BOOKLET

Volume 14, Issue 3, May 2024

Online J. Anim. Feed Res., 14 (3): 165-217; May 25, 2024

Editorial Team

Advisory Board

Join OJAFR Team

As an international journal we are always striving to add diversity to our editorial board and operations staff. Applicants who have previous experience relevant to the position may be considered for more senior positions (Section Editor, SE) within OJAFR. All other members must begin as Deputy Section Editors (DSE) before progressing on to more senior roles. Editor and editorial board members do not receive any remuneration. These positions are voluntary.

Download OJAFR Application Form

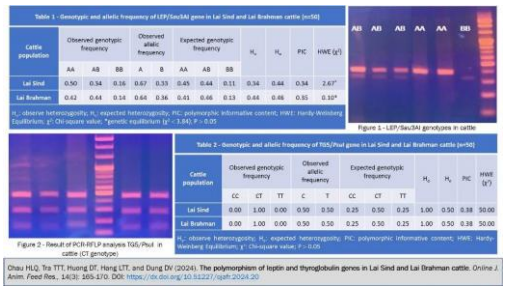
crossref metadata search

Volume 14 (3); May 25, 2024

Research Paper

The polymorphism of leptin and thyroglobulin genes in Lai Sind and Lai Brahman cattle

Chau HLQ, Tra TTT, Huong DT, Hang LTT, Dung DV.
Online J. Anim. Feed Res., 14(3): 165-170, 2024; pii: S222877012400020-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.20

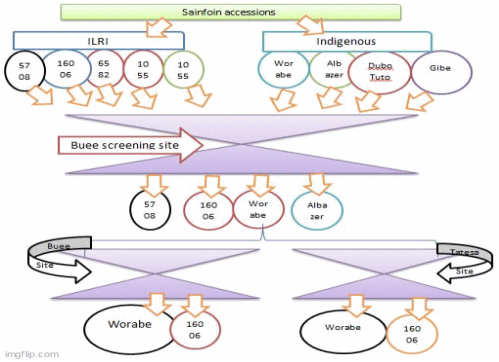
Abstract

The aim of this study was to investigate the single nucleotide polymorphisms (SNPs) present in intron 2 region of leptin (LEP) and 5' untranslated region of thyroglobulin (TG5) genes in Lai Sind and Lai Brahman cattle populations raised in the Central Vietnam. For each cattle group, fifty hair root samples were collected and extracted genomic DNA. The LEP/*Sau*3AI and TG5/*Psu*I gene polymorphisms were analyzed using PCR-RFLP technique. The results showed that the SNPs of LEP/*Sau*3AI and TG5/*Psu*I were found in the both cattle groups. Three LEP/*Sau*3AI genotypes were detected, including LEP^{AA}, LEP^{AB} and LEP^{BB}. All of investigated cattle carried $TG5^{CT}$ genotype. The Hardy-Weinberg equilibrium was reached in the both cattle populations for LEP/*Sau*3AI, but not for TG5/*Psu*I. The polymorphisms at these two loci were moderate in the both cattle populations. It can be concluded that the SNPs LEP/*Sau*3AI and TG5/*Psu*I can be used as genetic markers for molecular selection in these cattle groups. A selection program is needed to increase the frequency of $TG5^{T}$ allele in Lai Sind and Lai Brahman cattle groups to improve beef marbling score.
Keywords: Cattle, Lai Brahman, Lai Sind, Leptin gene, Polymorphism, Thyroglobulin gene.

[Full text-PDF]

Research Paper

Nutritional and chemical composition of sainfoin (*Onobrychis viciifolia*) accessions in mid-altitude of Soddo and Abeshgie Woredas of Ethiopia

Abebe DG, Cherkos SD, Ejeta TT, Dejene M, Shignato TK and Geletu AS.
Online J. Anim. Feed Res., 14(3): 171-184, 2024; pii: S222877012400021-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.21

Abstract


Sainfoin (*Onobrychis viciifolia*) is a perennial herbaceous leguminous forage crop with a high content of crude protein, palatability, nutritive value and non-bloating. It can be offered in the form of green forage, grazing pasture, hay, or silage. To mitigate the dry season feed shortage, the agronomic performance and chemical composition of sainfoin (*Onobrychis viciifolia*) accessions were studied in the mid-altitude of Soddo and Abeshgie Woredas of Garage Zone of Ethiopia. For the screening, five International Livestock Research Institute (ILRI) accessions (No 5708, 16006, 6582, 10558, and 10556) and four wild sainfoin accessions were collected from Worabe, Albazer, Meskan Dubo Tuto and Gibie river basin with morphological variation. They were subjected to initial screening followed by field trials for a period of fifteen months per session. Among the twenty-nine agronomic parameters (for screening purpose), based on the dendrogram results, fifteen data points were used for location wise (Buee and Tatesa) evaluation purposes. The results indicated that, with the exception leaf length, leaf width, and annual seed yield, all parameters were affected by the accession by location interaction. The highest ($P<0.001$) cumulative dry matter, seed yield, and crude protein (CP%) contents were recorded for Worabe sainfoin, ILRI 5708 and ILRI 16006, respectively. Lower ($P<0.05$) condensed tannin (CT) was recorded in ILRI 5708, Worabe's and Albazer's sainfoin without any significant difference. During the first 24-hour incubation period, the highest and least ($P<0.001$) gas were produced from ILRI 16006 and Albazer, and ILRI 5708, respectively. The highest and least ($P<0.001$) methane gas was recorded from ILRI 16006 and ILRI 5708 respectively. The results indicated that Worabe sainfoin was superior to the result in all evaluated parameters. So, Worabe sainfoin hay mixture with crop residues can be used to enhance the nutritional value of crop residue-based poor feed resources.
Keywords: Agronomic performance, Alternative feedstuffs, Crude protein, Dry matter yield, Sainfoin accessions.

[Full text-PDF]

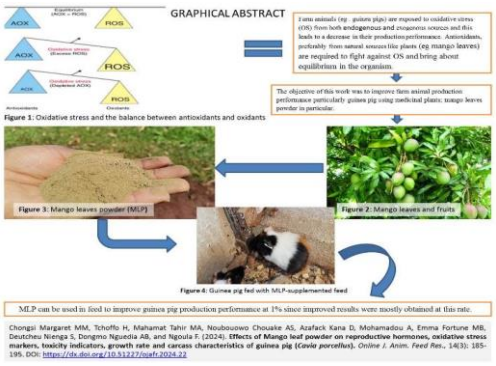
Research Paper

Effects of Mango leaf powder on reproductive hormones, oxidative stress markers, toxicity indicators, growth rate and carcass characteristics of guinea pig (*Cavia porcellus*)

Mary Momo CM, Hervé T, Markhous Adam MT, Arthur Stella NC, Dorice AK, Adamou M, Fortune Magloire BE, Sorelle DN, Arius Baulland DN, and Ferdinand N.

Online J. Anim. Feed Res., 14(3): 185-195, 2024; pii: S222877012400022-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.22

Abstract
The present work aimed to evaluate the effects of *Mangifera indica* leaf powder (MLP) on reproductive hormones, biomarkers of oxidative stress and toxicity, growth and carcass characteristics of female *Cavia porcellus*. 40 female guinea pigs (*Cavia porcellus* L.) aged 2 months with an average weight of 257.65±11.28 g were used. These guinea pigs were weighed, then randomly divided into 4 groups of 10 animals each and subjected to the following rations: T0 (basic diet), T1, T2 and T3 (basic diet + 0.50; 0.75 and 1% MLP, respectively). For 45 days, the guinea pigs of all groups received *ad libitum* drinking water and the corresponding experimental rations. Reproductive hormones analyses showed a significant (P<0.05) rise in serum progesterone concentration in the highest dose of MLP-treated guinea pigs. Malondialdehyde level was lowest in guinea pigs given feed with the highest dose of MLP while superoxide dismutase and catalase activities were maximum in animals that received the highest doses of MLP. Total Peroxidase activity was greatest in animals fed with the higher MLP dose. The lowest level of cholesterol was noticed in the group that received the greatest dose of MLP. Feed consumption was higher in guinea pigs receiving 0.75% and 1% MLP. The body weight gain and average daily gain had higher values in the subjects fed with MLP feed than those of the control group. Feed efficiency values were improved in animals that that were given 0.5% and 1% MLP with regards to those fed without MLP, the length of small intestine was higher (P<0.05) in 0.75% MLP-treated animals than the other groups, while the greatest value for the density of big intestine was recorded in those that received 0.25% MLP. Based on these values (1% of diet as optimum level), MLP can be used in feed to improve animal production performance.
Keywords: Antioxidant, Average weight, *Mangifera indica*, Oxidative stress, Reproductive hormones.



[Full text-PDF]

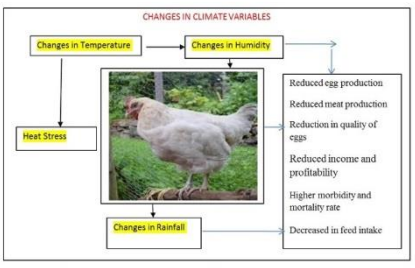
Research Paper

Effect of climate variables on poultry production efficacy in Nigeria

Osuji E, Ahamefule B, Ben-Chendo G, Osuji M, Nwose R, Eleazar A, Opaluwa H, Ukoha I, Nwaiwu I, Ajibade Y, Opeyemi G, Ibeagwa O, Tim-Ashama A, Offor E, Nwachukwu E, Anyanwu U, Anyiam K, and Iwezor-Magnus D.

Online J. Anim. Feed Res., 14(3): 196-203, 2024; pii: S222877012400023-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.23

Abstract
The paper examined the effect of climate variables on poultry production efficacy in Nigeria with emphasis on broilers and layers. Multi-stage sampling was used to select 401 poultry farmers who provided useful information with the aid of a questionnaire. Data were analyzed using descriptive statistics, and logit regression model. Result shows that free range accounted for 22.7%, battery cage 11.2%, deep litter 54.6%, and semi-free range 11.5% were notable systems of poultry rearing in the state. Heat stress 75.1%, reduced egg 99.8% and meat production 88.5%, and reduction in quality of eggs 51.9% were some of the climate change effects on poultry production. Adaptation strategies of poultry farmers include; proper housing system 100%, proper feed formulation 99.8%, right stocking density 96.8% and adequate water and feed supply 82.8%. Age, education, off-farm activities, size of poultry pen and poultry farming experience were important adaptation determinants of poultry farmers to climate change. High price of feed 100%, lack of access to credit services 77.1%, disease outbreak and parasites 100% and high cost of poultry inputs 99.5% constrained poultry farming. The study concludes that climate variable affects poultry farming. Farmers were recommended to practice climate smart poultry production to mitigate adverse effects.
Keywords: Climate Change, Farm Efficacy, Poultry Farmers, Poultry Production



[Full text-PDF]

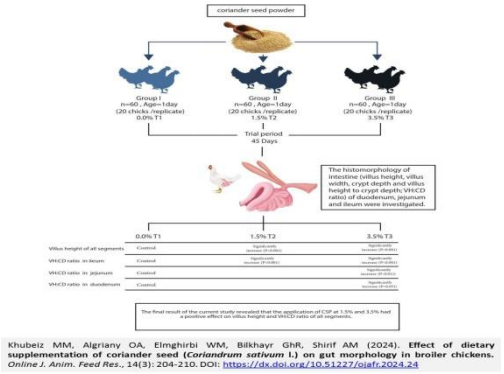
Research Paper

Effect of dietary supplementation of coriander seed (*Coriandrum sativum* l.) On gut morphology in broiler chickens

Khubeiz MM, Algriany OA, Elmghirbi WM, Bilkhayr GhR, Shirif AM.

Online J. Anim. Feed Res., 14(3): 204-210, 2024; pii: S222877012400024-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.24

Abstract
Several herbal plants have demonstrated remarkable efficacy in modulating the morphology of the gut, leading to improved nutrients absorption, enhanced growth, and reduced susceptibility to diseases. Aim of this study was to investigate the effects of different levels of coriander seed powder (CSP; 0% as T1, 1.5% as T2 and 3.5% as T3) in modulating of broiler chickens gut morphology. A total of 180 one-day-old broiler chicks were randomly allocated into 3 treatment groups 60 chick/group in 3 replicates. The trial was designed according to a completely randomized design for 45 days. The histomorphology of duodenum, jejunum and ileum [villus height (VH), villus width, crypt depth (CD) and villus height to crypt depth; VH:CD ratio] were investigated. The result showed a significant increase (P<0.01) of villus height in two treatments (1.5% and 3.5%) as compared to control in all segments. Moreover, the significant increase (P<0.01) on VH:CD ratio is observed between treatments as compared to control in ileum and dietary use of 3.5% CSP had significant effects on the jejunum and duodenum as compared to the control group. In conclusion, the application of CSP at 1.5% and 3.5% had a positive effect on villus height and VH:CD ratio of all segments. However, further research is required to understand the precise mechanisms underlying medicine plant effects as well as what the ideal percentage for each plant will be added for a best positive effect on performance.
Keywords: Broiler chicken, Coriander Sativum, Gastrointestinal functions, Gut Morphology Herbal additives.



Khubeiz MM, Algriany OA, Elmghirbi WM, Bilkhayr GhR, Shirif AM (2024). **Effect of dietary supplementation of coriander seed (*Coriandrum sativum* l.) on gut morphology in broiler chickens.** *Online J. Anim. Feed Res.*, 14(3): 204-210. DOI: https://dx.doi.org/10.51227/ojafr.2024.24

[Full text-PDF]

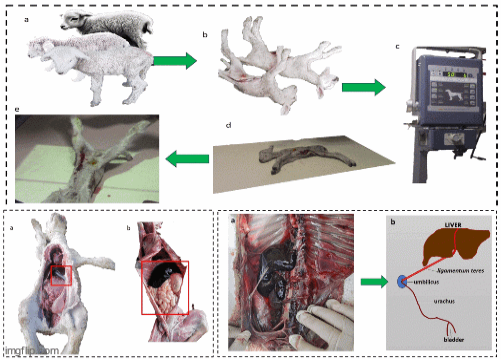
Case Report

Urachus anomaly in sheep: Incidence and considerations presented in neonatal lambs in the Peru

Carhuas JN, Napoleon MS, Villar FA, Garcia-Olarte E, Eulogio CQ, Mauricio-Ramos Y, and Payano IU.

Online J. Anim. Feed Res., 14(3): 211-217, 2024; pii: S222877012400025-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.25

Abstract
Over the years, an anomaly has been observed in newborn lambs, manifesting itself as a curvature in their posture and a slight dampness in the navel, known by the locals as "pupote". For this reason, the present study was carried out to document and present for the first time to the scientific community an anomaly of the urachus in sheep and its incidence. In the sampling process, five lambs who died of starvation were selected. The specimens were transported to the Animal Health Laboratory of the Faculty of Zootechnics of the National University of Central Peru. Radiographic analyses were carried out there. Then the incidence was found in the collected records. The incidence of the anomaly is an average of 0.825±0.09%. This case highlights the persistence of urachus in newborn lambs, evidenced by the identification of a ligament that establishes a connection between the umbilicus and the liver. Specifically, the ligament identified in the described anomalies corresponds to the *ligamentum teres*.
Keywords: Congenital disease, Ligament of the liver, Pupote, Sheep breeding, Urachus.



[Full text-PDF]

Archive

Online Journal of Animal and Feed Research

ISSN: 2228-7701

Frequency: Bimonthly

Current Issue: 2024, Vol: 14, No: 3 (May 25)

DOI Prefix: 10.51227

Publisher: SCIENCELINE

Online Journal of Animal and Feed Research is an international peer-reviewed journal, publishes the full text of original scientific researches, reviews, and case reports in all fields of animal and feed sciences, bimonthly and freely on the internet ...view full aims and scope

www.ojafr.ir and www.ojafr.com

» OJAFR indexed/covered by Scopus, AGRIS, EBSCO, Ulrich's™, HINARI, NSD, AKSTEM, BASE, ZDB, ICV, EZB ...details

Journal metrics: h5-index=9; h5-median=12

» Full texts and XML articles are available in Crossref and AGRIS.

» Digital Archiving: Journal Repository (eprints)

» This journal is in full compliance with BOAI and ICMJE's Recommendations.

» High visibility of articles over the internet.

» Publication Ethics and Policies ...details

» High visibility of articles over the internet through Gold Open Access.

» Publisher Item Identifier ...details

» This journal encourage the academic institutions in low-income countries to publish high quality scientific results, free of charges... Peer Review Process

Scienceline Publication, Ltd.
Ömer Nasuhi Bilmen Road, Dönmez Apart., G Block, No:1/6, Yakutiye, Erzurum/25100, TURKEY
Phone: +90 538 770 8824 (TURKEY)
Homepage: www.science-line.com
Emails: administrator@science-line.com ; saeid.azar@atauni.edu.tr

2024 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 14, Issue 3: 165-170; May 25, 2024

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2024.20

THE POLYMORPHISM OF LEPTIN AND THYROGLOBULIN GENES IN LAI SIND AND LAI BRAHMAN CATTLE

Ho Le Quynh CHAU✉, Than Thi Thanh TRA, Duong Thi HUONG, Le Thi Thu HANG, and Dinh Van DUNG

College of Agriculture and Forestry, Hue University, 102 Phung Hung St., Hue city, Vietnam

✉Email: hochauhuaf@hueuni.edu.vn

Supporting Information

RESEARCH ARTICLE

PII: S2228770124000020-14

Received: April 10, 2024

Revised: May 15, 2024

Accepted: May 16, 2024

ABSTRACT: The aim of this study was to investigate the single nucleotide polymorphisms (SNPs) present in intron 2 region of leptin (LEP) and 5' untranslated region of thyroglobulin (TG5) genes in Lai Sind and Lai Brahman cattle populations raised in the Central Vietnam. For each cattle group, fifty hair root samples were collected and extracted genomic DNA. The LEP/*Sau*3AI and TG5/*Psu*I gene polymorphisms were analyzed using PCR-RFLP technique. The results showed that the SNPs of LEP/*Sau*3AI and TG5/*Psu*I were found in the both cattle groups. Three LEP/*Sau*3AI genotypes were detected, including LEP^{AA}, LEP^{AB} and LEP^{BB}. All of the investigated cattle carried $TG5^{CT}$ genotype. The Hardy-Weinberg equilibrium was reached in the both cattle populations for LEP/*Sau*3AI, but not for TG5/*Psu*I. The polymorphisms at these two loci were moderate in the both cattle populations. It can be concluded that the SNPs LEP/*Sau*3AI and TG5/*Psu*I can be used as genetic markers for molecular selection in these cattle groups. A selection program is needed to increase the frequency of $TG5^{T}$ allele in Lai Sind and Lai Brahman cattle groups to improve beef marbling score.

Keywords: Cattle, Lai Brahman, Lai Sind, Leptin gene, Polymorphism, Thyroglobulin gene.

INTRODUCTION

Beef has been the third most consumed meat worldwide after poultry and pork. The beef consumption per person in Vietnam amounted to about 8.73 kilograms in 2023, and was forecast to increase to 9.6 kilograms per capita annually (Statista Research Department, 2023). The Vietnamese yellow cattle is an indigenous breed well adapted to tropical condition and poor nutrition. However, the disadvantages of this cattle breed were small size, low carcass and milk yield. In Vietnam, from the 1960s to 1970s, with the implementation of the program of "Red-Sindhization" and afterward of "Zebuization", the ratio of crossbred cattle was increased. Today, the two cattle groups of Lai Sind (a crossbred breed between Red Sindhi bulls and local cows) and Lai Brahman (a crossbred breed between Brahman bulls and local cows) have been used as dam lines for crossing with beef cattle to improve growth performance and carcass yield in many provinces in Vietnam (Bang et al., 2022).

Breeding values predicted by Best Linear Unbiased Prediction for selection economic indices have become widely applied in animal breeding over the last few decades. The discovery of genetic markers related to the phenotypic expression of particular traits -has raised hopes for successful animal breeding based on candidate genes. Advancements in genotyping technologies for single nucleotide polymorphisms (SNPs) at different loci in the genome to estimate the breeding values are the strong basis for marker-assisted selection (Binh, 2019). Most of the economically important traits in cattle are quantitative traits and environmental factors (Getaneh and Alemayehu, 2022). Until now, many scholars have identified great quantities of candidate genes associated with economically important traits in cattle, such as LEP, TG5, CAST-T1, Calpain 316-T2, Calpain 4751-T3 (De Carvalho et al., 2012; Sedykh et al., 2016; Coria et al., 2018).

The leptin (LEP) gene is located on chromosome 4 in cattle (Pomp et al., 1997). It consists of three exons separated by two introns. The exon 1 and four nucleotides of exon 2 are not translated. The remaining part of exons 2 and 3 are translated into the functional 16kDa leptin protein of 146 amino acids (Haruna et al., 2020). Leptin hormone is produced by adipose tissue and the small intestine, plays an important role in lipid accumulation, and regulates the energy balance by suppressing hunger, resulting in the reduction of fat in adipocytes (Al-hussaniy et al., 2021). Many study results also found leptin roles in regulating body mass, reproductive and immune functions (Santos-Alvarez et al., 1999; Kadokawa et al., 2000; Block et al., 2001).

The SNP LEP/Sau3AI (g.1926C>T) in intron 2 region was changed the amino acid at position 2059 of the chain protein from arginine to cysteine (Moravčíková et al., 2012; Trakovická et al., 2013; Putra et al., 2019). This SNP has been used as molecular marker for selection many economically traits in cattle, such as milk yield (Moravčíková et al., 2012; Trakovická et al., 2013), reproductive performance (Moussavi et al., 2006; Öner et al., 2017; Ferchichi et al., 2018), body

Citation: Chau HLQ, Tra TTT, Huong DT, Hang LTT, and Dung DV (2024). The polymorphism of leptin and thyroglobulin genes in Lai Sind and Lai Brahman cattle. *Online J. Anim. Feed Res.*, 14(3): 165-170. DOI: https://dx.doi.org/10.51227/ojafr.2024.20

weight (Almeida et al., 2003; Nobari et al., 2010; Hussain et al., 2017), fat percentage (Sedykh et al., 2016) and feed intake (Liefers et al., 2002).

Thyroglobulin is a glycoprotein hormone synthesized in thyroid follicular cells. It is a precursor of triiodothyronine (T3) and thyroxine (T4) hormones, playing an important role in metabolic regulation and can influence adipocyte development, differentiation and adipose tissue homeostasis (Ailhaud et al., 1992; Casas et al., 2005). Also, Mears et al. (2001) demonstrated that both T3 and T4 are involved in fat marbling in Japanese black cattle. Findings of Barendse et al. (2004) indicated that the SNP TG5/*Psu*I (g.422C>T) had been associated with marbling in cattle. The SNP TG5/*Psu*I has been used for genetic markers for selection marbling index (Barendse, 1999; Barendse et al., 2004; Casas et al., 2005), fat percentage (Sedykh et al., 2016) and backfat thickness (Gan et al., 2008; Mears et al., 2001; Moore et al., 2003) in some beef cattle breeds.

The aim of this study was to identify the LEP/*Sau*3AI and TG5/*Psu*I gene polymorphism of Lai Sind and Lai Brahman cattle. The study result could aid in the marker-assisted selection program to improve productivity and beef quality.

MATERIALS AND METHODS

Sample collection and genomic DNA extraction

A total of 100 hair follicle samples was collected from Lai Sind and Lai Brahman cattle (n=50) raised in Quang Ngai province, Vietnam (Latitude: 14°32'-15°25' North, Longitude: 108°06'- 109°04' East). The samples were kept separately in a plastic bag and transported to the Laboratory of Molecular Biology, College of Agriculture and Forestry, Hue University. For increasing DNA extraction efficiency, the hair follicles were homogenized in the lysis buffer within 5 minutes using Bullet blender (Next Advance, USA). The further steps were followed the kit manufacturer's instruction. Nanodrop system (Thermo Scientific, USA) was used for DNA quantity and quality measurement. The research protocol was approved by the Scientific Committee of Hue University dated 30th September 2021, Decision No: 1472/QĐ-DHH.

Leptin and Thyroglobulin gene polymorphism analysis

The intron 2 fragment of LEP gene and the TG5 gene were amplified by polymerase chain reaction (PCR) technique. Following specific primers described by Barendse (1999) and Liefers et al. (2022) were used in this study, including: LEPF: 5'-TGGAGTGGCTTGTTATTTTCTTCT-3'; LEPR: 5'-GTCCCCGCTTCTGGCTACCTAACT-3'; TG5F: 5'-GGGGATGACTACGAGTATGACTG-3'; TG5R: 5'-GTGAAAATCTTGTGGAGGCTGTA-3'. The PCR reaction was performed in PCR thermal cycler (Axygen® MaxyGene™) in a total volume of 20µL containing 50ng genomic DNA, 1.25µM of each primer, 200µM dNTP, 1× PCR buffer, and 0.75 unit Taq polymerase (Solgent, Korea). The optimized cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 40 sec, 40 sec for primer annealing at 62°C (for LEP gene) or 55°C (for TG5 gene), extension at 72°C for 40 sec, and a final extension at 72°C of 7 min (for LEP gene) or 10 min (for TG5 gene). About 8µL of PCR products were electrophoresed on a 2.0% agarose gel with 6× GelRed (ABT, Vietnam) and then was analyzed using Gel DocTM XR+ (Bio Rad, USA). The LEP or TG5 gene amplification was restricted by *Sau*3AI or *Psu*I endonucleases, respectively. The restriction reaction mixture of 12 µL containing 10 µL of PCR product, 1x buffer and 3 units of restriction enzyme was digested at 37°C overnight. The number and lengths of the restriction fragments were determined by 2%-agarose gel electrophoresis, 6× GelRed staining and UV-visualization, then analyzed by gel-documentation.

Statistical analysis

Genotypic frequency, allele frequency, expected heterozygosity (He), observed heterozygote (Ho) were calculated based on Nei and Kumar (2000). Polymorphic informative content (PIC) was calculated as Roychoudhury and Nei (1988). Chi-squared (χ^2) was used to test for Hardy-Weinberg equilibrium.

RESULTS AND DISCUSSION

Polymorphism of LEP/*Sau*3AI gene in cattle

The 422bp fragment of LEP gene was amplified from genomic DNA of the two cattle populations and digested with restriction enzyme *Sau*3AI. Three genotypes of LEP/*Sau*3AI gene were detected in Lai Sind and Lai Brahman cattle, included LEP^{AA} (390 and 32 bp), LEP^{AB} (390, 303, 88 and 32 bp), and LEP^{BB} (303, 88 and 32 bp) (Figure 1). The observed allelic frequencies of LEP^{A} ranged from 0.64 to 0.67, higher than those of LEP^{B} allele (0.33-0.36) (Table 1). This result agrees with the previous reports in various cattle populations (Sharifzadeh and Doosti, 2010; Jecminkova et al., 2016; Hussain et al., 2017). In the Lai Sind cattle population, the observed frequency of LEP^{AA} genotype was highest, followed by LEP^{AB} and LEP^{BB} genotypes. Meanwhile, the observed genotypic frequencies of LEP^{AA} and LEP^{AB} were similar in Lai Brahman cattle group. Only 7/50 investigated Lai Brahman cattle had LEP^{BB} genotype. Some study results on LEP gene polymorphisms in other cattle breeds also indicated that LEP^{AA} and LEP^{AB} were two main genotypes (Nassiry et al., 2008;

Hussain et al., 2017). According to Trakovická et al. (2013), the SNP LEP/*Sau*3AI had a significant impact on milk yield and first calving age. The highest milk yield, protein and fat content in milk and the lowest age at first calving associated with LEP^{AA} genotype cows. The authors also indicated that the highest age at first calving was found in the LEP^{AB} genotype cattle (Trakovická et al., 2013), but the lowest milk production was observed in the LEP^{BB} genotype cows. However, the cattle carrying LEP^{BB} genotype had superior growth ability (Yang et al., 2007).

The expected genotypic frequencies of three genotypes in the both cattle populations were also calculated based on the Hardy-Weinberg formulas. The expected genotypic frequencies of LEP^{AA} and LEP^{BB} were similar in Lai Sind cattle group (Table 1). Meanwhile, the expected genotypic frequency of LEP^{AB} was higher than those of LEP^{AA} and LEP^{BB}. The expected frequency for LEP^{BB} genotype was lowest in the both investigated cattle populations. The calculated Chi-squared test values in Table 1 indicated that LEP/*Sau*3AI gene polymorphism in the both cattle populations were in Hardy-Weinberg equilibrium ($\chi^2<3.84$). The polymorphism of the LEP/*Sau*3AI gene was moderate (0.25<PIC<0.50) in the both cattle populations. Therefore, LEP/*Sau*3AI polymorphism can be used as molecular selection in these cattle groups.

Polymorphism of TG5/*Psu*I gene in cattle

The genotypes of TG5/*PsuI* gene consisted of $TG5^{CC}$ (295, 178 and 72 bp), $TG5^{CT}$ (473, 295, 178 and 72 bp), and $TG5^{TT}$ (473 and 72 bp; Anwar et al., 2017). All surveyed cattle carried $TG5^{CT}$ genotype (Table 2). Therefore, the allelic frequencies of $TG5^{C}$ and $TG5^{T}$ were equal. The representative result of PCR-RFLP analysis TG5/*Psu*I was shown in Figure 2. On the basis of the Hardy-Weinberg equation, the expected frequency of $TG5^{CT}$ genotype in both crossbred cattle groups were 0.5. The expected heterozygosity coefficient (H_e) was lower than the observed heterozygosity coefficient (H_o) in the both cattle groups. The χ^2 value in Table 2 indicated that Hardy-Weinberg equilibrium was not reached in these two cattle populations for this investigated locus ($\chi^2 > 3.84$). The PIC value of the TG5/*Psu*I gene in both cattle groups was 0.38, indicating that the level polymorphism in genetic marker was moderate. Therefore, TG5/*Psu*I gene polymorphism can be used as molecular selection to improve the productivity and meat quality in Lai Sind and Lai Brahman cattle.

In Hereford and Limousine breeds, Sedykh et al. (2016) indicated that the cattle had significant potential for increased beef taste and nutritional qualities associated with a high proportion of desirable $TG5^{TT}$ genotype. Also, Casas et al. (2005) reported the increase in fat yield in cattle carried $TG5^{TT}$ genotype. Meanwhile, some authors reported that $TG5^{CC}$ genotype cattle had tendency increase pre-slaughter live weight, hot carcass yield, carcass output, dressing weight and slaughter yield (De Carvalho et al., 2012; Sedykh et al., 2016).

Dolmatova et al. (2020) indicated a clear tendency of an effect of the TG5 genotype on milk productivity in dairy cattle. Cows with the $TG5^{TT}$ genotype had the highest milk yield and fat content in milk (Dolmatova et al., 2020), and had significantly higher lipid content in the loin muscle (Thaller et al., 2003) than the $TG5^{CT}$ or $TG5^{CC}$ genotypes. The $TG5^{TT}$ genotype was the only genotype that showed differences in the distribution of marbling, increasing marbling in beef (Burrell et al., 2004).

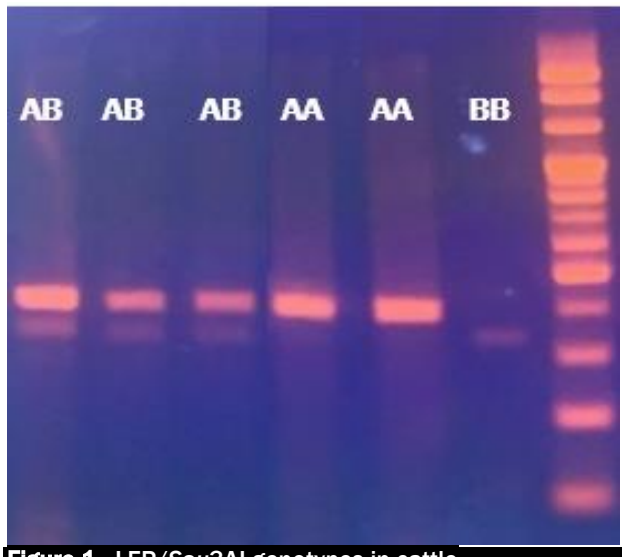


Figure 1 - LEP/*Sau*3AI genotypes in cattle

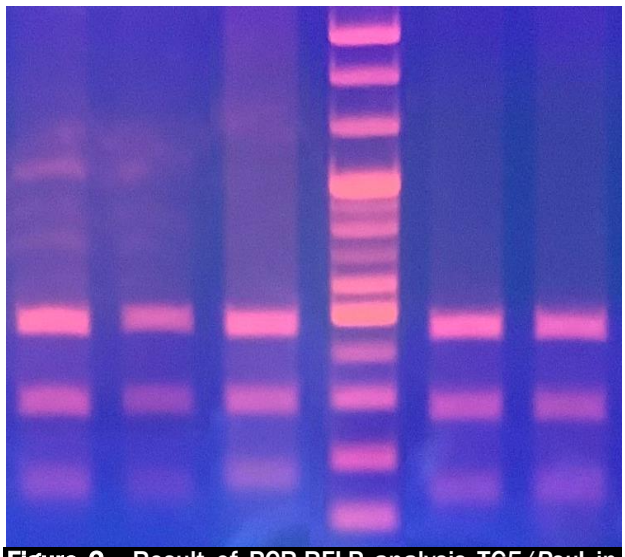


Figure 2 - Result of PCR-RFLP analysis TG5/*Psu*I in cattle

Table 1 - Genotypic and allelic frequency of LEP/*Sau*3AI gene in Lai Sind and Lai Brahman cattle (n=50)

Cattle population	Observed genotypic frequency			Observed allelic frequency		Expected genotypic frequency			H_o	H_e	PIC	HWE (χ^2)
	AA	AB	BB	A	B	AA	AB	BB				
Lai Sind	0.50	0.34	0.16	0.67	0.33	0.45	0.44	0.11	0.34	0.44	0.34	2.67*
Lai Brahman	0.42	0.44	0.14	0.64	0.36	0.41	0.46	0.13	0.44	0.46	0.35	0.10*

H_o: observe heterozygosity; H_e: expected heterozygosity; PIC: polymorphic informative content; HWE: Hardy-Weinberg Equilibrium; χ^2: Chi-square value; *genetic equilibrium ($\chi^2 < 3.84$), $P < 0.05$.

Table 2 - Genotypic and allelic frequency of TG5/*Psu*I gene in Lai Sind and Lai Brahman cattle (n=50)

Cattle population	Observed genotypic frequency			Observed allelic frequency		Expected genotypic frequency			H_o	H_e	PIC	HWE (χ^2)
	CC	CT	TT	C	T	CC	CT	TT				
Lai Sind	0	1.00	0	0.50	0.50	0.25	0.50	0.25	1.00	0.50	0.38	50.00
Lai Brahman	0	1.00	0	0.50	0.50	0.25	0.50	0.25	1.00	0.50	0.38	50.00

H_o: observe heterozygosity; H_e: expected heterozygosity; PIC: polymorphic informative content; HWE: Hardy-Weinberg Equilibrium; χ^2: Chi-square value. $P < 0.05$.

CONCLUSION

It can be concluded that the polymorphisms in LEP/*Sau*3AI and TG5/*Psu*I genes were observed in Lai Sind and Lai Brahman cattle populations. The allelic frequency of LEP^A was higher than LEP^B in both cattle populations. All of investigated cattle had $TG5^{CT}$ genotype. The Hardy-Weinberg equilibrium was reached in the both cattle populations for LEP/*Sau*3AI, but not for TG5/*Psu*I. The SNPs LEP/*Sau*3AI and TG5/*Psu*I can be used as candidate genes for molecular selection in these cattle groups. A selection program is needed to increase the frequency of $TG5^T$ allele in Lai Sind and Lai Brahman cattle groups to improve beef marbling score.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Ho Le Quynh Chau; E-mail: hochauhuaf@hueuni.edu.vn; ORCID: https://orcid.org/0009-0000-9930-5776

Authors' contribution

Ho Le Quynh Chau wrote the manuscript, designed the experiment, collected and analyzed data. Duong Thi Huong, Than Thi Thanh Tra and Le Thi Thu Hang collected sample, performed the experiment. Dinh Van Dung designed the experiment, revised the draft of the manuscript. All authors read and approved the final manuscript.

