

BOOKLET





Online J. Anim. Feed Res., 16 (2): 49-124; March 2026

Editors-in-Chief

Habib Aghdam Shahryar, PhD, Professor of Animal Nutrition; Department of Animal Science, Islamic Azad University, Shabestar, **IRAN** ([Google Scholar](#), [SCOPUS](#), Email: ha_shahryar@yahoo.com)

Saeid Chekani Azar, PhD, Faculty of Veterinary Medicine, Animal Physiology, Atatürk University, **TURKEY** ([Google Scholar](#), [SCOPUS](#), [WoS Metrics](#), Email: saeid.azar@atauni.edu.tr)

Managing Editor

Alireza Lotfi, PhD, Animal Physiology, Islamic Azad University, **IRAN** ([Google Scholar](#), [SCOPUS](#), [ResearchGate](#), Email: arlotfi@gmail.com)

Section Editors

Arda Yildirim, PhD, Professor Dr., Department of Animal Science, Faculty of Agriculture, Gaziosmanpasa University, 60240 Tokat, **TURKEY** (Email: arda.yildirim@gop.edu.tr); Animal Science, Nutrition-non Ruminants, Breeding, Nutritive Value

Çağrı Kandemir, PhD, Assistant Professor, Institute of Science, Department of Animal Science, Ege University, Izmir, **TURKEY** ([Website](#); Email: cagri.kandemir@ege.edu.tr); Animal Science, Nutrition – Ruminants, Animal Reproduction

Ehsan Gharib Mombeni, DVM, PhD in Bacteriology; Department of Pathobiology, Shahid Chamran University of Ahvaz, **IRAN** (Emails: e.mombeni@hotmail.com; e-gharibmombeni@stu.scu.ac.ir)

İbrahim Çakmak, Prof. Dr., Director of Beekeeping Development Application and Research Center, Animal Science Department, Faculty of Agriculture, Bursa Uludag University, Gorukle Campus, Nilüfer, Bursa, **TURKEY** (E-mail: icakmak@uludag.edu.tr); Apiculture, Honey bee biology, genetics, behavior, health and products, pollination, beekeeping materials

John Cassius Moreki, PhD, Department of Animal Science and Production, College of Agriculture, **BOTSWANA** (Email: jcmoreki@gmail.com); Nutrition - Non-Ruminants, Breeders, Livestock management

Mohamed Shakal, Professor & Head of Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, **EGYPT**; Director of the Endemic and Emerging Poultry Diseases Research Center, Cairo University, Shek Zaed Branch, **EGYPT**; Chairman of The Egyptian Poultry Forum Scientific Society. Representative for Egypt & Mena Region. (Email: shakal2000@gmail.com)

Muhammad Saeed, PhD, Northwest A&F University, Yangling, 712100, **CHINA** (Email: muhammad.saeed@nwsuaf.edu.cn), Nutrition – Ruminants

Language Editors

Mehrdad Ehsani-Zad, MA in TEFL, Takestan, Islamic Azad University, **IRAN** (Email: mehrdad_single2004@yahoo.com)

Samuel Stephen Oldershaw, Master of TESOL, The Humberston School & The Grimsby Institute, North East Lincolnshire, **UK** (Email: s.s.oldershaw@hotmail.com)

Statistical Editor

Ömer Eltas, PhD, Assist. Prof., Atatürk University, Faculty of Veterinary Medicine, Department of Biometry, Erzurum, **TURKEY** (Email; omer.eltas@atauni.edu.tr); Health Sciences, Veterinary Sciences, Zootechnical and Animal Feed, Biometry

Technical Editor

Alireza Lotfi, PhD, Animal Physiology, Islamic Azad University, **IRAN**

Editorial Team

Abdelfattah Y.M. Nour, DVM, PhD, Professor of Veterinary Physiology, Purdue University, **USA** (Email: nour@purdue.edu)

Adnan Yousaf, DVM, MPhil of Poultry Science (Gold Medalist), Ph.D. of Avian Embryology; Sindh Agricultural University Tandojam, **PAKISTAN** (E-mails: dr.adnan011@gmail.com; dr.adnan@salmanpoultry.com)

Ahmad Yildiz, PhD, Professor, Animal Science and Production Department, Faculty of Veterinary Medicine, Atatürk University, **TURKEY** (Email: ahmtstar@gmail.com); Nutrition – Ruminants

Ali Nobakht, PhD, Assistant Professor, Animal Science Department, Islamic Azad University, Maragheh, **IRAN** (Email: anobakht20@yahoo.com); Nutrition - Non-Ruminants

Alireza Radkhah, PhD, Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, **IRAN** (Email: alirezaradkhah@ut.ac.ir); Aquatic Biology, Aquaculture and Fisheries Biotechnology

Bahareh Hafezi, DVM, PhD Candidate for Veterinary Surgery, Ferdowsi University Veterinary, Mashhad, **IRAN** (Email: hafezibahareh@yahoo.com); Nutrition - Non-Ruminants: Small Animal and Poultry Internal Surgery

Dilem Gülece Ermutlu, Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, **TURKIYE** ([Website](#), [Google Scholar](#), [ORCID](#); Email: dilemermutlu@gmail.com); Histology and Embryolog

Erol Aydin, PhD, Professor Dr., Department of Animal Health Economics and Management, Faculty of Veterinary Medicine, Kafkas University, TR-36100 Kars, **TURKEY** ([Website](#), [Google Scholar](#), [SCOPUS](#), Email: dr-erolaydin@hotmail.com; ORCID: <https://orcid.org/0000-0001-8427-5658>);

Fazul Nabi Shar, PhD, Lecturer, Faculty of Veterinary & Animal Sciences, Lasbela University of Agriculture Water & Marine Sciences, Uthal Balochistan, **PAKISTAN** (Email: fazulnabishar@yahoo.com); Clinical Veterinary Medicine

Ferdaus Mohd. Altaf Hossain, DVM, Sylhet Agricultural University, **BANGLADESH** (Email: ferdaus.dps@sau.ac.bd); Microbiology, Immunology, Poultry Science, and Public Health

Godadaw Misganaw, PHD; Department of Animal Science, College of Veterinary and Animal Sciences, University of Gondar, P.O.Box 196, Gondar, **ETHIOPIA** ([SCOPUS](#); Email: godadaw@gmail.com; ORCID: <https://orcid.org/0000-0001-5624-7983>); Nutrition - Ruminants

Hazim Jabbar Al-Daraji, PhD, Professor, University of Baghdad, College of Agriculture, Abu-Ghraib, Baghdad, **IRAQ** (Email: prof.hazimaldaraji@yahoo.com); Avian Reproduction and Physiology

Mohammed Yousuf Kurtu, Associate Professor, Animal Sciences Department, Haramaya University, Dire-Dawa, **ETHIOPIA** (Email: mkurtu2002@yahoo.com); Animal Science, Nutrition

Mohamed M. El-Deeb, PhD, Animal Nutrition Research Department, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, **EGYPT**; Email: deep121eq@yahoo.com; ORCID

Moshood Adewale Belewu, PhD, Professor, Department of Animal Science, University of Ilorin, **NIGERIA** ([SCOPUS](#), [Google Scholar](#); Emails: mabel@unilorin.edu.ng; moshoad.belewu@uniabuja.edu.ng); Nutrition - Ruminants; Dairy Science

Nilüfer Sabuncuoğlu, PhD, Professor, Department of Animal Science and Production, Faculty of Veterinary Medicine, Atatürk University, **TURKEY** ([Website](#); Email: ncoban@atauni.edu.tr); Animal Hygiene and Welfare, Physiology

Ömer Çoban, PhD, Professor, Department of Animal Science and Production, Atatürk University, **TURKEY** ([Website](#); ocoban@atauni.edu.tr); Nutrition - Ruminants

Paola Roncada, PhD, Associate Professor, Veterinary Pharmacology and Toxicology, University of Bologna, **ITALY** (Email: paola.roncada@unibo.it); Pharmacokinetics

