The number and diameter of the bursa Fabricius follicle in the starter phase of native chicken

Parameter \ Treatment	P0	P1	P2
Number of Follicle	38.80 ±5.26	31.40 ± 12.25	41.87 ± 23.70
Follicle Diameter	1063.46 ± 219.63	1566.36 ± 221.83	1717.59 ± 489.97

P0 (Commercial feed as control); P1 (5% *Indigofera* flour + 25% BSF larvae flour + 2.5% turmeric flour) and P2 (10% *Indigofera* flour + 20% BSF larvae flour + 2.5% turmeric flour).

Table 6– Orthogonal contrast test of chicken native follicle diameter starter phase

Parameter	Folicle Diameter
P0 Vs P1 and P2	9.91*
P1 Vs P2	8.88*

*significant. ns non significant

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CONCLUSION

The combination of *Indigofera zollingeriana syn.* flour up to 10% and 25% black soldier fly larvae flour in feed did not produce native chicken performance (feed consumption, body weight gain and FCR) which was equivalent to commercial feed as a control. While the histomorphology of bursa Fabricius showed that the combination of *Indigofera zolliengeriana syn* flour up to 10% and 25% black soldier fly larvae flour in the feed identified the medulla width, cortex thickness and follicle width can increase lymphocyte cells to produce antibodies for Native chickens at starter phase.

It is necessary to be conducted trials on native chickens of other breeds to see the functionality of the prepared feed.

DECLARATIONS

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Authors' contribution

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

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Conflict of Interests

The authors declare that there is no conflict of interest.

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EFFECTS OF FODDER TREE LEAVE SUPPLEMENTATION FOR BASAL RICE STRAW DIET ON RUMEN AMMONIA, pH, AND DEGRADATION CHARACTERISTICS IN SHEEP

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Supporting Information

ABSTRACT: Fodder tree leaves (FTLs) contain high levels of protein, vitamins, and minerals that play a major role in enhancing roughage intake by ruminants, thus improving low-quality roughage utilization. The study sought to measure the rumen degradation characteristics, pH, and ammonia N concentration of sheep fed rice straw (RS) and supplemented with FTLs. Four forest-type rumen-fistulated rams of an average weight of 19.0±1.2 kg were randomly assigned to one of four treatments in a 4 × 4 Latin Square design. Treatment diets consisted of urea-ammoniated straw (UAS; control), RS+100% *Leucaena leucocephala* (L), RS+100% *Samanea saman* (S), and RS+50% L+50% S (LS). Data obtained were subjected to the Glimmix procedure of SAS (2016) and significant means were separated using Tukey's test at (P<0.05). Treatments differed significantly (P<0.001) in the quantity of readily soluble materials (a), rate of degradation (c), and a potentially degradable fraction (P) with LS recording the highest a, c, and P among the treatments. Ruminal pH and ammonia concentrations differed significantly (P<0.0001) among the treatments. Overall mean rumen pH values obtained ranged from 6.44 in UAS to 6.72 in the S-supplemented diet whereas mean rumen ammonia values ranged from 4.59 mg/100 ml in sheep fed UAS diet to 9.15 mg/L in sheep fed L diet. The pH values obtained imply that the experimental diets could improve rumen fermentation and, hence, serve as good sources of feed for ruminants. The rumen DM degradation values indicated that sufficient amounts of DM would be degraded over a period of time, thus releasing substantial quantities into the small intestines for digestion to provide essential nutrients needed for better animal performance. The rumen ammonia values obtained were higher than the minimum values recommended for optimal microbial activity for animals fed lignocellulosic materials. This indicated that such FTLs could be utilized for moderate animal performance, especially during the dry seasons when natural pastures are qualitatively and quantitatively poor.

Keywords: Ammonia concentration, Degradation, *Leucaena leucocephala*, *Samanea saman*, Sheep.

RESEARCH ARTICLE

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INTRODUCTION

Fodder tree leaves contain high levels of protein, vitamins, and minerals which play a major role in enhancing roughage intake by ruminants (Larbi et al. 1993; Idan et al., 2020), and hence improve low-quality roughage utilization (Idan et al., 2021; Adogla-Bessa et al., 2022). Sarkwa et al. (2020a) fed dried sole tree leaves to sheep and reported higher weight gain and lower methane emission than sheep fed urea-treated rice straw. However, feeding the same tree leaves in combinations resulted in higher weight gain and lower methane emission compared to feeding sole tree leaves (Sarkwa, 2020a). Improved weight gain of small ruminants as a result of feeding combinations of tree leaves has also been reported by Papachristou and Plastis (2011) and Idan et al. (2023).

The nylon-bag technique (*in sacco* degradability) is one of the powerful tools for ranking feeds according to the rate and extent of degradation of dry matter, organic matter, nitrogen, or other nutritional parameters (Osuji et al., 1993; Fonseca et al., 1998). This technique as reported by Abate and Kiflewahid (1991) is cheap, reliable, and easy to perform. Ørskov and McDonald (1979) described it as a major evaluation technique for determining the nutritive value of forages. A number of studies have demonstrated that the rumen's capacity for degrading feed provides a way of more thoroughly assessing the nutritional content of feedstuffs. For instance, Ørskov et al. (1980) indicated that because of its high degree of correlation with *in vivo* digestibility results, the *in sacco* nylon bag approach has been widely utilized for evaluating ruminal breakdown, screening feedstuffs, and estimating digestible organic matter intakes.

Ammonia concentration in the rumen fluid reflects the activities of fermentation of feed protein and protein synthesis by rumen microbes (Erdman et al., 1986; Gonzalez-Munoz et al., 2019; Tharwat et al., 2019). Ammonia production is the result of protein degradation from feed utilized by microbes as a source of protein (Gonzalez-Munoz et al., 2019; Tharwat et al., 2019). However, the minimum concentration of rumen ammonia for rumen microbes to thrive is

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5mg/100 ml (McDonald et al., 2012). Rumen ammonia level is necessary for efficient rumen microbial growth (Brooks et al., 2012) and gives an indication of dietary nitrogen adequacy. Moreover, rumen ammonia concentration is affected by the level of intake of protein, the extent of degradability, the duration of feed in the rumen, and the acidity of rumen fluid (McDonald et al., 2012). According to Gonzalez-Munoz et al. (2019) and Tharwat et al. (2019), the formation of ammonia in the rumen is a relevant factor influencing feed degradation.

Ruminal pH is an important factor influencing the rumen environment and subsequent performance of small ruminants. The degree of fiber in the diet influences rumen pH, with Castillo et al. (2006) indicating that feeding an excessively low-fiber or high-energy diet is associated with ruminal acidosis. In sheep, the normal ruminal pH ranges from 6.4 to 6.8. Previous studies have indicated that pH values of 5.5 or lower, or values of 7.0 or higher are deemed abnormal with the 5.5 or lower value resulting in subacute rumen acidosis (Beauchemin and Yang, 2005). Furthermore, continuous or occasional periods of low rumen pH are linked with animal health problems and can limit fiber digestion and hence microbial efficiency (Russell and Wilson, 1996; Faniyi et al., 2019).

Despite their relevance in predicting the nutritional value of feeds, rumen pH, ammonia, and degradation characteristics have not been extensively studied in Ghana. Therefore, the study, sought to ascertain the rumen degradation characteristics of sheep fed untreated rice straw and supplemented with sole and combined fodder tree leaves (*Leucaena leucocephala* and *Samanea saman*).

MATERIALS AND METHODS

Ethical considerations

The fistulated animals used for the *in Sacco* degradability trial were cared for using the guidelines of the Institutional and Federation of Animal Science Societies (FASS, 2010) for the care and use of agricultural animals in research and teaching. However, the Ethics Committee of the College of Basic and Applied Sciences, University of Ghana, Legon-Accra, granted ethical approval.

Study site

The experiment was conducted at the experimental station of the Livestock and Poultry Research Centre (LIPREC), University of Ghana (UG). LIPREC is located in the Coastal Savanna Zone in the Accra Plains of Ghana and lies at latitude 5˚ 68'N, 0˚10'W. The average annual precipitation is between 508 mm and 743 mm, whereas the average temperature is between 24.3°C and 32.9°C. While the minor rainy season lasts from September to October of each year, the main rainy season lasts from April to July of each year. November through March is considered the dry season.

Rice straw and forages

The Small-Scale Irrigation Agricultural Project in Ashaiman, in the Greater Accra Region of Ghana, provided the rice straw for the study. To ease and improve feed consumption, the rice straw was chopped into 30 mm lengths with an electric forage cutter (CeCoCo forage SFC1400, Central Commercial Company®, Osaka, Japan). Briefly, the urea-ammoniated straw (UAS) was prepared by distributing chopped RS into a concrete culvert that was lined with polythene sheets. Following the procedure outlined by Fleischer et al. (2000) and Idan et al. (2020), 1 kg of urea was dissolved in 10 liters of water and sprayed onto each layer of 16 kg of rice straw in the culvert. The mixture was then ensiled for a further two weeks. The ensiled rice straw was air-dried in a ventilated area before being fed to the sheep. From mature woodlots at the University of Ghana's LIPREC, the fodder trees replicated five times for each of the two species (*Leucaena leucocephala* and *Samanea saman*) were chosen at random and marked for harvesting during the experimental period. The leaves were then harvested by hand cutting and air-dried at room temperature in a well-ventilated room to reduce the moisture content before feeding them to the sheep.

Animals, dietary treatments, and experimental design

Four rumen-fistulated forest-type rams with an initial weight of 19.0 ± 1.2 kg were used. The rams were fitted with rumen cannulas (Nepean Rubber Mouldings Pty Ltd. - Macam Division, Baulkham Hills, Australia) and randomly assigned to one of four dietary treatments consisting of a urea-ammoniated straw (UAS), untreated rice straw (RS) supplemented with *Leucaena*, *Samanea*, or equal amounts of both *Leucaena* and *Samanea* over four periods in a repeated 4 × 4 Latin square design. The animals on the positive control diets were fed urea-ammoniated rice straw while those on the test received untreated rice straw as basal diets respectively. Animals were rested for a week after each 21-day period and allowed to accustom to new treatment diets in the subsequent period for another week.

Housing, management, and feeding

Individual pens measuring 2 m × 1.5 m (3 m^2) and constructed of a concrete floor, iron sheet roofing, and wooden sides were used to house the fistulated animals. The lighting program provided 12 h of light and 12 h of darkness per day. Over the course of the experiment, the average temperature of the pen house hovered around 25°C. The experimental diets were given to the animals twice daily. Nevertheless, water and mineral vitamin licks were made available at all times during the research period. Before rumen pH, ammonia, and in *Sacco* DM degradation measurements were made, the animals were allowed to adapt to the experimental diets for 14 days. After each period, the animals were allowed to

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rest for seven days to clear their GIT of the previous experimental diet. The sheep were fed urea-ammoniated straw during the resting period.

Rumen pH

Rumen liquor was collected from three different parts of the rumen of the fistulated animals through the cannula by inserting a tube connected to a suction pump (Sarkwa et al., 2021). About 20 ml of rumen liquor was collected at 0, 2, 4, 6, 8, 10, 12, and 24 hours after feeding and strained through cheesecloth into a beaker, and pH was measured immediately using a pH meter (Sarkwa et al., 2021). The fluid was then stored in a freezer for ammonia determination later.

Rumen ammonia

The stored fluid from rumen pH determination was strained through a four-layer cheesecloth and kept warm and anaerobic in a thermos. Rumen ammonia was determined by using the method described by Broderick and Kang (1980) as validated by Adjorlolo et al. (2014).

In Sacco DM degradation characteristics

Using the Dacron sample bags (Ankom, Macedon, NY, USA; 42 µm porosity), as reported by Orskov et al. (1980), the DM disappearance in *Sacco* was evaluated. The untreated rice straw samples used for the study were oven-dried at 55 °C and ground to pass through a 1 mm sieve. Two grams of the oven-dried samples were weighed into nylon bags (135 mm×75 mm). Triplicate samples in the bags were manually inserted into the ventral sac of the rumen's liquid phase of the fistulated animals and incubated for 96 hours. The bags were tied to a drop line which was made up of nylon cords (200 mm × 0.7 mm) with an 11 g steel bolt tied at one end to provide the required weight. The nylon bags were soaked in water to displace the air before they were inserted into the rumen. Incubation periods for the degradation studies were 3, 6, 9, 12, 24, 36, 48, 72, and 96 hours. After the required period, the bags were withdrawn sequentially and washed by hand under tap water until the water became clear and frozen to halt microbial fermentation. Samples in the bags were thawed and placed into a forced-dried oven at 55°C for 48 h and weighed. To determine the content of water-soluble material, bags representing 0 h degradation also underwent the same washing procedure as the incubated bags. Dried residues from 4 bags of each incubation time from each sheep were pooled together. The DM disappearance values were calculated as the difference between the weight before and after the incubation of each sample. The degradability data obtained for DM for each feed was fitted to the equation: $P = a + b\,(1\text{-}e^{-ct})$ (Orskov et al., 1980).

P = potential disappearance of DM at time "t"; a = rapidly soluble fraction; b = potentially degradable but insoluble fraction (%); t = time; c = rate of constant for degradation of "b" fraction; e = the natural logarithm.

Degradation curve

The percent DM disappearance was plotted against the time of incubation for each sample. Percent soluble fraction "a" value (the intercept of the graph on the y-axis) and the potentially degradable fraction "b" (the difference between the highest points on the graph less "a") were determined. The maximum rate of degradation, which represents the steepest section of the curves, was identified. The percentage degradation (P) and the incubation time (t) corresponding to the mid-point of this section were read off. The rate of constant degradation "c" was calculated from the exponential equation (Orskov et al., 1980).

Chemical analysis

Moisture and dry matter contents of the fodder tree leaves and rice straws (treated and untreated) were determined after drying the samples to constant weight in an oven at a temperature of 105°C. By ashing dried samples for 6 hours in a muffle furnace set to 600°C, the organic matter (OM) content was determined. The weight loss was calculated as organic matter and the residue as ash. The total nitrogen content was determined using the standard Kjeldahl method (Association of Analytical Chemists, 2016) and was converted into CP by multiplying the percentage of N content by a factor of 6.25. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to (Van Soest et al., 1991).

Statistical analysis

The data obtained from the study involving rumen ammonia, pH, and DM degradability were all subjected to a one-way Analysis of Variance of the Glimmix procedure of SAS (2016) in a replicated 4 × 4 Latin square design according to the following statistical model.

$Y_{ij(k)} = \mu + P_i + \tau_j + A_{(k)} + \varepsilon_{ij(k)}$

Where, Y_{ijkl} = measured dependent variable; μ = overall mean; Pi = fixed effect period i (i = 1,..,4); $\tau_{(j)}$ = fixed effect of diet j (j = 1,..,4); A_k = random effect of animal; $\varepsilon_{ij(k)}$ = residual variation

The mean separation was done using Tukey's pairwise comparison test at ($P \leq 0.05$).

RESULTS

Chemical composition of rice straws and fodder tree leaves

The chemical composition values of the experimental diets varied significantly ($P<0.0001$) between the rice straws and the fodder tree leaves (FTLs) fed to the experimental animals (Table 1). The forage DM content ranged from 965.0 to 987.0 g/kg in straws to 920.0 to 949.0 g/kg in FTLs with straws having higher ($P<0.0001$) DM values than the FTLs. Similar to the DM, the OM matter values for the straws were significantly higher ($P<0.0001$) than those for the FTLs. The fodder tree leaves and their combination had higher ($P<0.0001$) values for the CP levels compared to the straws. However, untreated rice straw had the lowest CP value (66.8 g/kg), while Leucaena had the highest value (271.1 g/kg). The NDF values ranged from 300.4 to 589.0 g/kg, whereas the ADF values varied from 175.6 to 517.0 g/kg, with the

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straws having higher values than the FTLs. The ash content varied greatly, ranging from 77.7 to 202.3 g/kg, with UAS having the highest and Samanea having the lowest.

Table 1 - Chemical composition of rice straws and fodder tree leaves.