Ethical consideration

The research was approved by the Scientific Committee of Hue University dated 30th September 2021, Decision No: 1472/QĐ-DHH. The experiment was conducted in accordance with ARRIVE guidelines (https://arriveguidelines.org). All methods were performed in accordance with the relevant guidelines and regulations.

Acknowledgements

This work was financially supported by Hue University – level research project, Code DHH2022-02-164. The authors also acknowledge the partial support of Hue University under the Core Research Program, Grant No. NCM. DHH.2018.04.

Competing interests

The authors have not declared any conflict of interests.

REFERENCES

Ailhaud G, Grimaldi P and Négrel R (1992). Cellular and molecular aspects of adipose tissue development. Annual Review of Nutrition, 12: 207-233. https://doi.org/10.1146/annurev.nu.12.070192.001231

Al-hussaniy HA, Alburghaif AH and Naji MA (2021). Leptin hormone and its effectiveness in reproduction, metabolism, immunity, diabetes, hopes and ambitions. Journal of Medicine and Life, 14(5): 600-605. https://doi.org/10.25122%2Fjml-2021-0153

Almeida SEM, Almeida EA, Moraes JFC and Weimer TA (2003). Molecular markers in LEP gene and reproductive performance of beef cattle. Journal of Animal Breeding and Genetics, 120: 106-113. https://doi.org/10.1046/j.1439-0388.2003.00377.x

Anwar S, Putra AC, Wulandari AS, Agung PP, Putra WPB and Said S (2017). Genetic polymorphism analysis of 5' untranslated region of thyroglobulin gene in Bali cattle (*Bos javanicus*) from three different regions of Indonesia. Journal of the Indonesian Tropical Animal Agriculture, 42(3): 175-184. https://doi.org/10.14710/jitaa.42.3.175-184

Bang NN, Hayes BJ, Lyons RE, Randhawa IA, Gaughan JB and McNeill DM (2022). Genomic diversity and breed composition of Vietnamese smallholder dairy cows. Journal of Animal Breeding and Genetics, 139(2): 145-160. https://doi.org/10.1111/jbg.12651

Barendse W, Bunch R, Thomas M, Armitage S, Baud S and Donaldson N (2004). The TG5 thyroglobulin gene test for a marbling quantitative trait loci evaluated in feedlot cattle. Australian Journal of Experimental agriculture, 44(7): 669-674. https://doi.org/10.1071/EA02156

Barendse WJ (1999). Assessing lipid metabolism. International Patent Application WO9923248 US6383751 (PCT/AU98/00882). https://patentimages.storage.googleapis.com/b3/e5/55/16c9b8b49f15ee/US6383751.pdf

Binh DV (2019). Genome selection – a new era of animal breeding. Journal of Animal Husbandry Science and Technics, 241: 2-9. http://116.97.88.11:86/Xem-Online/chon-giong-vat-nuoi-theo-bo-gen-%E2%80%93-ky-nguyen-moi-cua-khoa-hoc-chon-giong-vat-nuoi-958.html

Block SS, Butler WR, Ehrhardt RA, Bell AW, van Amburgh ME and Boisclair YR (2001). Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. Journal of Endocrinology, 171 (2):339–348. https://doi.org/10.1677/joe.0.1710339

Burrell D, Moser G, Hetzel J, Mizoguchi Y, Hirano T, Sugimoto Y and Mengersen K (2004). Meta analysis confirms associations of the TG5 thyroglobulin polymorphism with marbling in beef cattle. Proceedings of the 29th International Conference on Animal Genetics, 117 https://gyoseki1.mind.meiji.ac.jp/mjuhp/KgApp?detlId=28&detlUid=ymimgooiggy&detlSeq=55&Language=2

Casas E, White SN, Riley DG, Smith TP, Brenneman RA, Olson TA, Johnson DD, Coleman SW, Bennett GL and Chase CCJr (2005). Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in *Bos indicus* cattle. Journal of Animal Science, 83(1): 13–19. https://doi.org/10.2527/2005.83113x

Coria MS, Carranza PG and Palma GA (2018). Calpain system in meat tenderization: A molecular approach. Revista Mvz Córdob, 25(1): 6523-6534. http://hdl.handle.net/11336/81758

De Carvalho TD, Siqueira F, de Almeida Torres Júnior RA, de Medeiros SR, Feijó GLD, de Souza Junior MD, Blecha IMZ and Soares CO (2012). Association of polymorphisms in the leptin and thyroglobulin genes with meat quality and carcass traits in beef cattle. Revista Brasileira de Zootecnia, 41(10): 2162-2168. https://doi.org/10.1590/S1516-35982012001000004

Dolmatova I, Sedykh T, Valitov F, Gizatullin R, Khaziev D and Kharlamov A (2020). Effect of the bovine TG5 gene polymorphism on milk- and meat-producing ability. Veterinary World, 13(10): 2046-2052. https://doi.org/10.14202/vetworld.2020.2046-2052

Ferchichi MA, Jemmali B, Amiri S, Gara SB and Rekik B (2018). Effect of leptin genetic polymorphism on lameness prevalence in Tunisian Holstein cows. Archives Animal Breeding, 61: 305-310. https://doi.org/10.5194/aab-61-305-2018

Gan QF, Zhang LP, Li JY, Hou GY, Li HD, Gao X, Ren HY, Chen JB and Xu SZ (2008). Association analysis of thyroglobulin gene variants with carcass and meat quality traits in beef cattle. Journal of Applied Genetics, 49(3): 251-255. https://doi.org/10.1007/BF03195621

Getaneh M and Alemayehu K (2022). Candidate genes associated with economically important traits in dairy goats. Cogent Food & Agriculture, 8(1): 2149131. https://doi.org/10.1080/23311932.2022.2149131

Haruna IL, Hadebe SA, Oladosu OJ, Mahmoud G, Zhou H and Hickford JGH (2020). Identification of novel nucleotide sequence variations in an extended region of the bovine leptin gene (LEP) across a variety of cattle breeds from New Zealand and Nigeria. Archives Animal Breeding, 63(2): 241–248. https://aab.copernicus.org/articles/63/241/2020/aab-63-241-2020.pdf

Hussain DA, Abboud ZH and Abdulameer TA (2017). Genetic structure analysis of leptin gene/*Sau*3AI and its relationship with body weigh in Iraqi and Holstein Frisian cows population (Comparative study). IOSR Journal of Pharmacy and Biological Sciences, 12(3): 10-13. https://doi.org/10.9790/3008-1203041013

Jecminkova K, Kyselova J, Said Ahmed A, Zavadilov L, Matlova V and Majzlik I (2016). Leptin promoter region genotype frequencies and its variability in the Czech Fleckvieh cattle. Scientia Agriculturae Bohemica, 47(2): 54–59. https://doi.org/10.1515/sab-2016-0009

Kadokawa H, Blache D, Yamada Y and Martin GB (2000). Relationships between changes in plasma concentrations of leptin before and after parturition and the timing of first postpartum ovulation in high-producing Holstein dairy cows. Reproduction, Fertility and Development, 12(7-8): 405–411. https://doi.org/10.1071/rd01001

Liefers SC, te Pas MFW, Veerkamp RF and van der Lende T (2002). Associations between Leptin gene polymorphisms and production, live weight, energy balance, feed intake, and fertility in Holstein heifers. Journal of Dairy Science, 85: 1633–1638. https://doi.org/10.3168/jds.S0022-0302(02)74235-5

Mears GJ, Mir PS, Bailey DRC and Jones SDM (2001). Effect of Wagyu genetics on marbling, back fat and circulating hormones in cattle. Canadian Journal of Animal Science, 81: 65–73. https://doi.org/10.4141/A99-128

Moore SS, Li C, Basarab J, Snelling WM, Kneeland J, Murdoch B, Hansen C and Benkel B (2003). Fine mapping of quantitative trait loci and assessment of positional candidate genes for backfat on bovine chromosome 14 in a commercial line of Bos taurus. Journal of Animal Science, 81: 1919-1925. https://doi.org/10.2527/2003.8181919x

Moravčíková N, Trakovická A and Kasarda R (2012). Polymorphism within the intron region of the bovine leptin gene in Slovak Pinzgau cattle. Scientific Papers: Animal Science and Biotechnologies, 45(1): 211-214. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20123253568

Moussavi AH, Ahouei M, Nassiry MR and Javadmanesh A (2006). Association of leptin polymorphism with production, reproduction and

plasma glucose level in Iranian Holstein cattle. Asian-Australasian Journal of Animal Sciences, 19: 627-631. https://doi.org/10.5713/ajas.2006.627

Nassiry MR, Shahroudi FE, Moussavi AH, Sadeghi B and Javadmanesh A (2008). The diversity of leptin gene in Iranian native, Holstein and Brown Swiss cattle. African Journal of Biotechnology, 7(15): 2685-2687. https://www.ajol.info/index.php/ajb/article/view/59122

Nei M and Kumar S (2000). Molecular evolution and phylogenetics. Oxford University Press, New York. https://global.oup.com/academic/product/molecular-evolution-and-phylogenetics-9780195135855?cc=vn&lang=en&

Nobari K, Ghazanfari S, Nassiri MR, Tahmoorespur M and Jorjani E (2010). Relationship between leptin gene polymorphism with economical traits in Iranian Sistani and Brown Swiss cows. Journal of Animal and Veterinary Advances, 9(22): 2807-2810. https://profdoc.um.ac.ir/articles/a/1019041.pdf

Öner Y, Yılmaz O, Okut H, Ata N, Yılmazbaş-Mecitoğlu G and Keskin A (2017). Associations between GH, PRL, STAT5A, OPN, Pit-1, LEP and FGF2 genes polymorphisms and fertility in Holstein Friesian heifers. Kafkas Universitesi Veteriner Fakultesi Dergisi, 23: 527-534. https://vetdergikafkas.org/uploads/pdf/pdf_KVFD_2080.pdf

Pomp D, Zou T, Clutter AC and Barendse W (1997). Rapid communication: mapping of leptin to bovine chromosome 4 by linkage analysis of a PCR-based polymorphism. Journal of Animal Science, 75(5): 14–27. https://doi.org/10.2527/1997.7551427x

Putra WPB, Anwar S, Said S, Indarto SAA and Wulandari P (2019). Genetic characterization of thyroglobulin and leptin genes in Pasundan cattle at West Java. Buletin Peternakan, 43(1): 1-7. https://doi.org/10.21059/buletinpeternak.v43i1.38227

Roychoudhury AK and Nei M (1988). Human polymorphic genes world distributions. New York: Oxford University Press. https://doi.org/10.1002/ajpa.1330790412

Santos-Alvarez J, Goberna R and Sanchez-Margalet V (1999). Human leptin stimulates proliferation and activation of human circulating monocytes. Cellular Immunology, 194(1): 6–11. https://doi.org/10.1006/cimm.1999.1490

Sedykh TA, Kalashnikova LA, Gusev IV, Pavlova IY, Gizatullin RS and Dolmatova IY (2016). Influence of TG5 and LEP gene polymorphism on quantitative and qualitative meat composition in beef calves. Iraqi Journal of Veterinary Sciences, 30(2): 41-48. https://doi.org/10.33899/ijvs.2016.121382

Sharifzadeh A and Doosti A (2010). Genetic polymorphism at the leptin gene in Iranian Holstein cattle by PCR-RFLP. African Journal of Microbiology Research, 4(12): 1343-1345. https://academicjournals.org/article/article1380279572_Sharifzadeh%20and%20Doosti.pdf

Statista Research Department (2023). Per capita beef consumption in Vietnam 2017-2029. https://www.statista.com/statistics/757614/vietnam-beef-consumption-per-capita/

Thaller G, Kuhn C, Winter A, Ewald G, Bellmann O, Wegner J, Zühlke H and Fries R (2003). DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. Animal Genetics, 34: 354–357. https://doi.org/10.1046/j.1365-2052.2003.01011.x

Trakovická A, Moravčíková N and Kasarda R (2013). Genetic polymorphism of leptin and leptin receptor genes in relation with production and reproduction traits in cattle. Acta Biochimica Polonica, 60: 783-787. https://pubmed.ncbi.nlm.nih.gov/24432332/

Yang D, Chen H, Wang X, Tian Z, Tang L, Zhang Z, Lei C, Zhang L and Wang Y (2007). Association of polymorphisms of leptin gene with body weight and body sizes indexes in Chinese indigenous cattle. Journal of Genetics and Genomics, 34: 400-405. https://doi.org/10.1016/S1673-8527(07)60043-5

2024 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 14, Issue 3: 171-184; May 25, 2024

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2024.21

NUTRITIONAL AND CHEMICAL COMPOSITION OF SAINFOIN (*Onobrychis viciifolia*) ACCESSIONS IN MID-ALTITUDE OF SODDO AND ABESHGIE WOREDAS OF ETHIOPIA

Dinku Getu ABEBE[1], Solomon Demeke CHERKOS[2], Taye Tolemariam EJETA[2], Mesfin DEJENE[3], Tesfaye Kerga SHIGNATO[4] and Abel Sorsa GELETU[4]

[1]*Worabe Agricultural Research Centre, Worabe P.O. Box.21, Ethiopia*
[2]*College of Agriculture and Veterinary Medicine, Jimma University, Jimma P.O. Box 307, Ethiopia*
[3]*Holeta Research Centre, Holeta P.O. Box. 2003, Addis Ababa, Ethiopia*
[4]*Wolkite Research Centre, Wolkite, Ethiopia*

Email: fretse93@gmail.com

Supporting Information

RESEARCH ARTICLE

PII: S222877012400021-14

Received: November 07, 2023

Revised: April 27, 2024

Accepted: May 10, 2024

ABSTRACT: Sainfoin (*Onobrychis viciifolia*) is a perennial herbaceous leguminous forage crop with a high content of crude protein, palatability, nutritive value and non-bloating. It can be offered in the form of green forage, grazing pasture, hay, or silage. To mitigate the dry season feed shortage, the agronomic performance and chemical composition of sainfoin (*Onobrychis viciifolia*) accessions were studied in the mid-altitude of Soddo and Abeshgie Woredas of Garage Zone of Ethiopia. For the screening, five International Livestock Research Institute (ILRI) accessions (No 5708, 16006, 6582, 10558, and 10556) and four wild sainfoin accessions were collected from Worabe, Albazer, Meskan Dubo Tuto and Gibie river basin with morphological variation. They were subjected to initial screening followed by field trials for a period of fifteen months per session. Among the twenty-nine agronomic parameters (for screening purpose), based on the dendrogram results, fifteen data points were used for location wise (Buee and Tatesa) evaluation purposes. The results indicated that, with the exception of leaf length, leaf width, and annual seed yield, all parameters were affected by the accession by location interaction. The highest ($P<0.001$) cumulative dry matter, seed yield, and crude protein (CP%) contents were recorded for Worabe sainfoin, ILRI 5708 and ILRI 16006, respectively. Lower ($P<0.05$) condensed tannin (CT) was recorded in ILRI 5708, Worabe's and Albazer's sainfoin without any significant difference. During the first 24-hour incubation period, the highest and least ($P<0.001$) gases were produced from ILRI 16006 and Albazer, and ILRI 5708, respectively. The highest and least ($P<0.001$) methane gas was recorded from ILRI 16006 and ILRI 5708 respectively. The results indicated that Worabe sainfoin was superior to the result in all evaluated parameters. So, Worabe sainfoin hay mixture with crop residues can be used to enhance the nutritional value of crop residue-based poor feed resources.

Keywords: Agronomic performance, Alternative feedstuffs, Crude protein, Dry matter yield, Sainfoin accessions.

INTRODUCTION

In the crop-livestock mixed farming system of Ethiopia, livestock are subjected to crop residue over a long period of the year due to seasonal variation and diminished grazing land. Crop residues are poor in feed value to support production and reproduction processes (Mekuria and Mekonnen, 2018; Mekonnen et al., 2022). During the dry period, multipurpose perennial legume species enhance long term forage production and provide positive environmental effects such as improvement of soil fertility, reduction of erosion and control of nitrate leaching (McCallum et al., 2004). In the past, the introduction of improved forage plants comprised the Ethiopian forage development strategies. However, this option did not bring the anticipated change because of the exotic forage species failed under the local environment and inappropriate technical practices (Mekoya et al., 2008).

In Ethiopia, some indigenous legumes and browse species proved their availability and nutritive value at a time when feed is scarce or is of low quality (Salem et al., 2006). Unfortunately, most of the preferred and high quality indigenous legume species are under pressure (Chettri et al., 2009). Sainfoin is one of such indigenous legume species in the mid-altitudes of Ethiopia, specifically, around Soddo Woreda. Sainfoin is a perennial forage and fodder legume, tolerant to varying climatic conditions and poor soil fertility, with high nitrogen fixing capacity.

It represents a valuable pollen and nectar source for honey production. Although sainfoin is considered an important forage legume with high hay and pasture production potential (Bhattarai et al., 2018), information on agro-morphological and nutritional traits was lacking for Ethiopia. Hayot et al. (2011) summarized the sainfoin taxonomy, biology, and agronomy mainly from European perspective but not inclusive in Equatorial as well as Sub Saharan region. Sainfoin is distributed in northern temperate regions of the world and more specifically in the eastern Mediterranean and Western Asia with an emphasis on Iran and Turkey (Yildiz et al., 1999; Irani et al., 2016) but did not indicate the Ethiopian potential for sainfoin. Therefore, the objective of this research was to evaluate sainfoin accessions using adaptability,

Citation: Abebe DG, Cherkos SD, Ejeta TT, Dejene M, Shignato TK and Geletu AS (2024). Nutritional and chemical composition of sainfoin (*Onobrychis viciifolia*) accessions in mid-altitude of Soddo and Abeshgie Woredas of Ethiopia. Online *J. Anim. Feed Res*., 14(3): 171-184. DOI: https://dx.doi.org/10.51227/ojafr.2024.21

biomass yield, seed production and nutrient composition in the mid-altitude of Soddo and Abeshgie Woredas of Garage Zone.

MATERIALS AND METHODS

Description of the study area

The initial establishment of sainfoin accessions was undertaken in Soddo Woreda of Guragie Zone while the agronomic performance of selected accessions was carried out both in Soddo and Abeshgie Woredas of Guragie Zone. The Soddo experimental location is found within the geographical location of 8.31^0 98' to 8.31^0 99 'N and 37.95^0 31' to 37.95^0 62 'E while Abeshgie Woreda is found within 8.31^0 59 '-8.31^0 62 'N and 37.95^0 31 '-37.95^0 62 'E. Soddo has a mean annual temperature ranging from 10 to 25^{o}C and receives between 801-1200mm rain falls. Soddo Woreda is located at 103km south of Addis Ababa, while Abeshgie is 150km from Addis Ababa in south-west direction. Abeshgie Woreda has an altitude range from 1500-2900m above sea level, with an annual mean temperature in the range of 13^{o}C-25^{o}C and a mean annual rainfall in the range of 1000–1500 mm (Gugissa et al., 2022).

Screening sainfoin accessions

Five accessions from ILRI (International Livestock Research Institute) and four indigenous wild accessions with morphological variations were used as seed sources. Land size of 25m × 25m from black soil color was demarcated at Buee Construction and Industrial Collage compound. The land was ploughed and loosened manually. Each of the 5 ILRI accessions without replication and each of the 4 wild accessions on five plots (1 plot per block) was sawn on prepared 1m^2 (0.5m×2m) plot of land with a border side of 12. 5 cm (8.5m × 14m net demarcated plot size). During the wet season, on each plot, two parallel lines with a distance of 25cm were drawn for seed drilling. The space between blocks and plots was 1m and 0.5m respectively, as Alemayehu and Aklilu (2007). Seeds were dehulled and drilled at a depth of 2cm (Liu, 2006). The scholars' recommendations (Hume and Withers 1985; Shah et al., 1991 and Tufenkci et al., 2006) were applied for the conversion of available fertilizer into P_2O_5, N and K. To get the recommended types, 198.9kg ha^{-1} NPSB and 121 kg ha^{-1} KCl for initial and 212kg ha^{-1} NPSB and 161kg ha^{-1} KCl for regrowth were used. Plots were arranged using an Augmented Design.

Data collection for screening

Survival count was done on the 4th, 8th and 12th weeks after sowing, plant vigor, disease conditions, soil cover data, initial flowering date, date of 50% flowering, and plant height were recorded. Tiller number data until the first harvest, number of leaves per plant total stem, stem length, leaflet number, leaflet width, leaflet length, and the ratio of leaflet length to leaflet width data were collected. The leaf area was calculated using the formula used by Bianco et al. (2011).

$$LA = 0.691156 \times LWct + 0.3652754 \times LWlt \quad (1)$$

Leaf area = The central leaflet length x width (LWct) + 0.3652754 x [(left lateral leaflets length + right lateral leaflets length) x width].

Before reaching the 75% flowering stage, the method of Tarawali (1995) was applied for partitioning ILRI accessions in drilled lines. When the plants were at 75% flowering stage as Goplen et al. (1991), all plants from randomly assigned partitions were cut at a height of 10-15 cm and taken as standard cut (Tarawali, 1995). According to the Tarawali (1995) method, mowed partitions were again divided into two equal portions for minimum precipitation season yield evaluation. Before harvest, at 7th and 12th weeks of the regrowth during minimum precipitation season evaluation, soil cover, % of green leaf, pest incidence severity status data were recorded again. On randomly half of the above partitions, at 7th weeks, regrowth harvest was carried out as Bhattarai et al. (2018). Then after the 7th week's harvest, at 5th week, both the regrowth harvest and reserved halve were harvested independently, and these two were taken as the minimum precipitation season yield evaluation. The samples in every harvest season and week were weighted and sun - dried. Until reaching maximum precipitation season, data on diseases and coverage %, and vigor were repeatedly recorded. For maximum precipitation season evaluation, all randomly partitioned portions for biomass estimation including the border side were independently harvested just before maximum precipitation commenced. After border side demarcation (excluding the seed source part), all partitioned lines of the biomass estimation were divided equally into four for ILRI accessions and 2 parts for wild accessions. All four and the two partitions for ILRI and wild accessions, respectively from a single line were randomly assigned for 3rd and 15th, 6th and 15th, 9th and 15th and 12th and 15th week's herbage regrowth harvest. All the seasonal cuts were measured, sun-dried bulked, and analyzed for DM yield. Based on the suggestion of (Carleton et al., 1968), within every 4 days gap, the seed was collected. The biomass and seed yields from partitioned lines were extrapolated in to plot size and then in to hectares. As Tarawali (1995) suggestion, on the basis of the first year result, four accessions that set seeds with better biomass yield were transferred to agronomic field experimentation.

Land preparation and sowing methods for experimental establishment

Based on herbage mass and seed yield results, ILRI 5708, ILRI 16006, and wild sainfoin collected from Worabe and Albazer were selected for experimental field trial. Soil type, seed type, rate of fertilizer applications, sowing depth, and

spacing between plots and blocks were similar to that of the initial establishment work. Demarcated experimental land was ploughed repeatedly at both sites. An individual plot size of 6m² (2m×3m) with a border side of 25 cm was used for seeding with seed rate (Cupina and Erid, 1999). Plots were prepared in 9 rows. On prepared rows, dehulled seeds were drilled and fertilizer was applied in the screening phase. Sowing season was carried out based on available soil moisture season (Ethiopian moisture availability condition June-July). Each accession was randomly replicated on 6 plots. The 4 accessions were assigned to the plots in RCBD design with 6 replications.

Experimental establishment data collection

Biomass data was collected from randomly selected either left or right sides of each plot on 3 near sides' central rows of each plot. The first herbage harvest was carried out on the 12th week after sowing, when the average flowering stage reached 50-75%. This harvest was taken as Standard Cut. After the Standard Cut, the two - times regrowth herbage harvest was undertaken at 50-75% flowering stage. These herbage harvests were taken as the minimum precipitation season evaluation. Before entering the maximum precipitation season, Standard Cut was carried out on those 3 rows and then the three - times regrowth harvest at 50-75% flowering stage. Ripe seeds were collected from the left side of each plot on 3 near side rows. These 3 rows either for biomass and /or seed samples were extrapolated into hectares.

Sample preparation

Representative wet samples were taken during each cut period and season. Fresh samples were weighted, sun-dried, pooled and subsampled. The samples were sent to Hawassa College of Agriculture, Jimma University College of Agriculture and Veterinary Medicine and Holeta Agricultural Research Centre for the determination of chemical composition, digestibility, Phenolic, *in vitro* gas and methane production.

Chemical analysis

Coarsely chopped sward samples were dried in an oven for about 16 hours at 105^{o}C. Oven-dried samples were ground to pass through a sieve, a size of 1mm. Dry matter, total ash, ether extract, and crude proteins were determined as AOAC (2005). Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were determined using the method of Van Soest et al. (1991). The N content was determined by the Kjeldahl method and the CP content was calculated as N*6.25. The *in vitro* digestibility was determined by Tilley and Terry (1963) and applied as the modification of Van Soest and Robertson (1985).

Phenolic and tannin determination

The sample was dried at 50-55^{0}C for 48 hours. Determinations of total phenols, total tannins (TT) and condensed tannins (CT) were done as Makkar (2003). Butanol–HCl–iron, as Porter et al. (1985) was used for tannin determination. Hydrolysable tannins (HT) were estimated as the difference between TT and CT. Total phenols and tannins were expressed as tannic acid equivalent. The CT was expressed as leucocyanidin equivalent as Makkar (2003) and Porter et al. (1985)

$$CT = \frac{(Absorbance\ at\ 550\ nm \times 78.26 \times Dilution\ factor)}{(\%DM)} \quad (2)$$

This formula assumed the effective $E^{\%(\text{leucocyanidin equivalent}),\ 1\ cm,\ 550\ nm}$ of leucocyanidin at 460 (Porter et al., 1985). Phenolic and tannin determination was carried out in JUCAVM Post Harvest laboratory.

Methane production

Methane production was measured separately from duplicate bottles incubated for each sample at 24 hours. The procedure of Santoso et al. (2007) was applied for the conversion of CH_4 gas volume to energy and mass values by using the conversion factors of 9.45 kcal/l and 0.716 g/l respectively. The methane gas production analysis was carried out in Hawassa College of Agriculture Animal Nutrition Laboratory.

Relative feed value (RFV) of sainfoin

The Relative Feed Value (RFV) combines the estimates for forage digestibility and intake into a single number, and it was calculated from the estimation of ADF and NDF (Ward and Ondarza, 2008). The RFV index was estimated using the digestible dry matter (DDM) of the samples from ADF values and was calculated the dry matter intake (DMI) potential (as a percent of body weight, BW) from NDF values. The index was then calculated as Digestible Dry Matter (DDM) multiplied by Dry Matter Intake (DMI) as a % of body weight(BW) divided by 1.29 (Jeranyama and Garcia, 2004).

Digestible Dry matter (DDM) = 88.9 - (0.779x% ADF) (3)

DMI (% of BW) = 120/ (% NDF) (4)

Relative feeding value (RFV) = (DDM x DMI)/1.29 (5)

Statistical analysis

Analysis of variances were used to test the statistical significance of the treatments. Using the general linear model procedure of the SAS program version 9.3 (SAS, 2010) with a 5% probability with experimental model:

$Yijk = \mu + Li + \alpha j + \beta k + L\beta ik + eijk,$

Where: Yijk= An observation; μ = was the overall mean Li= is the i^{th} treatment locations (the 2 locations), i = 1Buee, 2 Tatesa; αj is the j^{th} block effect (the 6 blocks), j= 1...6; βk = is the k^{th} treatments(selected sainfoin accessions) , k= 1.....4 ; Lβik = The L^{th} site (i=2locations) and the β^{th} treatment (k= 4 accessions)interaction; eijk = was the random error.

RESULTS AND DISCUSSION

Classical separation of agronomic parameters

Among the 29 parameters, the 15 parameters (cover percent, infection level, plant height, mean tiller number, leaf length, leaf width, leaf area, regrowth score, fresh biomass weight, leaf and stem percent, air dried weight, air dried %, annual dry matter yield and annual seed yield) were taken for Dendrogram distance separation. The separation was conducted using principal component Analysis (SAS, 2010). Those first fifteen dependent components were found near 1.0 Eigenvalues, which encompassed 62%. Using these eighteen parameters, the four accessions were evaluated.

From the report of Thomson (1951), the seed height and width of the studied sainfoin' are in the range of 2.5–4.5 and 2.0-3.5 mm respectively. With the exception of 5708's, the values of the seed height for three accessions nearly agreed with their record. Minor variation is attributed to weather conditions and the seed collection period. *In* the study of Thomson (1951) 15.3g of milled and 21.4g of un milled sainfoin were recorded for 1000 seed weights.

Accessions' leaf and seed morphology

Accession 5708's leaflets were arranged separately, but those of 16006 were arranged laterally in 9 pairs, with a single pinnate leaflet at the tip (Figure 1). Similarly, Worabe and Albazer accessions had similar lateral arrangements in 13 paired leaflets with a single pinnate leaflet at the tip point. The entire accessions bear creamy to grey seed pods. The seed pod of 5708, Worabe's, and Albazer's were pinnate (spiny at endpoints) but that of 16006 was rough and resembled tortilis Keratein crust. Pod of 5708's hold pair seed per pod but that of 16006's was single seeded indehiscent. Seed pods of Worabe and Albazer were different from those of 5708's and 16006's. Both bear two-strand pods which stuck together at mid-rib until dry and bear seeds independently. The single lateral pod holds 3–5 seeds; and in a total, 6–10 seeds per pair pod. Seed pod length for 5708, 16006, and Worabe and Albazer was ranged from 33-34, 5-6, and 25-27mm respectively. Seed lengths of 5708, 16006, Worabe and Albazer were 6-7, 3-3.1, 2-2.3, and 2.9-3 mm respectively. Similarly, the seed width of 5708, 16006 and Worabe and Albazer were 5-5.7, 1.9–2 and 2-2.1 mm respectively. A Thousand seed weights for 5708, 16006, Worabe, and Albazer were 81, 16.9, 1.7, and 1.9 g respectively. Attacking weevils (Coleoptera: Curculionidae) fed sainfoin foliage and pods were assessed as pod fluid sucker. In the present study, seed weight of, 16006 is similar to the report of Thomson (1951) and Hayot et al. (2011) for milled giant and common types. Dis agreement with the other accessions were attributed to population, accession, harvesting stage and season and soil nutrient variability. The nature of sainfoin in seed setting is as single-seeded indehiscent with brown colored pods with olive to brown or black color observation of Goplen et al. (1991) is agreed with present study result for 16006 accession pod and seed color.

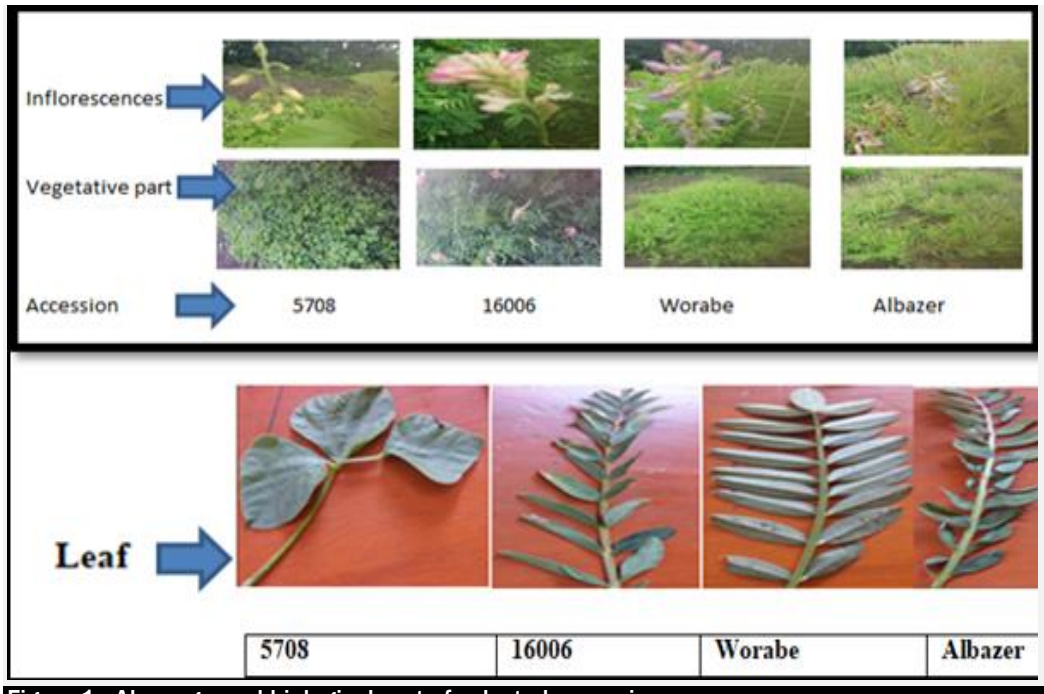


Figure 1 - Above ground biological part of selected accessions

Agronomic efficiency during screening phase

Measured plant phenology and yields during screening are presented in Tables 1 and 2. Germination failure was encountered for Meskan Dubo Tuto (MDDT), Gibie, ILRI 10558 and ILRI 10556 accessions. There were significant differences (P<0.001) in mean Phenological parts of the accessions. The mean disease tolerant score was higher for 16006, Worabe and Albazer without significant difference (P>0.05) but lower for 5708 accessions. Worabe was the tallest and 6582 was the shortest. Accession 16006 recorded the highest tiller-forming ability. Except for the accession 6582, a similar regrowth score was recorded (P>0.05) for the 4 accessions, with Worabe being the best compared to the others. As indicated in Table 2, significantly (P<0.0001) higher mean fresh herbage yield per square meter was recorded for Worabe followed by that of Albazer accession. The highest leaf percent was recorded for 6582, but the least was for Worabe and Albazer without showing a significant difference (P>0.05). The highest stem percent was recorded for Albazer but the least was for 16006. Air-dried weight per square meter was lower for Albazer and 6582 while all the others scored higher and/or comparable dried weight. Significantly (P<0.0001) higher annual DM (t ha^{-1}) yield was recorded for Worabe followed by 16006 accession. Relatively higher annual seed yield (kg ha^{-1}) was recorded for ILRI 5708 accession followed by Worabe, while seed setting ability failed in 6582 accession. Accession by block interaction was not significant (P>0.05) in all observed parameters. The effect of geographic location and moisture effect can cause differences among populations. The percentage of flowering plants in the sowing year and the speed of regrowth after the spring cut can help for differentiation of the two sainfoin types (giant/common) (Delgado et al., 2008). The results of the current study indicated that five accessions were able to flower in the sowing year, and the four accessions showed regrowth potential after successive harvest in dry and wet seasons. These characteristics led them to be giant type.

Agronomic efficiency of selected sainfoin accessions at two sites

Mean recorded accessions values are presented in Table 3 and Table 4. Tiller forming potential and leaf area records were found similar. Significantly higher (P<0.0001) mean fresh herbage mass per square meter was recorded for Worabe while the least was reported from ILRI accession 5708 (Table 4). Lower leaf and stem proportion was recorded for ILRI accession 16006. All the others found comparable in mean leaf stem percentage. The highest annual DM yield (t $h^{-1}y^{-1}$) and annual seed yield (kg $h^{-1}y^{-1}$) were obtained from Worabe whereas; all the other accessions were comparable in mean annual DM yield. The lowest mean annual seed yield was collected from ILRI accession 16006. In location wise, except for air-dried stem percentage, the Buee site was superior to all the others.

Table 1 - Agronomic performance of Sainfoin accessions during the screening phase.