Raga Mohamed Elzaki Ali, PhD, Assistant Professor, Department of Rural Economics and Development, University of Gezira, **SUDAN** (Email: ragaelzaki@yahoo.co.uk); Animal-feed interactions, Nutritive value

Rashid Habiballa Osman, PhD, Assistant Prof., in Department of Poultry Production, Faculty of Animal Production, West Kordofan University, **SUDAN** (E-mail: rashid@wku.edu.sd); Nutrition - Non-Ruminants

Raziye Raeesi, PhD in Fisheries Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, **IRAN** (Email: r.reisi2012@yahoo.com); Aquaculture, Fish nutrition

Serap İLHAN AKSU, Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, **TURKIYE** ([Website](#), [Google Scholar](#), [ORCID](#); Email: serapilhan01@gmail.com); Histology and Embryolog

Sesotya Raka Pambuka, MSc, Sinta Prima Feedmill, Poultry and Aqua Feed Formulation, Sulaiman Rd 27A, West Jakarta, **INDONESIA**

Shigdaf Mekuriaw, Andassa Livestock research center, **ETHIOPIA** (Email: shigdafmekuriaw@yahoo.com); Animal production and Nutrition

Terry Ansah, PhD, University for Development Studies-Ghana and Harper Adams University College, **UK** (Email: ansahterry@yahoo.com); Nutrition - Ruminants

Tohid Vahdatpour, PhD, Assistant Professor, Department of Physiology, Islamic Azad University, Shabestar, **IRAN** ([Scopus](#); [Google Scholar](#); Emails: vahdatpour@iaushab.ac.ir; tvahdatpour@gmail.com); Physiology and Functional Biology of Systems

Vassilis Papatsiros, PhD, Department of Porcine Medicine, University of Thessaly, Trikalon str 224, GR 43100, **GREECE** (Email: vpapatsiros@yahoo.com); Dietary input, Animal and Feed interactions

Wafaa Abd El-Ghany Abd El-Ghany, PhD, Associate Professor, Poultry and Rabbit Diseases Department, Cairo University, Giza, **EGYPT** (Email: wafaa.ghany@yahoo.com); Poultry and Rabbit Diseases

Wesley Lyeverton Correia Ribeiro, MSc, DVM, College of Veterinary, Medicine, State University of Ceará, Av. Paranjana, 1700, Fortaleza, **BRAZIL** (Email: wesleylyeverton@yahoo.com.br); Animal Health and Welfare, Veterinary Parasitology

Yonas Gizaw Habtemichae, DVM, MVSc; Jigjiga University, College of Veterinary Medicine, P.O.Box.1020 Jigjiga, **ETHIOPIA** (Email: yonasg5@gmail.com; ORCID: 0000-0003-4208-5682)

Advisory Board

Alireza Ahmadzadeh, PhD, Assistant Professor, Department of Animal Science, Islamic Azad University, Shabestar, **IRAN** (Emails: a.r.ahmadzadeh@gmail.com; ahmadzadeh@iaushab.ac.ir); Biometry - Plant Breeding (Biotechnology)

Daryoush Babazadeh; DVM, DVSc, PhD of Avian/Poultry Diseases, School of Veterinary Medicine, Shiraz University, Shiraz, **IRAN** (Scopus; ORCID ID; Publons; Full Member of WAME; Member of IAVE; Email: daryoush.babazadeh@shirazu.ac.ir)

Fikret Çelebi, PhD, Professor of Physiology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, **TURKEY** (Email: fnclebi@atauni.edu.tr); Physiology and Functional Biology of Systems

Mohamed Shakal, Professor, Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, **EGYPT**; Director of the Endemic and Emerging Poultry Diseases Research Center, Cairo University, Shek Zaed Branch, **EGYPT**; Chairman of The Egyptian Poultry Forum Scientific Society. REPRESENTATIVE FOR EGYPT & MENA REGION. Email: shakal2000@gmail.com

Naser Maheri Sis, PhD, Assistant Professor, Dept. Anim. Sci., Islamic Azad University, Shabestar, **IRAN** (Website; Emails: maherisis@iaushab.ac.ir; nama1349@gmail.com); Nutrition - Ruminants, Nutritive Value, Utilization of Feeds

Join OJAFR Team

Online Journal of Animal and Feed Research is always striving to add diversity to our editorial board and staff. Applicants who have previous experience relevant to the position they are applying for may be considered for more senior positions within OJAFR. All applicants should begin as section reviewers before progressing on to more senior roles. Editorial board members do not receive any remuneration unless in overtime working conditions. These positions are voluntary. If you are currently graduated from MSc, or PhD at university and interested in working for OJAFR, please fill out the application form below. Once your filled application form is submitted, the editorial board of the journal will review your request and inform you within a week of their decision for membership in the editorial board. The list of the editorial board will be updated yearly and the new members will be listed each year. If you are a PhD, assistant, associate professor, distinguished professor, or an active researcher, please send us a copy of your resume (CV) and your ORCID ID. You should briefly express any leadership positions, editorial or publishing activities, and other experiences you have had that are relevant to applied research, conducted studies, and published articles. Also, the volunteer editor/reviewer should declare if he/she has any conflict of interest for joining the journal editorial board in requesting time, and also during his/her activity as an editor or reviewer.

If you would like to represent the OJAFR at your university, join our volunteer staff today! OJAFR representatives can include any assistant students, teachers, instructors, researchers, and professors at university or international institutes. You can also register as a member of the journal for subsequent contacts by email and or invitation for joining educational webinars.

Editors affiliated with the *Online Journal of Animal and Feed Research* who are also serving on the editorial boards of other journals sharing similar goals and scope are expected to adhere to the policies of the *Online Journal of Animal and Feed Research* while they are involved in editorial responsibilities at the *Online Journal of Animal and Feed Research*. For such editors, it is important to declare any potential conflict of interest transparently. If at any stage of the journal's peer review process, it becomes apparent that a submitted article is under consideration in a journal where our editor also serves, the *Online Journal of Animal and Feed Research* immediately reassigns the article to another editor. Similarly, if such a situation involves the editor-in-chief, and the editor-in-chief collaborates with another journal, the responsibility for handling that article is delegated to the second editor-in-chief/associate editor-in-chief/handling editor/managing editor. In case of a conflict of interest between the editor-in-chief and any of the mentioned roles, the article will be handled by one of the editorial board members.

Download [OJAFR Application Form](#)

Volume 16 (2); March 2026

Research Paper

Growth, productivity, and meat quality of Mnu Buon chickens raised in Vietnam

Hanh TQ, Chi NTK, Dung VT, Duong LA, Thang LHV, Tran HNTH, Thuy DP and Hue PT

Online J. Anim. Feed Res., 16(2): 49-58, 2026; pii: S222877012600007-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.7>

Abstract

This study was conducted to evaluate the growth, carcass characteristics, and meat quality of the Mnu Buon chicken breed (a native breed) raised in Dak Lak province. A total of 200 one-day-old chicks were raised in confinement with a sports field to monitor cumulative growth, absolute growth (ADG), survival rate, feed efficiency, carcass characteristics, and meat quality. The Mnu Buon chickens adapted well to household farming conditions in Dak Lak, with a high survival rate of 91.5%. On Mnu Buon chickens belong to a group of native chicken breeds with small size and medium weight. At 1 day old, the chickens weigh 23.1g/chick, and at 24 weeks old, they reach a weight of 1572.8g (males) and 1233.8g (females). The absolute growth of the chickens gradually increases from week 1 to week 16 and then gradually decreased, absolute growth from 1-24 weeks old reaches 9.2g/day (males) and 7.2g/day (females). Feed intake of Mnu Buon chickens was at an average of 45.4g/day (males) and 39.7g/day (females). Feed consumption per kg of weight gain was 4.9kg (males) and 5.4kg (females). The carcass yield, thigh meat, and breast meat yield of Mnu Buon broiler chickens were average compared to other native chicken breeds in Vietnam. The breast meat color (CIE L*, a*, and b*) of the chickens was higher than some other native Vietnamese chicken breeds. The breast meat of the chickens has an average shrinkage rate during cooking compared to other native chicken breeds. In summary, the Mnu Buon chicken breed has several unique characteristics and many advantages in meat quality, high adaptability to the rearing conditions in Dak Lak, and this information can be helpful for breeders and consumers who prefer high-quality and healthy chicken meat.