Item / Forage	DM	OM	CP	NDF	ADF	Ash
	g/kg	(g/kg DM)				
RS	987.0[a]	826.9[d]	66.8[e]	589.0[a]	517.0[a]	173.1[b]
UAS	965.0[b]	797.7[e]	100.2[d]	542.0[b]	501.0[b]	202.3[a]
Leucaena (L)	920.0[e]	898.4[c]	271.1[a]	300.4[e]	175.6[e]	101.6[c]
Samanea (S)	949.0[c]	929.3[a]	240.5[c]	406.2[c]	313.5[c]	77.7[e]
[1]LS	937.0[d]	910.0[b]	256.8[b]	355.2[d]	261.8[d]	90.0[d]
SEM	0.678	0.624	0.571	0.429	0.432	0.503
P Value, <	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

[1]The combined foliage was derived from a mixture of an equal amount of individual fodder tree leaves and pooled together into a composite before analysis. RS = Untreated rice straw; UAS = Urea-ammoniated rice straw; DM = Dry matter; OM = Organic matter, CP = Crude protein, LS = 50% Leucaena and 50% Samanea supplement

Rumen degradation characteristics of DM

The DM degradability in the rumen of sheep fed the sole or combined fodder tree leaves as well as urea ammoniated rice straw differed as shown in Table 2. In the current study, supplementation of rice straw with sole or combined fodder tree leaves was hypothesized to influence rumen degradability due to improvement in the rumen environment for microbial fermentation. The experimental diets differed (P<0.0001) in the amount of readily soluble material, the potentially degradable fraction, and the rate of gas production. The LS treatment had the highest percentage of soluble material (30.65%) with Leucaena recording the least fraction (25.90%). The disappearance of DM increased progressively from 3 h up to 96 h incubation time where degradability peaked for all experimental diets (Figure 1). Similar pools of LS and UAS fiber fractions represented as "*b*" disappeared at the same rates and were higher in terms of fiber degradation than *Leucaena* and *Samanea*. The rate of constant degradation (c) was significantly (P<0.05) lower for LS (0.038) and UAS (0.038) when compared with *Leucaena* and *Samanea*, which recorded (0.049) and (0.043) respectively. The potential disappearance of dry matter (P %) was significantly (P<0.05) lower for L and S when compared with the UAS and LS. However, those of UAS and LS were similar (P>0.05).

Table 2 - Dry matter degradability parameters for *Leucaena*, *Samanea*, LS, and UAS

Treatment	a (%)	b (%)	c	P (%)
UAS	28.85[c]	47.33[a]	0.038[c]	76.15[b]
Leucaena	25.90[d]	39.20[b]	0.049[a]	65.35[d]
Samanea	29.70[b]	36.35[c]	0.045[b]	65.75[c]
LS	30.65[a]	47.35[a]	0.038[c]	78.55[a]
SEM	0.063	0.105	0.001	0.050
Significance	<0.0001	<0.0001	<0.0001	<0.0001

[a-d] Means in the same column with different superscripts are significantly different (P<0.05) UAS= Urea ammoniated straw; LS= 50% *Leucaena* + 50% *Samanea*; SEM= Standard error of the mean; a= initially degradable fraction; b= degradable DM fraction; c= rate constant for the disappearance of b; P= potential disappearance of DM; SEM = standard error of the mean

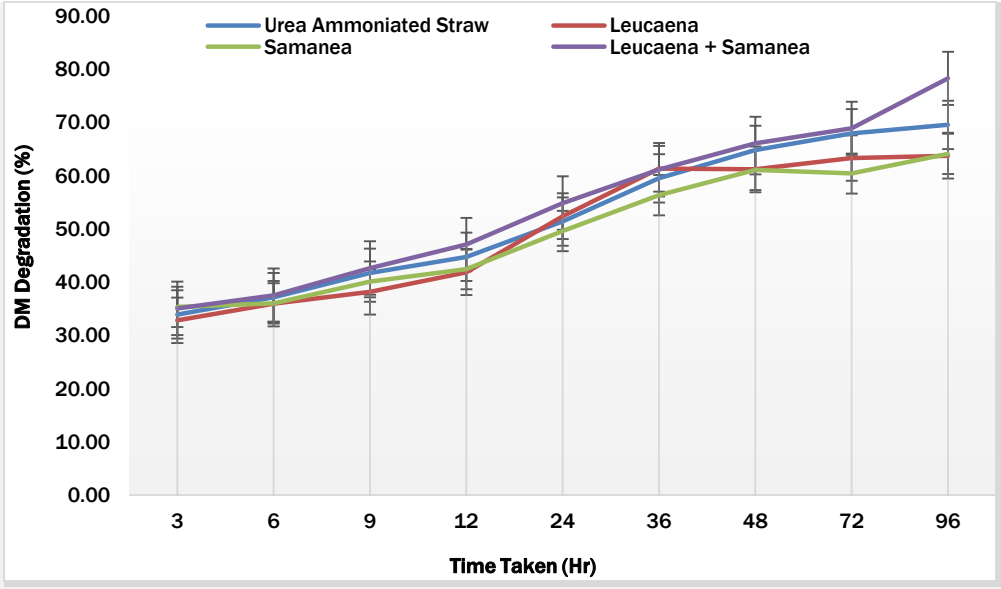

Figure 1 - DM Degradability of the Experimental Diets at various incubation times.

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Rumen pH

Experimental diets significantly influenced (P<0.0001) the pattern of rumen pH values (Table 3). The mean rumen pH values obtained in this current study over a 24 h period ranged from 6.28 to 6.64 (UAS); 6.35 to 6.95 (*Leucaena*); 6.55 to 6.89 (*Samanea*); and 6.18 to 7.01 in LS. There was no clear trend for the pH values in the four rumen environments. However, the pH tended to decrease shortly after feeding the experimental diets and increased at 12 hours, with the exception of the sole *Samanea*-supplemented diet, which did not exhibit any clear pattern (Table 3). However, the highest and lowest pH values of 7.01 and 6.18, respectively, were recorded in animals fed untreated rice straw and supplemented with LS. Nevertheless, the overall mean rumen pH values were highest (P<0.0001) in the sole S-supplemented diet compared to the other treatment diets.

Table 3 - Rumen pH for 24 hours after feeding the experiment diets

Hours / Treatment	0	2	4	6	8	10	12	24	Mean
	pH								
UAS	6.64^{d}	6.59^{d}	6.42^{c}	6.34^{d}	6.31^{c}	6.28^{c}	6.37^{c}	6.53^{d}	6.44^{c}
Leucaena	6.95^{b}	6.67^{c}	6.54^{b}	6.52^{b}	6.49^{b}	6.35^{b}	6.47^{b}	6.65^{b}	6.58^{b}
Samanea	6.89^{c}	6.86^{a}	6.82^{a}	6.80^{a}	6.71^{a}	6.58^{a}	6.54^{a}	6.55^{c}	6.72^{a}
LS	7.01^{a}	6.75^{b}	6.55^{b}	6.45^{c}	6.34^{c}	6.18^{d}	6.55^{a}	6.75^{a}	6.57^{b}
SEM	0.0071	0.0101	0.0043	0.0073	0.0069	0.0057	0.0054	0.0045	0.0058
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a-d}$ Means in the same column with different superscripts are significantly different (P < 0.05); UAS = Urea ammoniated straw; LS = 50% Leucaena + 50% Samanea; SEM = Standard error of mean

Rumen ammonia

The experimental diets significantly influenced the rumen ammonia concentrations in sheep (Table 4). The mean ammonia concentration ranged from 4.73 mg/100 ml in sheep fed UAS diet to 9.16 mg/100 ml in sheep fed *Leucaena*-supplemented diet. The highest ammonia concentration of 14.53 mg/100 ml was observed at 6 hours post-feeding in animals fed an LS-supplemented diet while the lowest value of 2.84 mg/100 ml was obtained in animals fed a UAS diet at 10 hours post-feeding. Apart from the sole *Samanea*-supplemented diet, which did not show any clear pattern for rumen ammonia concentration, the rest of the experimental diets showed a clear pattern. While the rumen NH_3 concentration peaked at 2 hours post-feeding for UAS and L, that of LS increased gradually after feeding and peaked at 6 hours (Table 4).

Table 4 - Rumen ammonia N concentration for 24 hours after feeding the experiment

Hours / Treatment	0	2	4	6	8	10	12	24	Mean
	Ammonia N concentration (mg/100ml)								
UAS	6.16^{d}	7.56^{c}	6.63^{c}	4.44^{d}	3.27^{d}	2.86^{d}	3.39^{c}	3.53^{c}	4.73^{d}
Leucaena leucocephala	8.49^{a}	12.61^{a}	12.45^{a}	11.04^{b}	9.63^{b}	7.68^{a}	6.18^{a}	5.17^{a}	9.16^{a}
Samanea saman	7.17^{c}	5.92^{d}	5.20^{d}	6.15^{c}	6.21^{c}	5.96^{c}	6.12^{a}	4.78^{b}	5.94^{c}
LS	7.33^{b}	9.84^{b}	12.25^{b}	14.55^{a}	9.84^{a}	7.44^{b}	5.36^{b}	3.48^{c}	8.76^{b}
SEM	0.008	0.013	0.008	0.0024	0.0054	0.011	0.030	0.067	0.006
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a-d}$ Means in the same column with different superscripts are significantly different (P<0.05); UAS = Urea ammoniated straw; LS = 50% Leucaena + 50% Samanea; SEM = Standard error of mean

DISCUSSION

Chemical composition of experimental diets

Previous research has shown that diets with CP levels of less than 6% are unlikely to provide the minimal ammonia levels necessary for the greatest possible microbial growth in the rumen (Norton, 1994). Except for the untreated rice straw, all of the experimental diets in the current study had CP levels well above 7%, indicating that they could be used as feed resources for small ruminants to achieve moderate-to-optimum growth performance.

Effects of sole and combined fodder tree leaves as supplements on *in sacco* degradation

The disappearance parameters examined in *in sacco* degradability studies are critical because they affect rumen fill and hence feed intake (Ørskov et al., 1988). The differences in the degradability parameters (a, b, c, and P) observed were due to variations in the chemical composition of the supplements. This agreed with the findings of Melaku et al. (2003), Anele et al. (2009), and Sarkwa et al. (2021), who all reported significant variations in the DM degradation parameters of multipurpose tree leaves. The degradation differences may also be associated with the structural and non-structural protein and carbohydrate fractions of the supplements used (Whetton et al., 1997) as well as the quality and content of fiber of the experimental diets (Smith et al., 1989, Antwi et al., 2014). The potential degradability of DM (P %) obtained in

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the current study (65.2–78.4 %) was lower than the values of 60.70–93.60 % reported by Attoh-Kotoku (2011) but comparable to those of Sarkwa et al. (2021) when similar browses were utilized. All the experimental diets had greater apparent DM degradability, indicating that they can be used to feed animals during the dry season when available feed is low in quantity and quality. However, the observed maximum (P%) value in LS suggested an optimal rumen environment for microbial breakdown. In addition, the (P%) value for UAS (76.40%) in the present study was higher than the value of 54.31% reported by Sarkwa et al. (2021). The soluble fraction (a %) for Samanea was consistent with results obtained by Lowry (1995) of (26.0 ± 4.8) but was higher than the value of (7.7 %) obtained by Attoh-Kotoku (2011). Reports by Tolera et al. (1997) and Melaku et al. (2003) suggested that (a %) and NDF were inversely related. This agreed with the results obtained in this study.

The potentially degradable but water-insoluble fraction (b %) of 39.30–47. 8% recorded in this study was lower than the 54.9–85.8 % reported by Attoh-Kotoku (2011) when similar browses were used to supplement rice straw. However, the (b %) for UAS was comparable to the results obtained by Sarkwa et al. (2021).

The greatest potentially degradable fraction was obtained by animals on 50% L: 50% S diets. The observed differences might be due to the variations in the chemical composition and the cell wall fractions of the various feedstuffs. Ramírez et al. (2000) reported that the slowly degradable fraction of plant cell walls was limited by ADL, organic matter, ash, and insoluble ash. However, several studies have reported a negative relationship between the extent of degradation of DM or CP with NDF, ADF, ADL, and CT (Siaw et al., 1993; Bonsi et al., 1995; Melaku et al., 2003). The findings of this study did not corroborate their observations. The variations in the (b %) and (P %) values in the rumen have also been attributed to variations in the NDF, ADF, lignin (Van Soest, 1994; Yan and Agnew, 2004) and tannins (Kamalak, 2006) or maturity (Khazaal et al., 1993; Kamalak, 2006). The rate of constant DM degradability of 'b' (0.038 to 0.049) observed in this study was similar to values reported by Sarkwa et al. (2021) but lower than those reported by Addo-Kwafo (1996). However, the 'c' values for *Samanea* were higher than the values obtained by Attoh-Kotoku (2011). The differences may be due to variations in the ecology, stage of maturity, and time of harvest of the fodder tree leaves (Giridhar et al., 2021; Navale et al., 2022). The significantly higher degradation rate of DM 'c' in 100 % *Leucaena* compared to the rest reflects differences in chemical composition between the plant species. For example, *Leucaena* had the highest CP compared to the rest of the forages used in this study. The result shows that 'c' is positively correlated with CP and is consistent with the findings of Tolera et al. (1997) and Kamalak (2006).

The results obtained in this study showed that 50 % L: 50% S had the highest potential disappearance of dry matter in the rumen and the slowest degradation rate. This means that a sufficient amount of it would be degraded over a period of time thus releasing a substantial amount into the small intestines for digestion to provide essential nutrients needed for better animal performance. This is consistent with the better growth rate recorded for LS reported by Idan et al. (2023).

Effects of sole or combined fodder trees as supplements on rumen pH and ammonia

The mean pH values obtained in this study (6.44 to 6.72) were similar to the 6.52 to 6.65 reported by Attoh-Kotoku (2011) when *Samanea saman* and *Stylosanthes hamata* were used as a supplement for sheep fed a basal diet of rice straw and the 6.15–6.60 reported by Sarkwa et al. (2021) when sheep were offered urea-treated rice straw. However, the values recorded in this current study were slightly lower than the optimum levels of 6.7-7.2 reported by Orskov (1982). It is established that a low pH of 5.5 reduces ruminal microbial fermentation (Cerrato-Sánchez et al., 2007) and as a result influences the activities of fibrolytic bacteria in digesting fiber in the rumen (Russell and Dombrowski, 1980). Russell and Dombrowski (1980) ascribed the reduced organic matter and fiber digestion and changes in the profile of volatile fatty acids to low pH resulting in the lowered activity of the fibrolytic bacteria. The pH values at various hours post-feeding within the minimum threshold as observed in the study imply that the effects of undigested fiber within the feed, which could have reduced the access of bacteria and enzymes to the protein, were eliminated. According to Devant et al. (2000), low fiber digestion leads to an increase in dietary N flow while decreasing CP degradation and ammonia N concentration. This suggests that low pH inhibits bacterial N flow, which subsequently reduces microbial growth. Generally, the mean pH of 6.58 reported for all the experimental diets in this study implied that optimal pH conditions existed in the rumen for cellulolysis and improved rumen fermentation.

The rumen NH_3 values observed in the study were comparable to the 4.93 to 6.36 mg/100 ml reported for sheep fed on rice straws with *Samanea saman* and *Sesbania sesban* supplementation by Attoh-Kotaku (2011). Furthermore, several studies with sheep revealed that the concentration of ammonia in the rumen ranged between 1 and 14 mg/100 ml (Fleischer et al., 2000; Miller and Thompson, 2003; Horadogado et al., 2008; Lascano and Heinrichs, 2009; Adjorlolo et al., 2014; Gumilar et al., 2018). However, Kaur et al. (2008) and Sarkwa et al. (2023) reported concentrations higher than 14 mg/100 ml. In the current study, no relationship was found between pH and rumen ammonia concentration, although Aguerre et al. (2009) found a negative relationship between pH and NH_3-N when all measurements were considered (r=-0.46, $P<0.001$). Their result agreed with Cajarville et al. (2006), who reported a low but significant negative relationship between rumen pH and NH_3-N concentration of cows grazing temperate pastures and supplemented with different sources of grains. The rumen NH3-N concentrations found in this study were higher than the suggested minimum values (1.65 to 3.79 mg/100 ml) for optimum microbial activity in animals fed lignocellulosic materials by Balcells et al. (1993). This shows that such feeds could be utilized for moderate animal performance, especially during the dry seasons.