Accession	Mean accession values							
	X1	X2	X3	X4	X5	X6	X7	
5708	40.74^{bc}	7.5^{b}	20.4^{c}	11.2^{e}	1.3^{a}	2.36^{a}	9.5^{a}	7.4^{b}
16006	56.7^{a}	9.0^{a}	15.2^{d}	40.2^{a}	1.3^{d}	0.8^{b}	1.93^{b}	8.06^{ab}
Worabe	41.7^{b}	9.0^{a}	38.7^{a}	15.4^{c}	1.6^{b}	0.48^{c}	1.06^{c}	8.1^{a}
Albazer	39.3^{cd}	9.0^{a}	34.2^{b}	12.7^{d}	1.5^{c}	0.42^{c}	0.98^{c}	7.6^{ab}
6582	38.9^{d}	8.0^{b}	13.7^{e}	32.1^{b}	1.3^{a}	0.74^{b}	1.97^{b}	7.3
MDDT	0	0	0	0	0	0	0	0
Gibie	0	0	0	0	0	0	0	0
10558	0	0	0	0	0	0	0	0
10556	0	0	0	0	0	0	0	0
SE	3.34	0.31	5.1	5.83	0.20	0.34	1.62	0.17
P	***	*	***	***	***	*	***	ns
Block	**Mean block value**							
1st	40.0^{b}	9.0^{ab}	36.3^{a}	14.4^{c}	1.32^{b}	0.46^{c}	1.06^{c}	7.75
2nd	41.0^{b}	9.0^{ab}	36.9^{c}	14.2^{c}	1.27^{a}	0.44^{c}	0.97^{c}	7.93
3rd	41.0^{b}	8.5^{b}	31.6^{b}	13.2^{d}	1.57^{bc}	1.1^{a}	3.9^{a}	7.47
4th	40.2^{b}	8.7^{ab}	29.1^{c}	20.3^{b}	1.54^{bc}	0.55^{b}	1.36^{c}	7.89
5th	45.3^{a}	9.0^{a}	28.4^{d}	22.2^{a}	1.27^{a}	0.56^{b}	1.30^{b}	8.02
SE	0.97	0.11	1.78	1.83	0.07	0.12	0.54	0.10
P	*	ns	*	*	*	*	***	ns
Accession by location interaction								
SE	1..31	0.13	2.54	2.41	0.08	0.15	0.65	0.11
P	ns	ns	ns	ns	ns	ns	ns	ns

a,b,c,d,e; Means within the column with different superscripts in accessions and blocks are differ significantly at P<0.05. 5708:Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; 6582: Accession collected from ILRI; MDDT: Accession collected from Meskan Dubotuto; Gibie: Accession collected from Gibie river basin; 10558: Accession collected from ILRI; 10556: Accession collected from ILRI; X1: Cover %; X2: Infection tolerance; X3: Plant height(cm); X4: Mean tiller number; X5: Leaf length(cm); X6:Leaf width(cm); X:Leaf area(cm^2); X8- Regrowth score. *: P<0.05; ***: P<0.0001; NS: not significant.

Table 2 - Yield efficiency of Sainfoin accessions during screening phase contd...

Accession	Mean accession values						
	9	10	11	12	13	14	15
5708	4.42^{d}	66.7^{c}	29.0^{c}	1.31^{a}	48.7^{a}	10.71	2163^{a}
16006	4.55^{c}	67.9^{b}	25.7^{e}	1.30^{a}	38.3^{b}	11.05^{b}	88.3^{d}
Worabe	5.24^{a}	52.2^{d}	32.9^{b}	1.33^{a}	31.6^{b}	12.49^{a}	927^{b}
Albazer	5.57^{b}	52.1^{d}	35.6^{a}	0.98^{b}	40.7^{c}	10.96^{c}	561^{c}
6582	4.90^{d}	70.1^{a}	26.6^{d}	1.03^{b}	46.8^{b}	0	0
MDDT	0	0	0	0	0	0	0
Gibie	0	0	0	0	0	0	0
10558	0	0	0	0	0	0	0
10556	0	0	0	0	0	0	0
SE	0.24	3.97	1.89	0.08	2.47	0.69	443.87
P	***	***	***	***	*	***	***
Block	**Mean block value**						
1st	5.49^{a}	51.97^{c}	34.5^{a}	1.20	35.6	11.75^{a}	767^{b}
2nd	5.45^{a}	52.3^{c}	34.3^{a}	1.19	36.4	11.74^{a}	691^{c}
3rd	5.16^{c}	57.07^{b}	32.4^{b}	1.45	40.39	11.39^{c}	1181^{a}
4th	5.17^{c}	58.21^{a}	31.7^{c}	1.14	39.7	11.73^{c}	750^{b}
5th	5.33^{b}	57.26^{a}	31.3^{d}	1.17	36.95	11.46^{b}	577^{d}
SE	0.07	1.33	0.66	0.01	0.82	0.22	102.53
P	*	*	*	ns	ns	ns	***
Accession by location interaction							
SE	0.12	1.97	0.97	0.05	0.89	0.33	144.6
P	ns	ns	ns	ns	ns	*	ns

a,b,c,d,e; Means within the column with different superscripts in accessions and blocks are differ significantly at P<0.05. 5708: Accession collected from ILRI, 16006- Accession collected from ILRI, Worabe- Accession collected from Worabe, Albazer- Accession collected from Albazer 6582- Accession collected from ILRI, MDDT- Accession collected from Meskan Dubotuto, Gibie- Accession collected from Gibie river basin, 10558-Accession collected from ILRI, 10556- Accession collected from ILRI. X9:Fresh biomass weight(kg) per meter square; X10:leaf%; X11:Stem %; X12:Air dried weight (kg); X13: Air dried %; X14: Annual dry matter yield(t); X15: Annual seed yield(kg). *: P<0.05; ***: P<0.0001; NS: not significant.

Table 3 - Agronomic efficiency of yield factors during experimental establishment

Accession	Mean accession values							
	1	2	3	4	5	6	7	8
5708	33.2^{c}	8.0^{b}	7.5^{d}	6.2^{d}	2.82^{a}	2.32^{a}	9.37^{a}	7.8^{c}
16006	41.6^{a}	7.8^{c}	19.1^{c}	33.3^{a}	2.08^{b}	0.76^{b}	1.81^{b}	4.5^{d}
Worabe	37.1^{b}	9.0^{a}	32.1^{a}	13.1^{b}	1.49^{d}	0.49^{c}	1.08^{c}	8.9^{a}
Albazer	30.6^{d}	9.0^{a}	26.1^{b}	12.0^{c}	1.59^{c}	0.40^{d}	1.07^{c}	8.3^{b}
SE	3.34	0.31	5.1	5.83	0.20	0.34	1.62	0.17
P	***	*	***	***	***	*	***	ns
Location	**Mean location value**							
Buee	41.1^{a}	8.5	29.1^{a}	25.6^{a}	2.06^{a}	1.01^{a}	2.79	8.5^{a}
Tatesa	30.1^{b}	8.4	13.4^{b}	6.6^{b}	1.94^{b}	0.97^{b}	2.73	6.4^{b}
SE	5.48	0.06	7.84	9.50	0.06	0.02	0.02	1.10
P	***	*	***	***	*	*	ns	***
Mean block								
SE	0.37	0.03	0.06	0.05	0.002	0.002	0002	0.04
P	ns	ns	ns	ns	ns	ns	ns	ns
Accession by location interaction								
SE	2.64	0.22	5.05	6.67	0.20	3.0	1.32	1.07
P	ns	ns	***	***	ns	ns	***	***

a,b,c,d; Means within the column with different superscripts in accessions and blocks are differ significantly at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; X1: Cover %; X2: Infection tolerance; X3: Plant height(cm); X4: Mean tiller number; X5: Leaf length(cm); X6:Leaf width(cm); X:Leaf area(cm^{2}); X8- Regrowth score;.*:P<0.05; ***:P<0.0001; NS: not significant.

Table 4 - Yield efficiencies during experimental establishment contd...

Mean accession values / Accession	9	10	11	12	13	14	15
5708	1.24^{d}	54.9^{a}	22.7^{a}	42.3^{a}	0.85^{a}	7.49^{b}	573.8^{b}
16006	1.08^{b}	35.7^{b}	7.4^{b}	21.4^{d}	0.42^{c}	3.69^{b}	90.91^{d}
Worabe	2.53^{a}	54.0^{a}	21.3^{a}	36.6^{b}	1.1^{d}	9.69^{a}	701.57^{a}
Albazer	1.87^{c}	55.6^{a}	22.6^{a}	36.5^{c}	0.89^{b}	7.85^{b}	389.5^{c}
SE	0.33	4.80	3.70	4.47	0.14	1.26	142.60
P	***	***	***	***	***	***	***
Location	**Mean location value**						
Buee	2.3^{a}	69.8^{a}	22.13^{b}	46.2^{a}	1.04^{b}	9.12^{a}	499.18^{a}
Tatesa	1.04^{b}	30.3^{b}	14.7^{a}	22.2^{b}	0.6^{a}	5.23^{b}	355.96^{b}
SE	0.63	19.79	3.63	11.99	0.22	1.94	71.61
P	***	***	*	***	**	***	***
Block mean							
SE	0.001	0.05	0.06	0.08	0.004	0.039	0.244
P	ns	ns	ns	ns	ns	ns	ns
Accession by location interaction							
SE	0.34	8.87	2.93	5.79	0.14	1.19	103.23
P	***	***	***	***	***	***	ns

a,b,c,d; Means within the column with different superscripts in accessions and blocks are differ significantly at P<0.05. 5708: Accession collected from ILRI, 16006- Accession collected from ILRI, Worabe- Accession collected from Worabe, Albazer- Accession collected from Albazer; X9:Fresh biomass weight(kg) per meter square; X10:leaf%; X11:Stem %; X12:Air dried weight(kg); X13: Air dried %; X14:Annual dry matter yield(t); X15: Annual seed yield(kg). *:P<0.05; **:P<0.01; ***:P<0.0001; NS: not significant.

Agronomic performance similarity and variations in two experimental sessions

During the screening phase, plant vigor record was highest for Worabe's than that of Albazer's. During the experimental session, the cumulative value of the accessions at both agro ecologies was found to be comparable. The total leaf count during the screening phase for ILRI 16006 accession was more vigorous than all the others. However, the cumulative value of the two agro ecologies showed Worabe's observed more vigorous. During the screening session, regrowth scores for Worabe, Albazer and ILRI 16006 were found similar. However, during the experimental session, Worabe became superior, followed by Albazer. During screening sessions, fresh biomass weight per square meter was higher for Worabe Sainfoin than Albazer sainfoin, but during experimental sessions ILRI 16006 replaced Albazer sainfoin. Cumulative seed yield for Worabe accession was highest during the experimental period, but during the screening period, the highest record was found in ILRI 5708. Variability could be attributed to agroecology variation.

The conclusion of Hanna (1993) indicates that the longest with the superior in leaf percent can score the highest DM yield, could contradict the present study in that the lowest leaf percent with highest scored the highest DM yield. The cumulative DM yield of sainfoin is within the range of 7–15 t ha^{-1} based on growing conditions (Tarawali, 1995). At seed filling and the beginning of flowering stages, 5.1 t ha^{-1} and 6.5 t ha^{-1} DM yields are reported by Turk et al. (2011) and Goplen et al. (1991) respectively. The results of the current study are in agreement with the ranges of monoculture or mixture stands, except for pick production time records. This result shows that the Ethiopian agroecology is suitable for either indigenous or exotic accessions. Several years of drought are the threat of Sainfoin on DM yield (Biligetu et al., 2014). Despite the determination of suitability of the Brown, Dark Brown and Black soils types for sainfoin, the report of Goplen et al. (1991) has limitation in determining the crack effect of Black Vertis that causes plant death as observed at the Tatesa site.

Chemical composition of sainfoin accessions

The chemical composition of the four sainfoin accessions is presented in Table 5. Higher crude protein contents were recorded for 5708 compared to that of 16006 and Albazer. Significantly (P<0.05) higher NDF and ADF were recorded for 16006 and 5708 compared with that of 5708 and Worabe respectively. The phenol content was 21.9 and 22.9 for 5708 and 16006 respectively. Higher total tannin was recorded from 5708 compared with that of 16006 and Albazer accessions. The crude protein content of sainfoin depends on the growth stage and population. The CP content of accessions in the present study was within the range of 16.36 -18.77 during the filling stage. This result was in agreement with that of the earlier reports presented by Turk et al. (2011) and Azuhnwi et al. (2012). Crude protein content in the range of 11.4–17.7% was also reported for the flowering stage (Kaplan, 2011). Differences in CP content with other reports could be attributed to the stage of maturity, soil type, and available chemical composition in the soil. The NDF content of sainfoin at flowering was reported to be in the range of 37.2-45% (Parker and Moss, 1981). In contradict to this, the lower NDF values of the regrowth stage reported by Bal et al. (2006) was agreed with the present result. On the other hand, the minimum content of ADF composition for the vegetative stage and flowering stages are agreed with the reports of Bal et al. (2006) and McCallum et al. (2004) respectively with the present result, but disagrees neither with the

maximum content nor the growing stages. Variation within fiber contents attributed to growth stage, population differences (Bhattarai et al., 2018), climatic conditions, and soil type, and soil chemical composition. Factors affecting the types, structures, and concentration levels of secondary metabolites in plants are many and complex. The two tannin types in plant parts and the CT distribution at the upper epidermis of leaf tissue varies from plant to plant (Jean-Blain, 1998). The tannin in sainfoin leaf, has a molecular weight of 17000–28,000 Da has lower efficiency in blood protein precipitation than the lower molecular weight (6000-7100 Da) present in Lotus species (Jones et al., 1976). The major causes of higher tannin concentration in plants are early growth stage (Bate-Smith, 1973), Phenological (leaf) (Mole et al., 1988), increased temperature (Lees et al., 1994), and poor soil fertility (Kelman and Tanner, 1990). On top of that, there is concentration variation in species and cultivars (Kelman and Tanner, 1990). Except for phenolic compounds, the present study indicates the existence of variations in secondary metabolite contents among accessions is in line with the reports of Kelman and Tanner (1990) and Roberts et al. (1993).

Roberts et al. (1993). The secondary metabolites in sainfoin is within the range of 5-8% /kg DM, and taken as a moderate level that has lower effects on digestion than the Lotus as reported by Waghorn et al. (1999). Waghorn et al. (1999) that has in agreement with Wang et al. (2015) report. This above range contradicts the report of Salem et al. (2006), who recorded 20–50 g/kg. DM and took it as a moderate level. Differences in accessions are attributed to leaf-to-stem ratio and growth stage variation. In the present study, the leaf percent ratio recorder holds the highest CT.

Table 5 - Chemical composition of experimental Sainfoin accessions

% DM / Accessions	DM%	Ash	OM	CP	NDF	ADF	ADL	TP (mg/GAE/g)	CT (mg/g)
5708	90.29^{a}	11.43^{d}	90.5^{ab}	18.77^{a}	46.3^{d}	31.09^{a}	17.7^{a}	21.90	7.64^{a}
16006	90.33^{a}	11.84^{b}	88.5^{a}	16.45^{c}	39.25^{a}	29.88^{c}	9.4^{bc}	22.14	7.47^{b}
Worabe	90.55^{a}	12.71^{c}	91.1^{b}	17.56^{b}	37.38^{c}	26.07^{d}	11.0^{b}	22.90	6.81^{a}
Albazer	89.17^{b}	13.53^{a}	91.4^{a}	16.36^{c}	37.78^{b}	30.03^{b}	9.1^{c}	22.11	7.12^{ab}
SE	0.31	0.03	0.55	0.05	0.26	0.03	0.50	0.50	0.15
p	*	***	***	***	***	***	***	ns	*

a,b,c,d; Means in the same column with different superscript letters are significantly different at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; DM: Dry matter; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; TP: Total phenol; GAE: Gallic acid equivalent; CT: Condensed tannin. *: P<0.05; **:P<0.01; ***:P<0.0001; NS: not significant

***In vitro* gas production potential of sainfoin accessions**

In vitro gas production of experimental sainfoin accessions is presented in Table 6 with its trend (Figure 2). There was a significant difference (P<0.05) in gas production and the characteristics of accessions in each incubation period. Accession 5708 was the lowest in gas production within the first 3 hours of the incubation period but 16006 and Albazer were found to be higher in gas production without showing a significant difference (P>0.05) between each other. Within 24 hour of incubation period, 16006 and Albazer sainfoin accessions significantly (P<0.0001) produced the highest gas, and the least was observed in 5708 accessions compared to others.

In vitro gas production is used for the estimation of the potential digestibility of the feed in the rumen (Getachew et al., 2000). However, there exist differences in gas production characteristics among laboratories that partially result from differences in chemical constituents (CP, NDF, ADL, TP, and CT), leaf-to-stem ratio, and cell wall composition, as indicated by Larbi et al. (2011) and Getachew et al. (2002). These causes for variations agree with the present study of gas production differences in the same species. Higher CP content is positively correlated with gas production extent, while higher fiber content reduces initial gas production and is negatively correlated with gas volume (Gasmi-Boubaker et al., 2005). The current study result contradicted the reports of Khazaal et al. (1994) and Tolera et al. (1997) because lower gas production was recorded from the highest CP content holder. The cause of low gas production vs higher CP could be attributed to the effect of higher affinities formed by CT and proteins than polysaccharides (Patra and Saxena, 2010). This affinity resulted the formation of insoluble CT-fibre and CT-protein complexes and implies fermentation reduction (Huyen et al., 2016).

The initial incubation period gas is related from the fermentation of the soluble and fast fermentable fraction of the substrate (i.e., soluble carbohydrates) and microbial protein synthesis, whereas the last portion of the incubation period is the result of fermentation of the insoluble but potentially degradable components (NDF) fraction (Groot et al., 1996). The NDF content in fermentation leads to proportionally greater amounts of gas production in the latter incubation periods (Lagrange and Villalba, 2016). Sainfoin is characterized by a very low fiber digestible legume at early incubation times (Niderkorn et al., 2011) due to the negative effect of its CT (Theodoridou et al., 2011). Accordingly, it can be concluded that along with other factors, a relatively higher CT in 5708 accessions may contribute to the reductions of the rate of gas production at initial as well as the potential gas production.

Sainfoin holds 12.6±0.6 g/kg CT with 5.5, 74.7, and 80.2 of the mean degree of polymerization (mDP), prodelphinidins percentage (%PD) and *cis* flavan-3-ols (%*cis*) respectively (Huyen et al., 2016). The percentage of

prodelphinidins within CT had the largest effect on fermentation characteristics followed by average polymer size and percentage of *cis* flavan- 3-ols (Huyen et al., 2016). The prodelphinidins (PD%), rather than procyanidins (PC) has the potential of inhibition of growth of proteolytic bacteria (Valentin et al., 1999). These listed cases for gas production variation are in lined with the present study gas production results of experimental accessions. In support of this, in *vitro* fermentation of sainfoin caused low VFA concentration and gas production than that of alfalfa (Niderkorn et al., 2011). Other than the CP content effect, methodology (Valentin et al., 1999), substrates and types of experimental animals, number of measurements and mathematical model; and also personnel error, laboratory chemical constituents, consistent working period and personnel execution rate could also be taken as the cause for gas record variations.

Table 6 - Chemical composition of experimental Sainfoin accessions.

Accessions	Incubation times, hour								Gas characteristics			
	3	6	9	12	18	24	48	72	a	b	c	a+b (%)
5708	6.7^{c}	9.6^{c}	12.4^{d}	15.0^{c}	19.6^{c}	23.6^{c}	35.1^{c}	41.6^{c}	3.49^{c}	46.7^{c}	0.0236^{c}	50.2^{b}
16006	16.6^{a}	25.3^{a}	32.1^{a}	37.4^{a}	44.7^{a}	49.2^{a}	55.4^{ab}	56.3^{a}	6.41^{a}	51.0^{ab}	0.082^{a}	57.4^{a}
Worabe	11.7^{b}	17.2^{b}	21.9^{b}	26.2^{b}	33.2^{b}	38.7^{b}	50.9^{b}	55.6^{ab}	5.44^{b}	53.3^{a}	0.0416^{b}	58.7^{a}
Albazer	15.3^{a}	23.6^{a}	30.1^{c}	35.1^{a}	41.9^{a}	46.0^{a}	51.4^{ab}	52.1^{b}	5.49^{b}	47.8^{bc}	0.0861^{a}	53.3^{b}
SE	0.29	0.45	0.56	0.63	0.70	0.72	0.65	0.56	0.04	0.60	0.003	0.57
p	***	***	***	***	***	***	***	***	***	***	***	*

a,b,c,d; Means within the column with different superscripts are differ significantly at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; a: Gas production from immediately soluble component (ml); b: Gas production from insoluble but potential degradable portion (ml); a+b(%): Potential gas production (ml); c: The rate constant of gas production (fraction/h). *: P<0.05; **: P<0.01; ***: P<0.0001.

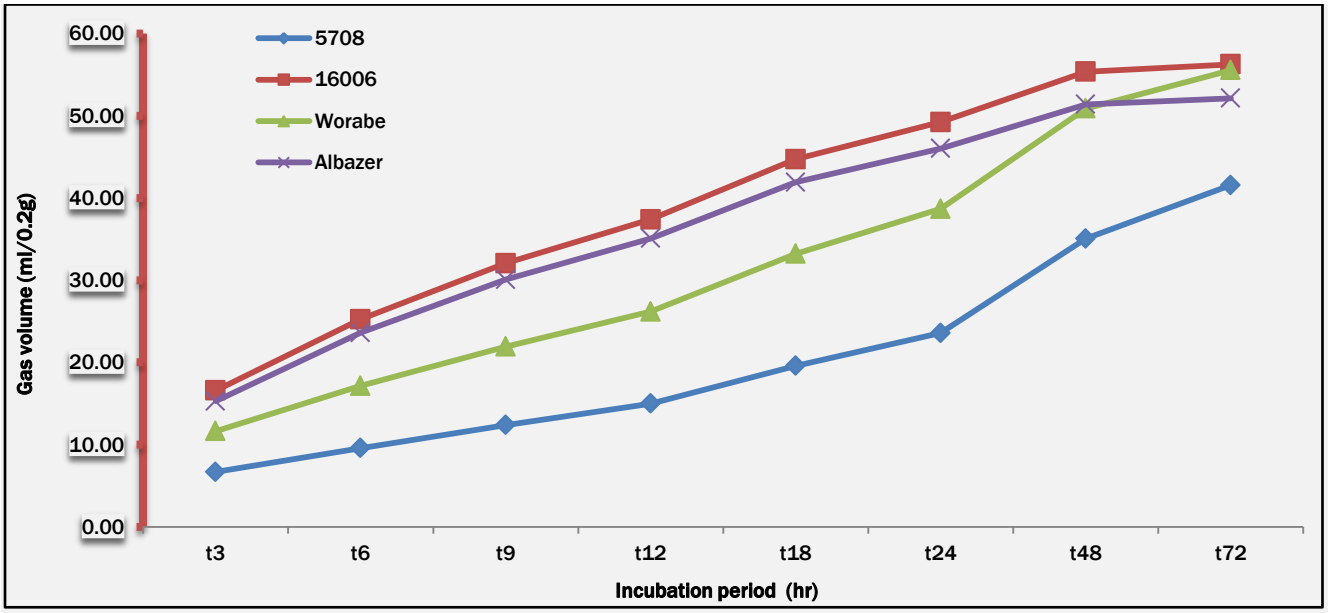


Figure 2 - *In vitro* gas production of experimental Sainfoin accessions

Nutritive value and methane emission potential of sainfoin accessions

As presented in Table 7, there were significant (P<0.05) variations between the experimental sainfoin accessions in chemical composition, estimated digestibility, energy value, and methane production potential. Except for 5708 accessions, all the others hold higher carbohydrate content. Significantly (P<0.001) lower *in vitro* dry matter digestibility (IVDMD) was recorded for Albazer followed by 16006 accessions. Organic matter digestibility (OMD) and metabolizable Energy (ME) were lower for 5708. Significantly (P<0.001) the highest methane gas (CH4), calculated energy, and its mass was recorded in Albazer while all the rest hold lower and similar value records (P>0.05).

The *in vitro* dry matter digestibility of sainfoin accession within the range of 55.6–73.3% is in agreement with the ranges reported by Yordanka and Viliana (2015). There exists higher OM degradability and rate of gas production for alfalfa than that of sainfoin (Lagrange and Villalba, 2016). The lower OM degradability and rate of gas production records for sainfoin are attributable to the greater contents of the cell wall and relatively more CT together with the advanced stage of maturity (Guglielmelli et al., 2011).

In the current study, metabolizable energy content in the range of 11.55-12.49 MJ/kg DM is higher than the report of Scharenberg et al. (2007). *Acacia tortilis* in mid-rift-valley grassland of Ethiopia holds lower ME as reported by Bezabih et

al. (2014) than the present study result. Such variation could be attributed to differences in morphological composition (Larbi et al., 2011), harvesting season (Zafu et al., 2020), stage of maturity (Guglielmelli et al., 2011), and differences in CT that causes fermentation and end-product variation (Huyen et al., 2016).

Methane production from 5.6-9.9ml/200mg for sainfoin accessions is reported by Aderao et al. (2018). There is a positive correlation between methane and gas volume production (Huyen et al., 2016). In contrast to this, there are reports presented by Bueno et al. (2020) indicating the potential effect of CT on methane production reduction through reducing methanogens, shifting metabolic pathways, and reducing the availability of protein for microbial activity. In support of this, Huyen et al. (2016) reported the ability of all CT sources on CH4 production reduction. As a result, the liberation of 2[H]-ions from the fermentation of organic matter to acetate and butyrate that uses for CH4 production is suppressed. In contrast, increased propionate production has a role as [H]-ion sinker (Tavendale et al., 2005). Moreover, condensed tannins have higher affinities for proteins than polysaccharides (Patra and Saxena, 2010). From the current study, accession with the highest CHO and least IVDMD produced higher methane than those hold higher CP, since higher CP can suppress acetate production.

Table 7 - Digestibility, nutritive value and methane emission potential of experimental Sainfoin accessions

Sample	Chemical composition and energy			Digestibility (%)				Methane characteristics in 24 hrs		
	SCFA (mmol/200 mg $^{-1}$ DM) at 24 hr	CHO	ME (at 24 hr)	*IVDMD*	TDN	DCHO	OMD at 48hr	Volume (ml/200 mg^{-1} DM)	Energy (k Cal)$^{-1}$	Mass (mg ml^{-1})
5708	0.63^{b}	47.5^{b}	6.51^{d}	73.3^{a}	73.8^{c}	52^{c}	62.0^{c}	5.60^{bc}	52.9^{bc}	4.03^{bc}
16006	1.24^{a}	59.3^{a}	9.86^{a}	62.7^{c}	88^{a}	70.6^{a}	79.8^{a}	7.0^{b}	66.2^{b}	5.0^{b}
Worabe	0.99^{ab}	56.9^{a}	8.49^{c}	71.1^{b}	85.5^{ab}	66.5^{b}	75.7^{b}	7.0^{b}	66.2^{b}	5.0^{b}
Albazer	1.16^{a}	58.9^{a}	9.42^{b}	55.6^{d}	84.2^{b}	66.9^{b}	76.7^{b}	9.9^{a}	93.6^{a}	7.1^{a}
SE	0.11	0.86	0.13	0.38	1.03	0.38	0.70	0.65	6.13	0.46
p	ns	***	***	***	***	***	***	***	***	***

a,b,c,d; Means within the column with different superscripts are differ significantly at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; SCFA: Short chain fatty acid; DM: Dry matter; CHO: Carbohydrate; ME: Metabolizable energy; IVDMD: *in vitro* dry matter digestibility; TDN: Total digestible nutrient; DCHO: Digestible carbohydrate; OMD: Organic matter digestibility. ***: P<0.0001; NS: not significant.

Relative feeding value

The Relative Feeding Value (RFV) of the four accessions is presented in Table 8. There was significant variation in relative feeding value among the accessions (P<0.05). The highest (P<0.001) relative feeding value was recorded for Worabe accessions. Significantly (P<0.001) the least RFV was observed for 5708.

Determination of RFV for legumes compared with that of grass mixture and grass forages bears limitations (Ward and Ondarza, 2008). Many scholars stated that, intake is a function of the NDF content. Alfalfa forage has greater than 100 RFV and is taken as a greater intake of digestible dry matter by the cow compared to more NDF holders (Ward and Ondarza, 2008). The result of the current study is in agreement with the above conclusions in that intake is inversely proportional to the NDF content but positively correlated with ADF content. The mean relative feeding value of alfalfa at the early and late cutting stages is within a range of 119-203 and 125-254 respectively, Hackmann et al. (2008). Having RFV of more than 100 in the present study shows the importance of sainfoin as feed source, and this result is in agreement with the report of Ward and Ondarza (2008). It can be concluded that the RFV of studied sainfoin accessions in the present study and studied alfalfa in another report are comparable with the existence of variability in secondary metabolites and their effect on animal production.

Table 8 - Relative feeding value of experimental Sainfoin accessions

Chemical composition and energy / Sample	DDM (%)	DMI (%BW)	RFV
5708	64.7^{c}	2.59^{c}	130.0^{c}
16006	65.6^{b}	3.06^{b}	155.5^{b}
Worabe	68.6^{a}	3.21^{a}	170.7^{a}
Albazer	65.5^{bc}	3.18^{ab}	161.3^{b}
SE	0.02	0.02	0.77
p	***	***	***

a,b,c,d; Means within the column with different superscripts are differ significantly at P<0.05. 5708: Accession collected from ILRI; 16006: Accession collected from ILRI; Worabe: Accession collected from Worabe; Albazer: Accession collected from Albazer; DDM: Digestible dry matter; DMI(%BW): Dry matter intake in % of body weight; RFV: Relative feeding value. ***: P<0.0001; NS: not significant.

CONCLUSION

Testing locations caused inconsistency result in agronomic performances collected during the screening phase at one testing site and the evaluation session at two testing sites. Location that boosts manifestation for some potential of some accession could suppress the others potential. The highest CP, together with the higher secondary metabolite (Phenol) content in 5708 accession, suppressed *in vitro* gas fermentation. The lowest ADL content, together with higher CHO composition in Albazer sainfoin caused a higher fermentability and led to higher methane production. In this study, the lowest NDF content indicated a level of digestibility but couldn't indicate the RFV. Compared with other accessions, the highest in disease tolerance ability, regrowth potential, annual herbage biomass and seed yield, and better in CHO and RFV, but lower in NDF content scores assisted Worabe sainfoin to be the primary accession. In areas where crop residues become the dominant feed resources and in moisture scarce areas of Ethiopia, the use of Worabe sainfoin to enhance the nutritive value of poor feed resources seems appealing.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dinku Getu ABEBE; E-mail: fretse93@gmail.com; ORCID: 0009-0000-6383-8909

Authors' contribution

D. Getu: Designed the experiment, set methodology, conducts the experiment, collect, analyses and interpret and prepare the manuscript. S. Demeke, T. Tolemariam and M. Dejene: Reviewed, edited and supervised the experimental design, performed methodology, data quality, data analysis, data interpretation and manuscript writing.

Acknowledgements

The authors would like to thank the South Agricultural Research Institute, Worabe Agricultural Research Centre, and Ethiopian Institute of Agricultural Research for funding; Jimma University College of Agriculture and Veterinary Medicine Department of Animal Science and Postharvest, Hawassa University College of Agriculture, Department of Animal and Range Sciences and Holeta Agricultural Research Centre for facilitating the laboratory execution; Soddo Woreda, Buee Municipality, Buee Secondary School, Buee Construction College and Wolkite Research Centre administrative heads and officers for facilitating experimental sites and the International Livestock Research Institute (ILRI), Ethiopian branch for availing initial Sainfoin Seed.

Data availability

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

Competing interests

We, the authors, have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Aderao G N, Sahoo A, Bhatt R S, Kumawat PK and Soni L (2018). In vitro rumen fermentation kinetics, metabolite production, methane and substrate degradability of polyphenol rich plant leaves and their component complete feed blocks. Journal of Animal science and Technology, 60: 1-9. https://doi.org/10.1186/s40781-018-0184-6

Alemayehu M and Aklilu M (2007). Measurements in Pasture and Forage Cropping Systems (Technical manual 18). EIAR. p.39.

AOAC (2005). Association of Official Analytical Chemists. Official Methods of Analysis , 18th Edition. Washington DC, Method 935.14 and 992.24.

Azuhnwi BN, Thomann B, Arrigo Y, Boller B, Hess HD, Kreuzer M and Dohme-Meier F (2012). Ruminal dry matter and crude protein degradation kinetics of five Sainfoin (Onobrychis viciifolia Scop.) accessions differing in condensed tannin content and obtained from different harvests. Animal Feed Science & Technology, 177(3-4): 135-143. https://doi.org/10.1016/j.anifeedsci.2012.08.004

Bal MA, Ozturk D, Aydin R, Erol A, Ozkan CO, Ata M, Eser k and Karabay P (2006). Nutritive Value of Sainfoin *(Onobrychis viciaefolia)* Harvested at Different Maturity Stages. Pp. 205-209. https://doi.org/10.3923/pjbs.2006.205.209

Bate-Smith EC (1973). Tannins of herbaceous leguminosae. Phytochemistry, 12(7): 1809-1812.

https://doi.org/10.1016/0031-9422(73)80409-1

Bezabih M, Pellikaan WF, Tolera A, Khan NA and Hendriks WH (2014). Chemical composition and in vitro total gas and methane production of forage species from the M id R ift V alley grasslands of E thiopia. Grass and Forage Science, 69(4): 635-643. http://dx.doi.org/10.1111/gfs.12091

Bhattarai S, Coulman B, Fu YB, Beattie AD and Biligetu B (2018). Genetic diversity and relationship of sainfoin *(Onobrychis viciifolia Scop.)* germplasm as revealed by amplified fragment length polymorphism markers. Canadian Journal of Plant Science, 98(3): 543-551. https://doi.org/10.1139/cjps-2017-0094

Bianco S, deCarvalho LB and Bianco MS (2011). Determination of alfalfa leaf area by non-destructive method. Communications in Plant Sciences, 1(2): 17-20. http://dx.doi.org/10.26814/cps201117

Biligetu B, Jefferson PG, Muri R and Schellenberg, MP (2014). Late summer forage yield, nutritive value, compatibility of warm- and cool-season grasses seeded with legumes in western Canada. Canadian Journal of Plant Science, 94(7): 1139-1148. https://doi.org/10.4141/cjps2013-269

Bueno ICS, Brandi RA, Fagundes GM, Benetel G and Muir JP (2020). The Role of Condensed Tannins in the In Vitro Rumen Fermentation Kinetics in Ruminant Species: Feeding Type Involved? Animals (Basel), 10(4): 635. https://doi.org/10.3390/ani10040635

Carleton AE, Cooper CS and Wiesner LE (1968). Effect of seed pod and temperature on speed of germination and seedling elongation of sainfoin *(Onobrychis viciaefolia Scop.).* Agronomy Journal, 60(1): 81-84. https://doi.org/https://doi.org/10.2134/agronj1968.00021962006000010026x

Chettri N, Sharma E and Thapa R (2009). Long Term Monitoring Using Transect and Landscape Approaches within Hindu Kush Himalayas. Proceedings On International Mountain Biodiversity Conference. International Center for Integrated Mountain Development(ICIMOD). Pp. 16-18, November, 2008.Kazmandu , Nepal.