Keywords: Carcass Characteristics; Growth Performance; Local breeds; Meat Quality; Mnu Buon Chicken.



Hanh TQ, Chi NTK, Dung VT, Duong LA, Thang LHV, Tran HNTH, Thuy DP and Hue PT (2026). Growth, productivity, and meat quality of Mnu Buon chickens raised in Vietnam. *Online J. Anim. Feed Res.*, 16(2): 49-58. DOI: <https://dx.doi.org/10.51227/ojafr.2026.7>

[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Research Paper

The effects of pen design for farrowing and boar breed on reproductive traits of sows

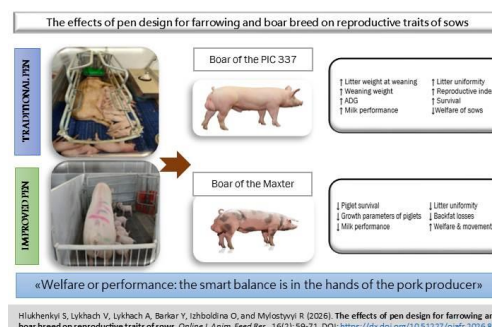
Hlukhenkyi S, Lykhach V, Lykhach A, Barkar Y, Izhboldina O, and Mylostyvyi R.

Online J. Anim. Feed Res., 16(2): 59-71, 2026; pii: S222877012600008-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.8>

Abstract

Modern pig production increasingly requires compliance with welfare-oriented housing standards, prompting farms to modernize farrowing systems while maintaining productive performance. This study aimed to evaluate the effects of farrowing pen design during the suckling period and boar line on the reproductive performance of sows, piglet growth, and survival under industrial conditions aligned with contemporary animal welfare requirements. The experiment was conducted using a 2 × 2 factorial design involving 192 sows (Large White × Landrace) of second to fourth parity and 2,443 piglets obtained through terminal crossbreeding with Maxter and PIC 337 boars. Animals were fed complete diets and housed under controlled microclimate conditions. Reproductive traits, piglet survival, and sow backfat thickness were assessed using standardized methods and subjected to statistical analysis. Sows housed in fixed traditional farrowing pens exhibited superior reproductive performance compared with those kept in improved welfare-oriented pens. The boar line significantly affected the total number of piglets born and the number born alive, with the highest total number of piglets born (14.1 piglets) recorded in sows inseminated with PIC 337 semen. Farrowing pen design had a significant effect on litter size, litter uniformity, stillbirth rate, litter weight at weaning, sow backfat thickness, and piglet weaning weight. The highest average daily gain and piglet live weight at weaning were observed in Group 1, whereas the



Hlukhenkyi S, Lykhach V, Lykhach A, Barkar Y, Izhboldina O, and Mylostyvyi R (2026). The effects of pen design for farrowing and boar breed on reproductive traits of sows. *Online J. Anim. Feed Res.*, 16(2): 59-71. DOI: <https://dx.doi.org/10.51227/ojafr.2026.8>

lowest values were recorded in Group 4. Piglet survival to weaning was the highest in Group 3 (97.3%). A combined effect of farrowing pen design and boar line was identified for milk performance (defined as total milk output estimated from litter weight gain), litter uniformity index (reflecting within-litter variation in piglet body weight), and piglet survival. The practical significance of these findings lies in providing science-based recommendations for selecting farrowing pen configurations and boar lines that support welfare-compliant and efficient pig production systems in Ukraine. The study demonstrates a performance-welfare trade-off, whereby traditional pens improved piglet growth parameters, while welfare-oriented improved pens enhanced sow mobility. This balance should be considered when implementing welfare-compliant farrowing systems.

Keywords: Breeding systems, Fertility, Performance, Pig, Welfare.

[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Research Paper

Physical properties and nutritional quality of wafer made from sugarcane bagasse-indigofera mixture as feed for ruminant

Ali A, Rodiallah M, Misrianti R, Harahap AE, Julianтони J, and Lase DHMS.

Online J. Anim. Feed Res., 16(2): 72-81, 2026; pii: S222877012600009-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.9>

Abstract

The purpose of this study was to determine the best composition of sugarcane bagasse and Indigofera (legume plant) as main ingredients for wafer formulation for ruminant feed based on physical properties, nutritional quality and palatability. The research was conducted using a complete randomized design (CRD) consisting of 3 treatments with 5 replicates. The treatment was a ration formulation consisting of 6 feed ingredients arranged by the trials and error method with estimated crude protein content (CP) of 12.1-14.2%. The use of Indigofera from 30% to 40% was significantly ($P < 0.05$) improved wafer color score. The use of 35% and 40% Indigofera was significantly ($P < 0.05$) increased the wafer texture score from 3.06 to 3.35. An increase in Indigofera used from 30% to 40% was significantly ($P < 0.05$) improved wafer odor scores. It was found that the difference in the composition of bagasse and Indigofera in wafer formulation gave a significant ($P < 0.05$) difference to the value of wafer density. The difference in the percentage of bagasse and Indigofera in the formulation was not significantly ($P > 0.05$) affecting the water absorption of wafers. The increase in the percentage of Indigofera was significantly ($P < 0.05$) increased the CP and ash content and decreased crude fibre (CF) content of wafer. The increasing in the percentage of Indigofera from 30% to 35% and 40% was significantly ($P < 0.05$) increased in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD), ammonia nitrogen ($\text{NH}_3\text{-N}$) and volatile fatty acid (VFA) total of wafer. The increase in the use of Indigofera from 30% to 35% and 40% in wafer formulations was significantly ($P < 0.05$) lowering wafer palatability in goats. Based on data from physical properties, nutrient content, and in vitro digestibility, it can be concluded that the best wafer formulation was 25% bagasse + 40% Indigofera.

Keywords: Legume, Nutritional quality, Physical treatment, Physical quality, Sugarcane by-product, Wafer formulation.

[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Research Paper

In situ degradation of heat-treated plant protein leaf meals to extrapolate rumen bypass potential in rumen - fistulated Brahman cattle

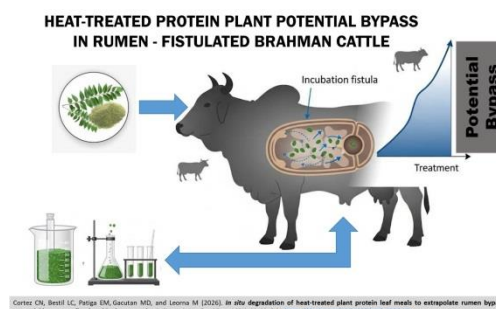
Cortez CN, Bestil LC, Patiga EM, Gacutan MD, and Leorna M.

Online J. Anim. Feed Res., 16(2): 82-89, 2026; pii: S222877012600010-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.10>

Abstract

Many plant protein leaf meals (LM) remain unassessed for their rumen bypass potential following heat treatment. This study aimed to evaluate whether heat treatment could reduce ruminal degradability and improve bypass protein availability in five LMs—ipil-ipil (ILM), kakawate (KaLM), moringa (MLM), kudzu (KuLM), and gumamela (GLM). A total of 60 nylon bags (3 replicates per treatment) containing untreated or heat-treated LMs were incubated for 24 h in a rumen-fistulated Brahman bull using a completely randomized design. Heat treatment reduced mean crude protein degradability (CPD) by 7.6% (81.41% untreated vs. 73.77% treated; $P = 0.006$), with the largest reductions recorded in ILM (-23.9%), KuLM (-14.7%), and GLM (-6.8%). In contrast, dry matter degradability (DMD: 69.76% vs. 68.65%) and organic matter degradability (OMD: 74.44% vs. 70.56%) were not significantly affected. Among the LMs, moringa consistently exhibited the highest degradability (CPD = 98.27%, DMD = 94.98%, OMD = 97.00%), whereas kudzu had the lowest (CPD = 68.69%, DMD = 48.52%, OMD =



54.21%). Degradability trends were influenced by drought-resistance, botanical classification (legume vs. non-legume), and growth form (tree, shrub, creeping). Legume LMs were 16% less digestible in protein content than non-legumes, and creeping types were 19% less digestible than non-creeping types. Heat treatment further lowered CPD in both categories, indicating its capacity to enhance bypass protein potential. These findings suggest that targeted application of heat treatment, especially for ipil-ipil, kudzu, and gumamela, offers a practical strategy to improve protein utilization efficiency in ruminant feeding systems by increasing post-ruminal protein supply.