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CONCLUSION

The chemical composition of fodder tree leaves varies greatly depending on plant species and edible plant sections. The current study demonstrates that all of the fodder tree leaves (FTLs) investigated can be used as potential protein banks to supplement grasses or fibrous crop residues during dry seasons. The mean pH values obtained from the current study were within the range of 6.2 to 7.0 required for optimal rumen function, indicating that the experimental diets could promote rumen fermentation and hence be good sources of feed for ruminants. The rumen ammonia levels measured in this study were greater than the reported minimum levels for optimum microbial activity in animals fed lignocellulosic materials. This shows that such diets might be suitable for improving animal performance, particularly during the dry seasons. The readily degradable and potentially degradable fractions and disappearance of DM were superior in the combined FTLs compared to the sole FTLs. However, the overall rumen dry matter degradation values recorded by the various diets indicate that a sufficient amount of dry matter could be degraded over time, releasing a significant amount into the small intestines for digestion to supply important nutrients required for improved animal performance. Therefore, *Leucaena leucocephala and Samanea saman,* or their combination, can be utilized as supplements to improve the usage of low-protein, high-lignin basal diets. Future research should use conventional feeding experiments to further understand the effects of different fodder tree leaves and their combinations on growth performance, carcass characteristics, hematological parameters, and meat quality.

DECLARATIONS

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Authors' contribution

F. Idan and T. Adogla-Bessa came up with the idea, planned the experiment, and wrote the manuscript; Ch. Antwi and F. O. Sarkwa worked together on the statistical analysis and took part in the manuscript review; O. Alhassan reviewed the manuscript and formatted it; Y. Abdul Aziz reviewed the manuscript; and F. Idan carried out the practical component and laboratory analysis. The final manuscript was read and approved by all authors.

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Conflict of interests

The authors have not declared any conflict of interest.

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EFFECT OF DIETARY CRUDE PROTEIN LEVELS ON FEED INTAKE AND NUTRIENT DIGESTIBILITY OF WAGYU CROSSBRED CATTLE

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ABSTRACT: The objective of the experiment was to determine the suitable crude protein level on feed intake and digestible nutrient value of Wagyu crossbred cattle from 13 to 20 months of age. The research included 2 experiments. Experiment 1: five male Wagyu x Zebu crossbred cattle (12.2±0.56 months of age and average live weight of 179±24.2 kg, Mean±SD). Experiment 2: five male Wagyu x Zebu crossbred cattle (16.7±1.05 month's old and live weight of 276±22.0 kg). Two experiments were Latin square design (5x5) with 5 treatments and 5 periods (21 days per period). The treatments were different crude protein levels at 210, 245, 280, 315, and 350 g per 100 kg live weight (LW) corresponding to CP210, CP245, CP280, CP315, and CP350 treatments, respectively. The basal diet was commercial concentrate (1.2 kg/day), fresh Elephant grass (5.0 kg/day) and *ad libitum* rice straw. While soybean meal was used to adjust the dietary CP level per 100 kg LW in diets. The result showed that increasing nutrient consumption and metabolism energy ($P<0.05$) but reduced fiber intakes ($P>0.05$) by increasing crude protein levels. Experiment 1: the CP digestibility was highest ($P<0.05$) of CP350 (72.8%) treatment compare to CP210 (58.8%) treatments, while the CP280 (67.2%) treatment was not significant ($P>0.05$) with CP245 (62.9%) and CP315 (71.7%) treatments. Experiment 2: the highest CP digestibility ($P<0.05$) of CP350 treatment as compared to CP315, CP280, CP245, and CP210 treatments (80.2, 77.4, 73.1, 70.5, and 65.0%, respectively). As a result, increasing CP levels per 100 kg BW could rise nutrients digestibility and digestible value for Wagyu crossbred cattle. The level of 245 g CP per 100kg live weight in Wagyu crossbred cattle diet from 13 to 20 months of age could be recommended for application.

Keywords: Beef production, Crude protein, Digestion, Rumen escape protein, Ruminants.

Abbreviations: CP210, CP245, CP280, CP315 and CP350: crude protein at 210, 245, 280, 315 and 350 g/100kgLW.

INTRODUCTION

A survey of nutrients intake of Zebu cross cattle showed that the crude protein (CP) intake (g/100 kg body live weight) consume of cattle at 6, 12, 18, 24, 30 and 36 months of age were 219, 196, 194, 192, 172 and 153, respectively (Truong and Thu, 2019). In previous studies, Nha et al. (2008) reported that the crude protein level of local growing cattle diet was 210 g CP/100 kg body weight/day. Similarly, Thu and Dong (2015) concluded that supplementing CP in diets by using multi-nutrient cake improved nutrient intake and digestibility. Further studies should increase the CP contained in diets more than 230 g/100 kg body weight. In Vietnam, the Wagyu crossbred beef cattle is produced from the artificial insemination between Zebu cattle groups and frozen semen of Wagyu (Pham-Thanh et al., 2020; Vu et al., 2021). The crossbred cattle have better beef performance compared to the local breeds, nevertheless, they require higher-quality diets (Favero et al., 2019; Mwangi et al., 2019). However, research on crude protein demands of crossbred beef cattle by each of the age periods is limited. Nutritionally, protein in the diet plays an essential role in the nutrition of ruminants. Preston et al. (2021) have proposed that the key factor in the design of diets or growing cattle is the supply of rumen escape protein. Soybean meal is used as the main protein source for beef cattle as it is richer in valuable rumen undegradable protein than most other protein sources (Keller et al., 2021). In addition, research from Truong and Preston (2021) proves that soybean meal is a source of rumen scape protein for fattening crossbred beef cattle. The combination of both local feed sources and scientific results for further studies to respond to the nutrient requirements of growth performance cattle is necessary. We hypothesized that the level of CP could act collectively on the intake and digestibility of crossbred beef cattle.

In this study, the objective was to evaluate the effects of different crude protein levels in the diets on feed intake and nutrient digestibility of Wagyu crossbred cattle from 13 to 20 months of age for applications.

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MATERIALS AND METHODS

Materials

This study was conducted from May 2020 to December 2020 at Sau Duc cattle farm, Vinh Gia commune, Tri Ton district of An Giang province and the Laboratory E205 of the Department of Animal Science, College of Agriculture of Can Tho University. All experimental procedure was under ethical regulations (animal welfare and animal care rules) of Can Tho University.

Experimental design

Five male Wagyu x Zebu crossbred cattle at 12.2±0.56 months of age (Mean±SD) with an average body weight of 179±24.2 kg were used in the first experiment. Five male cattle of Wagyu crossbred (50% Wagyu and 50% Zebu crossbred) at 16.7±1.05 months of age and 276±22.0 kg body weight were used in the second experiment. The experimental design was a Latin square with five treatments and five beef cattle in five periods (21 days/period). The five dietary was different crude protein levels including 210, 245, 280, 315, and 350 g/100 kg body weight for CP210, CP245, CP280, CP315, and CP350 treatments. The basal diet was commercial concentrate (1.2 kg/d), fresh Elephant grass (5 kg/d), *ad libitum* rice straw, and soybean meal was used to adjust CP levels in different treatments.

The commercial concentrate and soybean meal were bought from a Feed Company. The Elephant grass was planted on the farm. Rice straw was purchased from local farmers. The fixed quantities of commercial concentrate (1.2 kg/animal/day) and soybean meal were daily offered to the animals 2 times at 7:00 am and 1:00 pm. Elephant grass was supplied at a level of 5 kg/animal/day (in the fresh matter) at 7:30 am and 1:30 pm followed by the rice straw offered *ad libitum* at 8:00 am, 11:00 am 2:00 pm, 6:00 pm and the remainder given at 10:00 pm. Clean and fresh water were offered *ad libitum* during the whole experiment. Before the start of this study, each animal was treated for external and internal parasites (Ivemectin and given Albendazole, respectively).

Measurements taken

- *Feed, nutrient and energy intakes:* Feeds and refusals were daily measured for analyses of dry matter (DM), organic matter (OM), crude protein (CP) and ash following the procedure of AOAC (1990), acid detergent fiber (ADF) and neutral detergent fiber (NDF) according to Van Soest et al. (1991). The metabolic energy (ME) was determined according to Bruinenberg et al. (2002), in which ME (MJ/animal/day) = 14.2*DOM + 5.9*DCP (DOM/DCP<7.0); ME (MJ/animal/day) = 15.1 x DOM (with DOM/DCP>7.0, DOM is digestible organic matter and DCP is digestible crude protein).

- *Apparent nutrient digestibility:* Apparent DM, OM, CP NDF and ADF digestibility were employed with the animal feces that were daily collected and weighed according to McDonald et al. (2010). In present study has five periods. One experimental period was three weeks, including two weeks for dietary adaptation and another week for the sampling.

- *Daily weight gains (DWG):* Experimental cattle were weighed by an electronic scale (Model TPSDH, YAOHUA, Taiwan) and calculated by using cattle live weights, which were weighed for 3 consecutive days in the early morning before feedings at the beginning and at the end of each experimental period.

Statistical analysis

The data were analyzed by analysis of variance using the ANOVA of General Linear Model (GLM) of Minitab Reference Manual Release 16.1 (Minitab, 2010). The statistical equation for this model was $y_{ijk} = \mu + T_i + A_j + P_k + e_{ijk}$; where y_{ijk}: = the dependent variable, μ: the overall mean, T_i = the effect of treatment (i = 1 to 5), A_j: the effect of animal (j = 1 to 5), P_k= the effect of period (j = 1 to 5), and e_{ijk} = the random error. Then for the paired comparison of two treatments, Tukey test of the Minitab was used (P = 0.05).

RESULTS AND DISCUSSION

Feed characteristics of experimental diet is presented in Table 1. The results presented in Table 1 indicated that the CP content was higher in soybean meal (42.5-45.0%) than in concentrate (15.3-15.9%), elephant grass (8.13-8.76%) and dry rice straw (5.11-5.41%). The chemical composition of feeds in the present study agreement with the result of some previous studies. Don et al. (2020) reported that rice straw's CP, NDF, and ADF were about 2.0-6.6% CP, 66.3-73.2% NDF and 36.3-42.6% ADF. The nutrient of elephant grass was 7.20-12.1%, 57.4-75.4% and 30.6-51.7% corresponding to CP, NDF, and ADF (Rusdy, 2016). The CP, NDF, and ADF of soybean extraction meal in the present study agreed with that presented by Dong and Thu (2020) being 43.2% CP, 18.4% NDF and 11.3% ADF. However, the highest protein from soybean meal in the present study.

Experiment 1: Wagyu crossbred cattle from 13 to 16 months of age

Feed, nutrient and ME intakes of experimental cattle

Results presented in Table 2 indicated that the DM intake (kg/animal/day) was significantly different (P<0.05) among treatments, with the highest value for CP350 (5.51 kg) and the lowest value for CP210 treatment (5.02 kg). While the CP280 treatment (5.17 kg) was not different (P>0.05) compared to CP245 (5.09 kg) and CP315 treatments (5.33 kg).

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The DM intake of the experiment was higher than the result reported by Dung and Ngoan (2016) in crossbred beef cattle from 13 to 15 months of age was 4.98-5.58 kgDM/animal/day. The crude protein intake increased (P<0.05) by increasing soybean meal supplement levels in diets. It was 0.448, 0.520, 0.590, 0.670 and 0.743 kg corresponding to CP210, CP245, CP280, CP315 and CP350. According to Thu and Dong (2015), supplementing CP in diets by using multi-nutrient cake improved nutrient intake and further studies should increase the CP containing in diets more than 230 g/100 kg. Because the transformation of feed protein into body protein is an important process of nutrition and metabolism. While protein is needed to meet for cell repair and synthetic processes in the body (Dong and Thu, 2020). The CP intake of cattle in this experiment was similar to those reported by Kearl (1982) studied growing crossbred beef cattle (225 kg) being of 0.502-0.784 kg/day for daily weight gain 0.25-1.10 kg/animal/day.

The NDF consumption per day was not different (P>0.05) among treatments. It ranged from 2.96 to 3.00 kg/animal/day, which was similar to those reported by Quang et al. (2015) being 2.38-3.01 kg/animal/day. Similarly, the ADF intake ranged from 1.80-1.84 kg/animal/day (P>0.05). Metabolizable energy intake was different (P<0.05) with the highest value for CP350 (45.0 MJ) and the lowest value for CP210 (37.8 MJ). However, the CP280 treatment was not different (P>0.05) compared to CP245 and CP315 treatments (41.4, 39.8 and 43.9 MJ, respectively). The ME intake obtained in this experiment was consistent with the results of Kearl (1982) being 36.9-64.9 MJ/animal/day but lower than that reported by Dung and Ngoan (2016) being 45.3-49.7 MJ/animal/day. Theoretically, the cell wall is the main source of roughages in the diet because their rumen microbes can effectively digest fibrous feeds into energy sources. Thus, elephant grass and rice straw are often used for cattle feeding. In our study, we found that ME consumption was significantly affected by supplementation with soybean extraction meal. The DM intake per 100 kg LW was different (P<0.05) between treatments, the highest value for CP350 (2.59%) and the lowest value for CP210 (2.36%). The result in the experiments was similar to reported by Pimpa et al. (2019) in Wagyu crossbred cattle in Thailand about 2.41-2.64%. In general, improved nutrient intakes are found by increasing the CP in the diets.

Table 1 - Chemical composition of feeds (% DM basis) used in the experiment.

Feed	DM %	% in DM				
		OM	CP	NDF	ADF	Ash
Experiment 1						
Elephant grass	14.8	89.0	8.76	62.5	40.2	11.0
Rice straw	85.2	89.2	5.11	67.6	44.0	10.8
Concentrate	89.3	90.6	15.3	40.0	15.6	9.43
Soybean meal	83.3	93.5	45.0	14.2	10.9	6.50
Experiment 2						
Elephant grass	14.0	88.1	8.13	62.5	-	11.9
Rice straw	84.3	87.8	5.41	69.0	-	12.2
Concentrate	86.1	90.9	15.9	36.4	-	9.13
Soybean meal	86.2	93.5	42.5	19.3	-	6.52

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber

Table 2 - Effect of protein levels on feed, nutrient and metabolizable energy intakes of Wagyu crossbred cattle in the experiment 1.

Item \ Treatments	CP210	CP245	CP280	CP315	CP350	P	SEM
Feed intake, kg DM/animal/day							
Elephant grass	0.741	0.741	0.741	0.741	0.741	-	-
Rice straw	3.07	2.96	2.89	2.86	2.88	0.116	0.058
Concentrate	1.07	1.07	1.07	1.07	1.07	-	-
Soybean meal	0.137^{e}	0.311^{d}	0.474^{c}	0.655^{b}	0.815^{a}	0.001	0.022
Total nutrient intake, kg/animal/day							
DM	5.02^{c}	5.09^{bc}	5.17^{bc}	5.33^{ab}	5.51^{a}	0.001	0.055
OM	4.50^{c}	4.56^{c}	4.65^{bc}	4.79^{ab}	4.96^{a}	0.001	0.049
CP	0.448^{e}	0.520^{d}	0.590^{c}	0.670^{b}	0.743^{a}	0.001	0.009
NDF	3.00	2.95	2.92	2.92	2.96	0.616	0.039
ADF	1.84	1.82	1.80	1.80	1.83	0.674	0.025
ME, MJ	37.8^{d}	39.8^{cd}	41.4^{bc}	43.9^{ab}	45.0^{a}	0.001	0.662
DM/LW, %	2.36^{c}	2.39^{bc}	2.43^{bc}	2.50^{ab}	2.59^{a}	0.001	0.025

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ME: metabolizable energy, LW: live weight. CP210, CP245, CP280, CP315 and CP350: crude protein at 210, 245, 280, 315 and 350 g/100kgLW. $^{a, b, c}$ values with different superscript letters within one row are significantly different at the level of 5%.

Apparent nutrient digestibility and daily weight gain

The result of Table 3 showed that the CP350 treatment (57.7%) was higher DM digestibility (P<0.05) than CP210 treatment (52.9%) but it was not different (P>0.05) compared to CP315, CP280 and CP245 treatments (58.1, 56.3 and 55.0%, respectively).