Cupina B and Erid P (1999). The effect of sowing method and seeding rate on yield and quality of Sainfoin *(Onobrychis sativaL.)* forage. Scientia Agriculturae Bohemica (Czech Republic), 30(2):107-114. https://doi.org/https://cotswoldseeds.com/images/legumeplus/downloads/uns-ifvcns%20sci%20agric%20bohem%2030%20cupina%20&%20eric.pdf

Delgado I, Salvia J, Buil I and Andres C (2008). The agronomic variability of a collection of sainfoin accessions. Spanish Journal of Agricultural Research, 6(3): 401-407. https://doi.org/https://doi.org/10.5424/sjar/2008063-333

Gasmi-Boubaker A, Kayouli C and Buldgen A (2005). In vitro gas production and its relationship to in situ disappearance and chemical composition of some Mediterranean browse species. The in vitro Gas Production Technique: Limitations and Opportunities. Pp.123-124, 303-311. https://doi.org/10.1016/j.anifeedsci.2005.04.017

Getachew G, Crovetto GM, Fondevila M, Krishnamoorthy U, Singh B, Spanghero M, Steingass H, Robinson PH and Kailas MM (2002). Laboratory variation of 24 h in vitro gas production and estimated metabolizable energy values of ruminant feeds. 102: 169-180. https://doi.org/https://www.sciencedirect.com/science/article/pii/S0377840102002122

Getachew G, Makkar HP and Becker K (2000). Effect of polyethylene glycol on in vitro degradability of Nitrogen and microbial protein synthesis from tannin rich browse and herbaceous legumes. 84: 73-83. https://doi.org/https://pubmed.ncbi.nlm.nih.gov/10961163/

Goplen BP, Moyer JR and Richards KW (1991). Sainfoin for Western Canada. Communications Branch, Agriculture Canada. https://www.biodiversitylibrary.org/item/121280

Groot JCJ, Cone JW, Williams BA, Debersaques FMA and Lantinga EA (1996). Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. Animal Feed Science and Technology, 64(1): 77-89. https://doi.org/10.1016/S0377-8401(96)01012-7

Gugissa DA, Abro Z and Tefera T (2022). Achieving a Climate-Change Resilient Farming System through Push–Pull Technology: Evidence from Maize Farming Systems in Ethiopia. Sustainability, 14(5): 2648. https://www.mdpi.com/2071-1050/14/5/2648

Guglielmelli A, Calabro S, Primi R, Carone F, Cutrignelli M I, Tudisco R, Picolo G, Ronchi B and Danieli PP (2011). In vitro fermentation patterns and methane production of sainfoin *(Onobrychis viciifolia Scop.)* hay with different condensed tannin contents. Grass and Forage Science, 66(4): 488-500. https://doi.org/https://doi.org/10.1111/j.1365-2494.2011.00805.x

Hackmann TJ, Sampson JD and Spain JN (2008). Comparing relative feed value with degradation parameters of grass and legume forages1. Journal of Animal Science, 86(9): 2344-2356. https://doi.org/10.2527/jas.2007-0545

Hanna WW (1993). Improving forage quality by breeding. International crop science I. Pp. 671-675. https://doi.org/https://doi.org/10.2135/1993.internationalcropscience.c106

Hayot CC, Mueller-Harvey I, Brown T and Smith L (2011). Sainfoi*n (Onobrychis viciifolia):* A beneficial forage legume. Plant Genetic Resources, 9(1): 70-85. https://doi.org/doi:10.1017/S1479262110000328

Hume LJ and Withers NJ (1985). Nitrogen fixation in sainfoin *(Onobrychis viciifolia)* 1. Responses to changes in nitrogen nutrition. New Zealand Journal of AgriculturalResearch, 28(3): 325-335. https://doi.org/10.1080/00288233.1985.10430435

Huyen NT, Desrues O, Alferink SJJ, Zandstra T, Verstegen MWA, Hendriks WH and Pellikaan WF (2016). Inclusion of sainfoi*n (Onobrychis viciifolia)* silage in dairy cow rations affects nutrient digestibility, nitrogen utilization, energy balance, and methane emissions. Journal of Dairy Science, 99(5): 3566-3577. https://doi.org/10.3168/jds.2015-10583

Irani S, Majidi MM and Mirlohi A (2016). Genetic variation for clonal propagation and trait association with field performance in sainfoin. Tropical Grasslands-Forrajes Tropicales, 4(1):38-46. https://doi.org/10.17138/tgft(4)38-46

Jean-Blain C (1998). Nutritional and Toxicologial Aspects of Tanins. Journal of Veterinary Medicine, 149: 911-920. https://api.semanticscholar.org/CorpusID:89039320

Jeranyama P and Garcia AD (2004). Understanding Relative Feed Value (RFV) and Relative Forage Quality (RFQ)SDSU Extension Extra Archives. https://openprairie.sdstate.edu/extension_extra/352

Jones WT, Broadhurst RB and Lyttleton JW (1976). Condensed tannins of pasture legume species. Phytochemistry, 15: 1407-1409. https://doi.org/10.1016/S0031-9422(00)97128-0

Kaplan M (2011). Determination of otential nutritive value of Sainfoin *(Onobrychis viciifolia Scop.)* hays harvested at flowering stage. 10: 2028-2203. http://dx.doi.org/10.3923/javaa.2011.2028.2031

Kelman WM and Tanner GJ (1990). Foliar condensed tannin levels in lotus species growing on limed and unlimed soils in South-Eastern Australia. Proceedings of the New Zeabnd Grassland Association. P.52. https://doi.org/10.33584/jnzg.1990.52.1934

Khazaal K, Boza J and Ørskov ER (1994). Assessment of phenolics-related antinutritive effects in Mediterranean browse: a comparison between the use of the in vitro gas production technique with or without insoluble polyvinylpolypyrrolidone or nylon bag. Animal Feed Science and Technology, 49(1): 133-149. https://doi.org/10.1016/0377-8401(94)90087-6

Lagrange S and Villalba JJ (2016). 0609 Influence of forage diversity on feeding behavior and diet digestibility in lambs. Journal of Animal Science, 94(suppl_5): 289-289. https://doi.org/10.2527/jam2016-0609

Larbi A, Khatib-Salkin A, Jammal B and Hassan S (2011). Seed and forage yield, and forage quality determinants of nine legume shrubs in a non-tropical dryland environment. Animal Feed Science and Technology, 163(2): 214-221. http://dx.doi.org/10.1016/j.anifeedsci.2010.11.006

Lees GL, Hinks C F and Suttill NH (1994). Effect of high temperature on condensed tannin accumulation in leaf tissues of big trefoil (Lotus uliginosus Schkur). Journal of the Science of Food and Agriculture, 65(4): 415-421. https://doi.org/10.1002/jsfa.2740650408

Liu Z (2006). Developing a Modern Agronomy for Sainfoin *(Onobrychis viciifolia Scop.)* PhD Thesis, Coventry University in association with Royal Agricultural College]. P.182.

Makkar HPS (2003). Quantification of tannins in tree and shrub foliage: A laboratory Manual. Kluwer Academic Publishers, Sordrecht. Netherlands. Pp. 43-54.

McCallum MH, Kirkegaard JA, Green TW, Cresswell HP, Davies SL, Angus JF and Peoples MB (2004). Improved subsoil macroporosity following perennial pastures. Australian Journal of Experimental Agriculture, 44(3): 299-307. http://dx.doi.org/10.1071/EA03076

Mekonnen K, Bezabih M, Thorne P, Gebreyes MG, Hammond J and Adie A (2022). Feed and forage development in mixed crop–livestock systems of the Ethiopian highlands: Africa RISING project research experience. Agronomy Journal, 114(1):46-62. https://doi.org/10.1002/agj2.20853

Mekoya A, Oosting SJ, Fernandez-Rivera S and Van der Zijpp AJ (2008). Multipurpose fodder trees in the Ethiopian highlands: farmers' preference and relationship of indigenous knowledge of feed value with laboratory indicators. Agricultural System, 96(1-3): 184-194. https://doi.org/10.1016/j.agsy.2007.08.001

Mekuria W and Mekonnen K (2018). Determinants of crop–livestock diversification in the mixed farming systems: evidence from central highlands of Ethiopia. Agriculture and food security, 7:1-5. https://doi.org/10.1186/s40066-018-0212-2

Mole S, Ross JAM and Waterman PG (1988). Light-induced variation in phenolic levels in foliage of rain-forest plants : I. Chemical changes. Journal of chemical ecology, 14(1-21): 23-24. https://doi.org/10.1007/bf01022527

Niderkorn V, Baumont R, Le Morvan A and Macheboeuf D (2011). Occurrence of associative effects between grasses and legumes in binary mixtures on in vitro rumen fermentation characteristics. Journal of Animal Science, 89(4). 1138-1145.https://doi.org/10.2527/jas.2010-2819

Parker RJ and Moss BR (1981). Nutritional value of sainfoin hay compared with alfalfa hay. Journal of Dairy Science, 64: 206-210. https://doi.org/10.3168/jds.S0022-0302(81)82555-6

Patra AK and Saxena J (2010). A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry, 71(11): 1198-1222. https://doi.org/10.1016/j.phytochem.2010.05.010

Porter LJ, Hrstich LN, and Chan BG (1985). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry, 25: 223-230. https://doi.org/10.1016/S0031-9422(00)94533-3

Roberts CA, Beuselinck PR, Ellersieck, MR, Davis DK and McGraw RL (1993). Quantification of tannis in birdsfoot trefoil germplasm. Crop Science, 33(4): 675-679. https://doi.org/10.2135/cropsci1993.0011183X003300040005x

Salem AZM, Salem MZM, El-Adawy MM and Robinson, PH (2006). Nutritive evaluations of some browse tree foliages during the dry season: Secondary compounds, feed intake and in vivo digestibility in sheep and goats. Animal Feed Science and Technology, 127(3): 251-267. https://doi.org/10.1016/j.anifeedsci.2005.09.005

Santoso B, Mwenya B, Sar C and Takahashi J (2007). Methane production and energy partition in sheep fed timothy silage-or hay-based diets. Jurnal Ilmu Ternak dan Veteriner, 12(1): 27-33. https://doi.org/10.14334/JITV.V12I1.561

SAS, Analytical software. Statistical Analysis System for widows version 9.3, SAS Institute Inc., Cary, NC, USA, 2010.

Scharenberg A, Arrigo Y, Gutzwiller A, Soliva CR, Wyss U, Kreuzer M and Dohme F (2007). Palatability in sheep and in vitro nutritional value of dried and ensiled sainfoin (Onobrychis viciifolia) birdsfoot trefoil *(Lotus corniculatus),* and chicory (Cichorium intybus). Archives of Animal Nutrition, 61(6): 481-496. https://doi.org/10.1080/17450390701664355

Shah MH, Singh KN, Kachroo D and Khanday BA (1991). Performance of lucerne and sainfoin under different cuttings and levels of phosphorus. Indian Journal of Agronomy, 36(1): 61-66. https://library.iut.ac.ir/dL/search/default.aspx?Term=1944&Field=0&DTC=104

Tarawali SA (1995). Methods for the Evaluation of Forage Legumes, Grasses and Fodder Trees for Use as Livestock Feed (Vol. 1). ILRI (aka ILCA and ILRAD). https://hdl.handle.net/10568/481

Tavendale MH, Meagher LP, Pacheco D, Walker N, Attwood, GT and Sivakumaran S (2005). Methane production from in vitro rumen incubations with *Lotus pedunculatus and Medicago sativa, a*nd effects of extractable condensed tannin fractions on methanogenesis. The in vitro Gas Production Technique: Limitations and Opportunities, 123-124: 403-419. https://doi.org/10.1016/j.anifeedsci.2005.04.037

Theodoridou K, Aufrère J, Niderkorn V, Andueza D, Le Morvan A, Picard F and Baumont R (2011). In vitro study of the effects of condensed tannins in sainfoin on the digestive process in the rumen at two vegetation cycles. Animal Feed Science and Technology, 170(3): 147-159. https://doi.org/10.1016/j.anifeedsci.2011.09.003

Thomson JR (1951). Seed studies in sainfoin. Journal of the British Grassland Society, 6(3):147-159. https://doi.org/10.1111/j.1365-2494.1951.tb00923.x

Tilley JMA and Terry RA (1963). A two-stage technique for in vitro digestion of forage crops. Journal of British grasss land Society, 18(2): 104-111. https://doi.org/https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2494.1963.tb00335.x

Tolera A, Khazaal K and Ørskov ER (1997). Nutritive evaluation of some browse species. Animal Feed Science and Technology, 67(2): 181-195. https://doi.org/10.1016/S0377-8401(96)01119-4

Tufenkci S, Erman M and Sonmez F (2006). Effects of phosphorus and nitrogen effects of phosphorus and nitrogen applications and rhizobium inoculation on the yield and nutrient uptake of sainfoin *(Onobrychis viciifolia L.)* under irrigated conditions in Turkey. NewZealand Journalof Agricultural Research, 49(1): 101-105. https://doi.org/doi: 10.1080/00288233.2006.9513699

Turk M, Albayrak S, Tuzun CG and Yuksel O (2011). Effects of fertilisation and harvesting stages on forage yield and quality of sainfoin *(Onobrychis sativa L.).* Bulgarian Journal of Agricultural Science, 17(6): 789-794. https://www.agrojournal.org/17/06-11-11.pdf

Valentin SF, Williams PEV, Forbes JM and Sauvant D (1999). Comparison of the in vitro gas production technique and the nylon bag degradability technique to measure short- and long-term processes of degradation of maize silage in dairy cows. Animal Feed Science and Technology, 78(1): 81-99. http://www.sciencedirect.com/science/article/pii/S0377840198002624

Van Soest PJ, and Robertson JB (1985). A laboratory Manual for Animal Science. Cornell University, Ithaca, New York, USA.

Van Soest PJ, Robertson JB and Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74(10): 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Waghorn GC, Reed JD and Ndlovu LR (1999). Condensed tannins and herbivore nutrition. Proceedings of the XVIIth International Grasslands Congress, 3:153-156.

Wang Y, McAllister TA and Acharya S (2015). Condensed tannins in sainfoin: composition, concentration, and effects on nutritive and feeding value of sainfoin forage. Crop Science, 55(1): 13-22. http://dx.doi.org/10.2135/cropsci2014.07.0489

Ward R and Ondarza MB (2008). Relative feed value (RFV) vs. relative forage quality (RFQ). Cumberland Valley Analytical Services. Inc., Hagerstown. MD. p. 6. https://www.foragelab.com/Media/RFV_vs_RFQ-CVAS%20Perspective.pdf

Yildiz B, Çiplak B and Aktoklu E (1999). Fruit morphology of sections of the genus *Onobrychis Miller* (Fabaceae) and its phylogenetic implications. Israel Journal of Plant Sciences, 47(4): 269-282. http://dx.doi.org/10.1080/07929978.1999.10676784

Yordanka N and Viliana V (2015). Forage quality analysis of perennial legumes-subterranean clover mixtures. Science International, 3: 113-120. https://doi.org/https://scialert.net/abstract/?doi=sciintl.2015.113.120

Zafu A, Tolera A, Kochero Y and Nurfeta A (2020). Chemical composition, In vitro gas production and tannin biological activity of *Prosopis juliflora* pods varying with harvesting season. Ethiopian Journal of Applied Science and Technology, 11(1): 36-42.

2024 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 14, Issue 1: 185-195; May 25, 2024

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2024.22

EFFECTS OF MANGO (*Mangifera indica*) LEAVES POWDER ON REPRODUCTIVE HORMONES, OXIDATIVE STRESS MARKERS, TOXICITY INDICATORS, GROWTH AND CARCASS CHARACTERISTICS OF GUINEA PIG (*Cavia porcellus*)

Chongsi Margaret MARY MOMO[1], Tchoffo HERVÉ[1], Mahamat Tahir MARKHOUS ADAM[1,4], Noubouowo Chouake ARTHUR STELLA[1], Azafack Kana DORICE[2], Mohamadou ADAMOU[1,3], Bend Emma FORTUNE MAGLOIRE[1], Deutcheu Nienga SORELLE[2], Dongmo Nguedia ARIUS BAULLAND[1], and Ngoula FERDINAND[1]

[1]*Department of Animal science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 188, Cameroon*
[2]*Department of Animal Science, Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon*
[3]*Institute of Agricultural Research, Mbalmayo Agricultural Research Centre, Yaounde, Cameroon*
[4]*Department of Biology, Faculty of Science and Techniques, University of Adam Barka, Abéché, Chad*

Email: margaretchongsi@yahoo.fr

Supporting Information

RESEARCH ARTICLE

PII: S222877012400022-14

Received: February 18, 2024

Revised: May 12, 2024

Accepted: May 18, 2024

ABSTRACT: The present work aimed to evaluate the effects of *Mangifera indica* leaf powder (MLP) on reproductive hormones, biomarkers of oxidative stress and toxicity, growth and carcass characteristics of female *Cavia porcellus*. 40 female guinea pigs (*Cavia porcellus* L.) aged 2 months with an average weight of 257.65±11.28 g were used. These guinea pigs were weighed, then randomly divided into 4 groups of 10 animals each and subjected to the following rations: T0 (basic diet), T1, T2 and T3 (basic diet + 0.50; 0.75 and 1% MLP, respectively). For 45 days, the guinea pigs of all groups received *ad libitum* drinking water and the corresponding experimental rations. Reproductive hormones analyses showed a significant (P<0.05) rise in serum progesterone concentration in the highest dose of MLP-treated guinea pigs. Malondialdehyde level was lowest in guinea pigs given feed with the highest dose of MLP while superoxide dismutase and catalase activities were maximum in animals that received the highest doses of MLP. Total Peroxidase activity was greatest in animals fed with the higher MLP dose. The lowest level of cholesterol was noticed in the group that received the greatest dose of MLP. Feed consumption was higher in guinea pigs receiving 0.75% and 1% MLP. The body weight gain and average daily gain had higher values in the subjects fed with MLP feed than those of the control group. Feed efficiency values were improved in animals that that were given 0.5% and 1% MLP with regards to those fed without MLP, the length of small intestine was higher (P<0.05) in 0.75% MLP-treated animals than the other groups, while the greatest value for the density of big intestine was recorded in those that received 0.25% MLP. Based on these values (1% of diet as optimum level), MLP can be used in feed to improve animal production performance.

Keywords: Antioxidant, Growth, *Mangifera indica*, Oxidative stress, Reproductive hormones.

Abbreviations: MLP: mango leaf powder; **FR**: Free radicals; **ROS**: reactive oxygen species; **OS**: oxidative stress; **AO**: antioxidants; **FC**: feed consumption; **WG:** weight gain; **ADG**: average daily gain; **CI**: consumption index; **FE**: Feed efficiency; **CY**: carcass yield; **Ur**: urea; **Cr**: creatinine; **TC**: total cholesterol; **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase; **E2**: estradiol; **CAT**: Catalas; **SOD**: Superoxide dismutase; **POX**: Total peroxidase; **MDA**: malondialdehyde.

INTRODUCTION

Food security in general and meeting the needs in protein particularly, of populations is a great challenge for developing countries (Noumbissi et al., 2013; Miégoué et al., 2018c). In fact, population growth increases the need for proteins especially animal proteins. However, despite measures put to place by the governments, supplies are still not sufficient to meet demands not only at national but also at international levels (FAO, 2014). Small scale farming, especially caviar farming, seems to be another way to meet the needs in proteins from animals while preserving this animal resource at the time (Miégoué et al., 2018a). This farming is easy to practice and is also an important income source for Cameroonians (Miégoué et al., 2018b). Despite the many advantages of guinea pig, its production is still low. These animals like other are affected by endogenous and exogenous factors that cause oxidative stress (OS) which leads to a decline in productivity thereby leading to financial losses. Increasing genetic selection for maximum growth rate, and commercial animal agriculture conditions can also introduce oxidative stress which hinders health, optimum growth and reproduction, welfare as well as the quality of the product concerned (Mishra and Jha, 2019; Esposito et al., 2021; Ogawa et al., 2024).

Free radicals (FR) and reactive oxygen species (ROS) are molecules produced naturally from metabolic reactions in living organisms. They play some useful roles in the body like destroying bacteria and viruses. However, when overproduced they can cause OS in the body (Arts and Hollman, 2005).

Citation: Mary Momo CM, Hervé T, Markhous Adam MT, Arthur Stella NC, Dorice AK, Adamou M, Fortune Magloire BE, Sorelle DN, Arius Baulland DN, and Ferdinand N (2024). Effects of mango (*Mangifera indica*) leaves powder on reproductive hormones, oxidative stress markers, toxicity indicators, growth and carcass characteristics of guinea pig (*Cavia porcellus*). *Online J. Anim. Feed Res.*, 14(3): 185-195. DOI: https://dx.doi.org/10.51227/ojafr.2024.22

Various biomolecules have been shown to have the capability of hindering or slowing down OS in foods and living organisms. These molecules have the ability to donate their hydrogen atom in order to stabilize FR or ROS. These biomolecules are generally known as antioxidants (AO) (Djikeng et al., 2017). There are two different types of AO: synthetic and natural antioxidants. However, synthetic AO have side effects on human health, so the search for natural AO sources that can be used to slow down OS in organisms and extend the shelf-life of foods has been extensively studied (Womeni et al., 2016). Natural antioxidants in plants are mainly phenolic compounds like phenolic acids, flavonoids, tannins, carotenoids, anthocyanin, etc. (Habermann et al., 2016). Mango (*Mangifera indica*) belonging to Anacardiaceae family is an important tree species with many medicinal properties. It is famous for its fruits which are very juicy and rich in vitamin C. Its leaves also have many benefits. In fact, they are rich in compounds like phenols, alkaloids, phytosterols, tannins, triterpenoids, flavonoids and saponins. At the traditional level, mango leaves are used for the treating many diseases like pneumonia, diabetes, colds and fever (Adjanohoun and Dramane, 1993).

Its bioactive compound mangiferin has been shown to possess good antioxidant activities against oxidative stress (Shah et al., 2010); It possesses many health benefits like antibacterial, antiviral, anti-inflammatory, anticancer, antidiabetic, antioxidant, antiaging, immunomodulatory, hepatoprotective, antiparasitic and analgesic effects (Dar et al., 2005).

According to the above information, the leaves of *Mangifera indica* could be used as potential natural source of antioxidant, to reduce oxidative stress effects in guinea pigs thus improving health and consequently, reproductive and growth performances as well as meat quality (Djikeng et al., 2017). However, the activities of plant substances vary with doses, means of administration, form and part of plant, animal material, etc which lead to variations in results from their use. So far, results on the use of mango leaves powder as supplements in guinea pig feed are very rare.

It is within this framework that this study was initiated with the general goal of contributing to the promotion of the productivity of guinea pigs.

MATERIALS AND METHODS

Animal material and housing

A number of 40 female guinea pigs (*Cavia porcellus L.*) aged 2 months with an average weight of 257.65±11.28 g were used for this study. The animals were produced in a farm in the city of Dschang. Throughout the trial period, the latter were housed in the caviaculture building of the Teaching and Research Farm of the University of Dschang. This building was equipped with rectangular boxes, made of plywood mounted on the floor, equipped with a lighting device and mesh on top. Each of these boxes measuring 100 cm x 80 cm x 60 cm (length x width x height) was lined with litter of wood shavings, renewed every 3 days and equipped with a feeder (60 cm long, 10 cm wide and 5 cm deep) and a drinker (50 cl).

Feeding

Four experimental rations (T0, T1, T2 and T3) were formulated. Throughout the trial period, the guinea pigs of all groups received *ad libitum* drinking water and a compound feed whose ingredients were purchased from a local market. The percentage composition and the calculated bromatological characteristics of this ration are presented in the table 1.

Table 1 - Percentage and chemical composition of the compound feed

Ingredients (kg)	Quantity
Maize	22
Trypsacum laxum	26
Soybean cake	04
Cotton seed cake	03
Palm kernel cake	09
Fish meal	08
Bone meal	01
Wheat bran	22
Premix 2% *	02
Shell	01
Molasses	02
Total	100
Chemical composition of rations	
Digestible energy (kcal/kg DM)	2,803
Dry matter (%)	89.56
Organic matter (%DM)	75.14
Crude protein (%DM)	17.82
Gross cellulose (%DM)	17.60
Ash (%DM)	24.86
Lipids (%DM)	1.47

*Premix 2%: Vit. A=3000000 IU/kg, Vit. D3=600000 IU/kg, Vit. E=4000mg/kg, Vit. K3=500mg/kg, Vit. B1=200mg/kg, Vit. B2=1000mg/kg, Vit. B3=2400mg/ Biotin=10mg/kg, Vit. PP=7000mg/kg, Folic acid=200mg/kg, Choline chloride=10000mg/kg, Iron sulfate=8000mg/kg, Copper(II) sulphate=2000mg/kg, Mangano oxide=1400mg/kg, Calcium iodate=200mg/kg, Basic cobalt carbonate=200mg/kg, Sodium selenite=20mg/kg, Methionine=20000mg/kg, Lysine=78000mg/kg,

Plant material

The mango (*Mangifera indica*) leaves used were harvested from the same tree located in the locality of Dschang. They were cleaned and dried in an oven (45°C) to constant weight and crushed in a mill to obtain a homogeneous powder, used for the preparation of the various experimental rations.

Experimental procedure

The forty guinea pigs used in this trial were weighed using a scale with a capacity of 5 kg and a sensitivity of 1 g, then randomly divided into 4 groups, namely: T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder). These groups were comparable in terms of body weight. Each of the guinea pigs was marked with an earring bearing its code and considered as an experimental unit. The quantities of feed served and refusals were weighed daily, while the animals were weighed individually every 7 days. After 45 days of treatment, the guinea pigs of each group were sacrificed and eviscerated, then weighed for the evaluation of carcass yields 1 and 2.

Parameters studied and data collection

Evaluation of growth performance

Feed consumption: The feed was weighed and distributed daily (every morning between 8 am and 9 am), then the refusals of each treatment were weighed daily using an electronic scale with a capacity of 5 kg and an accuracy of 1 g. Subsequently, the feed consumption (FC) of the guinea pigs was determined by taking the difference between the quantity of feed served (Qs) and the refusals (Qr) of the period considered. FC = Qs – Qr

Evolution of weight: The animals were weighed at the start of each week using an electronic scale with a capacity of 5 kg and a precision of 1 g until the end of the trial.

Weight gain (WG): The weight gain of the guinea pigs was obtained by taking the difference between the live weight at the week considered (Pn) and that of the previous week (Pn-1). WG = Pn - Pn-1

Average daily gain (ADG): The average daily gain was obtained by relating the weight gain (WG) to the period considered in days. ADG = Weight gain/Duration (in days)

Consumption index: The consumption index (CI) was determined by calculating the ratio between the quantity of feed consumed during a period and the weight gain of the same period. CI= Feed consumption/Weight gain

Feed efficiency: Feed efficiency (FE) was determined by relating weight gain to the amount of feed consumed over a period. FE = Weight gain/Feed consumption

Assessment of carcass characteristics

Carcass yield 1

To obtain carcass yield 1 (CY1), the 40 guinea pigs were fasted for 24 hours in order to empty the gastrointestinal contents, then sacrificed and eviscerated. The internal organs were then removed and the carcass weighed and expressed as a percentage of live weight:

CY1 (%) = (Whole Gutted Carcass Weight) / (Animal Live Weight) × 100

Carcass yield 2

As for carcass yield 2 (CY2), in addition to evisceration, the skin, the head and the ends of the legs of the guinea pigs were removed: CY2 (%) = (Weight of the dressed and eviscerated carcass) / (Live weight of the animal) × 100

Relative weight of organs

The relative weight or proportion of the removed organs (skin, head, legs, liver, kidney, caecum) was calculated in relation to the live weight at slaughter according to the following formula:

Relative organ weight (%) = (Organ weight (g))/ (Live weight at slaughter (g)) × 100

Biochemical and hormonal analyses

Samples of blood were obtained using cardiac puncture and were collected without anticoagulant for biochemical dosages and with anticoagulant (EDTA) for complete blood count. Biochemical parameters analyzed from serum were urea (Ur), creatinine (Cr), total cholesterol (TC), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) performed using appropriate commercial Chronolab kit. Progesterone and estradiol (E2) were measured in serum with the use of AccuDiagTM ELISA kits from OMEGA DIAGNOSTICS LTD (Scotland, England).

Oxidative stress markers

Catalase (CAT), Superoxide dismutase (SOD), Total peroxidase (POX) activities and concentration of malondialdehyde (MDA) were measured with the use of protocols proposed by Aebi (1984), Misra and Fridovich (1972), Moron et al. (1979) and Botsoglou et al. (1994), respectively.

Statistical analyzes of data

The data obtained at the end of this work, were subjected to the analysis of the variance as a factor, to test the effects of the various rates of incorporation of the powder of leaves of *Mangifera indica* on the studied parameters in test-subjects. Waller Duncan's test was used to separate the means in case of significant difference. The significance threshold was set at 5% for these different tests. Results were expressed as mean ± standard deviation and SPSS 26.0 software was used for these analyses.

RESULTS

Effects of MLP on reproductive hormones in female guinea pigs

Serum concentration of estradiol

The effects of MLP on serum concentration of estradiol in female guinea pigs are shown in figure 1. It was observed that the concentration of estradiol in serum in the groups that received MLP was comparable (P>0.05) to that of the control group for those that received 0.75% and 1%. However, the animals that received 0.5% of MLP recorded a significantly lower serum concentration of estradiol with respect to the control guinea pigs.

Serum concentration of progesterone

Figure 2 demonstrates the eeffects of MLP on serum concentration of progesterone in female guinea pigs. It appears that, a significant (P<0.05) rise in serum progesterone concentration was registered in guinea pigs fed with the diet containing the highest dose of MLP as compared to the control animals.

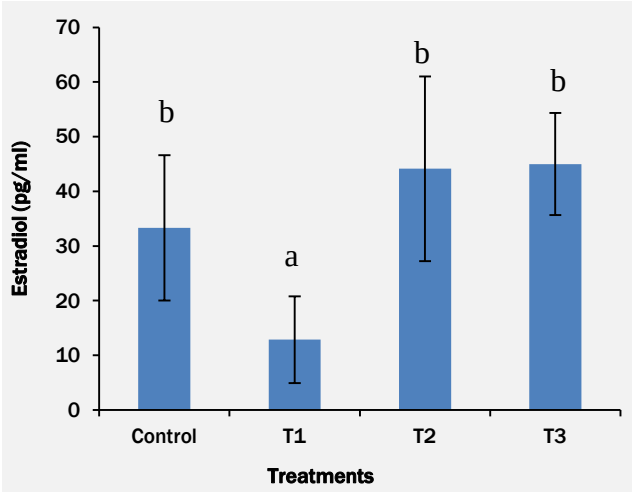


Figure 1 - Effects of MLP in the diet on serum concentration of Estradiol in female guinea pigs. a, b: bars affected with the same letter do not differ significantly (P>0.05). T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder).

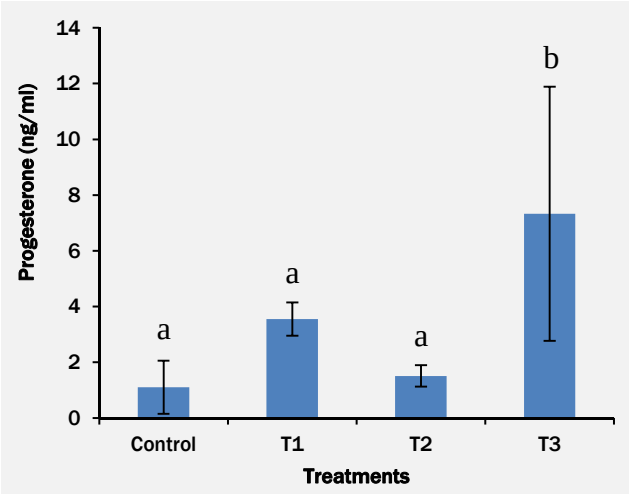


Figure 2 - Effects of MLP in the diet on serum concentration of progesterone in female guinea pigs. a, b: bars affected with the same letter do not differ significantly (P>0.05). T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder).

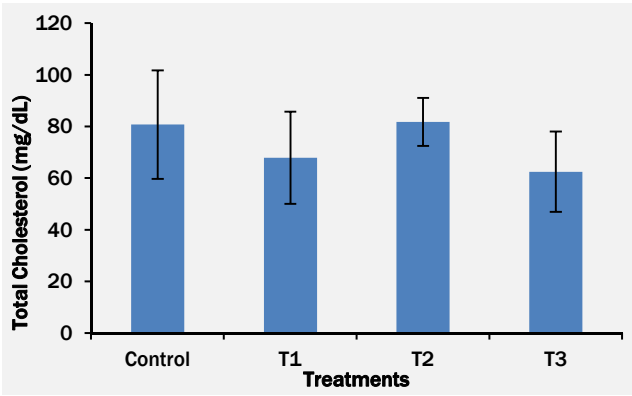


Figure 3 - Effects of MLP in the diet on cholesterol level in female guinea pigs. T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder).

Table 2 - Effects of MLP in the diet on oxidative stress biomarkers in female guinea pigs

Oxidative stress biomarkers	Control (n=10)	Doses *Mangifera indica* leaf powder			*P*-value
		T1 (n=10)	T2 (n=10)	T3 (n=10)	
MDA (µM)	1.31±0.15	1.86±0.92	1.51±0.90	0.96±0.87	0.36
CAT (µM/min/g PT)	3.13±1.06	2.73±0.77	2.39±1.06	3.22±1.99	0.73
SOD (U/min/g PT)	0.47±0.13	0.40±0.09	0.46±0.20	0.57±0.25	0.54
POX (mM/min/g PT)	43.08±3.60^{ab}	46.71±9.74^{ab}	53.78±6.42^{b}	41.95±10.41^{a}	0.04

a, b: values with the same letter per row are not significantly different (P> 0.05), n: number of animals per group, MDA: Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase; POX: Total Peroxidase; PT: T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder), Total proteins.

Table 3 - Effects of MLP in the diet on kidney weight and volume and biochemical markers of nephrotoxicity in female guinea pigs

Toxicity indicators	Control (n=10)	Doses *Mangifera indica* leaf powder			*P*-value
		T1 (n=10)	T2 (n=10)	T3 (n=10)	
Relative weight of the kidney (%)	0.40±0.14	0.40±0.05	0.40±0.08	0.41±0.02	0.99
Volume of the kidney (dm^3)	1.37±0.23^{ab}	1.20±0.19^{a}	1.27±0.15^{ab}	1.45±0.05^{b}	0.04
Creatinine (mg/dL)	0.96±0.24^{a}	0.93±0.16^{a}	1.57±0.40^{b}	1.21±0.05^{a}	0.001
Urea (mg/dL)	58.79±12.91	47.36±11.85	47.12±8.85	42.60±12.20	0.19

a, b: values with different letter per row are significantly different (P<0.05), n: number of animals per group, T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder).

Table 4 - Effects of MLP in the diet on liver weight and volume and biochemical markers of hepatotoxicity in female guinea pigs

Toxicity indicators	Control (n=10)	Doses *Mangifera indica* leaf powder			*P*-value
		T1 (n=10)	T2 (n=10)	T3 (n=10)	
Relative weight of the liver (%)	2.71±1.07	2.41±0.29	2.53±0.30	2.64±0.16	0.85
Volume of the liver (dm^3)	7.67±1.53	7.67±0.50	7.40±0.69	8.67±0.58	0.18
ALT (U/L)	60.06±18.57	72.28±16.31	76.82±19.14	78.57±11.91	0.33
AST (U/L)	78.57±12.11	87.47±14.15	81.54±20.44	76.65±15.06	0.72

AST: aspartate aminotransferase, ALT: alanine aminotransferase, n: number of animals per group. T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder).

Effects of MLP on biomarkers of oxidative stress in female guinea pigs

It is remarked from table 2 that, whatever the marker of oxidative stress taken into consideration, no statistical (P>0.05) difference was reported among the mango leaf powder-treated groups and the control. Nevertheless, malondialdehyde level was lowest in guinea pigs given feed with the highest dose of mango leaf powder. Also, superoxide dismutase and catalase activities were maximum in animals that received the highest doses of MLP in comparison with the other groups. Similarly, total Peroxidase activity was greatest in animals fed with the higher MLP dose, with respect to the other groups of animals.

Effects of the incorporation of MLP in the diet on toxicity indicators in female guinea pigs

Kidney weight and volume and biochemical markers of nephrotoxicity in female guinea pigs

The effects of MLP on the weight and volume of kidney and biochemical markers of nephrotoxicity in female guinea pigs is represented in table 3. The kidney weight and volume as well as urea level recorded no significant (P>0.05) difference in comparison to the control group. Likewise, creatinine levels in treated groups were comparable to those of the control except at the dose of 0.75% MLP which showed a significant (P<0.05) increase with respect to the control group.

Weight and volume of liver and biochemical markers of hepatotoxicity in female guinea pigs

The weight and volume of liver and biochemical markers of hepatotoxicity in female guinea pigs are summarized in table 4. No significant (P>0.05) difference was noticed in liver weight and volume, as well as AST and ALT whatever the dose, when compared with the control group.

Total cholesterol in female guinea pigs

Figure 3 illustrates the effects of MLP in diet on cholesterol level in female guinea pigs. The lowest level of cholesterol was noticed in the group that received the greatest dose of MLP, though the value was not statistically (P>0.05) different from that of the control animals.

Effects of the incorporation of MLP in the diet on growth parameters in guinea pigs

Table 5 presents the variation in the characteristics of growth in the guinea pig from the start and the end of the trial according to the levels of MLP in the different rations. It appears that the growth characteristics of the different treatments are all comparable (P>0.05). Despite this similarity in all values, feed consumption was higher in guinea pigs receiving 0.75% and 1% MLP as compared to the control. The body weight gain and average daily gain had higher values in the subjects fed with MLP feed than those of the control group. In addition, we note that feed efficiency values were improved in animals that that were given 0.5% and 1% MLP with regards to those fed without MLP.