Keywords: Brahman cattle, Heat-treatment, In situ degradation, Leaf meal, Rumen.

[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Research Paper

Influence of different levels of rumen content as a replacement for grass meal in compound feed on the productivity of Kazakh fine-wool sheep

Kenenbay G, Chomanov U, and Idayatova M.

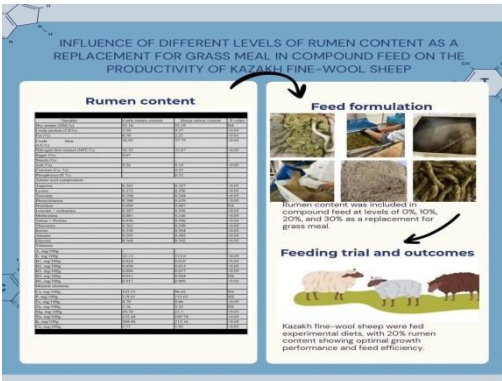
Online J. Anim. Feed Res., 16(2): 90-97, 2026; pii: S222877012600011-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.11>

Abstract

This study evaluated the effects of replacing grass meal with different inclusion levels of dried rumen content on the growth performance of Kazakh fine-wool sheep. Twenty five-month-old intact male sheep (initial body weight 32.1 ± 0.20 kg) were randomly allocated to four dietary treatment groups for 60 days following a fifteen-day adaptation period. The control diet contained no rumen content, while experimental diets included 10%, 20%, and 30% dried rumen content (a mixture of cattle and sheep rumen content at a ratio of 60:40). Inclusion of rumen content at 10–20% significantly improved average daily gain and feed conversion ratio compared to the control diet ($P < 0.05$), whereas a 30% inclusion level reduced feed palatability and productive performance. No adverse effects on animal health were observed during the experiment. The results indicate that dried rumen content can be safely used as a partial replacement for grass meal in sheep diets at inclusion levels up to 20%.

Keywords: Alternative feedstuffs; Feed palatability; Sheep; Rumen content; Ruminant nutrition



[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Research Paper

Bioconversion of cereal by-products through *Pleurotus ostreatus* mediated solid-state fermentation of three agro by-products

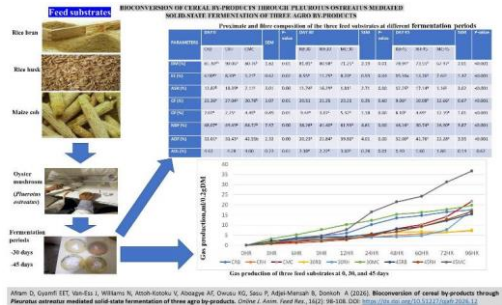
Afram D, Gyamfi EET, Van-Ess J, Williams N, Atttoh-Kotoku V, Aboagye AF, Owusu KG, Sasu P, Adjei-Mensah B, Donkoh A.

Online J. Anim. Feed Res., 16(2): 98-108, 2026; pii: S222877012600012-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.12>

Abstract

Solid-state fermentation (SSF) offers a promising approach to enhance the nutritional value of abundant but underutilized agro-industrial by-products in sub-Saharan Africa. The study investigated the effect of SSF using *Pleurotus ostreatus* on rice bran (RB), rice husk (RH) and maize cob (MC). The design used was a 3×3 factorial arrangement of treatments in a completely randomized design (CRD). The factors were three substrates (RB: rice bran, RH: rice husk, and MC: maize cob) and three fermentation periods (0, 30, 45 days). Unfermented and fermented samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash, crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). In vitro gas production (3-96 h) was measured using rumen liquor. In vitro organic matter digestibility (IVOMD), metabolizable energy (ME), net energy (NE), short-chain fatty acids (SCFAs), and microbial protein (MP) were estimated using established models. Significant differences ($P < 0.05$) in CP and EE contents were observed among RB, RH, and MC within fermentation periods. The CP contents at days 30 and 45 were 9.44%, 8.10% for RB, 3.07%, 4.69% for RH, and 5.32%, 12.19% for MC, respectively. Fibre fractions varied among substrates at each fermentation period, with NDF differing among RB, RH, and MC, and ADL showing significant differences among substrates at day 30. Potential gas production (b) rose in MC (52.7 ml) and RH (53.9 ml) after 45 days. The rate of gas production (c) ranged from 0.03-0.08 h^{-1} at day 0 and 30, but after 45 days, RH reached 0.08 h^{-1} , while RB and MC slowed to 0.01 h^{-1} . IVOMD, ME, NE,



SCFA and MP were all higher in fermented RH and MC, with peak improvements at day 30. The study results indicated that SSF for 30-45 days could improve the nutritional value of agro-industrial by-products.

Keywords: Chemical composition, In vitro gas production, Maize cob, Rice bran, Rice husk, Oyster mushroom, Solid-state fermentation.

[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Research Paper

Effect of *Nigella sativa* oil on liver function and lipid profile in brown, albino and local rabbits: a comparative study

Hasan WI, Hidayet HM, Azeez OH, Sefdeen SM, Yateem CA, Mustafa KN.

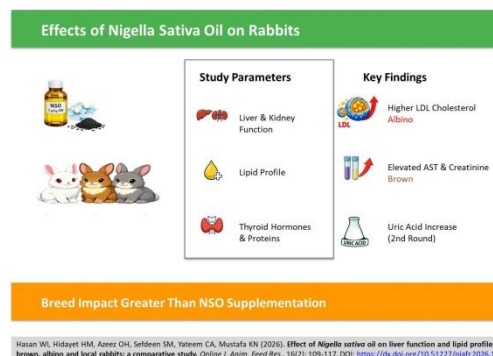
Online J. Anim. Feed Res., 16(2): 109-117, 2026; pii: S222877012600013-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.13>

Abstract

This study investigated the effects of *Nigella sativa* oil (NSO) on the metabolism of three rabbit breeds: Albino, Brown, and Local. A total of 48 rabbits were assigned to six groups and fed either a control diet or an NSO-supplemented diet (5 g/kg DM). Diets were alternated between groups every 15 days. Blood samples were analyzed for liver enzymes, kidney function parameters, lipid profile, thyroid hormones, and blood proteins. The results showed that NSO had no significant effect ($P > 0.05$) on blood glucose, liver enzymes, or lipid profile. However, numerical differences were observed among breeds. Albino rabbits showed higher total cholesterol 83.6% and low-density lipoprotein (LDL) cholesterol (177.6%) compared with the Local breed ($P \leq 0.01$). Brown rabbits exhibited higher aspartate aminotransferase (AST) activity (60-64%) than Albino rabbits ($P \leq 0.02$). Additionally, brown rabbits showed higher (51.2%) creatinine levels compared with Albinos ($P \leq 0.0001$). Uric acid levels slightly increased (15-18%) during the second treatment period. Protein levels remained relatively constant among groups; however, the brown breed had higher ($P \leq 0.02$) albumin levels (9%) than other breeds. In conclusion, rabbit breed had a greater influence on the measured parameters than dietary treatments which indicates that breed-specific genetic variations play an important role in such studies.

Keywords: Breeds, Liver function, Lipid profile, *Neglia sativa*, Rabbits.



[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

Research Paper

Effect of emulsified high-PUFA oils enriched with natural antioxidants on in vitro goat rumen fermentation parameters and nutrient degradability

Fassah DM, Alfiyyah MN, Azka SM, Damayanti SP, Pujiawati Y, Khotijah L, and Sholeha NA.