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The DM digestibility in the present study was lower than reported by Pimpa et al. (2019), who found that DM digestibility of Wagyu crossbred cattle in Thai Lan being 58.5-62.7%. In our study, the NDF and ADF digestibility were not (P>0.05) among treatments and they were about 55.6-58.3% and 48.8-51.9%, respectively. Seankamsorn and Cherdthong (2020) reported that NDF digestibility of Wagyu crossbred cattle in Thailand was about 53.0-56.4%. However, the rumen digestibility converts rice straw and elephant grass protein of roughage into the microbial protein of high biological value (Liu et al., 2019). The CP digestibility was different (P<0.05) among treatments. It was 58.8, 62.9, 67.2, 71.7 and 72.8% for CP210, CP245, CP280, CP315 and CP350 treatments. However, CP280 treatment was not significantly different (P>0.05) compare to CP245, CP315 and CP350 treatments. Although nitrogen available for rumen microbes for their growth and cell activities is equally vital, by-pass protein is advantageous for the host ruminant to receive good quality protein directly from the diet (Ngu et al., 2019). Because a high percentage of rumen undegradable protein sources can improve host protein utilization. Moreover, Sari et al. (2018) reported that higher digestibility of CP means the total amount of CP content that can be digested in the digestive tract. The CP digestibility (%) was increased as the offer level of protein was increased. A result digestible CP (kg/animal/day) was significantly different (P<0.05) among treatments. It was 0.226, 0.332, 0.397, 0.483 and 0.541 kg corresponding to CP210, CP245, CP280, CP315 and CP350 treatments. To our knowledge, the CP of soybean extraction meal is by-pass protein, which is advantageous for the host ruminant to receive good quality protein directly from the diet. Because protein will escape from fermentative processes increasing the total amino acid supply (from dietary sources and microbial cells) for more efficient digestion by mammalian enzymes in the small intestine (Preston and Leng, 2021). Besides providing a more protein diet, the daily weight gain was increased (P<0.05) among treatments. It was 579, 607, 645, 742, and 888 g/animal/day corresponding to CP210, CP245, CP280, CP315, and CP350 treatments. In another study, Vu (2019) found that daily weight gain of Wagyu crossbred cattle from 13 to 18 months of age being 411-577 g/animal/day.

Thus, increasing CP intake per 100 kg body weight from 210 to 350 g was improved nutrient intake, digestibility and daily weight gain. The CP intake was 245 g/100 kg BW which was potentially promising for applied studies in diets of crossbred beef cattle from 13 to 16 months of age.

Table 3 - Apparent nutrient digestibility and daily weight gain of cattle in different treatments in the experiment 1.

Item \ Treatments	CP210	CP245	CP280	CP315	CP350	P	SEM
Nutrient digestibility, %							
DM	52.9^{b}	55.0^{ab}	56.3^{ab}	58.1^{ab}	57.7^{a}	0.029	1.076
OM	55.7^{b}	57.6^{ab}	58.9^{ab}	60.8^{a}	60.0^{a}	0.020	0.957
CP	58.8^{d}	62.9^{cd}	67.2^{bc}	71.7^{ab}	72.8^{a}	0.001	1.193
NDF	55.6	55.9	57.0	58.6	58.3	0.057	0.764
ADF	51.4	50.3	50.2	51.9	48.8	0.265	0.984
Body weight, kg							
Initial	209	208	207	207	206	0.618	1.582
Final	220	221	220	222	224	0.160	1.028
Daily weight gain, g	579^{b}	607^{ab}	645^{ab}	742^{ab}	888^{a}	0.033	64.58

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, LW: live weight. CP210, CP245, CP280, CP315 and CP350: crude protein at 210, 245, 280, 315 and 350 g/100kgLW. $^{a, b, c}$ values with different superscript letters within one row are significantly different at the level of 5%.

Experiment 02: wagyu crossbred cattle from 17 to 20 months old

Feeds and feeding in the present study

The result in Table 4 showed that DM intake was not significantly different (P>0.05) among treatments. It was 6.57, 6.88, 6.70, 6.83 and 7.14 kg/animal/day for CP210, CP245, CP280, CP315 and CP350 treatments, respectively.

It was similar to that of 325 kg crossbred beef cattle reported by Filho et al. (2016) in Brazil being 6.10-7.57 kg DM with a daily weight gain of 0.5-1.0 kg/day. In a previous report, Thu (2010) recorded variation in DM intake of local cattle when the cattle received supplements having different levels of protein 150, 180, 210 and 240 g/100 kg BW. The DM intake per body weight ratio gradually increased from CP210 (2.11%) to CP350 (2.29%) and was not significantly different (P = 0.071) among the treatments. The results of the present study were similar to those of Mirattanaphra and Suksomba (2020), who report that DM/LW (%) was about 2.14-2.15% in Wagyu crossbred cattle. The daily CP intake was significantly different (P<0.05) among the treatments and increased for CP210, CP245, CP280, CP315, and CP230 treatments corresponding to 0.662, 0.758, 0.873, 0.983, and 1.094 kg/animal/day. The result in the experiments was higher than reported by Dung and Ngoan (2016) being 0.667-0.762 kg for crossbred beef cattle from 17 to 20 months of age. The ME consumption (MJ/animal/day) was significantly different (P<0.05) among treatments with the highest value for the CP350 treatment (63.7 MJ) and the lowest value for the CP210 treatment (51.0 MJ). While the CP245 treatment was not significantly different (P>0.05) with CP280 and CP315 (54.3, 57.7 and 59.6 MJ, respectively). The ME intake in our study was in agreement with that presented by Filho et al. (2016) (53.6-72.0 MJ/animal/day).

Our study confirmed that the increasing level of CP from 210 to 350 g/100 kg BW affects feed consumption and tended to improve protein and estimated dietary metabolism energy.

Citation: Binh Truong N and Thanh Trung T (2023). Effect of dietary crude protein levels on feed intake and nutrient digestibility of Wagyu crossbred cattle. *Online J. Anim. Feed Res.*, 13(4): 295-301. DOI: https://dx.doi.org/10.51227/ojafr.2023.44

Table 4 - Feed and nutrient intake of experimental crossbred beef cattle in the experiment 2.

Item \ Treatments	CP210	CP245	CP280	CP315	CP350	P	SEM
Feed intake, kg DM/animal/day							
Elephant grass	0.70	0.70	0.70	0.70	0.70	-	-
Rice straw	4.34	4.44	3.93	3.78	3.83	0.054	0.128
Concentrate	1.03	1.03	1.03	1.03	1.03	-	-
Soybean meal	0.49^{e}	0.71^{d}	1.04^{c}	1.32^{b}	1.57^{a}	0.000	0.032
Intake, kg DM/animal/day							
DM	6.57	6.88	6.70	6.83	7.14	0.070	0.116
OM	5.84^{b}	6.13^{ab}	5.99^{ab}	6.12^{ab}	6.40^{a}	0.039	0.101
CP	0.662^{e}	0.758^{d}	0.873^{c}	0.983^{b}	1.094^{a}	0.001	0.012
NDF	4.00	4.13	3.83	3.79	3.88	0.308	0.083
ME, MJ	51.0^{c}	54.3^{bc}	57.7^{ab}	59.6^{ab}	63.7^{a}	0.001	1.231
DM/LW, %	2.11	2.23	2.16	2.19	2.29	0.071	0.037

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ME: metabolizable energy, LW: live weight. CP210, CP245, CP280, CP315 and CP350: crude protein at 210, 245, 280, 315 and 350 g/100kgLW; $^{a, b, c}$ values with different superscript letters within one row are significantly different at the level of 5%.

Digestibility measurements and daily weight gain

The analysis statistics result of Table 5 showed that the difference (P<0.05) was found in the digestibility of DM, OM, and CP but NDF was not significantly different (P>0.05) among treatments.

The DM digestibility was significantly different (P<0.05) among treatments with the highest value at 62.5% (CP350 treatment) and the lowest value at 56.7% (CP210 treatment). However, no difference was found that from CP245 to CP350 treatments (56.9, 61.5 and 61.2%, respectively). The digestibility of OM was a different increase (P<0.05) from CP210 to CP350 treatment. It was 58.5, 58.7, 63.4, 63.3, and 64.4 % corresponding to CP210, CP245, CP280, CP315, and CP350 treatments. The highest CP digestibility of CP350 treatment (P<0.05) as compared to CP315, CP280, CP245, and CP210 treatments (80.2, 77.4, 73.1, 70.5, and 65.0%, respectively). However, there were no significant differences (P<0.05) between CP245, CP280 and CP315 treatments. In another study, Thu and Dong (2015) recorded variation in CP digestibility of Sind crossbred cattle (52.3% to 70.5%) when the cattle received having different levels of protein from 140, 170, 200, and 230 g per 100 kg live weight. According to Dong and Thu (2020), the transformation of feed protein into body protein is an important process of nutrition and metabolism. Moreover, Sari et al. (2018) reported that higher digestibility of CP means the total amount of CP content that can be digested in the digestive tract. The NDF digestibility was not significantly different (P>0.05) among treatments and it was about 56.9-61.4%. However, the rumen digestibility converts rice straw and elephant grass protein of roughage into microbial protein of high biological value (Liu et al., 2019). In the second study, increasing CP levels per 100 kg live weight could rise daily weight gain for Wagyu crossbred cattle. It was 597, 713, 730, 862, and 876 g/animal/day corresponding to CP210, CP245, CP280, CP315, and CP350, respectively. However, no difference was found from 245 to 315 g CP/100 kg body weight.

Table 4 - Apparent nutrient digestibility (%) and daily weight gain of crossbred beef cattle in the experiment 2.

Item \ Treatments	CP210	CP245	CP280	CP315	CP350	P	SEM
Digestibility, %							
DM	56.7^{b}	56.9^{ab}	61.5^{a}	61.2^{a}	62.5^{a}	0.008	0.881
OM	58.5^{b}	58.7^{ab}	63.4^{a}	63.3^{a}	64.4^{a}	0.008	1.040
CP	65.0^{c}	70.5^{bc}	73.1^{b}	77.4^{ab}	80.2^{a}	0.000	1.371
NDF	56.9	58.9	60.9	60.1	61.4	0.114	1.170
Body weight, kg							
LW Initial	306	304	304	304	304	0.783	1.551
LW Final	319	319	319	322	323	0.512	1.735
Daily weigh gain, g	597^{b}	713^{ab}	730^{ab}	862^{a}	876^{a}	0.016	48.50

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, LW: live weight. CP210, CP245, CP280, CP315 and CP350: crude protein at 210, 245, 280, 315 and 350 g/100kgLW. $^{a, b, c}$ values with different superscript letters within one row are significantly different at the level of 5%.

CONCLUSION

Increasing CP intake per 100 kg body weight from 210 to 350 g was gradually improved nutrient intake, digestibility and daily weight gain. The CP intake was 245 g per 100 kg BW of Wagyu crossbred cattle from 13 to 20 months of age could be recommended for farmers' application.

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DECLARATIONS

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Authors' contribution

Nguyen Binh Truong contributes on data analysis and the write up of the manuscript. Truong N.B and Trung TT conceived and designed the experiments; Truong N.B performed the experiments; Truong N.B analyzed the data; Truong N.B and Trung TT wrote the paper.

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Competing interests

Authors declared no conflict of interest.

Consent to publish

All authors reviewed and approved the final manuscript.

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CHEMICAL COMPOSITION AND *IN VITRO* GAS PRODUCTION OF *Brachiaria decumbens* HARVESTED AT DIFFERENT STAGES OF GROWTH IN THE HOT HUMID REGION

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The study evaluated the effect of harvesting date on the chemical composition and *in vitro* gas production of the botanic fractions of *Brachiaria decumbens* grass. The botanic fractions (leaf, stem and whole plant) of the grass at two maturities (60 and 120 days) in a Completely Randomised Design with factorial arrangement. Samples of botanic fractions at the different harvest dates were chemically analyzed for dry matter (DM), crude protein (CP), fibre concentrations and *in vitro gas* production (IVGP) was measured at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h to estimate the volume and rate of gas production. Short-chain volatile fatty acids, microbial protein production, *in vitro* organic matter digestibility, and metabolizable energy were estimated from established models. Organic matter, crude fibre, NDF, ADF and ADL increased (P<0.05) with increasing maturity whereas the reverse was so for CP and ash contents (P<0.05). Significant interactions (P<0.05) between harvest date and plant fraction were present for both '*b*' and '*c*' attributable to treatment effects. Potential gas production '*b*' elicited a negative response for all plant fractions across the two harvest dates as the values decreased linearly. The rate at which the gases were produced '*c*' also induced a negative response for the leaf and whole fraction but a positive one for the stem fraction. The nutrient composition and gas production characteristics of grasses harvested at day 60 offer a better potential as high quality forage for improved intake and digestibility. The leaf fractions performed relatively better based on the afore-mentioned methods of quality assessment at both maturity periods.

Keywords: Botanic fraction, *Brachiaria decumbens,* Chemical composition, Feedstuff, *In vitro* gas production.

INTRODUCTION

The shortage of forage and its varying availability in quantity and quality throughout the year has been the bane of increased ruminant productivity in the developing world (Makkar, 2002). For improved animal productivity, feeds with high dry matter digestibility that ensure a higher supply of protein post-ruminally should be the choice ruminant feed (Gusha et al., 2015; Rehman et al., 2020). This therefore calls for sustainable pasture establishment practices with high-yielding species and the characterization of their nutrient composition as well as their nutritive value (Muschler, 2016; Leiber, 2022). Chemical composition and digestibility of forages in the tropics are influenced by plant species, variety, levels of maturity, the prevailing climatic conditions and soil fertility (Minson, 1990; Moore et al., 2020). Plant leaves are known to have higher levels of crude protein and digestible carbohydrates (cell contents) but lower structural carbohydrates than the stems. There is rapid accumulation of cell wall carbohydrate as grasses mature which leads to a reduction in crude protein content thereby affecting digestibility (Seyoum et al., 1997). Different plant botanic fractions (leaf, stem and whole plant) have different chemical constituents and digestibility with an increase in maturity among plant species and varieties.

Grasses, crop residues and browse plants are the traditional feed resources for ruminants in the West African sub Region. Most ruminant farmers in developing countries have been reported to harvest grasses daily or at most weekly for feeding their animals owing in part to limited or unavailable storage facilities (Ansah et al., 2013) or nutrient losses in storage (Antwi et. al., 2010). This harvest practice may lead to increased fibre deposition as the grass stays on the field for subsequent harvests which may in turn affect the leaf to stem ratio and the quality of the grass as well. For farmers to choose the most suitable harvesting period for the various plant fractions, the assessment of the nutrient composition and the nutritive value of grasses at different stages of growth cannot be overemphasised. Several studies have however reported the chemical components and *in vitro* gas production of some Brachiaria cultivars including *Brachiaria decumbens* (Ribeiro et al., 2014: Nguku et al., 2016), the gap in the literature on the evaluation of the various botanic fractions of *B. decumbens* in the hot humid regions of Ghana remains wide. The study therefore sought to assess the influence of different harvest dates (60 and 120 days on the chemical composition and *in vitro* gas production profiles of the leaf, stem and whole plant fractions of *B. decumbens* grass.

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MATERIALS AND METHODS

Ethical considerations

All the necessary standard operating procedures outlined by the Animal Research Ethics Committee (AREC, 2018) of the Quality Assurance and Planning Unit of the Kwame Nkrumah University of Science and Technology, Kumasi were followed.

Location and climate of the experimental site

The study was conducted at the Dairy/Beef Cattle Research Station of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi Ghana. The experimental site lies between Latitude 06˚43'N and Longitude 1˚36'W and is within the humid semi-deciduous forest belt of Ghana with a bimodal rainfall pattern. Annual rainfall for the site averages about 1194 mm.

Management practices and Experimental design

A 200 m^2 established *B. decumbens* pasture demarcated by lining and pegging. All grasses within the demarcated plot were cut to a stubble height of 5 cm. The plot was then divided into four sub-plots with 1m alley. Weeding was carried out once at 30 days after the commencement of the experiment by pulling out all unwanted plants. A 2×3 factorial arrangement in a Completely Randomised Design with four replications was used. The harvest dates (60 and 120 days) and botanic fractions (leaf, stem and whole plant) were the factors. The treatments imposed were leaf at 60 days (designated as L60), stem at 60 days (S60), whole plant at 60 days (W60), leaf at 120 days (L120), stem at 120 days (S120) and whole plant at 120 days (W120) for treatments 1–6 in that order. The four sub-plots served as replications.