Characteristics of carcass and relative weights of some organs in the guinea pig according to the different rates of incorporation of the powder of the mango leaves

Table 6 shows the carcass characteristics and relative weights of some organs in guinea pigs according to the different rates of incorporation of MLP. It appears that the different treatments had no significant effect on these different characteristics when compared with the control group. However, it is noted that the carcass yields 1 and 2 as well as relative weight of big intestine and caecum were lower in guinea pigs having received 0.75% MLP in the ration. In addition, the length of small intestine was higher (P<0.05) in 0.75% MLP-treated animals than the other groups, while the greatest value for the density of big intestine was recorded in those that received 0.5% MLP.

Table 5 - Variation of growth characteristics according to different doses of MLP in guinea pigs

Toxicity indicators	Control (n=10)	Doses *Mangifera indica* leaf powder			*P*-value
		T1 (n=10)	T2 (n=10)	T3 (n=10)	
Initial body weight (g)	250.6±36.88	247.6±61.60	249.4±62.25	251.8±69.58	1.00
Final body weight (g)	319.8±52.54	339.6±53.52	319.8±42.99	339.6±40.09	0.83
Feed consumption (g)	809.2	807.4	892.2	835.4	–
Body weight gain (g)	69.2±27.59	92.0±13.08	70.4±21.63	87.8±32.06	0.37
Daily body weight gain (g)	1.98±0.79	2.63±0.39	2.01±0.62	2.51±0.92	0.37
Feed conversion ratio	13.34±5.28	8.93±1.31	13.83±4.80	10.79±4.45	0.28
Feed efficiency	0.09±0.03	0.11±0.02	0.07±0.02	0.11±0.04	0.25

n: number of animals per group, T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder), / (absence of P value since feed consumption was per group and not individually, thus no standard deviation)

Table 6 - Carcass characteristics and relative weights of some organs in guinea pigs according to the different rates of incorporation of mango leaf powder.

Toxicity indicators	Control (n=10)	Doses *Mangifera indica* leaf powder			*P*-value
		T1 (n=10)	T2 (n=10)	T3 (n=10)	
Carcass yield 1 (%)	70.81±3.99^{b}	63.80±3.60^{a}	64.77±3.68^{a}	67.51±3.90^{ab}	0.04
Carcass yield 2 (%)	41.88±5.37^{b}	36.67±3.65^{ab}	36.06±2.40^{a}	37.85±2.69^{ab}	0.04
Relative weight of the fifth quarter (%)	28.93±2.45	27.14±0.84	28.72±2.87	29.66±1.36	0.30
Relative weight of the stomach (%)	4.16±0.95	3.73±0.62	3.05±0.84	3.87±1.38	0.36
Relative weight of the big intestine (%)	4.15±1.09^{bc}	4.76±0.56^{c}	3.07±0.37^{a}	3.59±0.30^{ab}	0.00
Relative weight of the small intestine (%)	4.22±1.48^{ab}	3.71±0.85^{ab}	2.94±0.36^{a}	4.28±0.56^{b}	0.04
Relative weight of the caecum (%)	12.14±3.07^{b}	9.86±1.82^{ab}	7.20±1.66^{a}	8.84±1.43^{a}	0.01
Length of the big intestine (cm)	69.03±12.44	72.90±3.57	68.90±2.12	77.57±1.10	0.16
Length of the small intestine (cm)	134.67±5.35^{a}	141.43±3.90^{a}	152.33±7.09^{b}	141.80±4.27^{a}	0.00
Density of the big intestine (g/cm)	18.99±1.34^{c}	22.37±0.38^{d}	13.93±0.85^{a}	15.79±0.52^{b}	0.00
Density of the small intestine (g/cm)	9.64±1.53^{b}	8.99±1.74^{b}	6.03±0.30^{a}	10.31±1.24^{b}	0.00

a, b, c, d: values with the same letter per row are not significantly different (P> 0.05), n: number of animals per group, T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder).

DISCUSSION

Animals are exposed to oxidative stress on daily bases from both internal and external sources. This can cause general health problems which can hinder their reproduction and growth as well as the quality of animal products. Antioxidants are known to fight against the undesirable effects of oxidative stress. A very important function is carried out by the hypothalamic-pituitary-gonadal (HPG) axis in controlling reproduction. The function of oestrous cycle is under the direct control of the pituitary and ovarian hormones; Follicle stimulating hormone (FSH), estrogen, pregesterone, and Luteinizing hormone (LH) generally increase during the oestrus phase of the cycle (Campbell, 2009).

Progesterone and estradiol are steroid hormones naturally associated with female fertility and pregnancy (Henderson, 2018). The synthesis of steroid starts with the conversion of cholesterol to pregnenolone that is then converted to steroid hormones (Payne and Hales, 2004). FSH will stimulate folliculogenesis, thus increasing the level of estradiol which is secreted by the ovaries using cholesterol. Saponins under the influence of cAMP, activates LH receptors of the theca interna of the ovarian follicles to convert cholesterol into androstenedione which in turn becomes estrogens making female animals to go into heat (Ndam, 2023).

Progesterone concentration begins to increase during proestrus and reaches its peak during ovulation. After ovulation, progesterone works synergistically with oestrogen to inhibit gonadotropin secretion. Estradiol concentration increases during met-oestrus, peaks at proestrus and drops at oestrus (Joffe et al., 2020; Yaseen et al., 2023).

The results of the present work demonstrated that the concentration of estradiol in serum in the groups that received MLP was comparable to that of the control group for those that received 0.5% and 0.75%. However, the animals that received 0.25% of MLP recorded a significantly lower serum concentration of estradiol with respect to the control guinea pigs. On the other hand, a significant rise in serum progesterone concentration was registered in guinea pigs fed with the diet containing the highest dose of MLP as compared to the rest.

These results agree with those obtained by Djuissi et al. (2021) in female guinea pigs fed with ethanolic extract of *Dichrostachys glomerata* fruit; Bafor et al. (2015) in mice receiving orally, methanolic extract of *Alchornea laxiflora* leaf a dose of 1000 mg/kg for 6 consecutive days as well as Yakubu et al. (2008) in female rats treated for 7 consecutive days with *Cnidoscolous aconitifolius*. This can be attributed to the presence of some bioactive molecules like alkaloids and phenols in their extracts, which are also found in mango leaves and protect *corpus luteum* and placenta from reactive oxygen species attacks, and subsequently favor the growth and function of the cells.

However, they are in disagreement with the findings of Funmileyi et al. (2013) who obtained an increase in estradiol level in non- pregnant female rats treated with aqueous leaf extract of *Mangifera indica* with no difference in progesterone level among all groups. Levels of creatinine, urea and cholesterol as well as the hepatocellular enzymes (AST, ALT) are used to evaluate liver and kidney function. A decrease or increase from standard values in their activities in the liver and kidney could be expected to take place as associated to the pathology involving necrosis of theses organs. Rahman et al. (2001) suggested that the decrease in these parameters might show the stressed conditions of the treated animals.

However, the present study recorded no significant variation among the mango leaves powder-treated groups and the control except for creatinine level at the medium dose for these biochemical toxicity indicators. These results are in line with the report of Djuissi et al. (2021) in female guinea pigs fed with ethanolic extract of *Dichrostachys glomerata* fruit at doses of 50, 100 and 200mg/kg BW. The present study indicated a decreased level of urea and an increased level of creatinine which was in agreement with findings of Kothari et al. (2014) and Ebile et al. (2018). This observation can be directly related to the hepato-protective as well as nephron-protective activities of mango leaves powder.The obtained results of the present study could have resulted from protein metabolism which could make some changes in kidney cell function.

The changes in oxidative stress biomarkers have been reported to be an indicator of tissue's ability to cope with oxidative stress (Mansour et al., 2009). The antioxidant enzyme catalase (CAT) acts as defence against free radicals. It is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water. Glutathione (GSH) is normally present in millimolar concentrations in cells and is known to protect the cellular system against the toxic effects of lipid peroxidation. It is very important in maintaining cellular redox status (Rao and Shaha, 2001) and its depletion is considered as a marker of oxidative stress (Lu, 1999). The decreased superoxide dismutase (SOD) activity may lead to massive production of superoxide anion. The production of such anions overrides enzymatic activity and leads to a fall in its concentration in renal tissue.

Although statistically comparable, the level of malondialdehyde was lowest in guinea pigs given feed with the highest dose of MLP. Also, superoxide dismutase, catalase and total peroxidase activities were maximum in animals that received the highest doses of MLP in comparison with the other groups. Meanwhile, the decreased level of MDA levels can express the cell-protective effects of MLP. These results agree with those obtained by Kuate et al. (2010) who analyzed the in vitro antioxidant activity of *D. glomerata* extracts. In fact, the phytochemical screening of *Mangifera indica* leaves powder before the start of this experiment revealed the presence of alkaloids, saponins, phenolics, tannins, flavonoids, and triterpenes which have been recognized to have antioxidant properties (Sen et al., 2010).

The results of the present study showed that the growth characteristics considered were comparable in all the treatments though improved values were recorded for animals that received MLP. These results are in agreement with those obtained by Chongsi et al. (2023) who after administration of *Mangifera indica* leaf powder in Brahma hens obtained a non-significant increase in feed consumption, body weight gain, average daily gain, feed conversion and feed efficiency. Likewise, Pasupathi et al. (2020) reported no significant difference for these characteristics in rabbits fed with *Mangifera indica* leaves (25 %). However, the feed intake disagrees with Arthenice et al. (2019) who registered a lower feed intake in male guinea pigs co-exposed to acetamiprid and 50 mg/kg BW of aqueous extract of *M. indica* leaves compared to the control group.

Indeed, the quantity of feed consumed would have allowed all the animals especially those receiving the mango leaves to cover their maintenance and production needs, thus leading to an increase in the availability of nutrients after digestion and consequently the increase in live weight which is only a consequence of the increase in the reactions of anabolism. In addition, compounds with antimicrobial properties pathogenic to the digestive tract through their bactericidal and antifungal activities, while promoting the development of beneficial microorganisms would have facilitated the digestion, absorption and efficient digestion of nutrients (Idris et al., 2019) and increased weight gain and average daily gain, though not significantly. Indeed, it stands to reason that if *Mangifera indica* leaf powder induced a non-significant increase in live weight gain in young guinea pigs, the same would be true for daily weight gain as there is a positive correlation between these two characteristics of growth. Moreover, this increase could be due to the androgenic properties possessed by mango leaves due to the presence of compounds such as flavonoids, phenols, and terponoids that they contain (Yadav et al., 2022). These are likely to stimulate the increase in muscle weight by synthesis and accumulation of muscle proteins. These results are similar to those obtained by Mohamadou et al. (2023) after administration of avocado seed powder in female cavies. On the other hand, the results obtained by Mba (2021) after using ginger powder as a feed additive in male guinea pigs are different.

The conversion ratio being closely linked to feed consumption and weight gain, the variation of one and/or the other of these two parameters leads to that of the conversion ratio.

Characteristics of carcass and relative weights of some organs in this study are similar to those reported by Zhang et al. (2017) in which, although carcass weight reflects weight gain data, abdominal fat, percent dressing and relative weight of meat cuts were not influenced by dietary treatments, as well as mango leaf extract supplementation. Their weights of carcass did not differ significantly (P>0.05) between chickens fed on treatment and control diets, which are in line with the findings of Odunsi (2005) in broilers fed with *Mangifera indica L*. seed kernel meal and Yibrehu et al. (2012) broilers given *Magnifera indica L*. fruit waste. Similar studies were carried out by Adu et al. (2020) on leaves of *Syzygium aromaticum* and seeds of *Myristica fragrans* which did not affect the relative weight of carcass cut in broilers.

CONCLUSION

At the end of this study, it may be concluded that supplementation of female guinea pig feed with *Mangifera indica* leaf powder at 1% can be a natural source of antioxidant to limit the impact of oxidative stress damages in their production performance particularly and farm animals in general, because improved values were mostly obtained at this level.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Chongsi Margaret Mary Momo; E-mail: margaretchongsi@yahoo.fr; ORCID: https://orcid.org/0000-0003-2863-4905

Ethical considerations

The experimental procedure in this study was approved by the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, Cameroon and complies with internationally accepted guidelines for the care and handling of laboratory animals. They were also in compatible with the European Union guidelines 86/609/EEC adopted by the Ethics Committee of the Ministry of Scientific Research and Innovation of Cameroon.

Authors' contribution

Chongsi MMM: Data collection and interpretation, manuscript final writing, editing and approval;
Tchoffo H: Data collection, data interpretation, designed research methodology;
Deutcheu SN: Data collection and literature review;
Noubouowo CAS: Drafting of the article and Data collection;
Azafack KD: Drafting of the article, Data collection and literature review;
Mahamat TMA: Data collection and literature review;
Bend EFM: Data collection and literature review;
Dongmo NAB: Data analysis and literature review;

Mohamadou A: data collection and literature review;
Ngoula F: Conceptualization and supervision of the study.

Data availability

Data that was used for analyses during this study are available from the corresponding author upon reasonable request.

Acknowledgements

No financial support was received for this work.

Competing interests

The authors declare that there is no conflict of interests.

REFERENCES

Adjanohoun E and Dramane K (1993). Revue de médecines et pharmacopées africaines : *Mangifera indica L*. Agence de cooperation culturelle et technique, 7: 49-61.

Adu OA, Gbore FA, Oloruntola OD, Falowo AB and Olarotimi OJ (2020). The effects of *Myristica fregrans* seed meal and *Syzygium aromaticum* leaf meal dietary supplementation growth performance and oxidative status of broiler chicken. Bulletin of National Research Centre, 44 (1): 1-10. DOI: https://doi.org/10.1186/s42269-020-00396-8

Aebi H (1984). Catalase "*in vitro*". Methods in Enzymology, 105: 121-126. DOI: https://doi.org/10.1016/s0076-6879(84)05016-3

Arthenice JNG, Augustave K, Ferdinand N, Bertin NV, Kenmeuhe SN and Etienne PT (2019). Attenuating effects of Mangifera indica leaves ethanolic extract against acetamiprid induced reproductive toxicity in male guinea pigs. Veterinary Research Forum, 10 (3): 187-192. DOI: https://doi.org/10.30466/vrf.2019.95154.2292

Arts IC and Hollman PC (2005). Polyphenols and disease risks in epidemiologic studies. American Journal of Clinical Nutrition, 81(1 Suppl):317S-325S. DOI: https://doi.org/10.1093/ajcn/81.1.317s

Bafor EE, Eyohan ES, Omoruyi O, Elvis-Offial U, Ayinde B, Eze GI, et al. (2015). Preliminary endocrinological, histological and haematological investigation of Alchornea laxiflora (Euphorbiaceae) leal extract effects on the ovary, uterus and cervix of mouse models. Journal of Science and Practice of Pharmacy, 2(1): 55-63. http://www.jsppharm.org/home/abstract.php?id=26&abstractTitle=Preliminary%20endocrinological,%20histological%20and%20haematological%20investigation%20of%20Alchornea%20laxiflora%20(Euphorbiaceae)%20leaf%20extract%20effects%20on%20the%20ovary,%20uterus%20and%20cervix%20of%20mouse%20models

Botsoglou NA, Fletouris DJ, Papageorgiou GE, Vassilopoulos VN, Mantis AJ and Trakatellis AG (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. Journal of Agricultural and Food Chemistry, 42: 1931-1937. DOI: https://doi.org/10.1021/jf00045a019

Campbell BK (2009). The endocrine and local control of ovarian follicle development in Ewe. Animal Reproduction Science, 6(1): 159–171. https://www.animal-reproduction.org/article/5b5a606df7783717068b4765

Chongsi MMM, Mahamat TMA, Tchoffo H, Vemo BN, Noumbissi MNB, Deutcheu NS, et al. (2023). Effects of spirulina (*Spirulina platensis*) powder supplementation in feed on reproductive performance in rabbits (*Oryctolagus cuniculus*) does. Asian Journal of Animal and Veterinary Advances, 18: 113-120. DOI: https://doi.org/10.3923/ajava.2023.113.120

Dar A, Faizi S, Naqvi S, Roome T, Zikr-ur-Rehman S, Ali M, et al. (2005). Analgesic and antioxidant activity of mangiferin and its derivatives: the structure activity relationship. Biological and Pharmaceutical Bulletin, 28(4):596–600. https://doi.org/10.1248/bpb.28.596

Djikeng TF, Womeni HM, Anjaneyulu E, Boungo TG, Karuna MSL, Prasad RBN, et al. (2017). Performance of Green Tea leaves methanolic extract in stabilizing refined, bleached and deodorized palm olein during storage at frying temperature. European Journal of Nutrition and Food Safety, 7:144–154. https://journals.indexcopernicus.com/api/file/viewByFileId/293677.pdf

Djuissi NM, Ngoula F, Kouamo J, Vemo NB, Nono MFS, Lontio AF, et al. (2021). Reproductive Characteristics, Serum Metabolites, and Oxidative Status in Female Guinea Pigs (*Cavia porcellus)* Fed with Ethanolic Extract of *Dichrostachys glomerata* Fruit. World's Veterinary Journal, 11(1): 66-72. DOI: https://dx.doi.org/10.54203/scil.2021.wvj9

Ebile DA, Kana JR, Pimagha MHJ, Edie NLW, Nguefack DG, Ngouana TR, et al. (2018). Effects of Dichrostachys Glomerata feeding regimes on growth performance, gut microbiota and haemato-biochemical profile of Japanese quails. Journal of Animal Research and Nutrition, 3(2): 5. https://animalnutrition.imedpub.com/effects-of-dichrostachys-glomerata-feeding-regimes-on-growth-performancegut-microbiota-and-haematobiochemical-profile-of-japanese.php?aid=23298

Esposito L, Tafuri S, Cocchia N, Fasanelli R, Piscopo N, Lamagna B, et al. (2021). Assessment of living conditions in wild boars by analysis of oxidative stress markers. Journal of Applied Animal Welfare Science, 24(1):64-71. DOI: https://doi.org/10.1080/10888705.2020.1790365

FAO (2014). «L'état de l'insécurité alimentaire dans le monde». Food and Nutrition Technical Report 1. https://openknowledge.fao.org/bitstreams/dafa77bf-428f-4798-b45c-aa7d8999d744/download

Funmileyi O. Awobajo I, Olatunji-Bello L and Ogbewey (2013). Reproductive impact of aqueous leaf extract of Mangifera indica (Mango) on some reproductive functions in female Sprague-Dawley rats. Biology and Medicine, 5: 58–64. https://www.walshmedicalmedia.com/abstract/reproductive-impact-of-aqueous-leaf-extract-of-mangiferaindicahopemango-on-some-reproductive-functions-in-femalespraguedawle-20404.html

Habermann E, Imatomi M, Pontes FC and Guattieri SCJ (2016). Antioxidant activity and phenol content of extracts of bark, stems, and young and mature leaves of *Blepharocalyx salicifolius* (Kunth) O. Berg (Murta). Acta Biologica Colombiana, 20:153–162. DOI: http://dx.doi.org/10.1590/1519-6984.03815

Henderson VW (2018). Progesterone and human cognition. Climacteric Journal of International Menopause Society, 21: 33-340. DOI: https://doi.org/10.1080/13697137.2018.1476484

Idris A, Aliyu AB and Oyewale AO (2019). Phytochemical Screening and Antibacterial Activity of Centaurea senegalensis growing in Nigeria. Journal of Applied Sciences Environmental Management, 23 (6) 1087-1092. DOI: https://dx.doi.org/10.4314/jasem.v23i6.14

Joffe H, Wit de A, Coborn J, Crawford S, Freeman M, Wiley A, et al. (2020). Impact of estradiol variability and progesterone on mood in perimenopausal women with depressive symptoms. Journal of Clinical Endocrinology and Metabolism, 105(3): 642-650. DOI: https://doi.org/10.1210/clinem/dgz181

Kothari SC, Shivarudraiah P, Venkataramaiah SB, Gavara S, Arumugam SN and Soni MG (2014). Toxicologic evaluation of Dichrostachys glomerata extract: Subchronic study in rats and genotoxicity tests. Food and Chemical Toxicology, 69: 120-131. DOI: DOI: https://www.doi.org/10.1016/j.fct.2014.03.045.

Kuate D, Etoundi BCO, Soukontoua YB, Ngondi JL and Oben JE (2010). Antioxidant characteristics of *Dichrostachys glomerata* spice extracts Caracteristicas antioxidantes de los extractos de la especia *Dichrostachys glomerata*. CyTA - Journal of Food, 8(1): 23-37. DOI: DOI: https://www.doi.org/10.1080/19476330903129126

Lu SC (1999). Regulation of hepatic glutathione synthesis: current concepts and controversies. The FASEB Journal, 13: 1169-83. https://pubmed.ncbi.nlm.nih.gov/10385608/

Mansour SA, Mossa AT and Heikal TM (2009). Effects of methomyl on lipid peroxidation and antioxidant enzymes in rat erythrocytes: in vitro studies. Toxicology and Industrial Health, 25: 557-563. DOI: https://doi.org/10.1177/0748233709349829

Mba TLA (2021). Utilisation de la poudre de ginger comme additif alimentaire chez le cobaye (*Cavia porcellus*), *Thèse de Doctorat (PhD) Nutrition et Alimentation Animale*. Faculté d'Agronomie et des Sciences Agricoles, Université de Dschang , Cameroun.

Miégoué E, Tendonkeng F, Mweugang NN, Fossi J, Ntsafack P, Agwh ED, et al. (2018 a). Post-weaning growth performance of guinea pig (*Cavia porcellus L.*) fed on *Panicum maximum* supplemented with graded level of *Arachis glabrata* in the died. Journal of Veterinary Science and Animal Husbandry, 6 (6): 2348-9790. https://www.semanticscholar.org/paper/Post-Weaning-Growth-Performance-of-Guinea-Pigs-L)-Tendonkeng-Ebile/bf2951e81974bf3e6576e9badb09046d9cad3af1

Miégoué E, Tendonkeng F, Mweugang NN, Mna TLA, Ntsafack P and Pamo TE (2018 b). Physiological features of *Pennisetum purpureum* or *Panicum maximum* consumption in guinea pigs (*Cavia porcellus*). Journal of Zoological Research, 1(1): p5. DOI: https://doi.org/10.14302/issn.2694-2275.jzr-18-2474

Miégoué E, Tendonkeng F, Ngouopo NM, Lemoufouet J, Fossi J, Ntsafack P, et al. (2018 c). Effect of Arachis glabrata level in diet on reproductive and preweaning growth performance of guinea pig (*Cavia porcellus L.*) fed on *Panicum maximum.* International Journal of Animal Science and Technology, 2 (4): 36-44. https://typeset.io/pdf/effect-of-arachis-glabrata-levels-in-the-diet-on-2ikp17onwz.pdf

Mishra B and Jha R (2019). Oxidative stress in the poultry gut: potential challenges and interventions. Frontiers in Veterinary Science, 6: 60. DOI: https://doi.org/10.3389/fvets.2019.00060

Misra HP and Fridovich I (1972). The generation of superoxide radical during the autoxidation of haemoglobin. The Journal of biological chemistry, 247: 6960-6962. https://pubmed.ncbi.nlm.nih.gov/4673289/

Mohamadou A, Dongmo NAB, Fomo NLM, Messongue MNS, Menkem B, Ngaleu NCC, et al. (2023). Effects of the ethanolic extract of avocado (*Persea americana* M.) seed powder on growth characteristics and reproductive Performance of female cavies (*Cavia porcelus* L.). Biomedical Journal of Scientific & Technical Research, 50(2): 41506-41518. DOI: http://dx.doi.org/10.26717/BJSTR.2023.50.007928

Moron MS, Depierre JW and Mannervik B (1979). Levels of glutathione, glutathione reductase and glutathione - S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta- (BBA)-General Subjects, 582: 67-78. DOI: https://doi.org/10.1016/0304-4165(79)90289-7

Ndam FN (2023). Effects of ethanolic extract of *senecio biafrea* on the production performances of female African giant rat (*Cricetomys gambianus*). Master of Science Memoire in Biotechnology and Animal Productions Faculty of Agronomy and Agricultural sciences, University of Dschang, Cameroon.

Noumbissi MNB, Tendonkeng F, Zougou TG, Miégoué E, Lemoufouet J, Boukila B, et al. (2013). Effet de la complementation au *Tithonia diversifolia* sur l'évolution du poids post-partum et la croissance pré-sevrage des cobayes (*Cavia porcellus L.*) [Effect of *Tithonia diversifolia* supplementation on the evolution of postpartum weight and pre-weaning growth of guinea pigs (*Cavia porcellus* L.)]. Livestock Research for Rural Development, 25: Article #145. https://lrrd.cipav.org.co/lrrd25/8/noum25145.htm

Odunsi AA (2005). Response of laying hens and growing broilers to the dietary inclusion of mango (*Mangifera indica L.*) seed kernel meal. Tropical Animal Health and Production, 37 (2): 139-150. DOI: https://doi.org/10.1023/B:TROP.0000048455.96694.85

Ogawa NN, Silva GL, Barbon AP, Flaiban KK, Silva CA, Rocha LM, et al. (2024). Animal Welfare Assessment and Meat Quality through Assessment of Stress Biomarkers in Fattening Pigs with and without Visible Damage during Slaughter. Animals, 14(5):700. DOI: https://doi.org/10.3390/ani14050700

Pasupathi K, Gopu P and Balasubramanyam D (2020). Growth performance of rabbits on *Mangifera indica* tree fodder. Journal of Entomology and Zoology Studies, 8(5): 999-1000. https://www.entomoljournal.com/archives/2020/vol8issue5/PartN/8-4-543-802.pdf

Payne AH and Hales DB (2004). Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocrine Reviews, 25: 947- 970. DOI: https://doi.org/10.1210/er.2003-0030

Rahman MF, Siddique MK and Jamil K (2001). Acid and alkaline phosphatase activities in a novel phosphorothionate (RPR-11) treated male and female rats. Evidence of dose and time-dependent response. Drug and Chemical Toxicology, 32: 479-509. DOI: https://doi.org/10.1081/dct-100100131

Rao AV and Shaha C (2001). Multiple glutathione S-transferase isoforms are present on male germ cell plasma membrane. FEBS Letters, 507: 174-180. DOI: https://doi.org/10.1016/S0014-5793(01)02958-1

Sen S, Chakraborty R, Sridhar C, Reddy YSR and Biplab (2010). Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research, 3(1): 91-100. https://www.globalresearchonline.net/journalcontents/volume3issue1/Article%20021.pdf

Shah KA, Patel MB, Shah SS, Chauhan KN, Parmar PK and Patel NM (2010). Anti-hyperlipidemic activity of Mangifera indica leaf extract on rats fed with high cholesterol diet. Der Pharmacia Sinica, 1:156–161. https://www.imedpub.com/articles/antihyperlipidemic-activity-of-mangifera-indica-l-leaf-extract-on-rats-fed-with-high-cholesterol-diet.pdf

Womeni H, Djikeng FT, Karuna MSI, Prasad RSN and Linder M (2016). Oxidative stabilization of RBD palm olein under forced storage conditions by old Cameroonian green tea leaves methanolic extract. Nutrition and Food Science Journal, 3:33–40. http://dx.doi.org/10.1016/j.nfs.2016.03.002

Yadav D, Pal AK, Singh SP, and Sati K (2022). Phytochemicals in mango (*Mangifera indica*) parts and their bioactivities: A Review. Crop Research-HISAR, 57(1-2):79-95. http://dx.doi.org/10.31830/2454-1761.2022.012

Yakubu MT, Akanji MA, Oladiji AT, Olatinwo AWO, Adesokan AA, Yakubu MO, et al. (2008). Effect of Cnidoscolous aconitifolius (Miller) I.M. Johnston leaf extract on reproductive hormones of female rats. Iranian Journal of Reproductive Medicine, 6(3): 149-155. https://journals.ssu.ac.ir/ijrmnew/article-1-115-en.pdf

Yaseen A, Hussain T, and Hameed A (2023). Association of faecal volatiles and steroids with behavioural expression during oestrous cycle of sheep (Ovis aries). Reproduction in Domestic Animals, 58(6):708-716. https://doi.org/10.1111/rda.14340

Yibrehu E, Aberra M and Getinet A (2012). The Effect of Dietary Inclusion of Mango (*Magnifera indica* L.) Fruit Waste on Feed Intake, Growth and Feed Efficiency of Cobb-500 Broiler Chickens. Ethiopian Journal of Agricultural Sciences, 22:73-83. https://www.ajol.info/index.php/ejas/article/view/142950

Zhang YN, Wang J, Qi B, Wu SG, Chen HR, Luo HY, et al. (2020). Evaluation of mango saponin in broilers: Effects on growth performance, carcass quality, meat quality and plasma biochemical indices. Asian-Australasian Journal of Animal Sciences, 30(8):1143. https://doi.org/10.5713%2Fajas.16.0847

2024 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 14, Issue 3: 196-203; May 25, 2024

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2024.23

EFFECT OF CLIMATE VARIABLES ON POULTRY PRODUCTION EFFICACY IN NIGERIA

Emeka OSUJI[1], Blessing AHAMEFULE[2], Glory BEN-CHENDO[3], Maryann OSUJI[3], Roseline NWOSE[1], Amelia ELEAZAR[1], Haruna OPALUWA[5], Igwe UKOHA[3], Innocent NWAIWU[3], Yetunde AJIBADE[5], Gbenga OPEYEMI[5], Okwudili IBEAGWA[3], Akunna TIM-ASHAMA[4], Evelyn OFFOR[2], Esther NWACHUKWU[3], Uchechi ANYANWU[3], Kelechi ANYIAM[3], and Doris IWEZOR-MAGNUS[2]

[1]*Department of Agriculture, Alex Ekwueme Federal University Ndufu-Alike Abakaliki, Nigeria*
[2]*Department of Agricultural Economics, Michael Okpara University of Agriculture Umudike, Nigeria*
[3]*Department of Agricultural Economics, Federal University of Technology, Owerri, Nigeria*
[4]*Department of Agricultural Science, Alvan Ikoku University of Education Owerri, Nigeria*
[5]*Department of Agricultural Economics and Extension, Prince Abubakar Audu University Anyigba Kogi, Nigeria*

Email: osujiemeka2@yahoo.com

Supporting Information

ABSTRACT: The paper examined the effect of climate variables on poultry production efficacy in Nigeria with emphasis on broilers and layers. Multi-stage sampling was used to select 401 poultry farmers who provided useful information with the aid of a questionnaire. Data were analyzed using descriptive statistics, and logit regression model. Result shows that free range accounted for 22.7%, battery cage 11.2%, deep litter 54.6%, and semi-free range 11.5% were notable systems of poultry rearing in the state. Heat stress 75.1%, reduced egg 99.8% and meat production 88.5%, and reduction in quality of eggs 51.9% were some of the climate change effects on poultry production. Adaptation strategies of poultry farmers include; proper housing system 100%, proper feed formulation 99.8%, right stocking density 96.8% and adequate water and feed supply 82.8%. Age, education, off-farm activities, size of poultry pen and poultry farming experience were important adaptation determinants of poultry farmers to climate change. High price of feed 100%, lack of access to credit services 77.1%, disease outbreak and parasites 100% and high cost of poultry inputs 99.5% constrained poultry farming. The study concludes that climate variables affect poultry farming. Farmers were recommended to practice climate smart poultry production to mitigate adverse effects.

Keywords: Climate Change, Farm Efficacy, Poultry Farmers, Poultry Production

RESEARCH ARTICLE

PII: S222877012400023-14

Received: March 31, 2024

Revised: May 14, 2024

Accepted: May 15, 2024

INTRODUCTION

Poultry production is becoming the first priority in terms of protein sources and income opportunities for the involved poultry farmers. According to FAOSTAT (2023), poultry meat represents about 33% of the total global meat production in the world. This posits the relevance and importance of poultry production. However, poultry production across Africa and other world countries have been hit by climate change affecting production and supply of poultry products especially meat and eggs (Abioja and Abiona, 2021). The vulnerability of poultry production to climatic change in Nigeria in recent times has been overwhelming and worrisome to the poultry producers (Ahmad et al., 2022).

It should be noted that poultry birds can only tolerate mild atmospheric and weather conditions, and recent variations in climate and weather condition had caused enormous fatality and high mortality rate in poultry production (World Bank Group, 2023). Studies have shown that the level of performance of birds depends on environmental conditions; such as rainfall, temperature, relative humidity and sunshine prevailing at a given time. Also, housing systems, ventilation of poultry houses and other management procedures have in recent times impacted negatively on poultry farming (World Bank Group, 2023). High temperature and humidity for instance, increases body temperature of poultry birds, decreases feed consumption and feed efficiency, and reduction in body weight and size, while high mortality and decrease in productivity and quality of eggs are caused by high and variant rainfall patterns (Zhang, et al., 2023). There are recent concerns that the ongoing global warming and climate change in Africa will have more negative impacts on the future growth and wellbeing of poultry birds.

Thus, developing countries, in Africa, especially Nigeria is bound to face substantial risks in poultry production from climate change due to increased exposure and inadequate adaptive potentials (Attia et al., 2022). These outcomes created the gap in knowledge and induced the motivation for the study.

Citation: Osuji E, Ahamefule B, Ben-Chendo G, Osuji M, Nwose R, Eleazar A, Opaluwa H, Ukoha I, Nwaiwu I, Ajibade Y, Opeyemi G, Ibeagwa O, Tim-Ashama A, Offor E, Nwachukwu E, Anyanwu U, Anyiam K, and Iwezor-Magnus D (2024). Effect of climate variables on poultry production efficacy in Nigeria. *Online J. Anim. Feed Res.*, 14(3): 196-203. DOI: https://dx.doi.org/10.51227/ojafr.2024.23

MATERIALS AND METHODS

The study was conducted in Ebonyi State, Nigeria. The State has an estimated population of 3,242,500 persons. Multi-stage sampling technique was used to select the poultry farmers. In the first phase, four local government areas (LGA's) were picked from the agricultural zones (Ebonyi North, Ebonyi South, and Ebonyi Central), to make 12 LGA's. The second phase involved the random selection of four autonomous communities making a total of 48 communities. The third phase had two villages picked at random from the communities to make 96 villages. In the final phase, 5 poultry farmers were picked to sum 480 farmers. Primary data were collected using questionnaire and out of the selected 480 poultry farmers, only 401 provided useful information for data analysis. Data were analysed using descriptive statistics, and the logit regression model. The logit regression model is expressed as follows;

$Y_i = \log (p/1\text{-}p) = F (X_i,b) + e$ ———— equation. 1

i.e.,

The logit of a number p between 0 and 1 is given by;

$(p) = \log (p/1\text{-}p) = \log (p) = \log (1\text{-}p)$ ———— equation. 2

Where:

P, is the probability, while (1- p) is the corresponding odds, and the logit of the probability is the logarithm of the odds.

Y_i = Observable dummy variable that indexes adaptation to climate change (Adapted =1, otherwise =0)

F = Logistic cumulative distribution function

b = Vector of estimated parameter

X_i = Independent variables considered, which include;

X_1 = Age (Years)

X_2 = Education (No of years spent in school)

X_3 = Household size (No of persons)

X_4 = Off-farm activities (Engaged =1, otherwise=0)

X_5 = Size of poultry pen (Meters)

X_6 = Access to livestock extension services (No of visits)

X_7 = Poultry farming experience (No of years)

X_8 = Participation in climate change workshop (No of times participated)

X_9 = Access to climate change information (Accessed =1, otherwise=0)

X_{10} = Climatic events (Experienced =1, otherwise=0)

e = error term

RESULTS AND DISCUSSION

Socioeconomic characteristics of the poultry farmers

The socioeconomic characteristics of the poultry farmers are presented in Table 1. The result shows that majority of the farmers 51.6% were between age ranges of 41-50 years with a mean of 48 years. This implies that the poultry farmers were young and in their productive age (Adeyemo et al., 2019). Majority of the poultry farmers, 75.8% were married, implying the dominance of married poultry farmers. Educational level of the poultry farmers shows that the farmers had different levels of education with majority, 39.9% in primary cadre. This means that the farmers attempted formal education, which could help in understanding of poultry management principles (Brown and Vivian, 2018). Household size shows that majority, 51.1% the poultry farmers had household size of 5-8 persons. This implies that the farmers had relatively large household size that could aid their poultry production in terms of family labour provisions. Majority of the poultry farmers 51.4% reared broilers, 40.1% reared layers, while 8.5% reared both broilers and layers. This implies the preference of broilers over layers among the poultry farmers (Castro et al., 2023). Majority of the poultry farmers, 94.8% belongs to cooperative societies. The poultry farmers recorded various levels of poultry farming experience with the majority 72.3% having between 21-30 year's experiences.