Online J. Anim. Feed Res., 16(2): 118-124, 2026; pii: S222877012600014-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.14>

Abstract

This study aims to evaluate the effect of emulsified high-PUFA oil enriched with natural antioxidants derived from jackfruit leaf extract on in vitro goat rumen fermentability and degradability. The study used a completely randomized design with two treatments and 5 replications. The treatments were supplementation with high-PUFA oil + natural antioxidants separately (P1, as the control), and supplementation with emulsified high-PUFA oil + natural antioxidants (P2). The parameters observed were rumen pH, total volatile fatty acid (VFA) concentration, NH_3 concentration, bacterial population, protozoa population, dry matter digestibility (DMD), and organic matter digestibility (OMD). The results showed that supplementing goat feed ration with emulsified high-PUFA oil enriched with natural antioxidants did not alter ($P > 0.05$) rumen pH, total VFA, NH_3 concentration, bacterial and protozoal population, DMD, or OMD. Overall, emulsified high-PUFA oil with natural antioxidants did not alter the examined rumen fermentation parameters and degradability. Thus, emulsified high-PUFA oil with natural antioxidants can be potentially used as an effective feed supplement to support goats' performance.

Keywords: Emulsification, Feed supplement, Fermentation characteristics, Jackfruit leaves, Polyunsaturated fatty acid.

[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]

[Previous issue](#) | [Next issue](#) | [Archive](#)



Online Journal of Animal and Feed Research



ISSN: 2228-7701

Frequency: Bimonthly

Current Issue: 2026, Vol: 16, No: 2 (March)

DOI Prefix: [10.51227](https://doi.org/10.51227)

Publisher: [SCIENCELINE](https://www.science-line.com)

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](#), [CAS](#), [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](#)



ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS



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

[ABOUT US](#)

| [CONTACT US](#)

| [PRIVACY POLICY](#)

GROWTH, PRODUCTIVITY, AND MEAT QUALITY OF MNU BUON CHICKENS RAISED IN VIETNAM

Tran Quang HANH¹ , Ngô Thi Kim CHI¹ , Van Tien DUNG¹ ✉, Le Anh DUONG¹ , Luong Huyng Viet THANG¹ ,
Ho Nguyen Thi Huyen TRAN¹ , Doan Phuong THUY³  and Pham The HUE² 

¹Tay Nguyen University, Dak Lak Vietnam

²Institute of Ecology, Resources and Environment, Dak Lak Vietnam

³Bac Giang University of Agriculture and Forestry, Bac Ninh Vietnam

✉Email: vtdung@ttn.edu.vn

✉Supporting Information



ABSTRACT: This study was conducted to evaluate the growth, carcass characteristics, and meat quality of the Mnu Buon chicken breed (a native breed) raised in Dak Lak province. A total of 200 one-day-old chicks were raised in confinement with a sports field to monitor cumulative growth, absolute growth (ADG), survival rate, feed efficiency, carcass characteristics, and meat quality. The Mnu Buon chickens adapted well to household farming conditions in Dak Lak, with a high survival rate of 91.5%. On Mnu Buon chickens belong to a group of native chicken breeds with small size and medium weight. At 1 day old, the chickens weigh 23.1g/chick, and at 24 weeks old, they reach a weight of 1572.8g (males) and 1233.8g (females). The absolute growth of the chickens gradually increases from week 1 to week 16 and then gradually decreased, absolute growth from 1-24 weeks old reaches 9.2g/day (males) and 7.2g/day (females). Feed intake of Mnu Buon chickens was at an average of 45.4g/day (males) and 39.7g/day (females). Feed consumption per kg of weight gain was 4.9kg (males) and 5.4kg (females). The carcass yield, thigh meat, and breast meat yield of Mnu Buon broiler chickens were average compared to other native chicken breeds in Vietnam. The breast meat color (CIE L*, a*, and b*) of the chickens was higher than some other native Vietnamese chicken breeds. The breast meat of the chickens has an average shrinkage rate during cooking compared to other native chicken breeds. In summary, the Mnu Buon chicken breed has several unique characteristics and many advantages in meat quality, high adaptability to the rearing conditions in Dak Lak, and this information can be helpful for breeders and consumers who prefer high-quality and healthy chicken meat.

Keywords: Carcass Characteristics; Growth Performance; Local breeds; Meat Quality; Mnu Buon Chicken.

INTRODUCTION

Native chicken farming is facing many negative impacts from heavy competition with high-yield imported breeds, climate change, the shifting to industrial farming methods, and increasing diseases (Kpomasse et al., 2023; Mogano et al., 2025). Therefore, many countries have exploited the genetic resources of native chicken breeds to respond to these negative effects and aim for sustainable farming development (Chebo et al., 2022; Kanyama et al., 2024). Choo et al. (2014) argued that native chickens adapt better to areas with unfavorable natural conditions and harsh climates. Berthouly-Salazar et al. (2010) stated that native chickens are easy to raise, may possess disease-resistant genes, and their products are favored by consumers.

Minh et al. (2022) studied the conservation of the genetic resources of Mong ethnic chickens in Dong Hy and Vo Nhai districts, Thai Nguyen province, aiming to select and preserve this breed of chicken to maintain valuable genetic resources for effective exploitation and development of the ethnic chicken breeds. The Mnu Buon chicken is a native breed domesticated and raised in ethnic minority communities in Dak Lak province. Mnu Buon chickens have a small stature, slim bodies, small heads, and small legs. Both adult males and females have a tuft of feathers on their heads. Adult roosters mostly have dark red feathers (plum-colored), shiny yellow hackle feathers around the neck, well-developed single combs, and long tails. Most hens have ash-gray feathers with black streaks, which are clearest around the neck, while others have colors like black, white, "hoa mo" (speckled), or dark yellow. Newborn chicks usually have light yellow or light brown feathers, and some have black stripes. The tuft of feathers on the head at birth is a physical feature used to identify the breed. This breed is highly adaptable to the farming conditions of ethnic minorities in Dak Lak. They are excellent at foraging for food in nature. Mnu Buon chickens are raised on a small scale for self-sufficiency, serving daily food needs and local festivals. Their meat quality is fragrant and delicious, making them popular with consumers.

Quentin et al. (2003) noted that diet concentration affects growth, but carcass composition and some meat quality parameters do not change significantly. Only meat color changes due to diet, mainly because of carotenoid content. The ability to change the carcass composition of broilers without significantly changing growth performance is very limited or

RESEARCH ARTICLE
P-I: S222877012600007-16
Received: January 28, 2026
Revised: March 14, 2026
Accepted: March 15, 2026

non-existent. [Ngo et al. \(2021\)](#) showed that "Many toed" chickens (another native breed) have a survival rate of 90.70% – 90.90%, with adult body weight reaching 1693.02g – 1702.6g per bird. The feed conversion ratio (FCR) per kg of weight gain up to 22 weeks of age is 4.97kg and 5.01kg. The carcass percentage is 74.25% - 74.68%, breast meat rate reaches 15.06% - 15.14%, and thigh meat rate is 15.69% - 15.83%. This study aims to evaluate the growth, productivity, and meat quality of the breed, providing a basis for selecting and exploiting the valuable genetic resources of this chicken.

MATERIALS AND METHODS

This experiment was conducted on 200 Mnu Buon chickens (a native breed) from 1 to 24 weeks of age. The chickens were raised at the Buon Ma Thuot Livestock Service Cooperative's farm in Dak Lak province.

Animal experiments

A total of 200 one-day-old Mnu Buon chickens were fitted with leg bands and wing clips. The chickens were raised in naturally ventilated coops with a bedding layer of rice husks. They were fed a complete mixed feed according to Vietnam standards 2265:2007, with a free-feeding regime. Metabolizable energy (ME, Kcal/kg feed) and protein content (CP, %) in the diet were provided according to the growth stage of the chickens, specifically: 22% CP and 3,000 Kcal ME for the period from 0 to 4 weeks of age; 18% CP and 3,000 Kcal ME for the period from 5 to 10 weeks of age, and 16% CP and 3,030 Kcal ME for the period from 10 to 24 weeks of age. During the first 4 weeks, the chickens were raised in cages, and from the 5th week onwards, they were allowed to roam freely in the yard during the day.