Harvesting and sample fractionation

The grass samples were taken from the pasture at each harvest date (60 and 120 days), using a quadrant of 1m x 1m in area. Within quadrant grass samples were cut at a height of approximately 5 cm above ground on all sub-plots that served as replications. The harvested grass from each sub-plot was divided into 3 groups and separated into leaves, stems and whole plant fractions.

Chemical analyses

Samples of the botanic fractions of the grass at each harvest date were milled to pass through a sieve size of 2mm and analysed according to the procedures of AOAC (1990) for dry matter (DM), crude protein (CP), ash and ether extract (EE). Representative samples were also assessed for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) with sulphite without amylase (Van Soest et al., 1991). Hemicellulose and cellulose were estimated from ADF, NDF and ADL.

Gas production

Rumen digesta or liquor was obtained from two bulls (*Bos indicus*) at the Kumasi Abattoir, Ghana receiving grass hay before slaughter. The digesta was collected early in the morning after the bulls had been starved for 12 hours prior to slaughter. The digesta was collected immediately during evisceration into a flask and conveyed to the laboratory. The *in vitro* gas production was determined from gas production measurements using the method of Menke and Steingass (1988) in which 200mg±2mg dried samples were incubated in triplicate with 20 ml of artificial saliva (bicarbonate buffer, macro- and micro-minerals, resazurin and a reducing solution) and 10 ml of rumen liquor. Gas volume was measured as the displacement of the syringe plunger at 0, 3, 6, 12, 24, 48, 72 and 96 h of incubation. Gas readings were fitted into the model described as $Y = b\ (1-e^{-ct})$ using the SigmaPlot for windows (version 14.0) software (Systat Software Inc, 2017).

$Y = b\ (1-e^{-ct})$;

where: Y = volume of gas produced at time t (ml); b = potential gas production (ml/200 mg DM); c = rate at which gas was produced from the insoluble fraction (ml/hr); t = incubation time.

In vitro organic matter digestibility (IVOMD, g/kg DM), metabolisable (ME MJ/kg DM), net energy (NE, MJ/kg DM), short chain fatty acids (SCFA) and microbial protein (MP) were predicted according to the following stoichiometric equations of Menke and Steingass (1988) and Close and Menke (1986):

IVOMD (g/kg DM) = [14.88+0.889 IVGP24 +0.45CP (%DM) +0.0651 Ash]

ME (MJ/kg DM) = 2.20+(0.136 IVGP24) +0.057 X CP

NE (MJ/kg DM) = 2.20+(0.0272 X IVGP 24) +(0.057 X CP) +(0.149 X EE)/14.64

SCFA = [−0.00425+0.0222× IVGP24 (ml/0.5g DM)] X 100

MP = 1.93×IVOMD /10

Where, IVGP24= *in vitro* gas production after 24 hours

Statistical analysis

The data obtained from the gas production technique were analyzed using the Minitab Statistical Package, version 19.1 (Minitab Inc., 2019). The means were separated by Bonferroni Pairwise Comparisons test. Probability level of significance was set at 5%.

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RESULTS AND DISCUSSION

Effects of harvest date on the chemical composition and estimated metabolizable energy of plant botanic fractions

The nutrient characteristics of the leaf, stem and whole plant fractions as influenced by harvest date is presented in Table 1. The results revealed significant (P<0.05) plant fractions x harvest date interactions for chemical composition and ME except for EE, cellulose and hemicellulose. Harvest dates resulted in different (P<0.05) nutrient compositions, ME and the fibre concentrations (NDF and ADF). In addition, estimates of the chemical composition as well as NDF, ADF were influenced (P<0.05) by the various plant fractions except for ME which recorded no differences. Ether extract was similar among the plant fractions (P=0.832) but tended to approach significance (P=0.073) at different harvest dates.

Generally, estimates from the plant cell wall fractions (hemicellulose and cellulose) were not significantly different among the plant fractions (P>0.05) and the harvest dates (P>0.05) with the exception of ADL which tended to approach significance at the various harvest dates (P=0.063). The influence of harvest dates on plant fraction reported in this study supports the study by Crowder and Chheda (1982) who stated that as grasses advance in age, there is generally increase in dry matter which increases the cell wall contents with a concomitant decrease in the cell contents. The increase in DM content with maturity observed in this study is consistent with those of Ansah et al. (2010) and Osman et al. (2019). The linear increase (P<0.05) in DM of the various plant fractions from the study may be due to photosynthetic activity leading to the accumulation of dry matter with advancing age (Crowder and Chheda, 1982). On the contrary, McDonald et al., (1995) reported increases in fibre concentration as plants advance in age.

Similarly, the organic matter (OM) content significantly increased (P<0.05) among the botanic fractions from day 60 of harvest to day 120. This pattern of increase was reported by Tilahun et al. (2017) in a study that evaluated harvesting date on the chemical composition of *Pennisetum pedicellatum*. However, the OM contents reported during the 60-day harvest for all botanic fractions were lower than the 83.65 – 84.71% reported by Umami et al. (2017).

Conversely, CP and ash contents decreased (P<0.05) consistently for botanic fractions with advancement in maturity. Seyoum et al., (1997) reported a dilution effect of protein by swift build-up of cell wall carbohydrates towards the end of the grass growth phase. The decline in CP contents observed in this study was consistent with the results of Peiretti (2009), Ansah et al. (2010), Tilahun et al. (2017) and Osman et al. (2019).

It is however noteworthy that the CP contents recorded for all botanic fractions across the two harvest dates were higher than the 7% minimum CP level needed to sustain rumen microbes according to Lazzarini et al. (2009). The CP concentrations of the leaves at the harvesting dates were 1-1.32 times higher than the CP content reported by Faria et al., (2018) when bromatological characteristics of *B. decumbens* were evaluated. These differences in the chemical composition according to Low (2015) may partly be attributable to the season of grass establishment, application of fertilizer, combination with leguminous species, growing conditions and sampling procedures (i.e. plot harvest or picked selection). With regards to ash content, the decreasing trend observed with an increase in harvest date in the current study was in consonance with the findings of Tilahun et al. (2017).

Fibre content represents the amount of indigestible components in the feed and supplies the bulk required for the needed peristalsis in the rumen (McDonald et al., 2010, Bhardwaj et al., 2018). Fibers also support the structure of growing plants and their accumulation with age is inversely proportional to CP content (McDonald, 2011). The contents of NDF, ADF, cellulose and ADL reported in the study significantly increased (P<0.05) with increasing days of growth and was consistent with the findings of Anele et al. (2008) and McDonald et al. (2011). The trends observed with NDF, ADF and ADL in the current study are consistent with earlier findings (Zinash et al., 1995; Seyoum et al., 1997; Tilahun et al., 2017; Wassie et al., 2018).

NDF, according to Nguku et al. (2016), gives an indication of forage dry matter intake. Therefore, forages high in NDF content will consequently have a lower intake. ADF refers to the cell wall fractions composed of cellulose and lignin. Values for ADF are essential as they relate to the animal's ability to digest forage, that is, grasses that are high in ADF have low energy values and are less digestible. ADF content of all botanic fractions over the 3 harvest dates were all within the 200 and 450 g/kg DM reported for tropical forages by Asaolu et al. (2011), McDonald et al. (2011) and Martens et al. (2012). According to Nussio et al. (1998), forages with 40% or more of ADF are indicative of poor intake and subsequent digestibility. With the exception of treatment S120 (stem at 120 days), all other treatments registered ADF contents of less than 40% and could therefore promote intake and digestibility.

The increasing levels of ADL with increasing harvest dates observed in this study confirm the reports of Kidunda et al. (1990), Tesema et al. (2002) and McDonald et al. (2002). Lignin, according to Xu et al. (2003), is a complex, phenolic polymer found in plant cell walls indispensable for providing mechanical support and transporting water and minerals. That is, the stem continues to become lignified as plants grow to provide the needed support to the plant, hence the highest levels of lignin found in the stem fractions over the differing harvest dates. Digestibility of cellulose decreases with high lignin contents (Nguku et al., 2016). Plants produce fibrous tissues as they age according to McDonald et al. (1995) which lead to an increase in structural carbohydrates and lignin. Plant maturity also has been reported by Rambau et al. (2016) as the primary factor that affects the nutritive value of forages.

Metabolisable energy refers to the energy left after losses from fecal and urinary sources. It represents the energy used for growth or reproduction and for supporting metabolic processes. Forages with ME values beyond 2000.5 kcal/kg (8.37 MJ/kg DM), according to National Council of Science and Technology (1975), are regarded to be of high quality. The study recorded ME values ranging from 1781kcal/kg in S120 to 2066.58kcal/kg in L60.

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Table 1 - Effects of harvest date and plant botanic fraction on the chemical composition of *Brachiaria decumbens.*

	Treatments						Significance			
Harvest date	60			120			P values			
Plant fraction	Leaf (L60)	Stem (S60)	Whole (W60)	Leaf (L120)	Stem (S120)	Whole (W120)	SEM	HD	PF	HD*PF
Component										
DM (g/kg)	353.30^{e}	381.63^{cd}	370.40^{de}	403.18^{bc}	442.80^{a}	420.15^{ab}	6.590	0.0001	0.0001	0.042
OM (g/kg DM)	783.90^{d}	801.53^{bc}	792.50^{cd}	804.83^{b}	824.53^{a}	807.18^{b}	2.810	0.0001	0.0001	0.012
CP (g/kg DM)	129.03^{a}	98.88^{c}	113.83^{b}	100.08^{c}	78.58^{d}	88.88^{cd}	3.560	0.0001	0.0001	0.029
Ash (g/kg DM)	94.63^{a}	73.48^{bc}	82.40^{b}	71.83^{c}	51.90^{d}	69.78^{c}	2.820	0.0001	0.0001	0.028
EE (g/kg DM)	31.65	32.00	32.65	34.33	33.00	33.33	0.368	0.073	0.832	0.523
NDF (g/kg DM)	606.03^{b}	648.60^{a}	645.93^{a}	652.88^{a}	658.58^{a}	656.73^{a}	4.760	0.003	0.014	0.045
ADF (g/kg DM)	320.70^{b}	370.18^{ab}	368.73^{ab}	365.28^{ab}	405.98^{a}	391.43^{a}	7.610	0.007	0.011	0.025
Cellulose (g/kg DM)	257.10	299.30	299.20	295.08	314.98	317.95	7.260	0.083	0.103	0.754
Hemicellulose (g/kg DM)	285.33	278.43	277.20	287.60	252.60	265.30	6.630	0.439	0.509	0.747
ADL (g/kg DM)	63.60^{b}	70.88^{ab}	69.53^{ab}	70.20^{ab}	91.00^{a}	73.48^{ab}	2.610	0.039	0.063	0.032
ME (kcal/kg)	2066.58^{a}	2053.65^{ab}	1926.87^{abc}	1895.28^{bc}	1781.86^{c}	1912.96^{abc}	23.700	0.0001	0.129	0.005

^{a,b,c,} Mean values with different superscripts on the same row differ significantly (P<0.05).

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A declining pattern in ME with advancing age was observed in the study and it is in consonance with the observation by Umami et al. (2017) who reported a constant decline in ME when the nutritive value of *Brachiaria sp* was accessed.

It can also be seen from Table 1 that in respect of the plant botanic fractions, the leaf fraction registered the highest CP and ash contents for both harvest dates followed by the whole fraction with the stem fraction recording the least levels of CP and ash. The high content of CP in the leaf fraction is advantageous to microbes in the rumen that utilize dietary sources of nitrogen for the synthesis of their body proteins. Ansah et al. (2010) also found that the leaf fraction of Napier grass had more CP than the stem fractions. Levels of NDF, ADF and ADL were highest in the stem followed by the whole fraction with the leaf fraction registering the lowest values. The least content of lignin in the leaves is required as it suggests minimum binding of cellulose and hemicellulose thereby making them available to be utilized efficiently by rumen microbes. CP content of the leaf and whole fractions at 60 days met the recommended 110g/kgDM to 130g/kgDM required for maintenance and growth for ruminant livestock (Freer et al., 2007; Asaolu et al., 2011).

Effects of harvest date on gas production profile of plant botanic fractions

The cumulative *in vitro* gas production of the various botanic fractions over the 3 harvest dates as a function of incubation time is shown in figures 1 and 2. *In vitro* fermentation is generally a reflection of the extent of the fermentation and digestibility of feed (Getachew et al., 1998). Gas production generally increased cumulatively with increase in incubation time from 3 hours to 96 hours for all plant fractions across the two harvest dates.

It can be seen from Figures 1 and 2 that the maximum amount of gas was typically produced within the first 3 hours of incubation, which is consistent with Sarkwa et al. (2020)'s report on various browse plants. Gas produced by the various botanic fractions decreased consistently with an increase in harvest date throughout the incubation period. The decline in gas production associated with maturity is in harmony with the report of Kamalak et al. (2005) to the effect that maturity affects gas production which decreases with advancement in age of the plant. Since cumulative *in vitro* gas production is proportional to substrate degradation, it can be said that maturity decreases the proportion of easily degradable portions of the plant fractions.

It is also worth noting that at both harvest dates, leaf fractions produced more gas, followed by the whole plant fraction with the stem producing less gas throughout the incubation period as illustrated in figures 1 and 2. This observation agrees with the report of Tang et al. (2008) who recounted that cumulative gas production was constantly higher for the leaf sheath portion relative to the stem in maize.

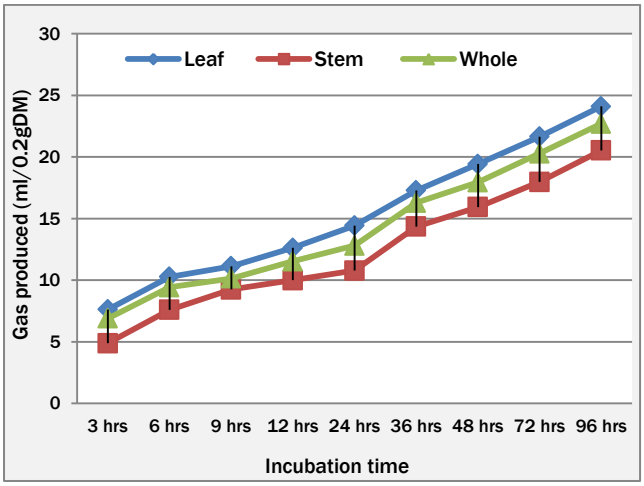

Figure 1 - *In vitro* gas production of leaf, stem and whole fractions at 60 days.

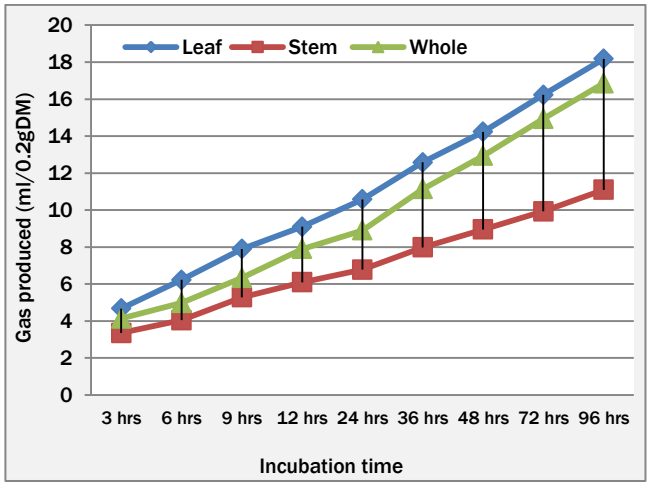

Figure 2 - *In vitro* gas production of leaf, stem and whole fractions at 120 days.

The highest gas production rate in the leaves relative to the stem and whole fractions suggests that the microbes in the rumen were able to degrade the leaf fractions better possibly because there was a higher level of fermentable nutrients present. Gasmi-Boubaker et al. (2005) found a positive correlation between crude protein and gas production, so the higher gas volume for the leaf fractions corresponded to the higher CP and lower cell wall contents. In addition, the leaf fractions recorded lower contents of NDF and ADF (Table 1), which are negatively correlated with gas production (De Boever et al., 2005) and fermentation parameters (Heidary and Kafilzadhe, 2012). Thus, the low fibre content according to van Soest (1994) can enhance feed utilization by rumen microbes which could lead to higher fermentation rates. This trend however conflicts the observation of Cone and Gelder (1999) that feed items rich in CP normally produce small amounts of gas during fermentation as the fermentation of protein results in ammonia production, which affects the carbonate buffer equilibrium by neutralizing H^+ ions from VFA without release of carbon dioxide.