Methods of poultry rearing adopted by the farmers

The methods of poultry rearing adopted by the farmers are presented in Table 2. The table shows that 22.7% practiced free range of poultry management, 11.2% battery cage system, 54.6% deep litter method, while 11.5% engaged in semi–free range method of poultry rearing. This implies that the most prominent method of rearing poultry in the state was the deep litter system, which does not require huge capital investment compared to battery cage (Cheng et al., 2022). In this system, birds are kept in large pens up to 250 birds in a house and the floor of the house covered with dry materials such as chopped straw, saw-dust, dried leaves, etc., which could be easily be replaced with new ones to enhance healthy management of the poultry birds (Evans et al., 2021).

Table 1 - Socio-economic characteristics of poultry farmers

Variables		Frequency	Percentage
Age	20-30	20	4.9
	31-40	159	39.7
	41-50	207	51.6
	51-60	15	3.7
	Mean	48	
Sex	Male	94	23.4
	Female	307	76.6
Marital status	Single	71	17.7
	Married	304	75.8
	Divorced	07	1.7
	Widowed	19	4.7
Level of education	Primary	160	39.9
	Secondary	139	34.7
	Tertiary	12	2.9
	Non formal	90	22.4
Household size	1-4	177	44.1
	5-8	205	51.1
	9-12	11	2.7
	13-16	08	1.9
	Mean	6.2	
Poultry reared	Broilers	206	51.4
	Layers	161	40.1
	Both	34	8.5
Cooperative membership	Yes	380	94.8
	No	21	5.2
Poultry experience	1-10	21	5.2
	11-20	78	19.5
	21-30	290	72.3
	31-40	12	2.9
	Mean	26	–

*Multiple responses

Table 2 - Methods of poultry rearing adopted by the farmers

Methods of poultry rearing	Frequency	Percentage
Free range	91	22.7
Battery cage	45	11.2
Deep litter	219	54.6
Semi-free range	46	11.5
Total	401	100

* Multiple responses

Effects of climate change on poultry production

The effects of climate change on poultry production are presented in Table 3. About 75.1% of the poultry farmers indicated heat stress (exceeding 41 °C) in poultry birds. This means that as temperature rises, chickens spend more time drinking water than consuming feed (Cheng et al., 2022). A high number of the poultry farmers 99.8% indicated drastic reduction (60%) in eggs produced. Empirical studies have revealed that high temperatures, intense sunrays, heat stress causes reduction in egg production (Castro et al., 2023). About 88.5% of the poultry farmers indicated reduced meat production (55%); rising temperatures causes poultry birds to consume less feed and concentrate in more water intake, this causes the birds to drop in weight and size (Evans et al., 2021). Furthermore, the table shows that the poultry farmers indicated 85% decline in income and profitability when compared to previous earnings (De-Sousa et al., 2023). Again 100% of the poultry farmers indicated a higher morbidity and mortality rate in poultry birds. Changes in temperature, relative humidity and rainfall patterns cause the outbreak of poultry diseases (coccidiosis) causing high death rate in poultry birds (Zhang et al., 2023). Increased in appetite of birds was indicated by 85.8%, while 51.1% of the poultry farmers indicated decreased in feed intake. This means that changes in precipitation such as increased rainfall causes poultry birds to consume more feed and drink less water and thus necessitating the provision of more poultry feed (Wasti et al., 2020). About 94.0% of the poultry farmers indicated high usage of drugs and vaccinations, this brings rising cost in farmers purchase of drugs and vaccinations administered to poultry birds (Sesay, 2022). Loss in body weight was indicated by 75.6%, rising temperatures causes poultry birds to consume less feed which leads to loss in body weight. Decrease in fodder/grain production was indicated by 51.6%, this result from unavailability of rainfall which hinders the optimal growth of fodder/grain used in poultry feed formulations (Wasti et al., 2020). About 90.0% of the poultry farmers reported increase in feed cost, which invariably resulted from a decrease in fodder/grain production causing a rise in cost of available poultry feeds (Shikwambana et al., 2021). Loss of strength and energy was observed by 76.1%. Invariably inability of the poultry birds to consume feed during high temperatures causes loss in strength and energy leading to body weaknesses and loss in feathers (Sesay, 2022). Sleepiness was indicated by 74.6%. Frequent rains result in extreme cold conditions in poultry environments which causes sleepiness. Also feather moult, mite infestation or worms can place an extra strain on their immune systems and induces sleepiness. Supply of poor quality chicks was indicated by 95.0%, changes in climate causes newly hatch pullets to shrink in body weight and size leading to sale and supply of poor quality chicks (Saeed et al., 2019). Change in taste of poultry meat and egg was indicated by 52.1%, implying that changes in climate and weather conditions cause a change in taste of poultry meat and eggs.

Table 3 - Effects of climate change on poultry production

Effects	Frequency	Percentage
Heat stress in both house and outdoor flocks	301	75.1
Reduced egg production	400	99.8
Reduced meat production	355	88.5
Reduction in quality of eggs	208	51.9
Reduced income and profitability	401	100.0
Higher morbidity and mortality rate	401	100.0
Increased in appetite of birds	344	85.8
Decreased in feed intake	205	51.1
High usage of drugs and vaccinations	377	94.0
Loss in body weight	303	75.6
Decrease in fodder/grain production	207	51.6
Rise in feed costs	361	90.0
Cold proneness leading to shivering	187	46.6
Loss of strength and energy (body weakness)	305	76.1
Sleepiness	299	74.6
Supply of poor quality chicks	381	95.0
Change in taste of poultry meat and egg	209	52.1

*Multiple responses

Climate change adaptation strategies adopted by poultry farmers

The climate change adaptation strategies adopted by poultry farmers is presented in Table 4. In Table 3, note that climate change negatively affected poultry production in the study area and this necessitated the adaptation strategies

and/ or measures deployed by the poultry farmers in mitigating these adverse effects on poultry birds. The table shows that all the poultry farmers adopted proper housing system. Proper housing allows for ventilation, cleaning, spacing and prevention of outbreak of disease in the pens (Salem et al., 2022). About 99.8% of the poultry farmers practiced proper feed formulation which includes essential nutrients required for optimal growth (Sabry et al., 2023). Right stocking density was adopted by 96.8%, this prevents transference of outbreak of diseases occasioned by climate change. About 82.8% adopted adequate water and feed supply; this involves the provision of adequate water and feed during the dry season to cushion heat stress (Osuji, 2019). Similarly, result shows that the poultry farmers practiced prompt vaccination of their birds as a strategic way of combating the effects of climate change (Pepper and Dunlop, 2021). About 51.9% of the poultry farmers adopted the use of resistant and improved varieties of pullets. Ensuring adequate ventilation was adopted, this ensures that the poultry pens and houses are well ventilated enough to prevent heat stress (Olutumise, 2023). The use of efficient energy bulbs was adopted by 64.1%. Use of energy bulbs emit less heat and promote healthy poultry environment. Raise of broods and sell was practiced by 77.6% of the poultry farmers; this involves the rearing and sale of early broods to avert the incidence of changing climate. Tree planting around poultry house was adopted by 92.0%, this involves the intentional planting of trees around poultry houses to provide shade thereby reducing heat stress (Olutumise, 2023). Similarly, the table reveals that 97.8% of the poultry farmers adopted prompt routine management services (FAOSTAT, 2023). Keeping of birds and other livestock was adopted by 77.8% of the poultry farmers; this involves a diversification method of averting the negative effects of climate change on poultry enterprise. Consultation of veterinary services was adopted by 74.8%, this involves the use of veterinary services in responding to the changing climate. Interestingly, these adaptation practices mitigated adverse effects of climate change on poultry birds and further enhanced poultry production in the area.

Table 4 - Climate change adaptation strategies adopted by poultry farmers

Adaptation strategies	Frequency	Percentage
Proper housing system	401	100.0
Proper feed formulation	400	99.8
Right stocking density	388	96.8
Adequate water and feed supply	332	82.8
Outright sale of early maturing birds	390	97.3
Prompt vaccination	376	93.8
Rearing of poultry varieties	208	51.9
Ensuring adequate ventilation	401	100.0
Use energy efficient bulb	257	64.1
Raising of broods and sell	311	77.6
Tree planting around poultry house	369	92.0
Prompt routine management	392	97.8
Keeping of birds and other livestock's	312	77.8
Consultation of veterinary services	300	74.8

* Multiple responses

Adaptation determinants of poultry farmers to climate change

The adaptation determinants of poultry farmers to climate change are presented in Table 5. The logit regression model was used in preference of other models because of its simplicity in handling binary values such as (1, 0). The table shows that age is positive and significant at 1% level. This implies that increasing age of the farmers increases likelihood capacity in adapting to climate change (Abioja and Abiona, 2021). Education is positive and significant at 1% level. This implies that the adaptation strategy to climate change is greater for those that have higher educational attainment compared to less-educated or illiterate farmers (Adeyemo et al., 2019). Off-farm activities were positive and significant at 5% level. This implies that farmers who engage in off-farm activities are more likely to adapt to climate change (Ahmad et al., 2022). Size of poultry pen was positive and significant at 10% level. This implies that as poultry pen size increases, adaptation to climate change increases too. Poultry farming experience was found positive and significant at 1% level. Experienced poultry farmers adapt more easily to climate change than inexperienced ones (Attia et al., 2022). Participation in climate change workshops was positive and significant at 5% level. This implies that increased participation in climate change workshops increases the likelihood of poultry farmers to adapt to changing climate (Aroyehun, 2023). Climatic events were positive and significant at 5% level. This means that the devastating effects of climate change on poultry enterprise increases the likelihood of the poultry farmers to adapt to climate change.

Table 5 - Adaptation determinants of poultry farmers to climate change

Variables	Coefficients	t-values	Std. Error
Constant	0.7019	1.6001*	0.4387
Age	19.0058	4.1043***	4.6307
Education	0.0889	3.8300***	0.0232
Household size	-10.0255	-0.3051	32.859
Off-farm activities	24.9602	4.1131***	6.0684
Size of poultry pen	0.6909	1.8901*	0.3655
Access of livestock extension services	-0.7803	-0.0650	12.005
Poultry farming experience	7.0501	3.0195***	2.3349
Participation in climate change workshops	15.4350	2.0017**	7.7109
Access to climate change information	-0.6784	-1.2194	0.5563
Climatic events	20.0008	2.5011**	7.9968
R^2	0.8699		
F-value	170.305***		

***p < 0.001; **p < 0.01; *p < 0.05

Constraints encountered in poultry production

The constraints encountered in poultry production are presented in Table 6. According to the table, high price of feed was indicated by all the poultry farmers (Brown and Vivian, 2018). About 77.1% of the poultry farmers indicated lack of access to credit services, this implies that the poultry farmers were unable to access credit facilities to purchase more poultry inputs (Castro et al., 2023). Disease outbreak and parasites was indicated by all the poultry farmers, this implies that the farmers experienced outbreak of disease and parasites that affected their poultry enterprise negatively. High cost of poultry inputs was indicated by 99.5% (Cheng et al., 2022). About 96.5% indicated inadequate extension services; this implies that the farmers had poor information dissemination. High cost of poultry drugs and vaccines was indicated by 85.8% (Evans et al., 2021). Other constraints faced includes, weather and climate change issues 97.0%, lack of government incentives 97.5%, high cost of improved bird varieties, 94.8% and unfavorable price fluctuations, 88.5%. This generally implies that these identified factors negatively affected poultry production and severely reduced both output and farm income of the poultry farmers (Wasti et al., 2020).

Table 6 - Constraints of poultry farming

Constraints	Frequency	Percentage
High price of feed	401	100.0
Lack of access to credit services	309	77.1
Disease outbreak and parasites	401	100.0
High cost of poultry inputs	399	99.5
Inadequate extension services	387	96.5
High cost of poultry drugs and vaccines	344	85.8
Weather and climate change issues	389	97.0
Lack of government incentives	391	97.5
High cost of improved bird varieties	380	94.8
Unfavorable price fluctuations	355	88.5

* Multiple responses

CONCLUSION

The findings of the study reveal that climate change had adverse effects on poultry production and its manifestations include; rising feed cost 90.0%, cold proneness 46.6%, body weakness 76.1%, and sleepiness 74.6%, and poor-quality

chicks 95.0%. Age, education, off-farm activities, size of poultry pen and poultry farming experience were important significant adaptation determinants of poultry farmers to climate change. Weather and climate change issues 97.0%, lack of government incentives 97.5%, high cost of improved bird varieties 94.8% and unfavorable price fluctuations 88.5% were identified poultry constraints. The study concludes that climate variable affects poultry farming. Farmers were recommended to practice climate smart poultry production to mitigate its adverse effects and boost poultry production in the state.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Emeka Osuji; E-mail:osujiemeka2@yahoo.com; ORCID: https://orcid.org/0000-0001-8605-834X

Authors' contribution

E. Osuji, B. Ahamefule, M. Osuji = Conceptualization, model design, data analysis, section writing and proof-read.

G.Ben-Chendo, R. Nwose, A. Tim-Ashama = Data analysis, results editing, proof-reading and grammar checking.

H. Opaluwa, E. Nwachukwu, A, Eleazar, I. Ukoha = Conceptualization, result design, section writing, reference sorting and editing.

E.Offor, U. Anyanwu, Y. Ajibade = Questionnaire design, methodology design, section writing, editing and proof-read.

D. Iwezor-Magnus, G. Opeyemi, K. Anyiam, I. Nwaiwu, O. Ibeagwa = Data collection, data processing, data curation, data sorting, and data coding.

Ethical consideration

There is no direct contact with animals.

Consent to publish

All authors consented to publish the article.

Acknowledgments

The authors acknowledged the role played by Prof. N.C. Ehirim, Department of Agricultural Economics, Federal University of Technology Owerri in proofreading the manuscript.

Conflict of interest

The authors declare that no conflict of interest exists.

REFERENCES

Abioja MO, and Abiona JA (2021). Impacts of climate change to poultry production in Africa: Adaptation options for broiler chickens. In: Oguge N, Ayal D, Adeleke L, da Silva I (eds) African Handbook of Climate Change Adaptation. Springer, Cham. https://doi.org/10.1007/978-3-030-45106-6_111

Adeyemo AJ, Akingbola OO, and Ojeniyi SO (2019). Effects of poultry manure on soil infiltration, organic matter contents and maize performance on two contrasting degraded alfisols in south-western Nigeria. International Journal of Recycle Organic Waste Agriculture, 8(1): 73–80. https://doi.org/10.1007/s40093-019-0273-7

Ahmad R, Yu YH, Hsiao FSH, Su CH, Liu HC, Tobin I, et al. (2022). Influence of heat stress on poultry growth performance, intestinal inflammation, and immune function and potential mitigation by probiotics. Animals, 12(17):2297. https://doi.org/10.3390/ani12172297

Aroyehun A (2023). Perceived effects of climate change on poultry egg production in Rivers State, Nigeria. Black Sea Journal of Agriculture, 6(1): 54-59. https://doi.org/10.47115/bsagriculture.1189204

Attia YA, Rahman MT, Hossain MJ, Basiouni S, Khafaga AF, Shehata AA, and Hafez HM (2022). Poultry production and sustainability in developing countries under the COVID-19 crisis: lessons learned. Animals, 3; 12(5): 644. https://doi.org/10.3390/ani12050644

Brown ED, and Vivian O (2018). Effect of poultry production on agricultural production in Nigeria. Economy, 5(1): 8–16. https://doi.org/10.20448/journal.502.2018.51.8.16

Castro FLS, Chai L, Arango J, Owens CM, Smith PA, Reichelt S, et al. (2023). Poultry industry paradigms: connecting the dots. Journal of Applied Poultry Research, 32(1): 100310. https://doi.org/10.1016/j.japr.2022.100310

Cheng M, McCarl B, and Fei C (2022). Climate change and livestock production: A literature review. Atmosphere, 13(1): 140. https://doi.org/10.3390/atmos13010140

De-Sousa KT, Deniz M, and Santos MP (2023). Decision support system to classify the vulnerability of broiler production system to heat stress based on fuzzy logic. International Journal of Biometeorology, 67: 475–484. https://doi.org/10.1007/s00484-023-02427-1

Evans M, Samuel N, and Samuel CM (2021).Production of indigenous poultry among smallholder farmers in Tigania West Meru County, Kenya. African Journal of Agricultural Research, 17(5):705-713. https://doi.org/10.5897/AJAR2021.15465

FAOSTAT (2023) Food and agriculture organization statistical data. https://www.fao.org/faostat/en/#data/. Accessed June 25, 2023

Olutumise AI (2023). Intensity of adaptations to heat stress in poultry farms: A behavioural analysis of farmers in Ondo state, Nigeria. Journal of Thermal Biology, 115:103614. https://doi.org/10.1016/j.jtherbio.2023.103614

Osuji MN (2019). Analysis of climate change perception on poultry production in Imo State, Nigeria. Advances in Research, 19(6): 1–8. https://doi.org/10.9734/air/2019/v19i630139

Pepper CM and Dunlop MW (2021). Review of litter turning during a grow-out as a litter management practice to achieve dry and friable litter in poultry production. Poultry Science, 100(6): 101071. https://doi.org/10.1016/j.psj.2021.101071

Sabry EI, Romeih MI, and StinoZU (2023). Water scarcity can be a critical limitation for the poultry industry. Tropical Animal Health Production, 55: 215-230. https://doi.org/10.1007/s11250-023-03599-z

Saeed M, Abbas G, Alagawany M, Kamboh AA, El-Hack ME, Khafaga AF, and Chao S (2019). Heat stress management in poultry farms: A comprehensive overview. Journal of Thermal Biology, 84: 414-425. https://doi.org/10.1016/j.jtherbio.2019.07.025

Salem HM, Alqhtani AH, Swelum AA, Babalghith AO, Melebary SJ, Soliman SM, et al. (2022). Heat stress in poultry with particular reference to the role of probiotics in its amelioration: An updated review. Journal of Thermal Biology, 108: 103302. https://doi.org/10.1016/j.jtherbio.2022.103302

Sesay AR (2022). Impact of heat stress on chicken performance, welfare, and probable mitigation strategies. International Journal of Environment and Climate Change, 12(11): 3120–3133. https://doi.org/10.9734/ijecc/2022/v12i111360

Shikwambana S, Malaza N, and Shale K (2021). Impacts of rainfall and temperature changes on smallholder agriculture in the Limpopo Province, South Africa. Water, 13(20): 2872. https://doi.org/10.3390/w13202872

Wasti S, Sah N, and Mishra B (2020).Impact of heat stress on poultry health and performances, and potential mitigation strategies. Animals, 10(8): 1266. https://doi.org/10.3390/ani10081266

World Bank Group (2023). Climate Change Knowledge Portal. https://climateknowledgeportal.worldbank.org/download-data.Accessed June 16, 2023

Zhang Y, Chen H, Cong W, Zhang K, Jia Y, and Wu L (2023). Chronic heat stress affects bile acid profile and gut microbiota in broilers. International Journal of Molecular Sciences, 24(12): 10238. https://doi.org/10.3390/ijms241210238

2024 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 14, Issue 1: 204-210; May 25, 2024

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2024.24

EFFECT OF DIETARY SUPPLEMENTATION OF CORIANDER SEED (*Coriandrum sativum* L.) ON GUT MORPHOLOGY IN BROILER CHICKENS

Mona Mohammed KHUBEIZ[1], Omran Ahmed ALGRIANY[1], Wafa Moftah ELMGHIRBI[1], Gheeda Riyadh BILKHAYR[2], and Abdelfettah Mohamed SHIRIF[1]

[1]*Department of Physiology, Biochemistry and Nutrition, Faculty Veterinary Medicine, University of Tripoli, Tripoli, Libya*
[2]*Faculty of Medicine, University of Tripoli, Tripoli, Libya*

Email: monakhubiez@gmail.com

Supporting Information

RESEARCH ARTICLE

PII: S2228770124000024-14

Received: March 16, 2024

Revised: May 18, 2024

Accepted: May 21, 2024

ABSTRACT: Several herbal plants have demonstrated remarkable efficacy in modulating the morphology of the gut, leading to improved nutrients absorption, enhanced growth, and reduced susceptibility to diseases. Aim of this study was to investigate the effects of different levels of coriander seed powder (CSP; 0% as T1, 1.5% as T2 and 3.5% as T3) in modulating broiler chickens gut morphology. A total of 180 one-day-old broiler chicks were randomly allocated into 3 treatment groups 60 chick/group in 3 replicates. The trial was designed according to a completely randomized design for 45 days. The histomorphology of duodenum, jejunum and ileum [villus height (VH), villus width, crypt depth (CD) and villus height to crypt depth; VH:CD ratio] were investigated. The result showed a significant increase ($P<0.01$) of villus height in two treatments (1.5% and 3.5%) as compared to control in all segments. Moreover, the significant increase ($P<0.01$) on VH:CD ratio is observed between treatments as compared to control in ileum and dietary use of 3.5% CSP had significant effects on the jejunum and duodenum as compared to the control group. In conclusion, the application of CSP at 1.5% and 3.5% had a positive effect on villus height and VH:CD ratio of all segments. However, further research is required to understand the precise mechanisms underlying medicine plant effects as well as what the ideal percentage for each plant will be added for a best positive effect on performance.

Keywords: Broiler chicken, Coriander Sativum, Gastrointestinal functions, Gut Morphology Herbal additives.

INTRODUCTION

The poultry production industry at a global scale has experienced significant growth and fluctuations in order to meet the increasing demand in recent years. Conversely, there has been a notable rise in both public demand and scientific interest for poultry production, specifically in relation to feeding practices involving medicinal botanicals (Jyotsana and Berwal, 2019). Apart from individual herbal plants, herbal blends have also emerged as promising alternatives for gut health modulation (Stefanello et al., 2020; Pham et al., 2022). The synergistic effects of herbal blends/mixtures can lead to even more pronounced improvements in gut morphology and overall broiler (Abudabos et al., 2018; Giannenas et al., 2018). This effect duo to multiple properties of these plants as anti-inflammatory, antioxidant, and antimicrobial activities (Windisch et al., 2008; Momin et al., 2012; Ulrikh et al., 2022).

One such botanical of interest is coriander (*Coriandrum sativum* L.), is an umbelliferous annual plant of parsley family, native of eastern Mediterranean and Southern Europe, but is extensively cultivated in North Africa (Ahmad et al., 2016). The whole dried seeds are ground and widely used as condiment or spice in the Mediterranean region and known as medicinal plants (Stefanaki and van Ande, 2022). Seeds are also employed to flavor foods like fish and meat, bakery and confectionery products due to antioxidant effects (Lin et al., 2022). The therapeutic properties and nutritional values of coriander seeds are due to the presence of many bioactive compounds such as monounsaturated fatty acids, especially of petroselinic and linolenic acids, flavonoids, β-carotene, phenolic compounds and volatile compounds (Bhat et al., 2014). These constituents play a crucial role in modulating the gastrointestinal environment of broiler chickens, thereby enhancing their feed digestion and overall utilization lead to increased growth performance (Ahmad et al., 2016). A study conducted by Khubeiz and Shirif (2020) has enhanced the increased immunity and dressing without skin percentage by adding seed powder 150g /1kg basal diet.

Since the small intestine has an essential role in the nutrient absorption and assimilation; therefore, the proper structure and function of the intestine is critical for poultry health and performance (Montagne et al., 2003; Othman et al., 2022). Based on these reports, the objective of the present study was to investigate the effects of application of three levels of coriander seed powder in various gastrointestinal morphometric. A specific emphasis is placed on the utilization of herbal plants as a supplement in poultry feed.

Citation: Khubeiz MM, Algriany OA, Elmghirbi WM, Bilkhayr GhR, Shirif AM (2024). Effect of dietary supplementation of coriander seed (*Coriandrum sativum* l.) on gut morphology in broiler chickens. *Online J. Anim. Feed Res.*, 14(3): 204-210. DOI: https://dx.doi.org/10.51227/ojafr.2024.24

MATERIAL AND METHOD

Chickens and experimental designs

The trial designed according to a completely randomized design to determine the effect of dietary use of coriander seed powder (CSP) on growth performance and the morphological changes of the intestinal parts parameters of (duodenum, jejunum and ileum) of broiler chickens reared in clean pens fitted with deep litter under a standard hygienic condition. One hundred sixty Ross 308 chickens were allocated in three different treatments/experimental groups; 0.0%T1, 1.5%T2, and 3.5%T3 plus the basal diet. Experimental diets (starter and finisher) were used in this trial according to the National Research Council (NRC) regulations (1994) and standard to meet the chickens' daily requirements. The ingredients and chemical composition of the experimental diets are presented in Table 1. Each treatment group involves 60 chickens in three replicate and each replicate has 20 chickens.

Samples collection

On the day 45, 6 chicks from each treatment were slaughtered, then, 3 cm lengths of duodenum (midpoint of the pancreatic loop), jejunum (midpoint of jejunum) and ileum (5 cm after *diverticulum vitellinum*) immediately after sample were cut the physiological saline was used to flush the sample in order to remove the lumen content. All samples were placed in individually labeled flasks containing 10% buffered formalin for fixation. Samples for histology were dehydrated in graded concentrations of ethyl alcohol (70%, 80%, 95%, 96% and 100%), cleared in xylene, and embedded in paraffin. After trimming, 5µm thick section of the samples was cut using a rotary microtome machine, each sample was placed onto a glass slide and dried then stained with hematoxylin-eosin and examined by Semi-light microscopy (2000) according to method of Hashemi et al. (2014). The intestinal morphometric variables, villus height, crypt depth and villus height: crypt depth ratio was evaluated in the duodenum, jejunum and ileum. Histological examinations were carried out according to the method of Iji et al. (2001). Histological sections were examined under magnification 2.5 X of light microscope and software for image analysis (Java image processing program inspired by NIH Image for the Macintosh) (Perić et al., 2015). Villus height was measured from the tip of the villi to the base between individual villi, and crypt depth measurements were taken from the valley between individual villi to the basal membrane.

Statistical analysis

Calculations and statistical analyses were carried out for all data by using Microsoft Excel (spreadsheet) and Minitab 21 version and significance was considered at $P<0.05$ and $P<0.01$ for analysis of variance. Preliminary analysis of data has been done by using a general linear model. Normality test was applied on all data residuals. Subsequent test used by Tukey's for comparing mean values of the variables (for more than two means).

Table 1 - Ingredients and chemical analyses composition of the starter and finisher diets

Ingredients (kg/1000kg)	Starter	Finisher
Yelow corn	470	522
Wheat	150	150
Soyabean (CP 47%)	322	265
Corn oil	10	15
Ground limestone	7.2	7.2
Salt	2.8	2.8
Vitamin and mineral premix	38	38
Total	1000	1000
Calculateded Chemical analysis of diet (%)		
Metabolizable energy (ME)	2930	2996
Crude protein (CP)	21.6	20
Ether extract (E E)	3	3
Crude fiber (CF)	3	3
Calcium	1	1
P (available)	0.4	0.40
Lysin	1.25	1
Methionin	0.50	0.46
Threonine	0.76	0.64
Tryptophan	0.25	0.23
Argenin	1.42	1.29
Valin	1.08	0.99

*Source: Two experimental diets (starter and finisher) were set by Al-Maraie Company according to the National Research Council (NRC) regulations (1994) and standard to meet the chickens' daily requirements.

RESULTS

In the study conducted by Khubeiz and Shirif (2020), the findings indicated that the addition of coriander seed powder at various levels did not have a significant effect (P>0.05) on the growth performance or mortality rates of the chickens throughout the experimental period. In the present study, the effect of coriander seed powder was evaluated on gut morphology, as completive work.

The mean villus height, crypt depth and villus height/crypt depth ratio of intestinal gut (duodenum, jejunum and ileum) of different groups are presented in Tables 2, 3 and 4 respectively. Representative intestinal histomorphology of different groups is shown in Figure 1.

The villus heights and villus width of duodenum were significantly (P<0.01) improved with the application of CSP at the two levels (1.5% and 3.5%) as compared to the control. Moreover, the villus heights/crypt depth ratio were significantly (P<0.05) increased only at the level of 3.5% as compared to the control. The Crypt depth of duodenum was significantly (P<0.01) increased only when the application rate of CSP at 1.5% as compared to 3.5% and the control as shown in Table 2.

The villus height of jejunum was significantly (P<0.01) increased as the application of CSP levels were increased and the rate of increases were similar for the both levels (1.5% and 3.5%). Moreover, the villus height/crypt depth ratio was significantly (P<0.01) augmented when the rate of CSP was applied at 3.5% as compared to the control. Whereas, the crypt depth of jejunum and the villi width of jejunum were not significantly affected (P>0.05) with the application of CSP at the two treatment levels as compared to the control as illustrated in Table 3.

The villus heights of ileum were remarkably (P<0.01) improved when the diet was fortified with CSP at 1.5% and 3.5% and the great improvement was observed at 1.5% as compared to 3.5% and control. Moreover, the villus width of ileum was significantly (P<0.01) increased at 1.5% as compared to 3.5% and the control. Whereas, the villus height/crypt depth ratio was significantly (P<0.01) increased at 1.5% and 3.5% where the rate of increases was similar for both treatment levels (1.5% and 3.5%). However, the crypt depth of ileum was not changed (P>0.05) in all treatment levels (0%, 1.5% and 3.5%) respectively as presented in Table 4.

Table 2 - Effect of the additional different levels of CSP to the broiler chicken diets on duodenum at 45 days of age.

Dependent variable (µm)	Control	1.5% CSP	3.5% CSP	SEM	*P*-Value
Villus height of duodenum	1150.2^{b}	1448.3^{a}	1459.8^{a}	15.0	**
Crypt depth of duodenum	194.9^{c}	235.1^{a}	215.3^{b}	2.90	**
Villus width of duodenum	189.6^{b}	233.3^{a}	228.2^{a}	3.47	**
Villus height/crypt depth ratio	6.5^{b}	6.8^{ab}	7.2^{a}	0.127	*

a, b and c: Means with different superscripts in the same row were significantly different at P<0.05. **: (P<0.01). SEM: standard error of means. CSP: coriander seed powder

Table 3 - Effect of offering different levels of CSP to the broiler chicken diets on jejunum at 45 days of age.

Dependent variable (µm)	Control	1.5% CSP	3.5% CSP	SEM	*P*-Value
Villus height of jejunum	601.9^{b}	833.3^{a}	817.2^{a}	10.9	**
Crypt depth of jejunum	157.4^{a}	174.6^{a}	175.8^{a}	2.92	NS
Villus width of jejunum	221.6^{a}	208.0^{a}	198.8^{a}	3.55	NS
Villus height/crypt depth ratio J	4.3^{b}	4.9^{ab}	5.3^{a}	0.10	**

a, b and c: Means with different superscripts in the same row were significantly different at P<0.05. **: (P<0.01). SEM: standard error of means. CSP: coriander seed powder

Table 4 - Effect of application of different levels of CSP to the broiler chicken diets on ileum at 45 days of age.

Dependent variable (µm)	Control	1.5% CSP	3.5% CSP	SEM	*P*-Value
Villus heights of ileum	369.3^{c}	750.0 ^{a}	599.5^{b}	13.7	**
Crypt depth of ileum	148.0^{a}	159.1^{a}	145.4^{a}	2.39	NS
Villus width of ileum	189.4^{b}	226.1^{a}	167.5^{b}	4.84	**
Villus height/crypt depth ratio I	2.6^{b}	5.0^{a}	5.0^{a}	0.12	**

a, b and c: Means with different superscripts in the same row were significantly different at P<0.05. **: (P<0.01). SEM: standard error of means. CSP: coriander seed powder.

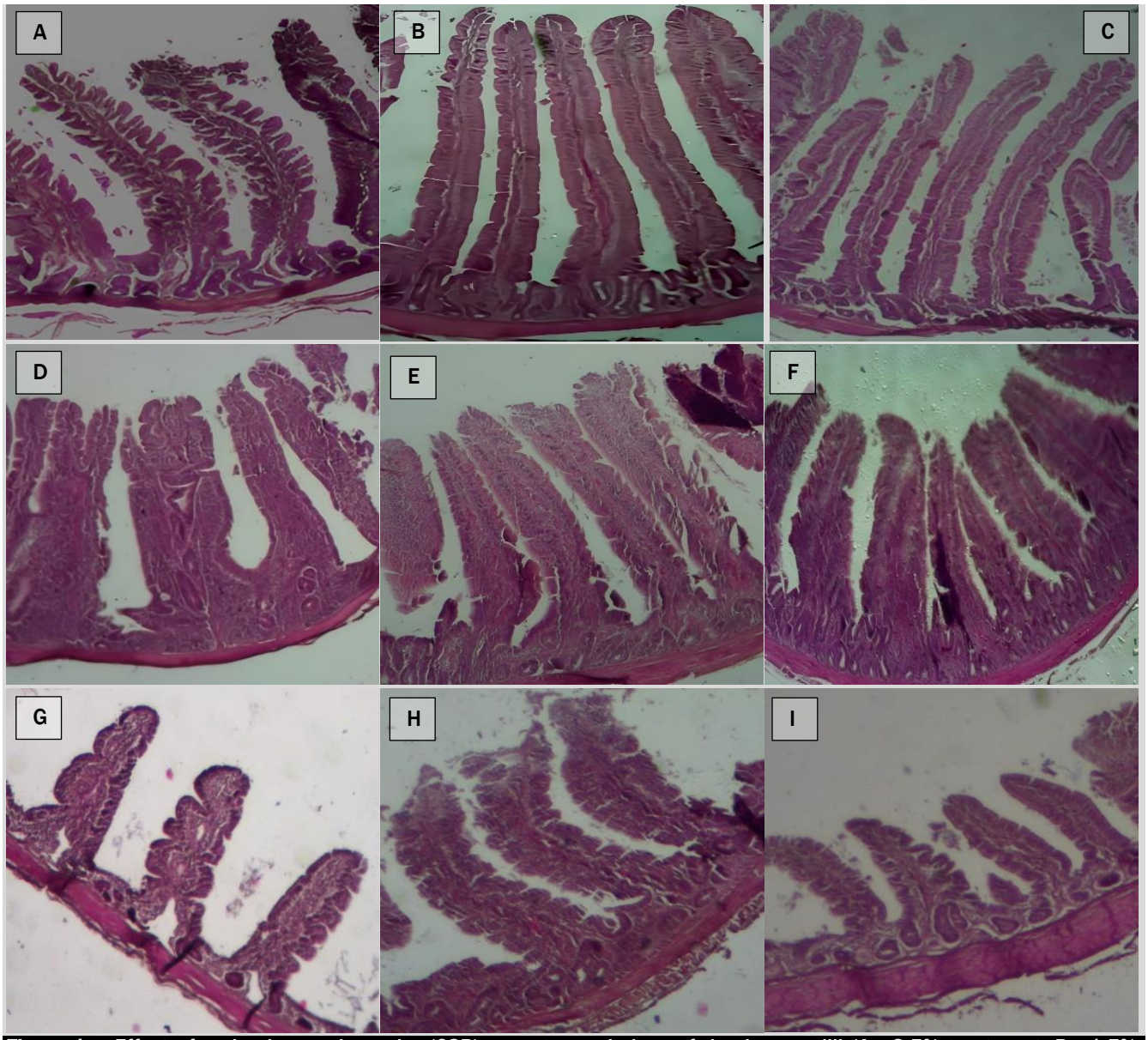


Figure 1 – Effect of coriander seed powder (CSP) on gut morphology of duodenum villi (A= 3.5% treatment: B= 1.5%, treatment; C= 0% treatment). Morphology of jejenum villi (D= 3.5% treatment; E= 1.5% treatment; F= 0% treatment). Morphology of ileum villi, G= 3.5% treatment; H= 1.5% treatment; I= 0% treatment). Histological sections were examined under magnification 2.5 X by Stemi light microscope.