Monitoring

Monitor the number of live, dead, and culled chickens from 1-24 weeks to calculate the survival rate. Weight (g): Weigh chickens from 1 day old to 24 weeks old, once every two weeks, weighing each individual chicken in the morning before feeding. 1-day-old chickens are weighed using a technical scale with an accuracy of ± 0.05 g; 1-4 weeks old chickens are weighed using a 2kg dial scale with an accuracy of ± 2 g; 5-24 weeks old chickens are weighed using a 5kg dial scale with an accuracy of ± 5 g. Cumulative growth, absolute growth, survival rate, and feed efficiency were calculated according to [Doan et al. \(2011\)](#). Productivity and meat quality indicators were also determined according to [Doan et al. \(2011\)](#). Daily feed intake (g/animal/day): The amount of feed given each day was weighed, and the remaining feed was weighed the following day to determine the total feed intake. Feed efficiency was assessed by the amount of feed consumed per 1 kg of weight gain at 1, 2, 4, to 24 weeks of age.

Meat yield and quality

Survey slaughter: Ten roosters and ten hens with average weight at 24 weeks of age were selected for slaughter survey. The carcass composition at slaughter was determined using the slaughter survey method for evaluation criteria as described by [Doan et al. \(2011\)](#). Some surveyed criteria included live weight (g), carcass weight (g), breast meat percentage (%), and thigh meat percentage (%), performed according to the method described by [Doan et al. \(2011\)](#). Breast meat was used to determine the percentage of water loss during storage and processing after 24 hours of slaughter, according to [Doan et al. \(2011\)](#). Meat quality was assessed according to the methods of [Doan et al. \(2011\)](#).

pH of meat

pH was determined by pH meter Testo 230 (Testo Co, German) at 15 minutes (pH15); 24 hours (pH24); after slaughter with 3 replicates. The pH15 was measured at 15 minutes after slaughter by taking 10g of minced loin muscle into a 400 ml beaker, adding 100 ml of distilled water, homogenizing the sample and centrifuging at 7000 rpm, and measuring the pH of the solution as quickly as possible. Similarly, values of pH24 were measured on meat samples stored at 4°C.

Drip loss

Meat samples were cut from the loin muscle with a size of thickness of 2.5 cm, width 2 cm and length 5 cm. They were weighed, put in a storage bag, sealed and stored at 2 – 4 °C according to [Brondum et al. \(2000\)](#). At 12, 24 and 48 hours after preservation, the sample should be taken immediately from the storage bag, lightly patted dry and weighed according to [Brondum et al. \(2000\)](#) and [Honikel \(1998\)](#). Drip loss was calculated formula;

$$\text{Drip loss (\%)} = (P1 - P2) \times 100 / P1$$

In which: P1 (g): initial weight; P2 (g): final weight.

Cooking loss

Meat samples were cut from the loin muscle with size of thickness of 2.5 cm, width 2 cm and length 5 cm, immediately weighed (initial weight), put in a polyethylene bag, heated in a water bath at 75°C for 60 minutes, taken and weighed again (final weight). Cooking loss was calculated as following:

$$\text{Cooking loss (\%)} = (P1 - P2) \times 100 / P1$$

In which: P1(g): initial weight; P2(g): final weight

Meat color

Meat color was measured in the loin sample with a Minolta CR-410 colorimeter (Japan) followed to Honikel (1998) and Baublits et al. (2006). The color was expressed as L*, a* and b* readings according to standard luminance D and standard angle of view 65° (Honikel, 1998; Baublits et al., 2006). - L* = 0 (black), L* = 100 white light (white light similar to BaSO₄ or MgO burnt) - b* = - 60 (green), +60 (yellow) - a* = - 60 (blue), + 60 (red)

Data analysis

Data processing: Statistical data were processed using Minitab 16 software. Data on growth, yield, and meat quality were analyzed using one-factor ANOVA (analysis of variance based on sex). Statistical parameters included mean and standard deviation (SD). The Tukey test was used to compare mean values at a significance level of $P < 0.05$.

RESULT

Survival rate

The survival rates presented in Table 1 show that Mnu Buon chickens have a high survival rate, reaching 91.5% from 1 to 24 weeks of age. The survival rate from 0 to 2 weeks of age was 100%, at 4-8 weeks of age was 97%, and at 91.5-96% in the remaining weeks.

Growth of Mnu Buon chickens

Table 2 shows that the weight of chickens at 1 day old was 23.1g/chicken, at 6 weeks old was 217.8g/chicken, with a coefficient of variation (Cv%) ranging from 3.3–10.6%. At 24 weeks old, the weight of males reached 1572.8g/chicken, and females reached 1233.8g/chicken. The difference in weight between males and females was statistically significant ($P < 0.05$) (Table 2 and Figure 1).

The absolute growth of Mnu Buon chickens is presented in Table 3 and Figure 2, showing that Mnu Buon chickens had an absolute growth rate from 1 day old to 24 weeks old of 9.2g/chicken/day for males and 7.2g/chicken/day for females, with statistically significant differences ($P < 0.05$). The highest absolute growth was reached at 14-16 weeks old in males at 15.3g/chicken/day and in females at 16-18 weeks old at 11.3g/chicken/day.

Feed intake and feed consumption of Mnu Buon chickens

Feed intake of Mnu Buon chickens gradually increased with age and weight gain. From 1 to 24 weeks of age, male chickens consumed 45.4g/chicken/day, while female chickens consumed 39.7g/chicken/day. The difference in feed intake was statistically significant ($P < 0.05$). Feed consumption per kg of weight gain was 4.9 kg feed/kg weight gain for male chickens and 5.4 kg feed/kg weight gain for female chickens.

Carcass yield of Mnu Buon chickens

The carcass yield of Mnu Buon chickens is presented in Table 5, showing that the chickens had a slaughter weight of 1220g (1020-1420g). The carcass weight was 1035g (870-1200g), the total carcass weight was 875g (720-1030g), the thigh weight was 200.5g (169.4-231.7g), and the breast weight was 163.1g (153.9-172.3g). Productivity indicators in roosters were higher than in hens (Table 5), with statistically significant differences ($P < 0.05$). The percentage of carcass, carcass percentage, thigh meat percentage, breast meat percentage, and thigh-breast meat percentage in the rooster group were 84.7%, 72.6%, 22.5%, 16.7%, and 39.3%, respectively, while in the hen group they were 85.4%, 70.6%, 23.5%, 21.3%, and 44.8%. The breast meat percentage and thigh-breast meat percentage were higher in hens than in roosters with $P < 0.05$.

The meat quality of Mnu Buon chicken

The pH₁₅ values in thigh and breast meat were 6.33 and 6.31, respectively. After 24 hours, the pH₂₄ values of the meat decreased to 5.81 and 5.78 (Table 6). There was no significant difference in the pH values of rooster and hen meat ($P > 0.05$).

The color of Mnu Buon chicken meat

The color of the thigh meat (Table 6) shows that the L* values in rooster and hen meat were 54.6 and 54.9, respectively, the a* values were 12.46 and 10.8, and the b* values were 12.5 and 14.7, respectively, with no statistically significant difference ($P > 0.05$). Similarly, for breast meat, the L* values were 59.6 and 59.9, the a* values were 11.75 and 10.78, and the b* values were 12.7 and 12.9. The color indicators for breast meat showed no significant difference ($P > 0.05$).