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Again, the least gas accumulation from the stem fractions was due to large amounts of cell wall constituents, that is, crude fibre and lignin. Jung and Deetz (1993) have reported lignin content to be negatively correlated with gas production. Lignification of cell walls, according to the authors, limits the activities of rumen microbes responsible for the fermentation or enzymatic breakdown of forage polysaccharides.

High lignin contents also limit access of rumen bacteria to easily fermentable cell contents (van Soest 1994). The least gas production from the stem fractions could also be linked to the low degradability of CP in the rumen. This ultimately affects the extent of ammonium nitrogen available for microbial cell synthesis thus resulting in low microbial fermentation and gas production.

Since the production of gas is positively correlated with feed fermentation, the stem fraction can be thought of as possessing a relatively low feeding value because of the low fermentative gas production. The differences observed in volume of gas produced in this study could be ascribed to constituents and method of preparation of culture used to incubate the test sample, size and variability of microbial inoculum, reading intervals used to record gas pressure and volume from the fermentation bottles and atmospheric pressure (Zinash et al., 1998).

Gas production kinetics of plant botanic fractions at different harvest dates

Results of gas production kinetics of the various plant botanic fractions over the two harvest dates are shown in Table 2. Kinetics of gas production deal with the quantities of gas produced by a material, '*b*' and the rate at which these gases are produced, '*c*'. These are indicative of how digestible a feeding material is and for that matter how long it will remain in the animal's gut (Getachew et al., 2005). Significant interactions ($P<0.05$) between harvest date and plant fraction were present for both '*b*' and '*c*' attributable to treatment effects. Potential gas production '*b*' elicited a negative response for all plant fractions across the two harvest dates as the values decreased linearly. The rate at which the gases were produced '*c*' also induced a negative response for the leaf and whole fraction but a positive one for the stem fraction.

The potential gas production *'b'* of the leaf fraction was consistently the highest followed by that of the whole plant fraction with the stem fraction registering the least at both harvest dates. Potential gas production ranged from 9.640 ml/0.2g DM in S120 (stem fraction at 120 days) to 20.759 ml/0.2g DM in L60 (leaf fraction at 60 days). The leaf fractions had the lowest levels of fibre contents which were indicative of higher levels of soluble carbohydrates which eventually translated to the uppermost potential gas production. A higher potential gas production is desirable as it can contribute significantly to energy supply through the synthesis of short chain fatty acid (Remesy et al., 1995). The gas production rate *'c'* was also constantly highest for the leaf fraction followed by the whole plant fraction with the stem fraction having the least. However, at 120 days, the rate for the stem fraction was higher than what was recorded for the whole fraction (Table 2). The whole plant fraction at 120 days (W120) was digested at the lowest rate 0.0481/hour while the leaf fractions at 60 days were digested at the highest rate of 0.0811/hour. The highest rate of fermentation which was observed in the leaf fractions was a result of the highest CP and lowest fibre (NDF, ADF and ADL) levels (Osuga et al., 2006) as well as carbohydrate portions that were easily available to microbes (Afshar et al., 2011).

The greater values obtained for the '*b*' and '*c*' for the leaf fractions across all harvest dates may be indicative of the fact that nutrients were much more easily available for the rumen microbes. The rates of fermentation for different chemical components reflect the growth of rumen microbes and access to feed by microbial enzymes (Getachew et al., 2004). The differences in the rate at which gases were produced are indicative of the fact that the plant botanic fractions used different times to degrade all potentially digestible materials and for that matter different rate of passage in the digestive system. The disparities in gas production kinetics across the various treatments were attributable to the treatments imposed as stages of growth and portions of plants used as feed among other factors have been reported by Akinfemi et al. (2009) to influence gas production.

Effects of harvest date on some estimated parameters of the plant botanic fractions

Effects of the different harvest dates on short chain fatty acids (SCFA), estimated energy values (ME and NE), *in vitro* organic matter digestibility (IVOMD) and microbial protein (MP) of the plant botanic fractions are given in Table 3. Harvest date by plant fraction interaction was present ($P<0.05$) for all parameters estimated. For SCFA, ME, IVOMD and MP, the interactions observed were due to the differing harvest dates. The said estimated parameters were significantly not affected ($P>0.05$) by plant fractions within the same harvest date (Table 3). In the case of NE, the interaction observed was due to the synergy between harvest date and plant fraction. Generally, all estimated parameters decreased with advancement in maturity.

Accordingly, SCFA, ME, NE, IVOMD and MP contents decreased linearly for all plant fractions from 60 to 120 days. SCFA is a major source of metabolic fuel while MP serves as a major portion of protein absorbed from in gut in ruminants. ME values recorded for the various botanic fractions at 60 days were all lower than the 5.3MJ/kg DM reported by Umami et al. (2017) at 60 days. The ME values recorded in the present work were higher compared to those (314.6–1406.9kJ/g DM) reported by Sarkwa et al. (2020) for some browse forages. The current ME values for all botanic fractions (3.79 – 4.79MJ/kg DM) were lower relative to the 9.35MJ/Kg DM recounted by Aung et al. (2019) for Mulato II grass. The differences in ME could be attributable to growing conditions (soil and climatic) and sampling methods (Low, 2015).

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Table 2 - Fermentation kinetics of experimental feedstuffs (plant botanic fractions at different harvest dates

	Treatments						Significance			
Harvest date	60			120			P values			
Plant fraction	Leaf (L60)	Stem (S60)	Whole (W60)	Leaf (L120)	Stem (S120)	Whole (W120)	SEM	HD	PF	HD*PF
GP parameters										
b	20.759^{a}	18.022^{b}	19.607^{a}	16.259^{c}	9.640^{d}	15.421^{c}	0.754	0.0001	0.0001	0.0001
c (h^{-1})	0.0811^{a}	0.0622^{ab}	0.0750^{ab}	0.0613^{ab}	0.0788^{a}	0.0481^{b}	0.003	0.066	0.250	0.007

^{a,b,c,} Mean values with different superscripts on the same row differ significantly ($p<0.05$). '*b*' quantities of gas produced by a material; 'c' the rate at which the gases were produced.

Table 3 - Effects of harvest day and plant part/fraction on some estimated parameters of *Brachiaria decumbens*.

	Treatments						Significance			
Harvest date	60			120			P values			
Plant fraction	Leaf (L60)	Stem (S60)	Whole (W60)	Leaf (L120)	Stem (S120)	Whole (W120)	SEM	HD	PF	HD*PF
GP parameters										
SCFA	27.94^{ab}	32.71^{a}	26.83^{b}	19.33^{c}	18.28^{c}	23.05^{bc}	1.100	0.0001	0.209	0.0001
ME (MJ/kgDM)	4.67^{a}	4.79^{a}	4.52^{a}	3.98^{bc}	3.79^{c}	4.14^{b}	0.080	0.0001	0.817	0.001
NE (MJ/kgDM)	3.32^{a}	3.20^{b}	3.22^{b}	3.05^{c}	2.91^{d}	3.03^{c}	0.029	0.0001	0.000	0.045
IVOMD (%)	32.66^{a}	33.08^{a}	31.45^{a}	27.76^{bc}	26.24^{c}	28.73^{b}	0.552	0.0001	0.410	0.001
MP	63.03^{a}	63.84^{a}	60.70^{a}	53.58^{bc}	50.65^{c}	55.46^{b}	1.070	0.0001	0.410	0.001

^{a,b,c,} Mean values with different superscripts on the same row differ significantly ($p<0.05$). SCFA: Short chain fatty acids, ME: Metabolisable energy, NE: Net energy, IVOMD: In vitro organic matter digestibility, MP: Microbial protein.

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Correlations between chemical composition and *in vitro* gas production

The correlation between chemical composition and *in vitro* gas production after 24 hours is presented in Table 4. The largest positive correlation was seen between 24 hr GP and '*c*' while the largest negative correlation was observed between CP and ADF. CP content correlated significantly and negatively with both NDF (r= -0.705; P=0.000) and ADF (r= -0.689; P=0.000). The content of CP was also significantly and positively correlated with ME (r=0.689; P=0.000) and GP at 24 hours (r=0.869; P=0.000). The observed relationship between CP and GP at 24 hours confirms the reports of Nsahlai et al. (1994), Gasmi-Boubaker et al. (2005), Basha et al. (2012) and Olfaz et al. (2018) who all found CP content to be positively correlated with gas production. Karabulut et al. (2007) however observed a negative correlation between CP and gas production at 24 hours. ME also correlated positively with CP which is one of the critical requirements for the growth of microbes (Larbi et al., 1998). A similar positive correlation between CP and *in vitro* gas production was found by Sarkwa et al. (2020) and Kulivand and Kafilzadeh (2015) for browse plants and pasture grasses respectively. A significant positive correlation (r= 0.861; P=0.000) between CP and potential gas production *'b'* was also observed. However, the correlation between CP and the rate of gas production *'c'* was not significant (r=0.289; P=0.171).

Table 4 - Correlations between chemical composition and *in vitro gas* production

	CP	NDF	ADF	ME	24hrsGP	b
CP						
NDF	-0.705***					
ADF	-0.689***	0.505*				
ME	0.689***	-0.526**	-0.615**			
24 hr GP	0.869***	-0.605**	-0.583**	0.612**		
b	0.861***	-0.533**	-0.616**	0.734***	0.912***	
c	0.289^{ns}	-0.299^{ns}	-0.072^{ns}	-0.032^{ns}	0.308^{ns}	-0.019^{ns}

CP: Crude protein (g/kg DM) NDF: Neural detergent fibre (g/kg DM) ADF: Aacid detergent fibre (g/kg DM) ME: Metabolisable energy (kcal/kg), *b*= potential gas production (ml/0.2 g DM) *c*= rate of gas production (/h). Level of significance: (*) P<0.05, (**) P<0.001, (***) P<0.0001, ns=not significant

There was also a positive and significant correlation (r=0.505; P=0.012) between the levels of NDF and ADF. Thus both NDF and ADF increased together with an increase in harvest dates for all botanic fractions. However, correlations between NDF and ME were negative and significant (r= -0.526; P=0.008). Correlation between NDF and 24hrGP followed a similar pattern (r= -0.605; P=0.002). NDF also correlated significantly and negatively with *'b'* (r= -0.533; P=0.007) and negatively but not significantly correlated with *'c'* (r= -0.299; P=0.156). Thus increased contents of NDF and ADF led to a decline in both potential gas production and the rate of fermentation. This trend agrees with the findings of Kamalak (2006) and Kulivand and Kafilzadeh (2015). The negative correlations observed between potential gas production *'b'* and both NDF and ADF could be a result of reduced growth of microbes and poor access to the feed by microbial enzymes as reported by Getachew et al. (2004). This trend contradicts the report of Abdulrazak et al. (2000) who reported positive correlations between NDF and ADF with gas production characteristics. However, the negative correlation between NDF and ADF with rate of gas production was in harmony with the results of the same authors. Nsahlai et al. (1994) and Basha et al. (2012) have both reported earlier that gas production of feeds negatively correlates with NDF content.

The concentration of ADF correlated significantly and negatively (r= -0.615; P=0.001) with ME as well as with GP (r= -0.583; P=0.003). Table 4 also shows that a very strong negative correlation was observed for ADF and *'b'* (r= -0.616; P=0.001). ADF correlated negatively but not significantly with *'c'* (r= -0.072; P=0.739). However, a significant positive correlation (r=0.583; P=0.001) was observed between ME and GP as well as between ME and *'b'* (r=0.73; P=0.0001). The correlation between ME and 'c' however, was not significant (r=0.032; P=0.882). Finally, 24hrGP correlated significantly and positively (r= 0.912; P=0.0001) with *b*'.

CONCLUSIONS

Harvest date influenced the chemical compositions of the leaf, stem and whole plant fractions of *B. decumbens* regrowth. While the CP and ash contents decreased with an increase in harvest date, OM, CF, ADF, NDF and ADL contents increased linearly with maturity. The leaf fraction that registered the highest CP and ash contents also had the lowest CF, ADF, NDF, cellulose and ADL contents across both harvest dates. The whole plant fraction consistently recorded values that were in between those of the leaf and stem fractions. The CP contents recorded for all botanic fractions across the two harvest dates met the least CP level of 7% required to sustain rumen microbes. In view of this when biomass yield is of interest, the whole plant fraction can be considered as the stem fraction also has some feeding value and could give bulk to the grass. At both harvest dates, the leaf fraction produced the most gas, followed by the whole plant fraction with the stem fraction producing the least amount of gas throughout the incubation period. Botanic fractions that were high in CP and low cell wall constituents (ADF, NDF, cellulose and ADL) showed better potential for gas production. *B. decumbens* regrowth should be harvested when it is 60 days in view of its comparatively superior chemical composition and highest *in vitro* gas production at that age.

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DECLARATIONS

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Consent

Not applicable

Author's contribution

E.L.K. OSAFO contributed to the design of the experiment, statistical analysis and interpretation, coordination of the study and the write up of the manuscript, A. OSMAN made substantial contribution to the conceptualisation and experimental design, data collection, statistical analysis and interpretation, laboratory analysis and write up of the manuscript, V. ATTOH-KOTOKU participated in the interpretation of results and contributed to the drafting of the manuscript and its revision, C. ANTWI was involved with interpretation of results and contributed to the drafting of the manuscript and its revision; Y. ABDUL AZIZ participated in the data collection, statistical analysis and drafting of the manuscript, F. IDAN contributed to data analysis and drafting of the manuscript and its revision. All authors read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interests.

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A NEW READING OF THE ANIMAL PRODUCTION SUBSIDY PROGRAM FOR THE SAHARAN REGIONS: OPPORTUNITIES AND CONSTRAINTS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Like in other Saharan regions, the Animal Production Subsidy Program has increased the herd in Ghardaia. The creation of breeding farms in such areas has generated specific dynamics through a set of measures that have had different impacts on the components of this sector. The data from the guides addressed to the various stakeholders illustrates that the management of the breeding farms presents shortcomings at different levels and even compromises its sustainability. In our model, around 49 % of farmers are renting their farmland, while 51 % are managing their own land. A significant portion of breeders (approximately 75.88 %) expresses the view that the main obstacle hindering the progress of these treatments is the insufficient availability of outreach programs. Applying this management results in young breeders abandoning the farms and using them for other activities. As a solution, the government must revise its agricultural programs and investments in order to achieve the long-term development goals that have been set. The measures to be taken are discussed to preserve the sector and explain the substantial investments made by the public authorities.

Keywords: Agricultural policies, Algeria, Animal production, Breeding farms, Saharan region, Subsidy program.

INTRODUCTION

In the Saharan region, various agricultural reforms and policies (socialist era) have discarded family farms in agrarian development (Baci, 1999; Abdelhedi and Zouari, 2020; Baghdad, 2022). So, the agricultural sector remains the third sector of the economy in terms of value-added formation (Djaafri and Abdelli, 2019). It represents 9.2% of the Gross Domestic Product, which does its ranking after the hydrocarbons (38%) and services (21%) sectors (Bedrani, 2008). In addition, it fulfills only 25% of the employment needs of 42% of the total labor force (Bensaha et al., 2015).

Bensaha et al. (2013) stated that, to implement this program was a decisive stage in the history of agricultural policies in Algeria; it promoted the role of the farmer as an active economic player. Moreover, the program mutated the traditional relationship between the farmer and agricultural administrations into a confident, responsible, and cooperative relationship (Adrian and Green, 2001).

So, these policies proved to be efficient ways to achieve higher production levels, mainly regarding food security (Dhehibi and Lachaal, 2006). For this reason, the government implemented a policy of aid and support for the agricultural sector by creating the National Fund for the Agricultural Regulation and Development (FNRDA) in 2000, with the objective to reduce the importation of foodstuffs and ensure the food security of the country (Benyoucef, 2005). However, food production has never satisfied domestic needs due to the scarce studies investigating the implemented profitability and sustainability strategy (Forbord and Vik, 2017; Govindan, 2018).

The incentive agricultural system has witnessed various revisions. The new subsidy system has been implemented in 2000, it aimed to improve the expansion of private investment in agriculture through the instauration of new economic supports and the encouragement of aggregation.