DISCUSSION

The application of coriander seed powder at 1.5% and 3.5% pulse the basal diet showed great augmentation of villus heights of the duodenum, jejunum and ileum as compared to control, which was in same line (Al-Tememy et al., 2011; Ghazanfari et al., 2015; Gurram et al., 2022). These results, in agreement with other studies, revealed the significant increase of jejunum villus height and villus height to crypt depth (VH: CD) ratio (Reuben et al., 2021; Othman et al., 2022) by adding herbal plants. This finding confirms the intestinal gut influence by active compounds present in diet. The main component of coriander oil, linalool, promotes increase in broiler villi height, and therefore, may enhance the activity of digestive enzymes, leading to change of its morphology (Misharina, 2001). Longer villi are associated with activated cell mitosis. It is assumed that increased villus height is paralleled by enhanced digestive and absorptive functions of the intestine due to larger absorptive surface area and higher expression of brush border enzymes and nutrient transport systems, which could influence intestinal morphology (Yamauchi, 2002). This result of increasing height of villus by adding fermented herbal plants was supported by Lokaewmanee et al. (2012) findings. Whereas there were no significant effect on jejunum crypt depth and these results were in agreement with other studies (Reuben et al., 2021; Gurram et al., 2022; Othman et al., 2022) by supplement with medical plant that opposite to finding revealed by Sholiha et al. (2023) added coriander to basal diet. Short villus and deep crypts may lead to poor nutrient absorption, increased toxin secretion in the gastrointestinal tract, and worse performance (Montagne et al., 2003) this could be attributed to either the

concentration levels added of herbs to the diet or due to chemical composition of basal diet, which contain anti nutritive factor as lignin, trypsin or phytates lead to reduce digestibility of other important nutrient (Brenes and Roura, 2010). In contrast, this result revealed that increases in VH: CD ratio is an indicator of the likely digestive capacity of the small intestine, which correlated with improved epithelial cell turnover this result was corresponding with Amad et al. (2013); Ghazanfari et al. (2015); Khan et al. (2020); Adegbeye et al. (2020); Reuben et al. (2021); Gurram et al. (2022). So, the increase in this ratio corresponds to an increase in digestion and absorption as well as reduced levels of pathogenic bacteria and may enhance immune status. The significant effect on VH: CD ratio may be related to antimicrobial and immune-modulating activities of coriander (Taha et al., 2019; Ulrikh et al., 2022). This result supported by Oso et al. (2019) that adding phytogenic blend to chicken diet. On the other hand, a non-significant effect in VH: CD ratio was agreed by Sholiha et al. (2023) which is indicative of a higher rate of enterocyte-cell migration from the crypt to the villus and poor absorption of nutrients. It is related to stress factor as ambient temperature leads to reduced growth performance of chickens (Welay et al., 2023) or due to secondary metabolites. A Recent study by Lin et al. (2022) revealed that the light quality and intensity influenced plant growth and secondary metabolites. These findings suggest that coriander has a positive impact on intestinal morphology in poultry, potentially contributing to improved gut health and overall performance.

CONCLUSION

The summarized conclusion of the current study revealed that the application of CSP at 1.5% and 3.5% had a positive effect on villus height of duodenum, jejunum and for the ileum was observed at 1.5% as compared with control and 3.5%. Moreover, the villus height/crypt depth ratio of duodenum and jejunum was at 3.5% and in the ileum were at 1.5% and 3.5% respectively. The dietary supplementation of coriander seed had a significantly positive effect on gut morphology in all segments of intestinal gut which this effect may enhance growth performance in broiler chickens. Future research should focus on exploring potential mechanisms underlying the observed change, as well as investigating the impact of coriander additives on other aspects of poultry health and performance.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Mona Khubeiz; E-mail: monakhubiez@gmail; ORCID: https://orcid.org/0009-0009-4534-7765

Data availability

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

Authors' contributions

M.M. khubeiz, collected data and samples, conducted laboratory analysis and prepared the original draft. O.A. Algriany, provided equipment and programming software. W. M. Elmghirbi, managed the fund of this research. G.R. Bilkhayr, provided chemicals for histological procedure. A. M. Shirif, designed and reviewed the writing. All the authors approved the final revision.

Ethical committee approval

This study was approved by the Graduate School of the University of Tripoli, Faculty of Veterinary Medicine, Department of Physiology, Biochemistry and Nutrition. All animal welfare protocols were followed.

Acknowledgment

The authors would like to acknowledge the support of University of Tripoli, Faculty of Veterinary Medicine, Department of Physiology, Biochemistry and Nutrition and the Technion Trkeia for helpful support on histological samples preparation.

Competing interests

The authors have declared no conflict of interest

REFERENCES

Abudabos AM, Alyemni AH, Dafalla YM and Khan RU (2018). The effect of phytogenics on growth traits, blood biochemical and intestinal histology in broiler chickens exposed to Clostridium perfringens challenge. Journal of applied animal research 46(1): 691-695. https://doi.org/10.1080/09712119.2017.1383258

Adegbeye M, Oloruntola O, Asaniyan E, Agunbiade B, Oisagah E and Ayodele S (2020). Pawpaw, black cumin, and mustard seed meals dietary supplementation in broiler chickens: effect on performance, gut microflora, and gut morphology. Journal of Agricultural Science and Technology, 22(5): 1235-1246. http://jast.modares.ac.ir/article-23-32233-en.html

Ahmad M, Kumar A and Singh P (2016). Effects of coriander (Coriandrum sativum L.) seed powder on growth performance of broiler chickens. Journal of Krishi Vigyan 5(1): 57-59. http://dx.doi.org/10.5958/2349-4433.2016.00034.9

Al-Tememy HS, Al-Jaff FK, Al-Mashhadani EH and Hamodi SJ (2011). Histological effect of inclusion different levels of coriander oil in broiler diet on small intestine. Diyala Agricultural Sciences Journal 3(2): 1-11. http://148.72.244.84:8080/jspui/handle/xmlui/10767

Amad AA, Wendler K and Zentek J (2013). Effects of a phytogenic feed additive on growth performance, selected blood criteria and jejunal morphology in broiler chickens. Emirates Journal of Food and Agriculture, 25: 549-554. http://dx.doi.org/10.9755/ejfa.v25i7.12364

Bhat S, Kaushal P, Kaur M and Sharma H (2014). Coriander (*Coriandrum sativum* L.): Processing, nutritional and functional aspects. African Journal of plant science 8(1): 25-33. https://academicjournals.org/article/article1390549943_Bhat%20et%20al.pdf

Brenes A and Roura E (2010). Essential oils in poultry nutrition: Main effects and modes of action. Animal feed science and technology 158(1-2): 1-14. https://doi.org/10.1016/j.anifeedsci.2010.03.007

Ghazanfari S, Mohammadi Z and Adib Moradi M (2015). Effects of coriander essential oil on the performance, blood characteristics, intestinal microbiota and histological of broilers. Brazilian Journal of Poultry Science 17: 419-426. https://www.redalyc.org/pdf/1797/179743242002.pdf

Giannenas I, Bonos E, Skoufos I, Tzora A, Stylianaki I, Lazari D, et al. (2018). Effect of herbal feed additives on performance parameters, intestinal microbiota, intestinal morphology and meat lipid oxidation of broiler chickens. British Poultry Science 59(5): 545-553. https://doi.org/10.1080/00071668.2018.1483577

Gurram S, Chinni Preetam V, Vijaya Lakshmi K, Raju M, Venkateswarlu M and Bora S (2022). Synergistic effect of probiotic, chicory root powder and coriander seed powder on growth performance, antioxidant activity and gut health of broiler chickens. Plos One, 17(6): e0270231. https://doi.org/10.1371/journal.pone.0270231

Hashemi S, Zulkifli I, Davoodi H, Hair Bejo M and Loh T (2014). Intestinal histomorphology changes and serum biochemistry responses of broiler chickens fed herbal plant (*Euphorbia hirta*) and mix of acidifier. Iranian Journal of Applied Animal Science 4(1): 95-103. https://journals.iau.ir/article_513723.html

Iji P, Saki A and Tivey D (2001). Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. Animal Feed Science and Technology 89(3-4): 175-188. https://doi.org/10.1016/S0377-8401(00)00223-6

Jyotsana PK and Berwal R (2019). Evaluation of the effect of supplementation of Ashwagandha (*Withania somnifera*) root powder in the broiler's ration on gut morphology and bacteriology: A. The Pharma Innovation Journal, 8(10): 86-89. https://www.thepharmajournal.com/archives/2019/vol8issue10/PartB/8-10-13-437.pdf

Khan I, Nawaz M, Anjum AA, Ahmad MD, Mehmood A, Rabbani M, et al. (2020). Effect of indigenous probiotics on gut morphology and intestinal absorption capacity in broiler chicken challenged with Salmonella enteritidis. Pakistan Journal of Zoology, 52(5): 1825-1831. https://dx.doi.org/10.17582/journal.pjz/20190518090547

Khubeiz MM and Shirif AM (2020). Effect of coriander (*Coriandrum sativum* L.) seed powder as feed additives on performance and some blood parameters of broiler chickens. Open veterinary journal, 10(2): 198–205. https://doi.org/10.4314/ovj.v10i2.9

Lin H-H, Lin K-H, Yang M-J, Nguyen HC, Wang H-J, Huang H-X and Huang M-Y (2022). Physiological responses and antioxidant properties of coriander plants (*Coriandrum sativum* L.) under different light intensities of red and blue lights. Scientific Reports 12(1): 21139. https://doi.org/10.1038/s41598-022-25749-3

Lokaewmanee K, Yamauchi K and Thongwittaya N (2012). Effects of fermented plant product on growth performance, some blood variables, carcase characteristics, and intestinal histology in broilers. British Poultry Science 53(2): 215-223. https://doi.org/10.1080/00071668.2012.665435

Misharina T (2001). Effect of conditions and duration of storage on composition of essential oil from coriander seeds. Prikladnaia biokhimiia i mikrobiologiia 37(6): 726-732. PMID: 11771329

Momin AH, Acharya SS and Gajjar AV (2012). *Coriandrum sativum*-review of advances in phytopharmacology. International Journal of Pharmaceutical Sciences and Research 3(5): 1233-1239. http://dx.doi.org/10.13040/IJPSR.0975-8232.3(5).1233-39

Montagne L, Pluske J and Hampson D (2003). A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Animal feed science and technology 108(1-4): 95-117. https://doi.org/10.1016/S0377-8401(03)00163-9

Oso A, Suganthi R, Reddy GM, Malik P, Thirumalaisamy G, Awachat V, et al. (2019). Effect of dietary supplementation with phytogenic blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. Poultry Science 98(10): 4755-4766. https://doi.org/10.3382/ps/pez191

Othman SM, Naser KMB, Kanoun AH, Salim AA, Sherif BM and Asheg AA (2022). Effect of adding clove buds powder in feed on performance and jejunum morphology in broiler chickens. Open Veterinary Journal 12(6): 995–999. https://doi.org/10.5455/ovj.2022.v12.i6.28

Perić L, Ivković M, Žikić D and Jajić I (2015). Effect of Rapeseed Meal on Nutrient Digestibility and Gut Morphology in Broiler Chickens. Scientific Papers: Animal Science & Biotechnologies/Lucrari Stiintifice, 48(1): 38-39. https://spasb.ro/index.php/public_html/article/view/194

Pham VH, Abbas W, Huang J, He Q, Zhen W, Guo Y, et al. (2022). Effect of blending encapsulated essential oils and organic acids as an antibiotic growth promoter alternative on growth performance and intestinal health in broilers with *necrotic enteritis*. Poultry Science, 101(1):101563. https://doi.org/10.1016/j.psj.2021.101563

Reuben R, Onimisi P, Sekoni A and Salihu E (2021). Growth performance, carcass characteristics and villi morphometry of broiler chickens fed diets containing Mentha piperitaand Adansonia digitataleaves meals as phytobiotics. Nigerian Journal of Animal Production 48(6): 217-228. https://doi.org/10.51791/njap.v48i6.3296

Sholiha K, Dono N and Ariyadi B (2023). Growth Performance and Intestinal Health of Broiler Chickens Supplemented with Coriander Oil Nanoemulsion in Drinking Water. Tropical Animal Science Journal 46(1): 55-62. https://doi.org/10.5398/tasj.2023.46.1.55

Stefanaki A, van Andel T (2021). Mediterranean aromatic herbs and their culinary use. In Aromatic Herbs in Food. Academic Press, Elsevier Inc. pp. 93-121. https://doi.org/10.1016/B978-0-12-822716-9.00003-2

Stefanello C, Rosa DP, Dalmoro YK, Segatto AL, Vieira MS, Moraes ML, et al. (2020). Protected blend of organic acids and essential oils improves growth performance, nutrient digestibility, and intestinal health of broiler chickens undergoing an intestinal challenge. Frontiers in Veterinary Science, 6:491. https://doi.org/10.3389/fvets.2019.00491

Taha AE, Hassan SS, Shewita RS, El-seidy AA, Abd El-Hack ME, Hussein E, et al. (2019). Effects of supplementing broiler diets with coriander seed powder on growth performance, blood haematology, ileum microflora and economic efficiency. Journal of animal physiology and animal nutrition 103(5): 1474-1483. https://doi.org/10.1111/jpn.13165

Ulrikh E, Smolovskaya O and Pak N (2022). Study of antibacterial and antioxidant properties of medical plant extracts. BIO Web of Conferences. EDP Sciences, ID: 06001. https://doi.org/10.1051/bioconf/20224706001

Welay K, Amaha N, Demeke S, Debusho LK and Girma M (2023). Growth performance and carcass characteristics of Koekoek chickens exposed to temperature variation with supplementary Coriander seed powder. Journal of Thermal Biology, 116: 103674. https://doi.org/10.1016/j.jtherbio.2023.103674

Windisch W, Schedle K, Plitzner C and Kroismayr A (2008). Use of phytogenic products as feed additives for swine and poultry. Journal of animal science 86(suppl_14): E140-E148. https://doi.org/10.2527/jas.2007-0459

Yamauchi K (2002). Review on chicken intestinal villus histological alterations related with intestinal function. The Journal of Poultry Science, 39(4): 229-242. https://doi.org/10.2141/jpsa.39.229

2024 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 14, Issue 3: 211-217; May 25, 2024

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2024.25

URACHUS ANOMALY IN SHEEP: INCIDENCE AND CONSIDERATIONS PRESENTED IN NEONATAL LAMBS IN THE PERU

Jordan Ninahuanca CARHUAS[1,3], Michue Salguedo NAPOLEON[2], Fernando Arauco VILLAR[1], Edgar GARCIA-OLARTE[1], Carlos Quispe EULOGIO[3], Yakelin MAURICIO-RAMOS[1], and Ide Unchupaico PAYANO[1]

[1]*Departamento Académico de Zootecnia, Universidad Nacional del Centro del Perú, Av. M.C. 3909, Huancayo, 12000, Perú*
[2]*División de Servicio Veterinario, SAIS Pachacútec SAC, CAP, Corpacancha 12780, Marcapomacocha, Perú*
[3]*Escuela Profesional de Medicina Veterinaria y Zootecnia, Facultad de Ciencias de la Salud, Universidad Peruana Los Andes, Huancayo, 12002, Perú*

Email: jninahuanca@uncp.edu.pe

Supporting Information

CASE REPORT

PII: S222877012400025-14

Received: February 16, 2024

Revised: May 17, 2024

Accepted: May 19, 2024

ABSTRACT: Over the years, an anomaly has been observed in newborn lambs, manifesting itself as a curvature in their posture and a slight dampness in the navel, known by the locals as "pupote". For this reason, the present study was carried out to document and present for the first time to the scientific community an anomaly of the urachus in sheep and its incidence. In the sampling process, five lambs who died of starvation were selected. The specimens were transported to the Animal Health Laboratory of the Faculty of Zootechnics of the National University of Central Peru. Radiographic analyses were carried out there. Then the incidence was found in the collected records. The incidence of the anomaly is an average of 0.825±0.09%. This case highlights the persistence of urachus in newborn lambs, evidenced by the identification of a ligament that establishes a connection between the umbilicus and the liver. Specifically, the ligament identified in the described anomalies corresponds to the *ligamentum teres*.

Keywords: Congenital disease, Ligament of the liver, Pupote, Sheep breeding, Urachus.

INTRODUCTION

A Chilean livestock complex stands out as a pre-eminent player in the sheep production sector, housing a considerable population of around 60,000 sheep (Valenzuela et al., 2019; Carhuas et al., 2022). Its role in Peruvian livestock farming is of significant economic relevance. It originated as a cooperative of surrounding communities, and later consolidated under the Agrarian Reform Law, as an Agrarian Society of Social Interest (SAIS) (Hurtado, 2020). Given the considerable magnitude of sheep production in this entity, several diseases and rare cases have been recorded that deserve attention from the scientific community.

The characterization of the patent urachus is established by the permanence of the tubular connection between the urinary bladder and the umbilicus after the delivery process, as has been noted by previous studies (Buddha et al., 2019; Wilson et al., 2019; Shi et al., 2022). Throughout the gestational period, this phenomenon plays a crucial role in facilitating the drainage of the bladder into the allantoic sac, as documented by Perondi et al. (2020). This process proves to be an essential component in the understanding of fetal physiology, establishing a structural connection that persists beyond birth and thus contributes to physiological dynamics during prenatal and postnatal development.

After birth and subsequent rupture of the umbilical cord, the urachus is expected to close, allowing urine to pass through the urethra (Steiner and Lejeune, 2009). In situations of patent urachus, a constant dribbling of urine from the umbilical stump is evident (Naiem et al., 2022). The process of involution of the umbilical arteries, which connect the internal iliac arteries to the placenta, results in their transformation into the round ligaments of the bladder (Sarmiento et al., 2021). For its part, the umbilical vein, establishing the connection between the placenta, the liver, and the porta cava, regresses to become the round ligament of the liver within the falciform ligament (Mottet et al., 2017).

This anatomical phenomenon, on which the present review is based, highlights the importance of the urachus in the perinatal context and its anatomophysiological repercussions according to the current scientific literature.

CASE PRESENTATION

The study was carried out in the company "SAIS Pachacútec S.A.C", in the facilities of the Production Unit: Corpacancha (11021'46" S; 76013'11" W), located in the district of Marcapomacocha, Province of Yauli, Junín Region - Peru (Figure

Citation: Carhuas JN, Napoleon MS, Villar FA, Garcia-Olarte E, Eulogio CQ, Mauricio-Ramos Y, and Payano IU. (2024). Urachus anomaly in sheep: Incidence and considerations presented in neonatal lambs in the Peru. *Online J. Anim. Feed Res.*, 14(3): 211-217. DOI: https://dx.doi.org/10.51227/ojafr.2024.25

1). In Peru, especially in large livestock companies such as SAIS, lambing usually begins in November. This company belongs to the livestock sector, producing more than 60,000 sheep of the Corriedale breed (Carhuas et al., 2023), 17,000 alpacas, and 4,000 cattle.

During the sampling process, five lambs that had died of starvation and exhibited the anomaly were selected. All of them had similar weights, belonged to the Corriedale breed, and were offspring of ewes from the cattle section, with a dental age of "4D." All lambs were disinfected with iodine at birth and their first lactation was ensured by the "huateros" (field personnel in charge of ensuring the first lactation of the rejected lambs). To evaluate the incidence, lambing records were collected for the 2023 lambing season from the Corpacancha, Santa Ana, and Conocancha production units.

We proceeded to identify lambs with peculiarities in their birth and physiology, manifesting themselves as hunched neonates (Figure 2a), without evidence of fever and with a slight dampness in the umbilicus. Subsequently, the deceased lambs identified with the aforementioned peculiarities underwent an autopsy (Figure 2b) which confirmed the reason for their death, known since the 1980s by the workers as "pupote". This term refers to lambs that, on post-mortem examination, showed a ligament connecting the liver to the umbilicus, characterized by a hunched posture and listless behavior. After that, the specimens were kept in a refrigerated thermal box for transport to the Animal Health Laboratory of the Faculty of Animal Science of the Universidad Nacional del Centro del Perú. In this phase, radiographic analyses were carried out with the El Detector Careray equipment (Figure 2c), using analog radiography to obtain images that facilitated the detailed description of the pathology.

Finally, a thorough autopsy was performed, during which tissues, veins, ligaments, and organs were identified and examined. This was done with the support of the research group to ensure proper identification and description of the anomaly.

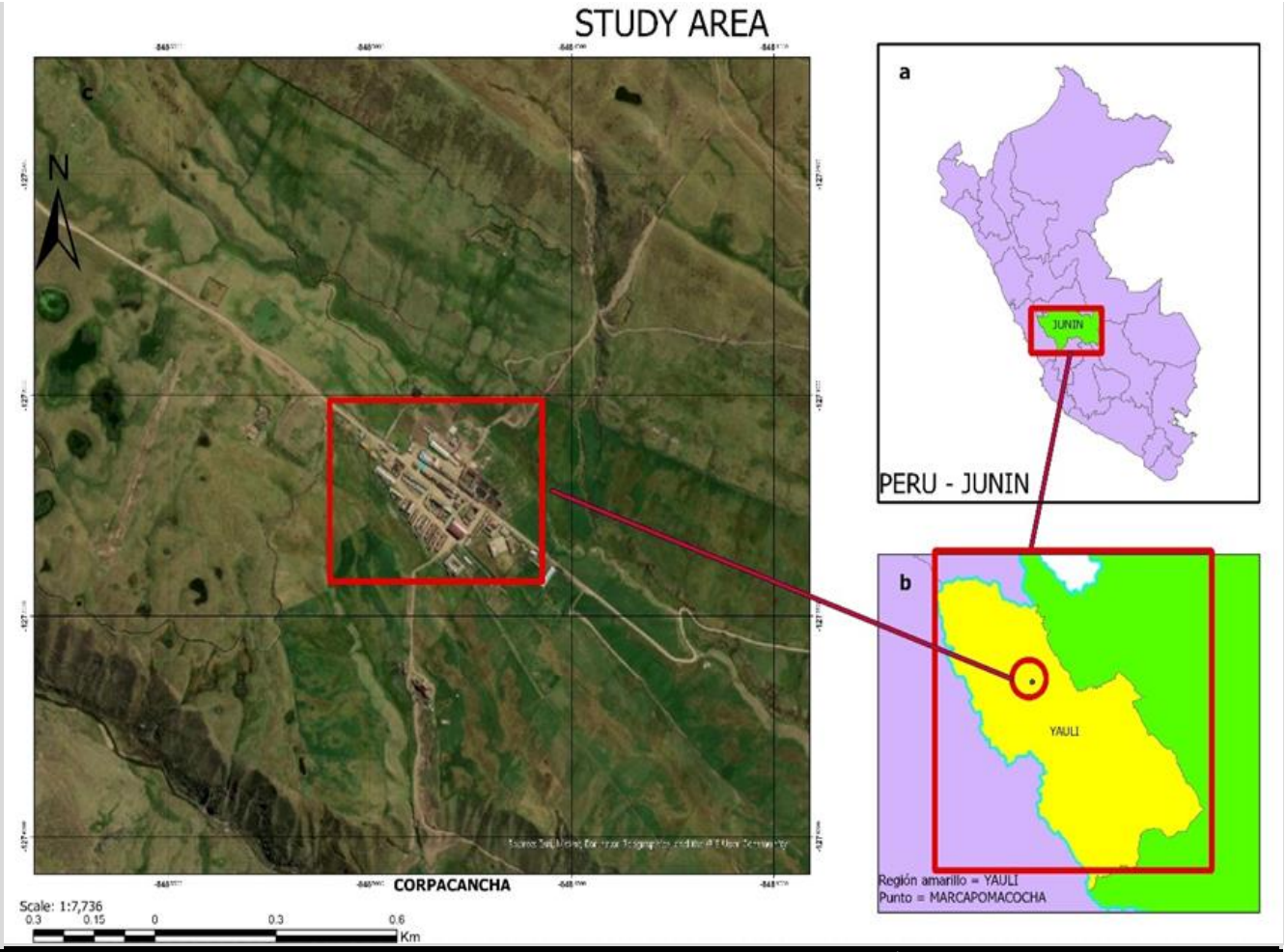


Figure 1 - Location of the study; a. map of Peru by region (green color, shows the Junín region); b. map of the Junin region (yellow color, shows the province of Yauli). District of Marcapomacocha; c. Satellite image, shows the study site, which belongs to the SAIS "PACHACUTEC" SAC - U.P. Corpacancha.

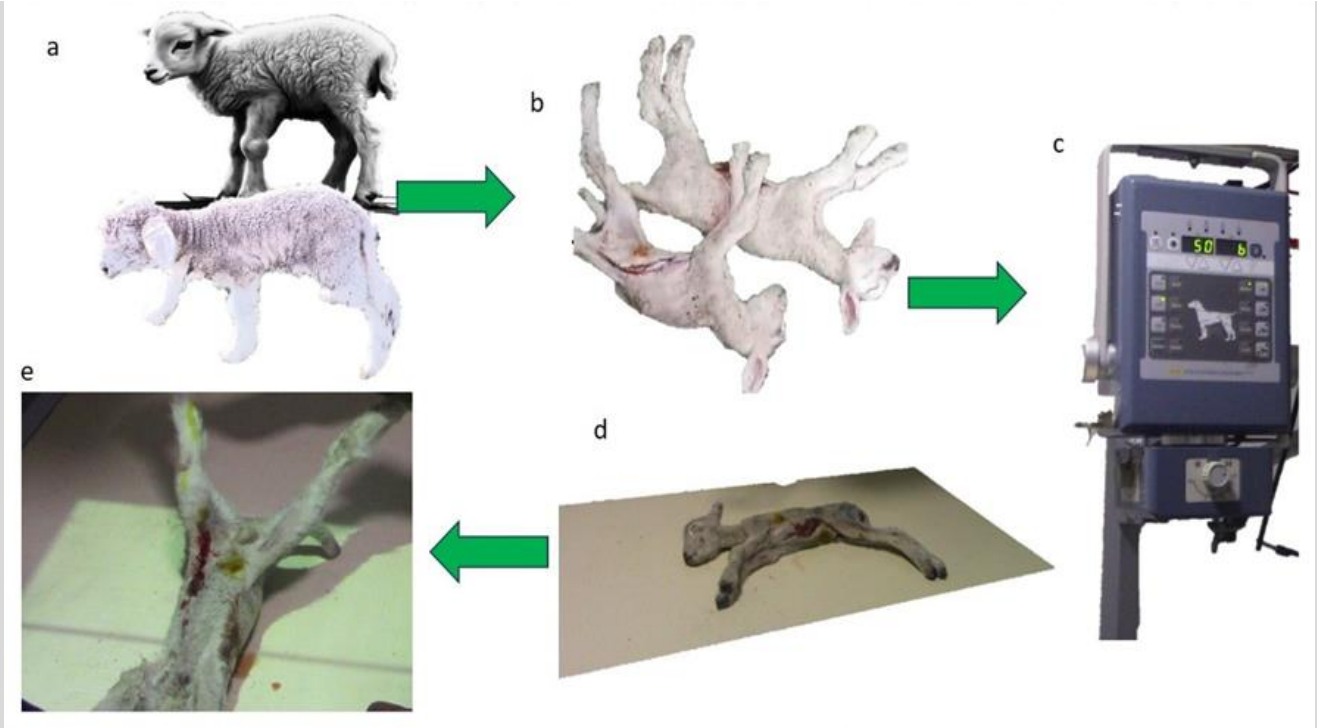


Figure 2 - Study procedure. A. Lambs identified by their hunched morphology and apathetic behavior; b. Autopsy to check for the presence of the abnormality; c. Careray Detector; d. Analogue radiograph of the lamb in lateral recumbency; e. Analogue radiograph of the lamb in dorsal decubitus.

DISCUSSION

The incidence of the anomaly averages 0.825 ± 0.09 % (Table 1) show similarities with the findings reported in Canada, where incidental incidences of urachal anomalies were recorded in a pediatric center caring for newborns (Gleason et al., 2015). There is also agreement with previous studies addressing this issue in infants, such as the work of Chiarenza et al. (2009). Similarly, our results are consistent with those of Dhillon et al. (2015), who claim that urachal abnormalities do not exceed 1% of the population. A comprehensive review of the literature evidences, through parenthetical citations, consistency in the paucity of reported cases of persistent urachus (Hirose et al., 2017; Rojas-Quintero et al., 2023;), underscoring the infrequency of this condition. Diagnostics and some treatment approaches, such as surgical incisions via laparoscopy and cauterizations (Sarmiento et al., 2021), appear to be the best options. These results highlight the importance of undertaking comprehensive longitudinal studies in the future, as indicated by Wilson et al. (2019).

The results obtained from the analog radiographs revealed the presence of the anomaly, commonly referred to by villagers as "pupote", in the lambs submitted for analysis and positioned in dorsal recumbency (Figure 3a,b). In contrast, an X-ray of a lamb without the above-mentioned anomaly, which was in the lateral decubitus position, was included. For ease of comparison, Figure 4 illustrates both a lamb with the presence of the anomaly (Figure 4a) and a lamb with uncomplicated natal development (Figure 4b).

The clinical manifestations identified corroborated the diagnosis of persistent urachus. In the analysis, the presence of transitional epithelium in the inner layer was evident (Figure 5a), corresponding to the same type of epithelium characteristic of the tunica interna of the urinary bladder, as established by Bacha and Bacha (2001), Zamora (2006), and Del Cid et al. (2023). Persistence of the urachus, which results in the non-complete obliteration of this structure after birth, may be due to various causes (abnormal development, genetic, urachal regression problems, and environmental factors; Cappele et al., 2001; Gelikman et al., 2023). It is important to note that persistent urachus can vary in severity and presentation, and is not always associated with significant health problems. However, in some cases, it may predispose to urinary tract infections or other urinary tract complications (Kim et al., 2021). Moreover, to explain the detected anomaly, the configuration of the systemic venous system has been the subject of analysis by several scholars (CFW, 1925; Beaubien-Souligny et al., 2020; Ziętek, 2022), who have sought to understand the anomaly present in the connection between the umbilicus and the liver. One possible explanation for the formation of this anomaly is visualized in Figure 4a. It is postulated that, during the early stages of embryonic development, the venous system is characterized by the persistence of the left vitelline venous connection to the liver (Azuma et al., 2002). Later, the left vitelline vein disappears, and blood from the placenta is redirected to the right sinus horn (Hikspoors et al., 2017; Terasaki et al., 2020), apparently giving rise to the presence of the round ligament of the liver (*ligamentum teres*).

Terasaki et al. (2020) mentioned that surgeons should be aware of portal and hepatic vein anomalies in patients with alterations in the *ligamentum teres*, as this could carry significant risks. Persistent urachus in sheep is a rare phenomenon that has been identified as an exceptional anatomical case. The urachus, a tubular structure that connects the urinary bladder to the umbilicus during fetal development, normally closes after birth, forming the umbilical ligament (Ziętek, 2022). However, this case report highlights an unusual variation in which a persistent ligament connecting the umbilicus to the liver is observed in newborn lambs identified as *ligamentum teres*, which, from the literature reviewed, lambs with persistence of the urachus have this persistence of the *ligamentum teres* (Figure 5b). Clarifying the terminology used by the field shepherds of "Pupote".

Table 1 - The incidence of the anomaly averages

Place*	Lambs born (n)	Lambs with the presence of the anomaly (n)	Incidence
U.P. Corpacancha	10501	98	0.933
U.P. Conocancha	3502	26	0.742
U.P. Santa Ana	3004	24	0.799

Explanatory notes: *epidemiological units.

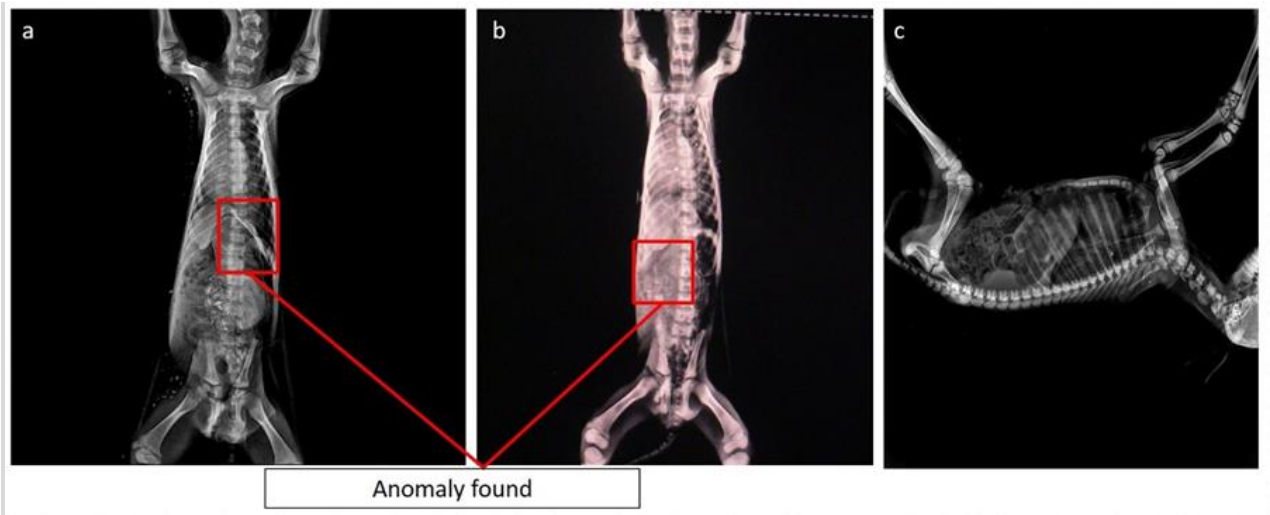


Figure 3 - Analogue radiographs of three lambs. a. Analogue radiograph of lamb in dorsal recumbency (lamb with abnormality); b. Analogue radiograph of lamb in dorsal recumbency (lamb with abnormality); c. Analogue radiograph of lamb in lateral decubitus (lamb without abnormality).

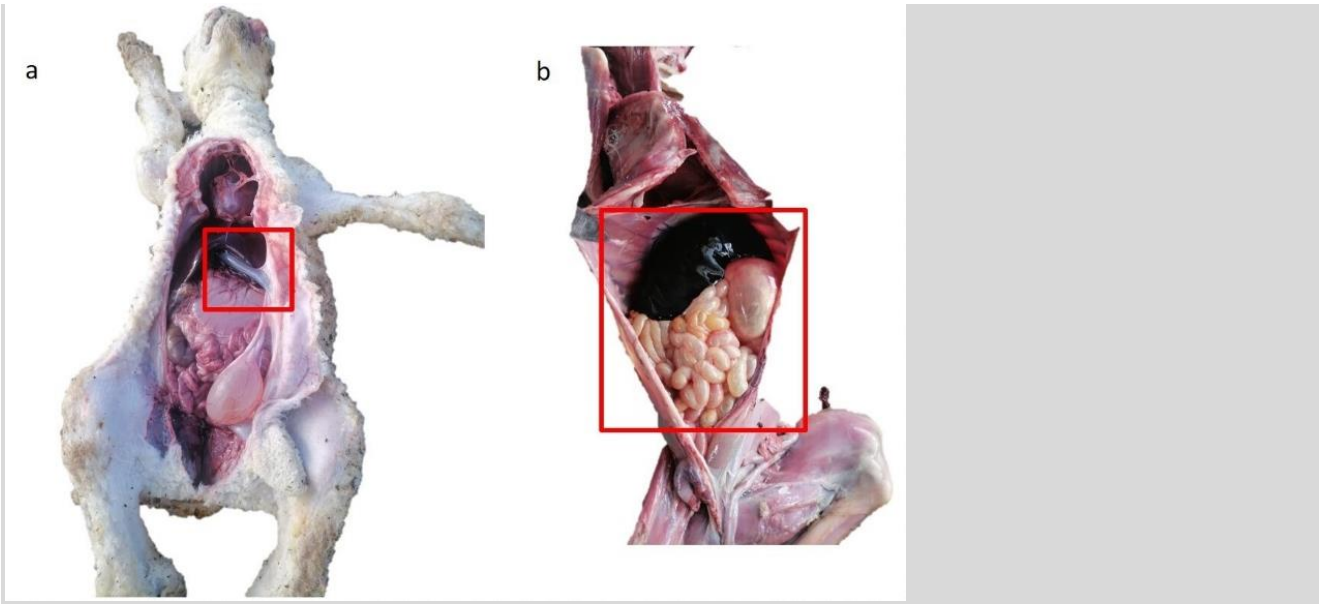


Figure 4 - Autopsy of two lambs. (a) Autopsy of lamb with abnormality; (b) Autopsy of normal lamb.