Table 1 - Survival rates of Mnu Buon chickens across different age groups (%)

Age (Week)	Beginning of the week (chicken)	Weekend (chicken)	Weekly survival rate (%)	Overall survival rate (%)
1	200	200	100.0	100.0
2	200	200	100.0	100.0
3	200	195	97.5	97,5
4	195	194	99.5	97
5	194	194	100.0	97
6	194	194	100.0	97
7	194	194	100.0	97
8	194	194	100.0	97
9	194	193	99.5	96.5
10	194	193	99.5	96.5
11	194	193	99.5	96.5
12	194	192	99.0	96
13	192	192	100.0	96
14	192	192	100.0	96
15	192	187	97.4	93.5
16	187	187	100.0	93.5
17	187	187	100.0	93.5
18	187	187	100.0	93.5
19	187	185	98.9	92.5
29	185	185	100.0	92.5
21	185	185	100.0	92.5
22	185	183	98.9	91.5
23	183	183	100.0	91.5
24	183	183	100.0	91.5
Survival rate up to 24 weeks of age				91.5

Table 2 - Cumulative weight of Mnu Buon chickens (g)

Weeks old	Total weight (Roosters + Hens)			
	Mean±SD (g)		Cv (%)	
Newly hatched	23.1±1.4		5.9	
1	41.1±1.4		3.3	
2	62.2±2.9		4.7	
3	87.4±9.2		10.5	
4	120.4±8.7		7.26	
5	162.2±13.9		8.6	
6	217.8±23.2		10.6	
	Roosters		Hens	
	Mean±SD (g)	Cv (%)	Mean±SD (g)	Cv (%)
8	326.9 ^a ±9.9	3.0	253.1 ^b ±8.9	3.5
10	437.8 ^a ±11.6	2.7	327.4 ^b ±10.0	3.1
12	589.1 ^a ±13.2	2.2	416.6 ^b ±11.2	2.7
14	779.7 ^a ±13.9	1.9	521.7 ^b ±11.4	2.2
16	994.1 ^a ±17.5	1.8	649.7 ^b ±12.7	2.0
18	1177.2 ^a ±20.8	1.7	808.5 ^b ±15.5	1.9
20	1312.5 ^a ±22.0	1.8	962.7 ^b ±18.6	1.9
22	1443.7 ^a ±26.3	2.1	1101.8 ^b ±18.0	1.6
24	1572.8 ^a ±32.7	1.8	1233.8 ^b ±18.0	1.5

Note: ^{a, b} Values within the same row that have different letters differ significantly (p<0.05).

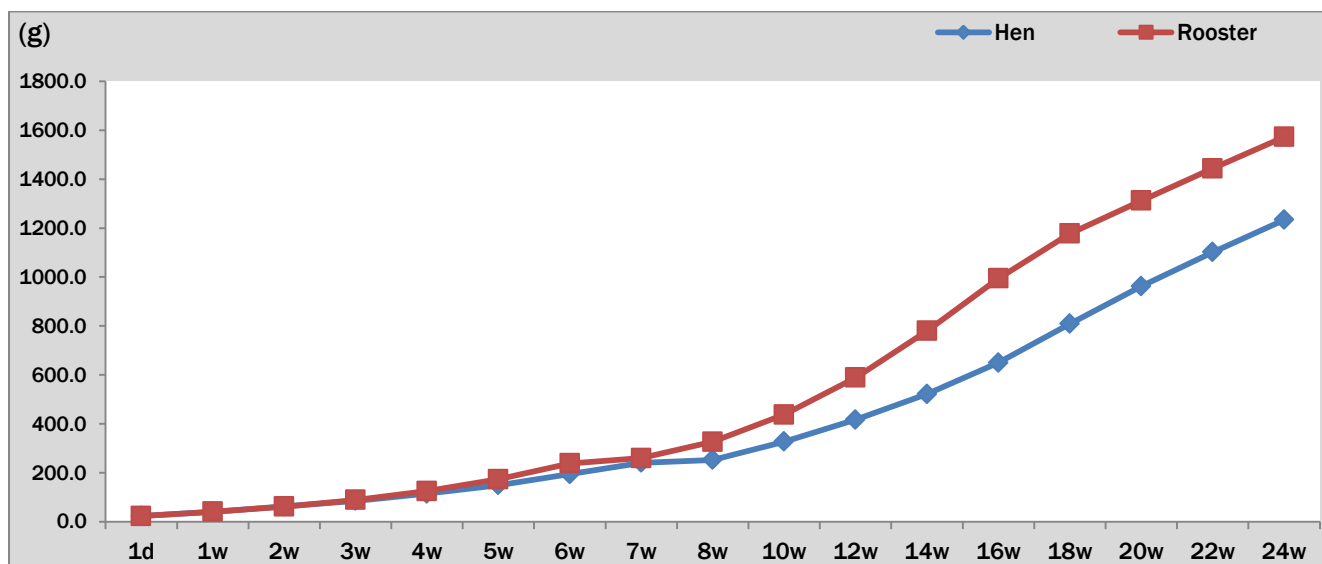


Figure 1 - Cumulative growth of Mnu Buon chickens from 1 day old to 24 weeks old.

Table 3 - Absolute weight gain of Mnu Buon chickens (g/day)

Weeks old	Increase in absolute weight (Roosters + Hens)			
	Mean±SD (g)	Cv (%)		
Newly hatched - 1	2.6±0.3	9.87		
1-2	30.0±0.5	15.5		
2-3	3.6±1.6	43.8		
3-4	4.7±1.2	26.15		
4-5	6.0±1.0	17.4		
5-6	7.0±1.5	19.4		
	Roosters	Hens		
	Mean±SD (g)	Cv (%)	Mean±SD (g)	Cv (%)
6-8	6.3 ^a ±0.6	9.8	4.2 ^b ±0.4	10.6
8-10	7.9 ^a ±0.4	5.4	5.3 ^b ±0.4	7.4
10-12	10.8 ^a ±0.3	2.4	6.4 ^b ±0.2	3.4
12-14	13.6 ^a ±0,5	3.6	7.5 ^b ±6.6	6.6
14-16	15.3 ^a ±0.5	3.4	9.1 ^b ±0.5	5.3
16-18	13.1 ^a ±0.5	4.1	11.3 ^b ±0.5	4.7
18-20	9.7 ^a ±0.5	5.4	11.0 ^b ±0.5	4.9
22-22	9.4 ^b ±0.8	8.6	9.9 ^a ±0.4	3.6
22-24	9.2 ^b ±0.7	7.9	9.4 ^a ±0.3	4.5
1 day old -24	9.2±0.2		7.2±0.1	

Note: ^{a, b} Values within the same row that have different letters differ significantly (P<0.05).

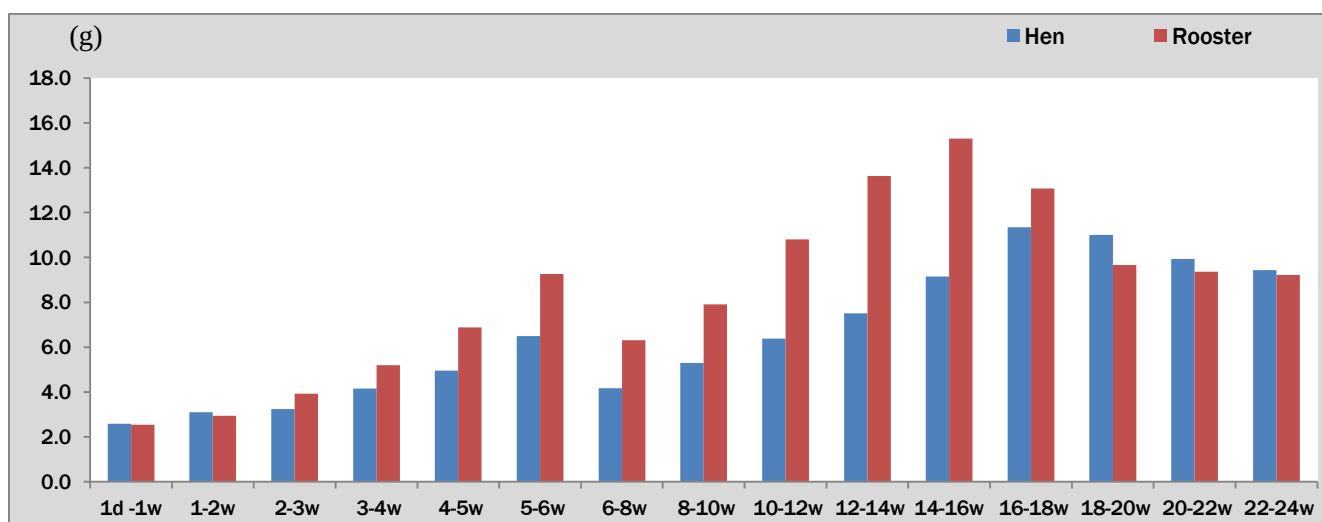


Figure 2 - Absolute growth of Mnu Buon chickens from 1 day old to 24 weeks old.