A significant proportion of "young" farmers and a territory in which agricultural activities occupy an important place a priori reflect a region where the agricultural sector is important, which would be rather favorable to an increase in milk production. According to MADR (2018), another strategy was fostered between 2000 and 2018, called the National Plan for Agricultural and Rural Development (NPARD), where a substantial budget of 350 billion Algerian dinars (DZD) was granted and 14 billion of the debts were annulled for the farmers. However, the exported agricultural products only increased by $ 3 billion in 2003, $ 8 billion in 2008 and $ 11 billion in 2011. Moreover, Thabet et al. (2002) found that approximately 80% of food subsidies went to urban areas, at the expense of the rural ones, despite the fact that more than 40% of the Algerian population lived in rural areas. This showed that food subsidies were ineffectually administrated and unequally distributed between rural and urban consumers. Therefore, specific policies had been implemented to secure the supply of food and create wealth and labor opportunities through the use of the natural resources: arable and range lands, animal wealth and irrigation water. However, in the absence of public policies tradition assessment in

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Algeria, the management authorities hardly establish physical and financial assessments instead of an actual evaluation (Akerkar, 2015).

This study presents the data of the obtained results related to breeder's investment in the region of Ghardaia (North Sahara of Algeria) in the era between 2005-2018 the need for evaluation of the various aids received by breeders is highly important. It also attempts to investigate the actors for the production development, and to provide recommendations that can be used by the actors concerned with the sustainable development of the Saharan regions.

MATERIALS AND METHODS

Presentation of the study area

Our study area (Ghardaia region) is located in the north of the Saharan Algerian; it is represented by thirteen communes (municipalities), as indicated in figure 1. It is an agricultural zone and a dairy basin, with an area of 1 370 911 ha. The total population of the Ghardaia region is estimated at 363,598 inhabitants, with a density of 4.3 inhabitants per km² General Census of Population and Habitat (GCPH, 2008), 53% of the population live in rural areas, and 61% are between 20 and 60 years of age. Agricultural potentials of this region have produced significant changes in Saharan agriculture thanks to the fostering of specialized breeding systems (Table 1). The study was used to point out the current situation of the farms and to provide a retrospective study considering the previous decades to identify the transformations in Saharan agriculture related to the climatic condition's changings. The choice of this region is based on the importance of agriculture and the ruminants breeding in their economic activity.

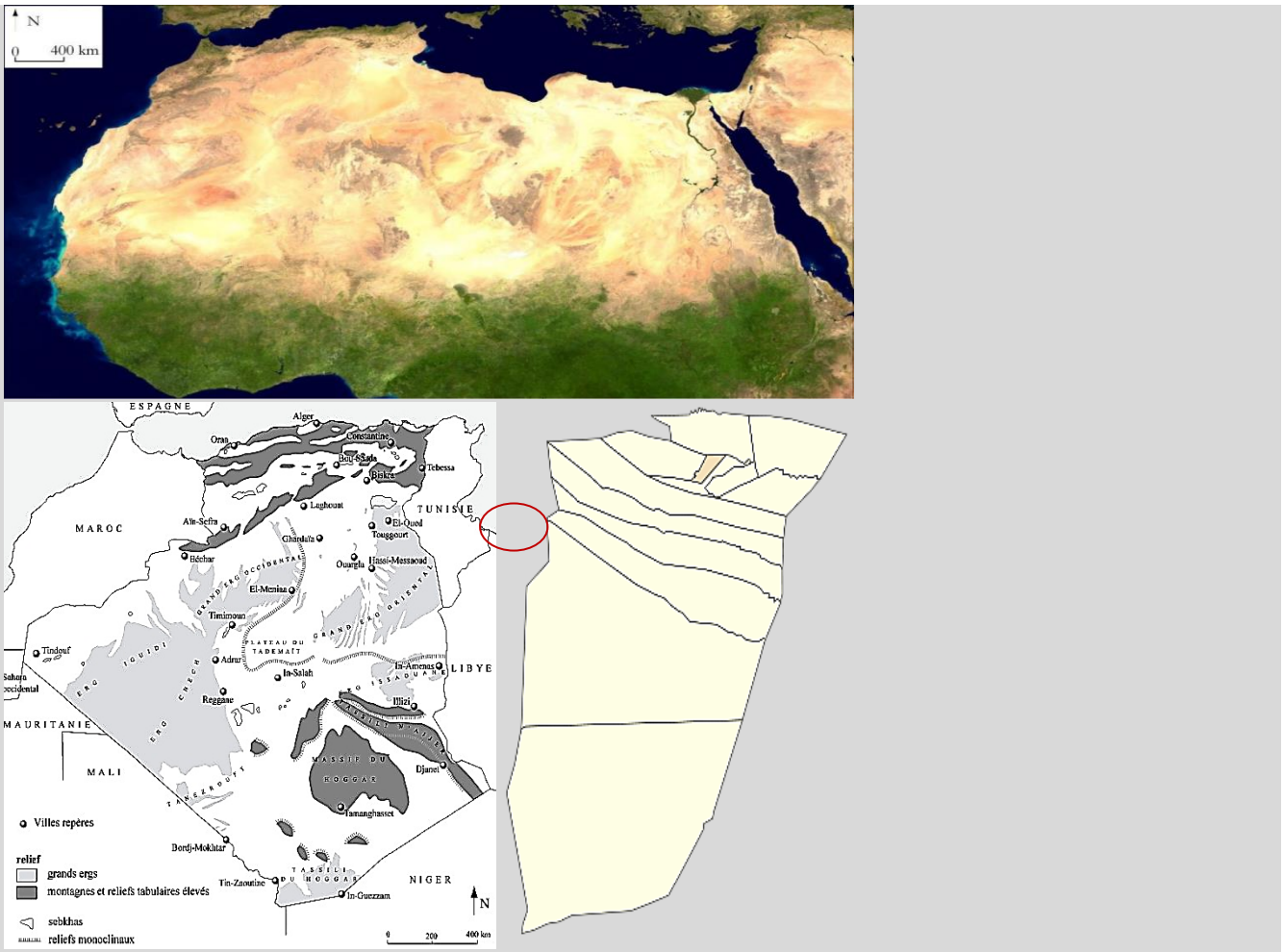

Figure 1 - Situation of the study area.

Table 1 - Overview of the agricultural potential in the study region of Ghardaia.

Animal production		Livestock	
Production	Quantities	Sheep	365 000 heads
Red meats	45 223 Qx	Cattle	4 000 heads
White meats	2 415 Qx	Goats	161 400 heads
Milk (10^3 liters)	28 990 L	Camels	11 650 heads
Of which milk collected (10^3 liters)	11 287 L	-	-
Honey	6000 kg	Beekeeping	2 020 hives

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Data sources and estimation procedure

In order to carry out the annual data on the value of projects number by activity, investment amount, jobs, and overlapping percentage are subjected to be investigated. Data collection is based on the responses of the farmers, on our own observations and on farm records if they exist, as well as on engineers from the Agricultural Services Direction. It is based on an analysis of the database provided by the Agricultural Services Direction (DSA) of Ghardaia region. It registers the exploitations received from the state aid under the agricultural development fund. This database contains several projects presented at this program (2005-2018). Our efforts are focused on monitoring and evaluating the physical implementation rate of the subsidized projects. The results are compared with the referred objective. The size of the sample, which is admittedly small, helps to carry out an adequate detailed analysis of the practical situations. Data processing is conducted through a descriptive analysis via XL-Stat.

Brief overview of the subsidy program: dynamic trigger

The agricultural policies of the last thirty years have been marked by a clear liberal orientation and a frank choice for the development of Saharan agriculture, which is considered as an essential part to solve food dependency problem of the country (Mutin, 2000). These agricultural policies have contributed to lifting the constraints of access to productive resources and have significantly boosted agricultural development in some areas of the Sahara, as is the case in the region of Ghardaia. The Algerian government has long given priority to the development of the rural sector. It allocated a significant portion of the planned investment budgetary funds to it. Thus, many milestones at the institutional, legislative, regulatory and organizational levels have been settled to pave the way for the gradual and harmonious achievement of sustainable development.

As a reminder, the program is based on three pillars, namely:

1. Increased food supply and reduced hunger.
2. Agricultural research, extension and adoption of technologies enabling sustainable growth in production.
3. Sustainable development of livestock, fisheries and forests.

Development programs also address socioeconomic imperatives. In short, it is about improving the incomes of farmers and the fight against poverty (Khiati, 2007). It also includes close extension and technical support programs for farmers, involving specialized technical institutes, the local agricultural administration and chambers of agriculture. According to Maghni (2013), the primary purpose of the new policy is the national food security and revitalization of rural areas.

In 2021, the Minister of Agriculture noted that: “Bureaucracy is the first obstacle that the farmer faces in Algeria”. Our study shows that, it is necessary to improve administrative procedures, where large farmers more easily put together the files to apply for a subsidy. Also, used to establishing oral contracts (informal land and water markets), smallholders perceive the use of written documents as a cumbersome and time-consuming process; it is also necessary to call on someone well-informed and to commute to various administrations. To cope, such the government is preparing to launch a new financing mechanism for farmers, while promulgating an instruction to simplify the procedures for obtaining credit (Figure 2). The services of the agricultural sector are currently working on the creation of new financing mechanisms in favor of farmers in order to revive agricultural activity and finance all sectors.

The Bank of Agriculture and Rural Development (BADR), which is responsible for supporting farmers, examine the obstacles that compromise obtaining credit for a short period. At the same time, the conditions of access to the subsidy are simplified by the creation of a one-stop shop. Nonetheless, the gaining of subsidies and farming strengthening did prove successful due to the intermittent of actions in time and space. The outcome of the current study indicated that farm improvement policies in drylands should be compliant and account for the heterogeneity of agriculture, in particular socio-economic factors.

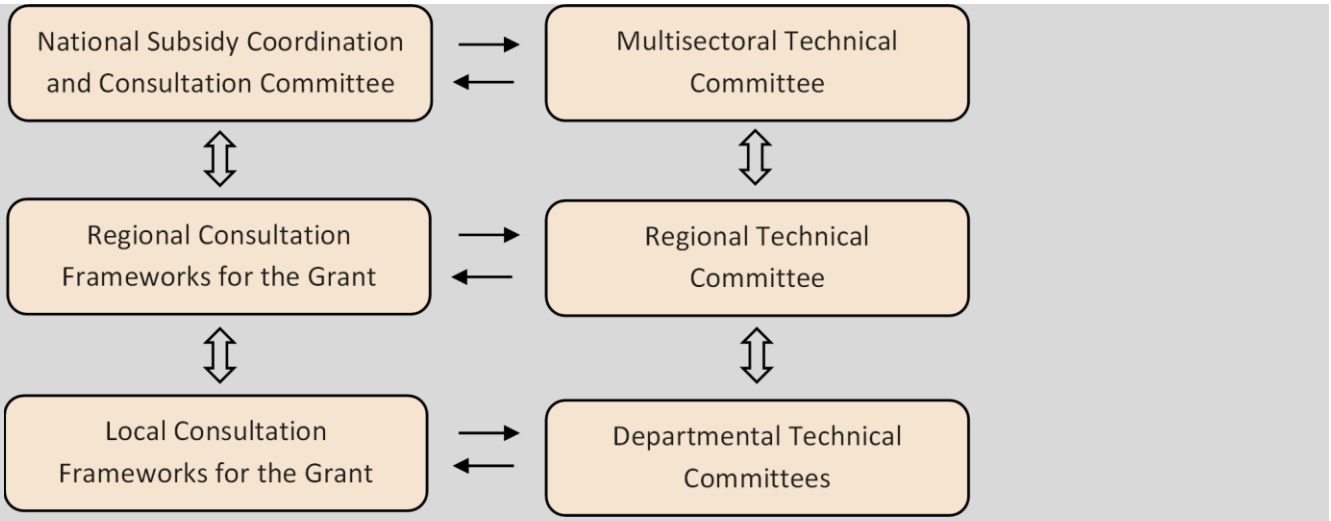

Figure 2 - The key steps for applying for and obtaining a subsidy

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RESULTS AND DISCUSSION

Integration of young people and availability of the workforce

Agricultural employment poses a dilemma for policy makers in Algeria (Bensaha et al., 2015). In the current global context, family farms represent more or less 1.4 billion people, and 96% of family farms are sited in southern countries (Ferraton and Touzard, 2009).

As discussed in Figure 3, the common age of farmers receiving aid in our study is 40 years, and the excessive standards ranged from 20 to 70 years. Approximately 49% of farmers in our model are tenants, and 51% operated their own farmland. The latest overall employment situation contributes to the diminution of the joblessness rate by 9% in rural areas and 6% in the area. The employment rate Feminine represents 13% in the rural setting, mainly crafts and small livestock, and 10% in the municipal environment, particularly in the administration. At present, the employment tendency recorded during the period (2005-2018) remains not worth mentioning in view of the growth of the rural workforce predictable at 7%, even though the number of farms augmented by 4.66% but they are mostly small (51% with an area of less than 3 ha), which is shaped as a single unit occupied by a single employment post, with low returns and incapable to turn out the minimum essential for the safeguarding of families; agriculture remains a less critical and seasonal activity for a considerable part of the agricultural population.

In this organizational context, the countryside employment condition in the Ghardaia region is similar to the common of Saharan areas, which is characterized by the dim training of the workforce, the lack of an alteration of farmers in old age, poor integrating circumstances of agricultural institutions and organizations (30% adhering to the Chamber of Agriculture, 16% insurance, 5% to both at the level of institutions and organizations bank and represented only by 5 associations of low activity), these are young investors, university graduates, the beneficiary of the aid program. They employ one or two permanent workers in order to free themselves up for farm management tasks. This wrong observation reflects the fact that the Algerian labor market is characterized by early-life migration from the countryside to metropolitan areas, many leaving agricultural farms in the hands of women and elderly workers.

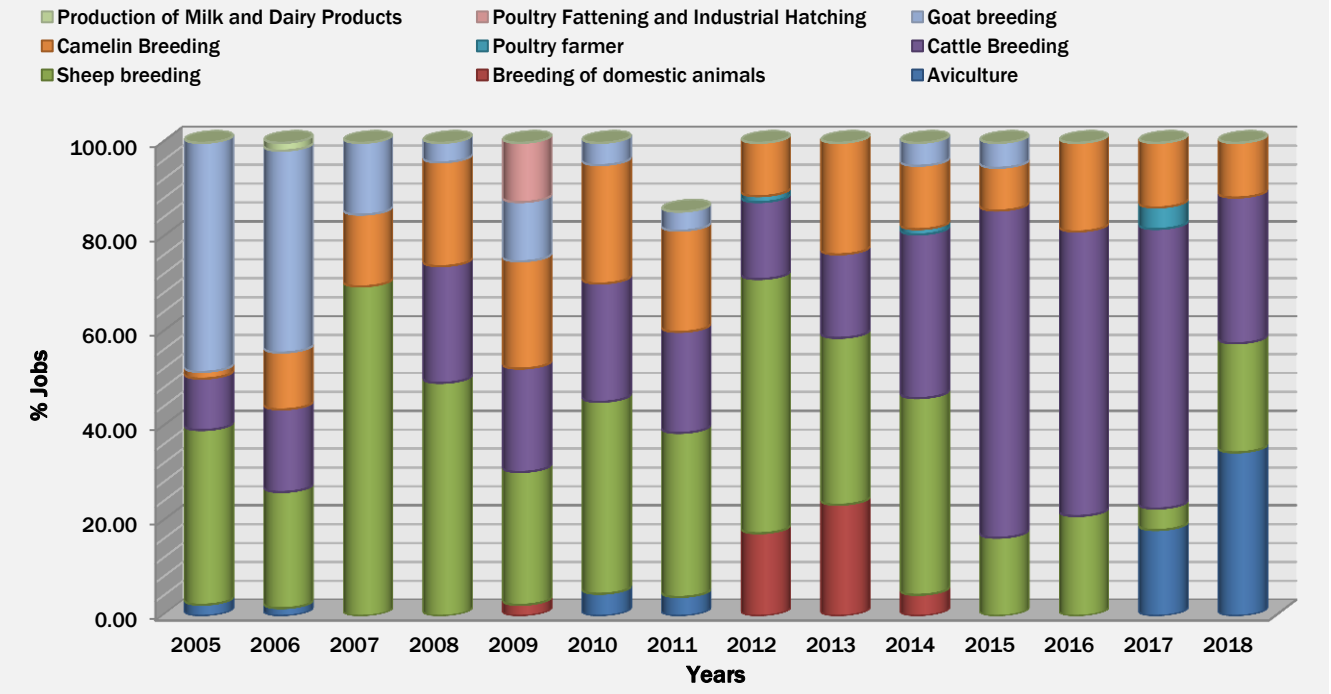

Figure 3 - Integration of young farmers in the sector

The number of projects by activity and the investment: dynamic trigger

Starting from the purpose of the subsidy program is to progress the farming and living standards of countryside populations (Bensaha et al., 2013). As well as, an immense flow in food demand has surfaced (Lampietti et al., 2011), mainly in animal products (meat and milk), implying, as in many other developing countries the need for a "Livestock Revolution" (Delgado, 2003). These programs have also powerfully supported the renewal and industrialization of the farm animals' sector and have motivated the start-up of livestock production farms (Figure 4).