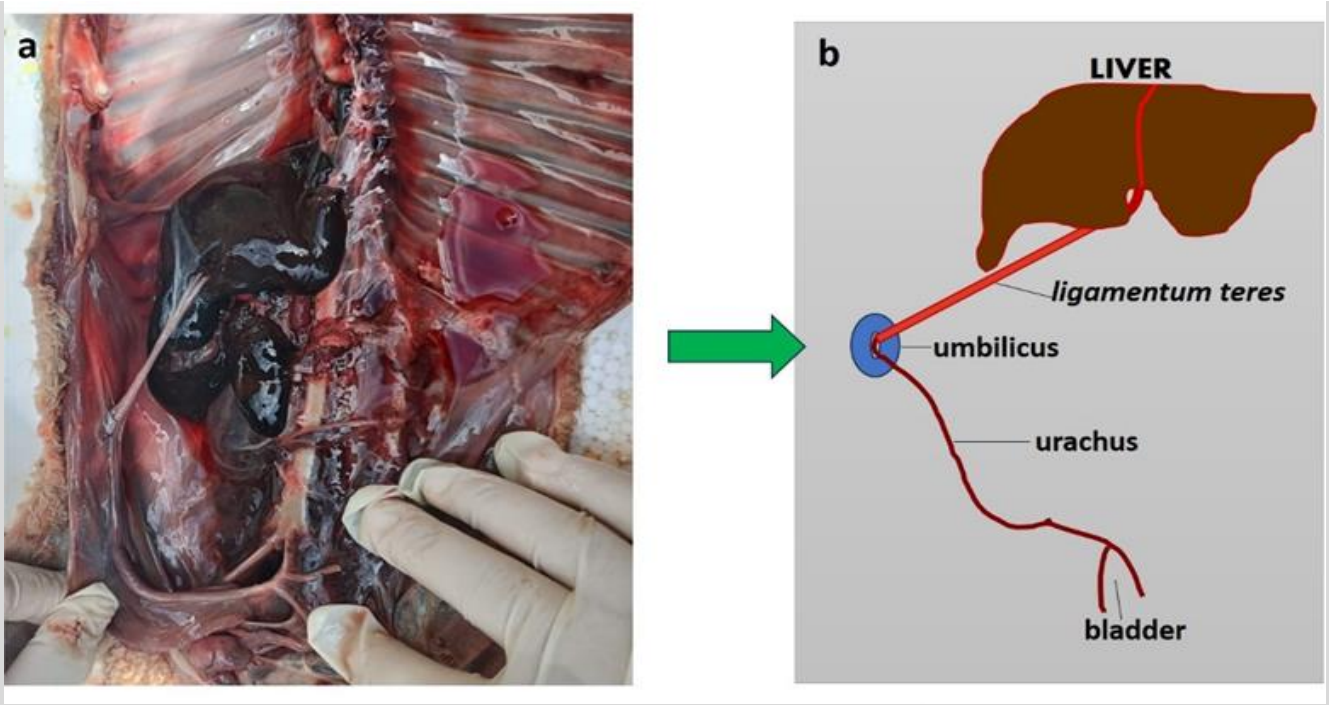


Figure 5 - Identification of the abnormality. a. Lamb with persistent urachus, ligamentum teres present; b. Identifying the components observed in the Lamb.

CONCLUSION

This "pupote" case highlights the persistence of urachus in newborn lambs, evidenced by the identification of a ligament that establishes a connection between the umbilicus and the liver. Specifically, the ligament identified in the described anomalies corresponds to the *ligamentum teres*. The incidence of persistent urachus, represented by persistent *ligamentum teres*, in neonatal lambs is 0.82%. This condition is classified as a congenital disease, possibly influenced by several factors, including abnormal development, genetic factors, problems in urachal regression, and environmental variables.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Jordan Ninahuanca Carhuas. E-mail: jninahuanca@uncp.edu.pe; ORCID: https://orcid.org/0000-0002-0137-0631

Ethical regulations

The present research is subject to the rules and ethical codes of Peruvian Law No. 30407, article 19, which establishes the animal welfare protocols and the conditions under which specimens should be included for academic-scientific purposes only. In addition to having the approval by the ethical charter of animal welfare LETTER N° 003-GRJ-DRA-AAC-PERÚ-2023.

Authors' contribution

J.N. Carhuas: conceptualization, research, writing-revision and editing. N.M. Salguedo: conceptualization, sampling and research. F.A. Villar: writing the original draft, visualization, and revision. E. García-Olarte: visualization, formal analysis, writing, revision and editing. C.Q. Eulogio: visualization, formal analysis, and conceptualization. Yakelin Mauricio Ramos: methodology, photo editing, and revision. I.U. Payano: Redaction.

Acknowledgements

The authors would like to thank the staff of the U.P. Corpacancha, especially Don Lucas Aliaga and Ángel Artica Capcha, for their collaboration at all times. We would also like to thank the workers of the U.P. Santa Ana for their hospitality throughout the research process.

Consent to publish

All authors agree to the publication of this manuscript.

Competing Interests

The authors have not declared any competing interest.

REFERENCES

Azuma C, Tohno Y, Tohno S, Moriwake Y, Utsumi M and Yamada M (2002). Persistent left hepatic venous connection with the right atrium and the ductus venosus. Anatomical science international, 77: 124-127 doi: https://doi.org/10.1046/j.0022-7722.2002.00001.x

Bacha WJ and Bacha LM (2001). Color atlas of veterinary histology: Inter-Médica, from https://apunteca.usal.edu.ar/id/eprint/3000/

Beaubien-Souligny W, Rola P, Haycock K, Bouchard J, Lamarche Y, Spiegel R and et al. (2020). Quantifying systemic congestion with point-of-care ultrasound: development of the venous excess ultrasound grading system. The ultrasound journal, 12: 1-12. doi: https://doi.org/10.1186/s13089-020-00163-w

Buddha S, Menias CO and Katabathina VS (2019). Imaging of urachal anomalies. Abdominal Radiology, 44: 3978-3989. doi: https://doi.org/10.1007/s00261-019-02205-x

Cappele O, Sibert L, Descargues J, Delmas V and Grise P (2001). A study of the anatomic features of the duct of the urachus. Surgical and Radiologic Anatomy, 23: 229-235. doi: https://doi.org/10.1007/s00276-001-0229-1

Carhuas JN, Dionisio CC, Napoleón MS, Augusto SP and Marcial BH (2022). Efficiency of the technique "chumir" on labor time and personnel required for sheep dosing. Voz Zootecnista, 2(1): 20-25. https://revistas.uncp.edu.pe/index.php/vozzootecnista/article/view/1826

Carhuas JN, Paco JLC, Martínez FG, Gomez EA, Camargo AC, Aquino VS and et al. (2023). Inclusion of whey in the diet of fattening rams. Revista de Investigaciones Veterinarias del Perú, 34(6): e25132-e25132. doi: https://doi.org/10.15381/rivep.v34i6.25132

CFW M (1925). The development of the inferior vena cava in man. Animal Journal Anatomic, 35: 331-383. doi: https://doi.org/10.1002/aja.1000350302

Chiarenza SF, Scarpa MG, D'Agostino S, Fabbro MA, Novek SJ and Musi L (2009). Laparoscopic excision of urachal cyst in pediatric age: report of three cases and review of the literature. Journal of Laparoendoscopic & Advanced Surgical Techniques, 19(S1): s183-s186. doi: https://doi.org/10.1089/lap.2008.0184.supp

Del Cid VAL, Vargas ÁDV and Herrera MAO (2023). Adenocarcinoma and Urachal Lithiasis, Review of the Literature and Report of a Case. Revista Guatemalteca de Urología, 11(1): 17-21. doi: https://doi.org/10.54212/27068048.v11i1.144

Dhillon J, Liang Y, Kamat AM, Siefker-Radtke A, Dinney CP, Czerniak B and et al. (2015). Urachal carcinoma: a pathologic and clinical study of 46 cases. Human pathology, 46(12): 1808-1814. doi: https://doi.org/10.1016/j.humpath.2015.07.021

Gelikman DG, Ibanez KR, Ghattas YS, Craver EC, Casas-Melley AT, Ellsworth P and et al. (2023). Management of urachal anomalies in pediatric patients: A comparison of treatment strategies between pediatric urology and general surgery. Journal of Pediatric Urology, 20(1):75-e1. doi: https://doi.org/10.1016/j.jpurol.2023.09.013

Gleason JM, Bowlin PR, Bagli DJ, Lorenzo AJ, Hassouna T, Koyle MA and et al. (2015). A comprehensive review of pediatric urachal anomalies and predictive analysis for adult urachal adenocarcinoma. The Journal of Urology 193(2): 632-636. doi: https://doi.org/10.1016/j.juro.2014.09.004

Hikspoors JP, Peeters MM, Mekonen HK, Kruepunga N, Mommen GM, Cornillie P, and et al. (2017). The fate of the vitelline and umbilical veins during the development of the human liver. Journal of anatomy, 231(5): 718-735. doi: https://doi.org/10.1111/joa.12671

Hirose Y, Shikino K and Ikusaka M (2017). An abscess of urachal remnant. Journal of General Internal Medicine, 32: 360-360. doi: https://doi.org/10.1007/s11606-016-3804-1

Hurtado AD (2020). The agrarian reform and communal processes: the communities of the Cahuide and Túpac Amaru SAIS in Peru's central highlands. Revista del Instituto Riva-Agüero, 5(2): 299-337. doi: https://doi.org/10.18800/revistaira.202002.010

Kim DS, Vaquer S, Mazzolai L, Roberts LN, Pavela J, Watanabe M, and et al. (2021). The effect of microgravity on the human venous system and blood coagulation: a systematic review. Experimental Physiology, 106(5): 1149-1158. doi: https://doi.org/10.1113/EP089409

Mottet N, Chaussy Y, Arbez-Gindre F and Riethmuller D (2017). Physiology and pathologies of the umbilical cord. EMC-Ginecología-Obstetricia, 53(4): 1-12. doi: https://doi.org/10.1016/s1283-081x(17)86887-9

Naiem MEA, Mohammed NIM and Mohammed R (2022). Infected urachal sinus with de novo stone and peritonism in a young athlete adult: A case report. International Journal of Surgery Case Reports, 101: 107784. doi: https://doi.org/10.1016/j.ijscr.2022.107784

Perondi F, Puccinelli C, Lippi I, Della Santa D, Benvenuti M, Mannucci T and et al. (2020). Ultrasonographic diagnosis of urachal anomalies in cats and dogs: retrospective study of 98 cases (2009–2019). Veterinary Sciences, 7(3): 84. doi: https://doi.org/10.3390/VETSCI7030084

Rojas-Quintero K, Collazos-Malagón M, Velásquez JC and Merchán-Galvis A (2023). Persistence of urachus in a young patient. Case report. Revista Colombiana de Cirugía, 38(3): 549-555. doi: https://doi.org/10.30944/20117582.2193

Sarmiento RH, Marroquín A, Cárdenas CAC and Cardona AMF (2021). Omphalitis and umbilical cord cyst associated with patent urachus in a newborn. Pediatría, 54(2): 78-81. doi: https://doi.org/10.14295/rp.v54i2.152

Shi Z-Y, Hou S-L and Li X-W (2022). Silver dressing in the management of an infant's urachal anomaly infected with methicillin-resistant Staphylococcus aureus: A case report. World Journal of Clinical Cases, 10(8): 2629. doi: https://doi.org/10.12998/wjcc.v10.i8.2629

Steiner A and Lejeune B (2009). Ultrasonographic assessment of umbilical disorders. Veterinary Clinics: Food Animal Practice, 25(3): 781-794. doi: https://doi.org/10.1016/j.cvfa.2009.07.012

Terasaki F, Yamamoto Y, Sugiura T, Okamura Y, Ito T, Ashida R, an et al. (2020). A case of perihilar cholangiocarcinoma with bilateral ligamentum teres hepatis treated with hepatopancreatoduodenectomy. Surgical Case Reports, 6: 1-4. doi: https://doi.org/10.1186/s40792-020-0793-4

Valenzuela H, Vaquerizo M and Ramírez I (2019). Influence of the activity of the Andean fox (Pseudalopes Culpaeus) on sheep breeding in a livestock enterprise in central Peru. Ciencia y Desarrollo, 21(1): 15-22. doi: https://doi.org/10.21503/cyd.v21i1.1717

Wilson AL, Gandhi J, Seyam O, Rahmani B, Patel S, Joshi G, and et al. (2019). Urachal anomalies: a review of pathological conditions, diagnosis, and management. Translational Research in Anatomy, 16: 100041. doi: https://doi.org/10.1016/j.tria.2019.100041

Zamora GE (2006). Urachus: Developmental anomalies, anatomical features and presentation of two cases. Revista chilena de radiología, 12(1): 9-11. doi: https://doi.org/10.4067/s0717-93082006000100004

Ziętek Z (2022). Unusual variability of the superficial venous system of the upper limb and its consequences for deep venous system. Folia Morphologica, 81(1): 254-257. doi: https://doi.org/10.5603/FM.A2020.0147

Instructions for Authors

OJAFR EndNote Style | Word Template | Declaration form | Authorship Agreement Form

Manuscripts as Original Research Paper, Review, Short Communication and Case Reports are invited for peer-review publishing in the *Online Journal of Animal and Feed Research (ISSN 2228-7701)*.

Papers can be in any relevant fields of Animal Sciences (Animal Nutrition, Physiology, Reproduction, Genetics and Breeding, Behavior, Health, Husbandry and its economy, Animal products and Veterinary medicines of domestic animals) and relative topics. The journal does encourage papers with emphasis on the nutritive value and utilization of feeds that is depended to methods of Improvement, Assessment, Conserving and Processing feeds, Agronomic and climatic factors, Metabolic, Production, Reproduction and Health responses to dietary inputs (e.g., Feeds, Feed Additives, Specific Feed Components, Mycotoxins). Also, Mathematical models relating directly to animal-feed interactions, Analytical and experimental methods for Feed Evaluation as well as Animal Production studies with a focus on Animal Nutrition that do have link to a feed (Food Science and Technology) are acceptable relative topics for OJAFR. ...view full aims and scope

Submission

The manuscripts should be submitted using our online submission forms (Scienceline Online Submission Form; OJAFR Online Submission Form). For facile submission, please embed all figures and tables at the end of the manuscript to become one single file for submission. Once submission is complete, the system will generate a manuscript ID and password sent to the author's contact email. If you have any difficulty in submitting the manuscript, kindly send via emails: editors@ojafr.com ; editorojafr@gmail.com. All manuscripts must be checked (by an English native speaker) and submitted in English for evaluation in a totally confidential and impartial way.

Supplementary information:

Author guidelines are specific for each journal. Our MS Word template can assist you by modifying your page layout, text formatting, headings, title page, image placement, and citations/references such that they agree with the guidelines of the journal. If you believe your article is fully edited per journal style, please use our Word template before submission. Supplementary materials may include figures, tables, methods, videos, and other materials. They are available online linked to the original published article. Supplementary tables and figures should be labeled with a "S", e.g. "Table S1" and "Figure S1". The maximum file size for supplementary materials is 10MB each. Please keep the files as small as possible to avoid the frustrations experienced by readers with downloading large files.

Submission to the Journal is on the understanding that:

1. The article has not been previously published in any other form and is not under consideration for publication elsewhere;
2. All authors have approved the submission and have obtained permission for publishing work.
3. Researchers have proper regard for conservation and animal welfare considerations. Attention is drawn to the 'Guidelines for the Treatment of Animals in Research and Teaching'. Any possible adverse consequences of the work for populations or individual organisms must be weighed against the possible gains in knowledge and its practical applications. If the approval of an ethics committee is required, please provide the name of the committee and the approval number obtained.

Ethics declarations

If experimental research includes animal subjects (involving live vertebrates and/or higher invertebrates), the authors will need to include one of the following appropriate ethics declarations in the Methods section of manuscript.

1. A statement that identifies the institutional and/or licensing committee that approved the experiments, including any relevant details (e.g. the board/committee names that gave the approval).
2. The authors confirm that all experiments were performed in accordance with relevant named guidelines and regulations.
3. A statement confirms that the authors complied with the ARRIVE guidelines and or the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee.

If the manuscript contains photos or parts of photos of patients, informed consent from each patient should be obtained. Patient's identities and privacy should be carefully protected in the manuscript.

Presentation of the article

Main Format

First page of the manuscripts must be properly identified by the title and the name(s) of the author(s). It should be typed in Times New Roman (font sizes: 12pt in capitalization for the title and the main text, double spaced, in A4 format with 2cm margins. All pages and lines of the main text should be numbered consecutively throughout the manuscript. The manuscript must be saved in a .doc or .docx formats. Abbreviations in the article title are not allowed except the well-known ones.

Manuscripts should be arranged in the following order:

a. TITLE (brief, attractive and targeted)
b. Name(s) and Affiliation(s) of author(s) (including postcode) and corresponding Email
c. ABSTRACT
d. Key words (separate by semicolons; or comma,)
e. Abbreviations (used in the manuscript)
f. INTRODUCTION
g. MATERIALS AND METHODS
h. RESULTS
i. DISCUSSION — The sections "RESULTS AND DISCUSSION" can be presented jointly.
j. CONCLUSION — The sections "DISCUSSION AND CONCLUSION" can be presented jointly.

k. DECLARATIONS
l. REFERENCES
m. Tables
n. Figures

Article Sections Format

Title should be a brief phrase describing the contents of the paper. Title Page should include full names and affiliations of the author(s), the name of the corresponding author along with phone and email information. Present address(es) of the author(s) should appear as a footnote.

Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The abstract should be 150 to 300 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 7 **key words** should be listed.

Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and Methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the author(s)'s experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the results but should be put into the discussion section.

Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

Conclusion should be brief and tight, providing a few specific tasks to accomplish: 1-Re-assert/Reinforce the Thesis; 2-Review the Main Points; 3-Close Effectively. The Conclusion section should not be similar to the Abstract content.

Declarations including Ethics, Consent to publish, Competing interests, Authors' contributions, and Availability of data and materials are necessary.

Acknowledgments of persons, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph forms or repeated in the text.

The Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or PowerPoint before pasting in the Microsoft Word manuscript file. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

DECLARATIONS

Please ensure that the sections: Ethics (and consent to participate), Consent to publish, Competing interests, Authors' contributions, and Availability of data and materials are included at the end of your manuscript in a Declarations section.

Consent to Publish

Please include a 'Consent for publication' section in your manuscript. If your manuscript contains any individual person's data in any form (including individual details, images or videos), consent to publish must be obtained from that person, or in the case of children, their parent or legal guardian. All presentations of case reports must have consent to publish. You can use your institutional consent form or our consent form if you prefer. You should not send the form to us on submission, but we may request to see a copy at any stage (including after publication). If your manuscript does not contain any individual person's data, please state "Not applicable" in this section.

Authors' Contributions

For manuscripts with more than one author, OJAFR requires an Authors' Contributions section to be placed after the Competing Interests section. An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship. We suggest the following format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript. For authors that equally participated in a study please write '**All/Both authors contributed equally to this work**.' Contributors who do not meet the criteria for authorship should be listed in an acknowledgements section.

Competing Interests

Competing interests that might interfere with the objective presentation of the research findings contained in the manuscript should be declared in a paragraph heading "Competing interests" (after Acknowledgement section and before References). Examples of competing interests are ownership of stock in a company, commercial grants, board membership, etc. If there is no competing interest, please use the statement "The authors declare that they have no competing interests.". *Online Journal of Animal and Feed Research* adheres to the definition of authorship set up by the International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to

conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3. It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Change in authorship

We do not allow any change in authorship after provisional acceptance. We cannot allow any addition, deletion or change in the sequence of author names. We have this policy to prevent fraud.

Acknowledgements

We strongly encourage you to include an Acknowledgements section between the Authors' contributions section and Reference list. Please acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include their source(s) of funding. Please also acknowledge anyone who contributed materials essential for the study. Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements. Please list the source(s) of funding for the study, for each author, and for the manuscript preparation in the acknowledgements section. Authors must describe the role of the funding body, if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Data deposition

Nucleic acid sequences, protein sequences, and atomic coordinates should be deposited in an appropriate database in time for the accession number to be included in the published article. In computational studies where the sequence information is unacceptable for inclusion in databases because of lack of experimental validation, the sequences must be published as an additional file with the article.

REFERENCES

OJAFR initially accepts the manuscripts in PDF, Word or TeX/LaTeX formats; Word files are preferred, especially those prepared using **EndNote®**. However, our team will reformat the articles of non-EndNote users via EndNote in Galley proof stage, if accepted.

An OJAFR reference style for EndNote may be found here.
How to install additional styles? Please click here
How to turn on "Jumping" from a citation to the bibliography? Please click here

1. All references to publications made in the text should be presented in a list with their full bibliographical description.
2. In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s surname should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lowercase letter like ’a‘ and ’b‘ after the date to distinguish the works.
3. References in the text should be arranged chronologically (e.g. Kelebeni, 1983; Usman and Smith, 1992 and Agindotan et al., 2003). 'et al.' should not be italic. The list of references should be arranged alphabetically on author's surnames, and chronologically per author. If an author's name in the list is also mentioned with co-authors, the following order should be used: Publications of the single author, arranged according to publication dates - publications of the same author with one co-author - publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 1992a, l992b,etc.
4. Names of authors and titles of journals published in non-latin alphabets should be transliterated in English.
5. A sample of standard reference is "1st Author surname A, 2nd Author surname B and 3rd Author surname C (2013). Article title should be regular, in sentence case form, and 9 pt. Online Journal of Animal and Feed Research, Volume No. (Issue No.): 00-00." (Journal titles should be full and not italic.)
6. If available please add DOI numbers or the link of articles at the end of each reference.

Examples (at the text)

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001).

Examples (at references section)

a) For journal

Graulet B (2014). Ruminant milk: A source of vitamins in human nutrition. Animal Frontiers, 4(2):24-30. Link, DOI

Miller BA and Lu CD (2019). Current status of global dairy goat production: An overview. Asian-Australasian Journal of Animal Sciences, 32(8): 1219. Link, DOI

Xu P, Zhang Z, Peng P, Yang J, Li X, Yuan T, et al. (2022). Study on vacuum drying kinetics and processing of the *Lonicera japonica* Thunb. aqueous extracts. LWT - Food Science and Technology. 167: 1-9. Link, DOI

b) For symposia reports and abstracts

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (Pampus argentens euphrasen) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. Asian Fisheries Society Manila, Philippine, 13: 191-199. Link, DOI

c) For edited symposia, special issues, etc., published in a journal

Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science, 31: 17-27. Link, DOI

d) For books

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88. Link, DOI

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603. Link, DOI

e) Books, containing sections written by different authors

Kunev M (1979). Pig Fattening. In: A. Alexiev (Editor), Farm Animal Feeding. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg). Link, DOI

In referring to a personal communication the two words are followed by the year, e.g. (Brown, J. M., personal communication, 1982). In this case initials are given in the text. Where available, URLs for the references should be provided.

Formulae, numbers and symbols

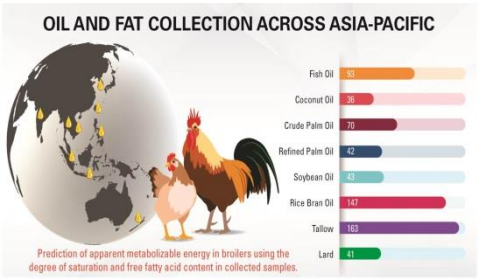
1. Typewritten formulae are preferred. Subscripts and superscripts are important. Check disparities between zero (0) and the letter O (0 vs. O), and between one (1) and the letter I (1 vs. I).
2. Describe all symbols immediately after the equation in which they are first used.
3. For simple fractions, use the solidus (/), e.g. 10 /38.
4. Equations should be presented into parentheses on the right-hand side, in tandem.
5. Levels of statistical significance which can be used without further explanations are *P <0.05, **P <0.01, and ***P <0.001.
6. In the English articles, a decimal point should be used instead of a decimal comma.
7. Use Symbol fonts for "±"; "≤" and "≥" (avoid underline).
8. In chemical formulae, valence of ions should be given, e.g. Ca2+ and CO32-, not as Ca++ or CO3.
9. Numbers up to 10 should be written in the text by words. Numbers above 1000 are recommended to be given as 10 powered x.
10. Greek letters should be explained in the margins with their names as follows: Aα - alpha, Bβ - beta, Γγ - gamma, Δδ - delta, Eε - epsilon, Zζ - zeta, Hη - eta, Θθ - theta, Iι - iota, Kκ - kappa, Λλ - lambda, Mμ - mu, Nν - nu, Ξξ - xi, Oo - omicron, Ππ - pi, Pρ - rho, Σσ - sigma, Tτ - tau, Yυ - ipsilon, Φφ - phi, Χχ - chi, Ψψ - psi, Ωω - omega. Please avoid using math equations in Word whenever possible, as they have to be replaced by images in xml full text.

Abbreviations

Abbreviations should be presented in one paragraph, in the format: "term: definition". Please separate the items by ";".
E.g. ANN: artificial neural network; CFS: closed form solution; ....

Graphical Abstract

Authors of accepted articles should provide a graphical abstract (a beautifully designed feature figure) to represent the paper aiming to catch the attention and interest of readers. Graphical abstract will be published online in the table of content. The graphical abstract should be colored, and kept within an area of 12 cm (width) × 6 cm (height) or with similar format. Image should have a minimum resolution of 300 dpi and line art 1200dpi.
Note: Height of the image should be no more than the width. Please avoid putting too much information into the graphical abstract as it occupies only a small space. Authors can provide the graphical abstract in the format of PDF, Word, PowerPoint, jpg, or png, after a manuscript is accepted for publication.
If you have decided to provide a Professional Graphical Abstract, please click **here**.



Thng A, Ting JX, Tay HR, Soh CY, Ong HC and Tey D (2020). **The use of predicted apparent metabolizable energy values to understand the oil and fat variability in broilers.** *Online J. Anim. Feed Res.*, 10 (4): 85-92.

Review/Decisions/Processing

Firstly, all manuscripts will be checked by one of the plagiarism finding tools (Docol©c, iThenticate and PlagScan). A double-blind peer-reviewing model is used by OJAFR for non-plagiarized papers. Two reviewers selected by section editor (SE) or deputy SE of OJAFR, who are research workers specializing in the relevant field of study. We always try to avoid delays in the reviewing process, but it relies on the time and cooperation of the referees that work without any remuneration, hence, it may take 2 weeks to 4 months. One unfavorable review means that the paper will not be published and possible decisions are: accept as is, minor revision, major revision, or reject. The corresponding authors should submit back their revisions within 14 days in the case of minor revision, or 30 days in the case of major revision.

To submit a revision please click here, fill out the form, and mark "Revised", mention the article code (for example OJAFR-1108), attach the revision (MS word) and continue submission. Manuscripts with significant results are typically reviewed and published at the highest priority. After review and editing the article, a final formatted proof is sent to the corresponding author once again to apply all suggested corrections during the article process. The editor who received the final revisions from the corresponding authors shall not be held responsible for any mistakes shown in the final publication. Manuscripts with significant results are typically reviewed and published at the highest priority.

Language editing

No paper will be rejected for poor language. However, if you would like assistance with writing your manuscript, you can consider asking colleagues for their input and/or use a professional editing service such as those provided by our affiliates American Journal Experts (USA) and or London Proofreaders (UK). In addition, we may offer a Scienceline service (English editing, additional scientific editing, and translation) in a modest fee, for those articles that are in the revision stage, upon request. For more information please visit here. The use of a language editing service has no bearing on editorial decisions and is not a requirement for publication.

Plagiarism: There is an instant policy towards plagiarism (including self-plagiarism) in our journals. Manuscripts (main text not including references list and title page) are screened for plagiarism by iThenticate and or PlagScan with default sensitivity before or during publication, and if found they will be rejected at any stage of processing.

Declaration: After the manuscript is accepted for publication, a declaration form will be sent to the corresponding author who is responsible for coauthors' agreements to publication of submitted work in OJAFR after any amendments arising from the peer review. All the authors should also approve any change in authorship (i.e., adding, removing or reordering existing authors) after initial submission. Authors should determine the order of authorship among themselves. In addition, any alterations must be clarified to the Editor/Editor-in-chief via the Authorship Agreement Form. For more information please read Authorship and Authors' Responsibilities.

Date of issue

All accepted articles are published bimonthly around 25th of January, March, May, July, September and November, each year in full text on the Internet.

Publication charges

The publication costs are covered through article processing charges (APCs) and No submission fee, or any other processing fees are required for the publication of the accepted article. There is a modest APC of 180 Euro(€) editor fee for the processing of each primary accepted paper (1000-4000 words) to encourage high-quality submissions. APCs are only charged for articles that pass the pre-publication checks and are ready to be published. A surcharge will be placed on any article that is over 4000 words in length to cover the additional processing costs. We encourage the authors to submit manuscripts with no more than 4000 words (not including Abstract, Methods, References and figure legends). Payment can be made by credit card, bank transfer, money order or check. Instruction for payment is sent during the publication process as soon as the manuscript is accepted. Meanwhile, this journal encourages the academic institutions in low-income countries to publish high quality scientific results, free of charge.

WORD COUNT	PRICE*
1000-4000 words (medium article)	€180
over 4000 words (long article)	€280

* The prices are valid until 30th December 2024.

The Waiver policy

The submission fee will be waived for invited authors, authors of hot papers, and corresponding authors who are editorial board members of the *Online Journal of Animal and Feed Research*. The Journal will consider requests to waive the fee for cases of financial hardship (for high quality manuscripts and upon acceptance for publication). Requests for waiver of the submission fee must be submitted via individual cover letter by the corresponding author and cosigned by an appropriate institutional official to verify that no institutional or grant funds are available for the payment of the fee. Letters including the manuscript title and manuscript ID number should be sent to editors@ojafr.com. It is expected that waiver requests will be processed and authors will be notified within two business day.

The OA policy

Online Journal of Animal and Feed Research is an Open Access journal which means that all content is freely available without charge to the user or his/her institution. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the *BOAI definition of Open Access*.

Scienceline Language Editing Services

We suggest that authors whose first language is not English have their manuscripts checked by a native English speaker before submission. This is optional, but will help to ensure that any submissions that reach peer review can be judged exclusively on academic merit. We offer a Scienceline service, and suggest that authors contact as appropriate. Please note that use of language editing services is voluntary, and at the author's own expense. Use of these services does not guarantee that the manuscript will be accepted for publication, nor does it restrict the author to submitting to Scienceline journals. You can send the article/s to the following Emails: administrator@science-line.com ; info@science-line.com

For more information about editing services please visit here.

Submission Preparation Checklist

Authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to the following guidelines:

- The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in -Comments to the Editor).
- The submission file is in Microsoft Word, RTF, or PDF document file format.
- Where available, URLs for the references have been provided.
- The text is double-spaced; uses a 12-point font; and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
- The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.

Scienceline Publication, Ltd.
Ömer Nasuhi Bilmen Road, Dönmez Apart., G Block, No: 1/6, Yakutiye, Erzurum/25100, TURKEY
Phone: +90 538 770 8824 (TURKEY)
Homepage: www.science-line.com
Emails: administrator@science-line.com ; saeid.azar@atauni.edu.tr

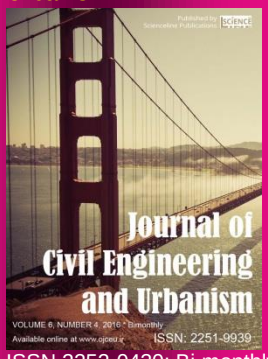
SCIENCELINE PUBLISHING CORPORATION

Scienceline Publication Ltd. is a limited liability non-profit non-stock corporation incorporated in Turkey (Company No. 0757086921600001). Scienceline journals that concurrently belong to many societies, universities and research institutes, publishes internationally peer-reviewed open access articles and believe in sharing of new scientific knowledge and vital research in the fields of life and natural sciences, animal sciences, engineering, art, linguistic, management, social and economic sciences all over the world. Scienceline journals include:

Online Journal of Animal and Feed Research

ISSN 2228-7701; Bi-monthly
View Journal | Editorial Board
Email: editors@ojafr.ir
Submit Online >>

Journal of Civil Engineering and Urbanism

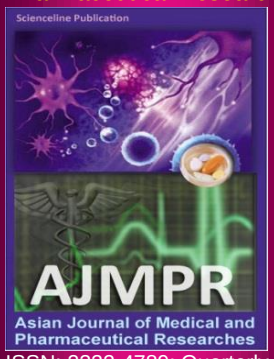


ISSN 2252-0430; Bi-monthly
View Journal | Editorial Board
Email: ojceu@ojceu.ir
Submit Online >>

Journal of Life Sciences and Biomedicine

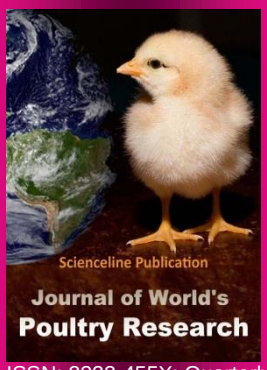
ISSN: 2251-9939; Bi-monthly
View Journal | Editorial Board
Email: editors@jlsb.science-line.com
Submit Online >>

Asian Journal of Medical and Pharmaceutical Researches



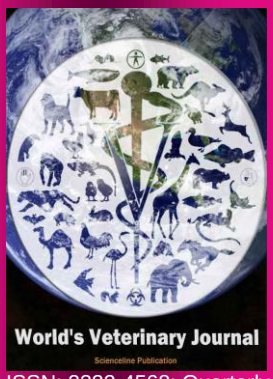
ISSN: 2322-4789; Quarterly
View Journal | Editorial Board
Email: editor@ajmpr.science-line.com
Submit Online >>

Journal of World's Poultry Research



ISSN: 2322-455X; Quarterly
View Journal | Editorial Board
Email: editor@jwpr.science-line.com
Submit Online >>

World's Veterinary Journal

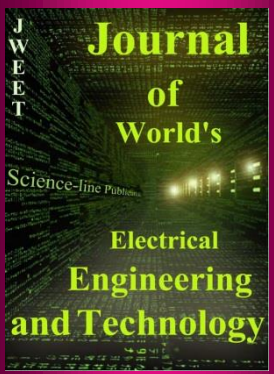


ISSN: 2322-4568; Quarterly
View Journal | Editorial Board
Email: editor@wvj.science-line.com
Submit Online >>

Journal of Educational and Management Studies

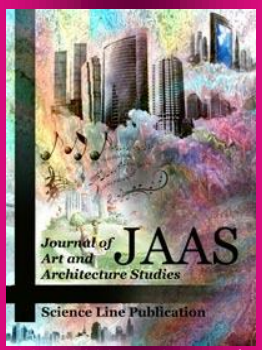
ISSN: 2322-4770; Quarterly
View Journal | Editorial Board
Email: info@jems.science-line.com
Submit Online >>

Journal of World's Electrical Engineering and Technology



ISSN: 2322-5114; Irregular
View Journal | Editorial Board
Email: editor@jweet.science-line.com
Submit Online >>

Journal of Art and Architecture Studies



ISSN: 2383-1553; Irregular
View Journal | Editorial Board
Email: jaas@science-line.com
Submit Online >>

Asian Journal of Social and Economic Sciences

ISSN: 2383-0948; Quarterly
View Journal | Editorial Board
Email: ajses@science-line.com
Submit Online >>

Journal of Applied Business and Finance Researches

ISSN: 2382-9907; Quarterly
View Journal | Editorial Board
Email: jabfr@science-line.com
Submit Online >>

Scientific Journal of Mechanical and Industrial Engineering

ISSN: 2383-0980; Quarterly
View Journal | Editorial Board
Email: sjmie@science-line.com
Submit Online >>

Scienceline is a non-profit organisation inspired by research funders and led by scholars. Our mission is to help researchers accelerate discovery and innovation by operating a platform for research communication that encourages and recognises the most responsible behaviours in science.

Scienceline Publications, Ltd is a limited liability non-profit non-stock corporation registered in the State of Erzurum, Turkey, with company number 0757086921600001, and branch number 18677/25379 at the address: Scienceline Publications, Ltd., Ömer Nasuhi Bilmen Road, Dönmez Apart., G1/6, Yakutiye, Erzurum 25100, Turkey