As shown in Figure 4, many reported operator's loans receiving support through the PNDR program. This need for external financing is explained by the significant cost of activity financing and by the vast sum that is necessary to start a new farm. This has been combined with the fact that breeding in Ghardaia has breathed new life into Saharan agriculture by allowing the diversification of agricultural production systems and the extension of other speculation. According to Daoudi and Lejars (2016), the development of animal husbandry has been accompanied by the development of the entire supply chain for agricultural inputs and equipment. Changes in livestock numbers are positively related to changes in the number of farmers and the degree of micro-credit.

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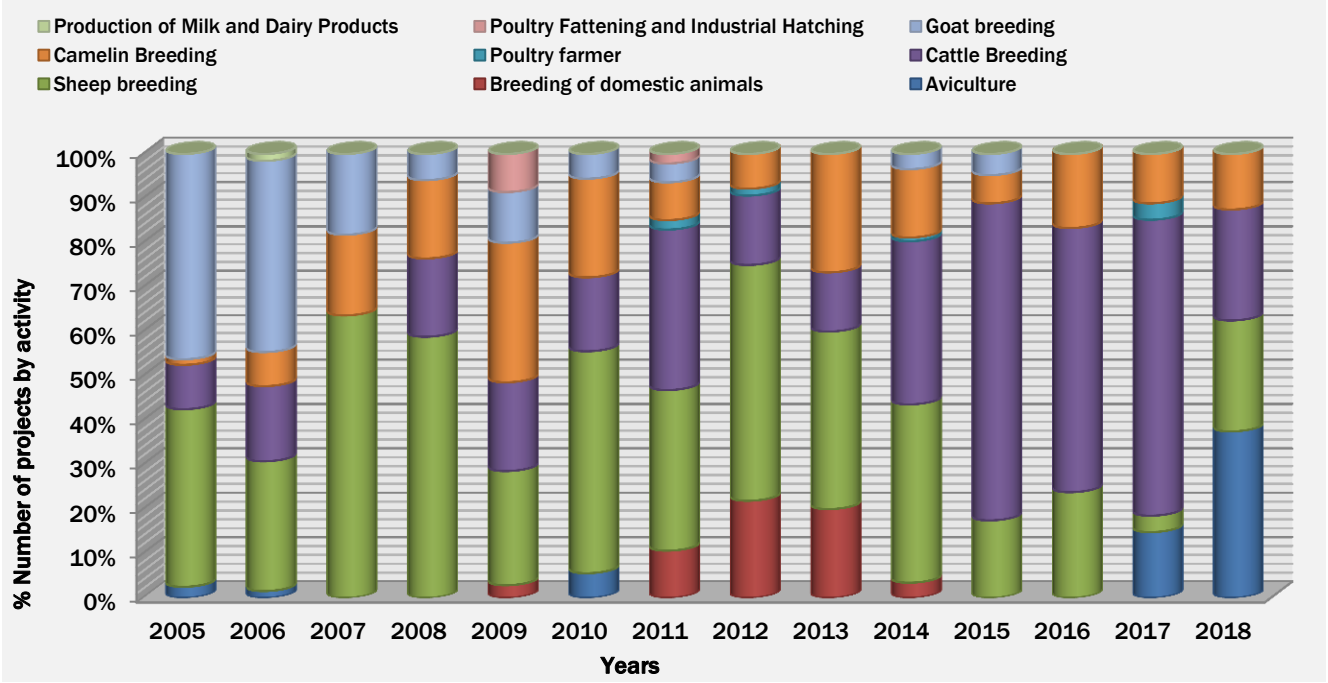

Figure 4- Number of projects for different activities

For our study, many breeders ignore their positive impact and those wishing to apply them do not master the technique. The majority of breeders (75.88%) believe that the lack of outreach programs is the major impediment to the development of these treatments. For this purpose, they should provide the necessary resources to the actors in charge of monitoring / evaluating agricultural investment programs (vehicle, computer, bonus ...) in order to help breeders for improving their production strategies. Such subsidies are mostly used to finance buildings or machinery. For livestock buildings in our case, breeders have built 30 sheepfold housing and 57 stables housing. Conversely to the situation of the vegetal production, for the rearing, the farmers (the area is <05 hectares) have benefited more than 50% of subvention distributed between 63% for the stables rearing, 77% for equipment and 25% for genetic amelioration.

It is worth mentioning that the remarkable evolution of production was observed from one commune to another. The good financial income obtained by large farms can be explained by the good management of breeding which is reflected by the low veterinary costs compared to the total costs. Large farms also potentially benefit from economies of scale that reduce unit production costs (Mosheim and Lovell, 2009) and promote increased regional production.

Inventory of investments and subsidies by type of project: Intensification of plant production, subsidies for agricultural equipment represent two-thirds of aid granted to this category, followed by units for the enhancement of agricultural products. In this context, breeders primarily invested in dairy and meat production, followed by forage farming for animal feed (Figure 5).

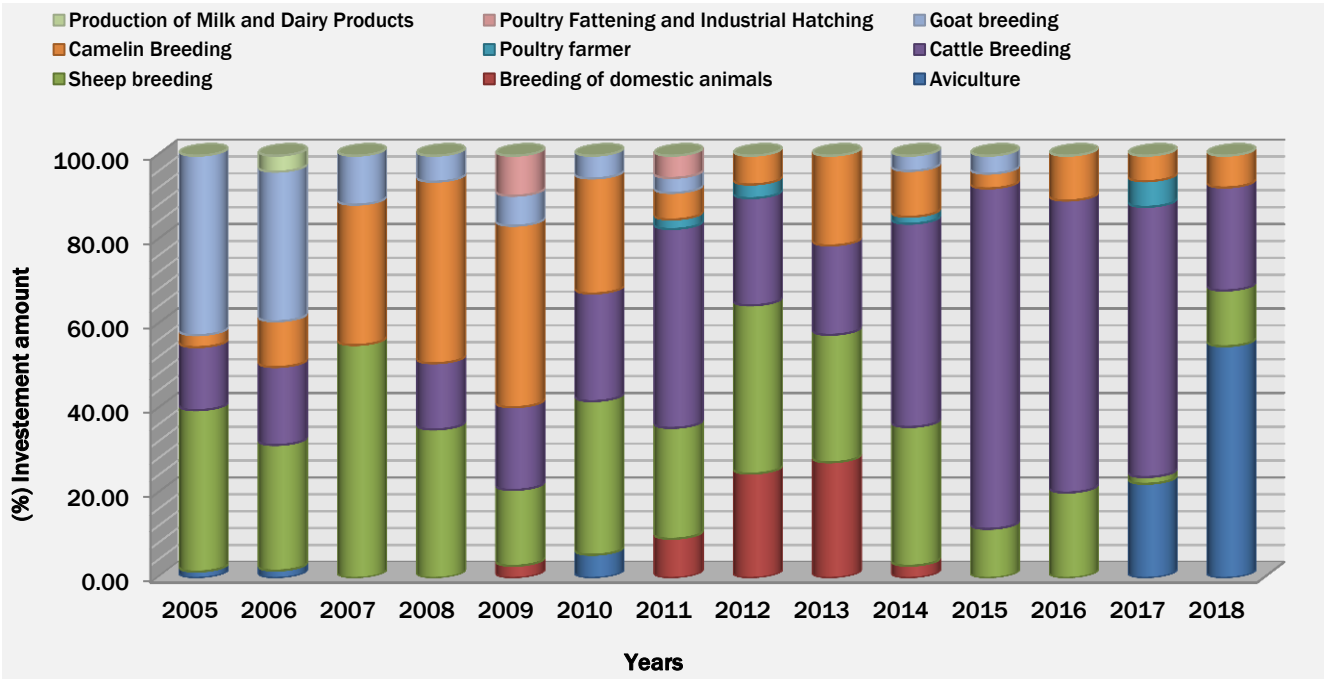

Figure 5 - Amount of investment for different activities

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According to our study, the major investments of the exploitations in equipment linked to the attainment of battery farming (Hens, Rabbits) with 58 new units, equipment for slaughter units, and equipment for heifer nurseries with 09 units. These three types represented more than 90% of the investment and financial support quantity (Figure 5). Private investment incorporates a great vehicle of innovation, economic development, and paucity diminution (Musvoto et al., 2015; Tomich et al., 2019).

Indeed, 41% of investors' states that they enhanced their income due to investment, and 59% of the investors are implicated in future investment projects. Even though there are numerous investment practices, the most frequent technique is to boost the amount of land under cultivation, particularly forage (alfalfa) and orchards, and to boost livestock husbandry or increase the number of heads. Bensaha et al. (2020) noted that the importance of livestock mortality hazard management for breeders is enlarged by the obvious presence of low income in Algeria, characterized by multiple herd size balances such that losses push a breeder below a critical threshold.

Development of associative movement and scientific research

As part of training in the field of animal husbandry, the region has a number of training activities (Table 2). These associations will aim to rehabilitate the niche for better production and better support and moralization of farmers who have specialized in the agri-food industry. According to MADR (2018), these types of associations are for the maintenance of agriculture, and they present a form of a special partnership that is established between an agricultural producer and a group of consumers.

Table 2 - Training activities in the agricultural and agri-food sectors.

	Distribution across the 13 municipalities of the region
Agricultural associations	38
Friends of nature and animal protection club.	02
Agricultural Vocational Training School	01

This communication device includes multimedia information campaigns on agricultural development programs which will be carried out under the aegis of the National Agricultural Extension Institute (MADR, 2018). This system also includes, in terms of information and communication, multimedia information campaigns on agricultural development programs to be carried out under the aegis of the National Agricultural Extension Institute (Bensaha and Arbouche, 2015).

The number of farmers who gain from training and expansion is still not sufficient. It does not go beyond 10% of the objective for training or a collective of 111 farmers, 42% in crop production, and 58% in animal production. Serge and Jacqueline (2009) specify that the associations and the state have thus formed a decisive couple. Associations have often played pioneering roles in detecting new needs of producer farmers and transferring know-how. They are dedicated mainly to technical vocational training. These training courses are based on a network of partner farms in order to be as close as possible to the needs of farmers. According to DSA (2018), this helps farmers to organize themselves into associations, cooperatives, and unions in order to influence decisions during market negotiations and also to fully integrate into the activities of the date sector taken as a whole.

However, our study shows that the objectives of these agricultural training activities through associations, clubs, and schools have yet to prove to be satisfactory. This is due to the negligence of young farmers by their low levels of education. As well as these activities need to be endowed with appropriate logistics. According to Tebani et al. (2019), this meager result is justified by the big number of farmers in old age, uneducated and indifferent to agricultural technology, such as the restricted experience of expansion workers, the lack of means of travel, and of partnership between local authorities.

In addition, it should be noted that there is no effective involvement of actors and partners to take into account the issues of farms in their specificity, as well as the absence of articulation and a global vision Research / Training / Agricultural Council, which induces a low rate of adoption and dissemination of knowledge in terms of improved technologies. The main interventions to be developed will focus on the reorganization of the research system as well as the strengthening of its human, logistical, and financial capacities (Bensaha et al., 2013).

Finally, the current professional organizations of breeders should be restructured and strengthened for greater efficiency in the supervision and promotion of the sector. In addition, the poultry sector (family and semi-industrial) is likely to experience faster development through financial support actions for investments and training and supervision of breeders (Bensaha et al., 2020).

CONCLUSION AND POLICY IMPLICATIONS

Through its subsidy program, the State intends to encourage animal husbandry and the intensification of production by modernizing the livestock systems. The significant contribution of the state at exploitations investment for the putting in place of heifer nurseries encourages farmers to put into practice breeding techniques. Nevertheless, the prospects for

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extending this existing policy and the revival of the agricultural sector remain possible, but for the objectives to be realized on the ground, it will be important to:

• Determine the practical objectives along with the real latent of each region

• Jointly address the economic and social justified through realistic projects

• Repair the administration responsible for the implementation of the programs and supply it with suitable means, defining the responsibility of each element responsible for the job awaiting it.

• Reform the actors implicated in rural development with a view to improving the sector organization and the real participation of farmers in their own projects.

Promoting the technical skills of technicians for program assessment and training and raising farmers' attentiveness

The positive impact of public investment suggests that Algeria should now devote more comprehensively to its own agricultural infrastructure, mainly to efficient livestock building management technologies. To sum up, the animal farm remains the backbone of farming in the study region and continues to supply significant economic and social functions. Moreover, animal farms play a significant role in maintaining the rural population and developing dryland areas. However, animal farm development policies should be modified considering the heterogeneity of agriculture, such as socio-economic factors. Yet, subsidies and farming strengthening have proven significant due to the non-continuity of action in time and space. Policymakers should support such investments in the agricultural sector through by implementing well-targeted public-private partnerships that fund structural projects.

DECLARATIONS

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Authors' contribution
From my point of view, it is consistent and concise work.

Conflict of interests
The authors have not declared any conflict of interest.

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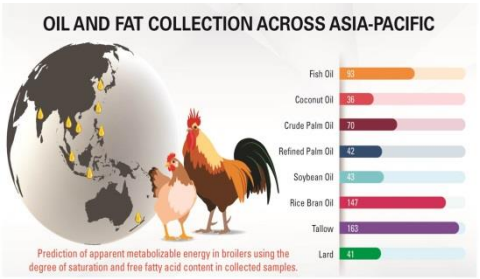

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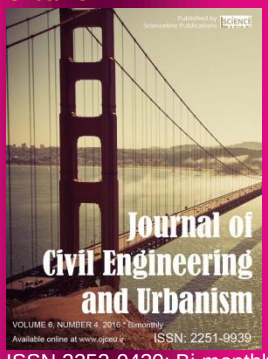

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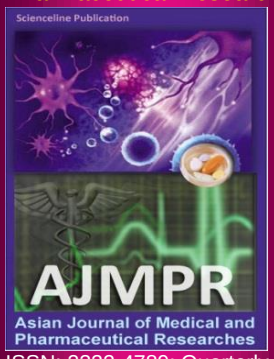

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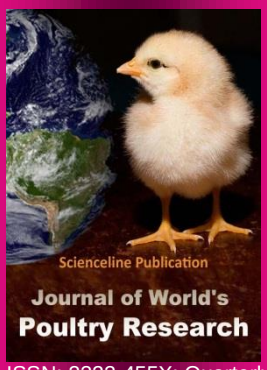

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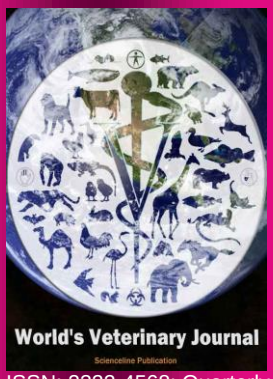

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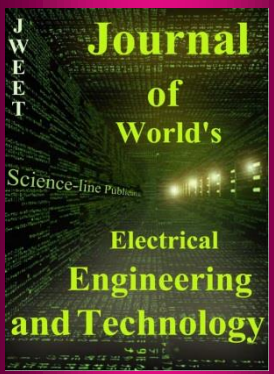

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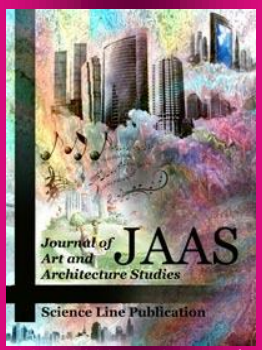

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