Table 4 - Feed intake from 0 to 24 weeks of age (g/chicken/day)

Weeks old	Mean±SD (n=150)		
1	2.7±0.2		
2	4.5±0.1		
3	6.3±0.5		
4	7.0±0.6		
5	8.7±0.7		
6	12.5±0.9		
	Roosters (n=79) Mean±SD	Hens (n=71) Mean±SD	P= Value
7	16.2 ^a ±1.1	14.9 ^b ±1.5	<0.001
8	20.8 ^a ±0.9	18.0 ^b ±0.8	<0.001
9	25.7 ^a ±0.9	20.6 ^b ±1.5	<0.001
10	29.8 ^a ±0.8	24.4 ^b ±1.9	<0.001
11	35.3 ^a ±1.1	29.6 ^b ±0.8	<0.001
12	38.2 ^a ±0.4	31.1 ^b ±1.3	<0.001
13	45.2 ^a ±0.7	38.9 ^b ±3.1	<0.001
14	48.4 ^a ±1.3	42.6 ^b ±1.7	<0.001
15	60.3 ^a ±1.9	52.0 ^b ±1.7	<0.001
16	66.2 ^a ±0.9	54.9 ^b ±1.3	<0.001
17	74.5 ^a ±0.5	65.0 ^b ±3.9	<0.001
18	78.3 ^a ±0.9	65.7 ^b ±2.3	<0.001
19	82.7 ^a ±0.5	73.7 ^b ±1.8	<0.001
20	83.3 ^a ±2.1	74.3 ^b ±2.5	<0.001
21	85.1 ^a ±1.0	75.0 ^b ±2.0	<0.001
22	85.6 ^a ±1.1	75.7 ^b ±2.1	<0.001
23	85.7 ^a ±0.9	77.0 ^b ±2.2	<0.001
24	86.4 ^a ±1.0	78.0 ^b ±2.1 ^b	<0.001
1-24	45.4±31.1	39.7±27.4	<0.076

Note: ^{a, b} Values within the same row that have different letters differ significantly (P<0.05).

Table 5 - Chicken carcass yield of local people in Dak Lak province.

Indicators	Total (n=10) Mean ± SD	Roosters (n=5) Mean ± SD	Hens (n=5) Mean ± SD
Live weight (g)	1220±74.2	1420 ^a ±58.3	1020.0 ^b ±37.4
Jaw hook weight (g)	1035±58.7	1200 ^a ±35.4	870.0 ^b ±25.50
Carcass weight (g)	875±57.5	1030 ^a ±46.4	720.0 ^b ±27.0
Thigh meat weight (g)	200.5±14.0	231.7 ^a ±16.5	169.4 ^a ±11.0
Breast weight (g)	163.1±6.3	172.3 ^a ±5.6	153.9 ^b ±10.1
Jaw hook ratio (%)	85.1±0.8	84.7±1.2	85.4±1.2
Carcass ratio (%)	71.6±0.9	72.6±1.6	70.6±0.4
Thigh meat ratio (%)	22.9±0.8	22.5±1.4	23.5±0.9
Breast meat ratio (%)	19.1±0.9	16.7 ^b ±0.6	21.3 ^a ±0.8
Ratio of thigh and breast meat (%)	42.1±1.3	39.3 ^b ±1.4	44.8 ^a ±1.2

Note: Values in the same row for male and female that have different letters are statistically significantly different (P<0.05).

Table 6 - Some quality indicators of chicken meat.

Type of meat	Indicator	Total (n=10)	Roosters (n=5)	Hens (n=5)
Thigh meat	pH ₁₅	6.33±0.02	6.31±0.01	6.4±0.04
	pH ₂₄	5.81±0.03	5.8±0.04	5.8±0.04
	L*	54.8±0.8	54.6±1.2	54.9±1.1
	a*	11.6±0.4	12.46±0.4	10.8±0.4
	b*	14.2±0.4	12.5±0.4	14.7±0.6
	Preservative water loss rate (%)	0.9 ^b ±0.2	0.7±0.1	1.1±0.3
	Processing water loss rate (%)	21.1±1.6	20.4±0.8	21.7±3.3
Breast meat	pH ₁₅	6.31±0.03	6.2±0.03	6.3±0.1
	pH ₂₄	5.78±0.02	5.8±0.04	5.8±0.1
	L*	59.8±0.8	59.6±1.2	59.9±1.1
	a*	11.2±0.4	11.8±0.6	10.8±0.4
	b*	13.7±0.6	12.7±0.9	12.9±0.9
	Preservative water loss rate (%)	1.5 ^a ±0.2	1.1±0.1	1.9±0.3
	Processing water loss rate (%)	18.9±0.6	19.8±0.8	17.9±0.6

Note: Values in the same column that have different letters are statistically significantly different (P<0.05).

DISCUSSION

Survival rate of Mnu Buon chickens

The survival rate of Mnu Buon chickens is high (Table 1). This is a long-domesticated breed that is highly adaptable to natural conditions and the care practices of ethnic minorities in Dak Lak province. These chickens can forage in nature and have high disease resistance, leading to a high survival rate. Compared to the results of Mui et al. (2012) on Long Cam chickens, which had an 80% survival rate from 0 to 15 weeks, Mnu Buon chickens show a higher rate. Thinh et al. (2016) stated that the survival rates for "Many-toed" chickens at 0-8 and 9-16 weeks were 90% and 88.3%, respectively. According to Thinh et al. (2020), Lac Thuy chickens reached 94.51% and 90.98% for the same periods. Dung et al. (2021) showed that Ri Dabaco chickens and golden-legged fighting rooster chickens have a high survival rate of 94-97%. Huong et al. (2023) noted a 95.83% survival rate for Van Linh chickens from 1 to 18 weeks. Our study shows that the survival rate of Mnu Buon chickens from 1 day to 24 weeks is 91.5%. This is quite high, similar to Dong Tao chickens and higher than Mia, Many-toed, and Lac Thuy chickens, but lowers than Van Linh chickens. These results prove that Mnu Buon chickens adapt well to the farming and natural conditions of Dak Lak.

Growth of Mnu Buon Chickens

The growth of Mnu Buon chickens increases with age from 1 to 24 weeks (Table 2), following the law of cumulative growth. At 1 day old, the weight is 23.1g per bird, which is lower than other breeds. For example, Van Linh chickens weigh 30.21g (Huong et al., 2023), and Ri Lac Son chickens weigh 25.28g (Thinh et al., 2020). At 24 weeks, the weight of roosters was 1305.5g and hens was 1104.8g). This weight is lower than Ri Lac Son, Van Linh, and Many-toed chickens. Mnu Buon chickens raised in Dak Lak are generally smaller than native breeds in other regions. The absolute growth rate was 9.2g/day for roosters and 7.2g/day for hens. The highest growth occurs at 14-16 weeks for roosters (15.3g/day) and 16-18 weeks for hens (11.3g/day). This result is lower than that of Ri Lac Son chickens at 20 years old, which was 14.1g/chicken/day in roosters and 10.7g/chicken/day in hens (Thinh et al. 2020); Huong et al. (2023) showed that Van Linh chickens at 18 weeks old had an absolute growth of 17.6g/chicken/day in roosters and 14.7g/chicken/day in hens. The results obtained in this study show that Mnu Buon chickens have a low body weight, only at the average level of native Vietnamese chicken breeds. This result is consistent with observations on breed that affect the growth performance of broiler chickens, indicating that breed has a significant influence on chicken body weight (Adebayo et al., 2023). The growth of Mnu Buon chickens is lower than that of other native chicken breeds raised domestically, possibly due to limitations in the selection and management of the breed. The decline in growth may be due to inbreeding and uncontrolled crossbreeding with many other breeds.

Feed Intake and Carcass Yield

The feed intake of Mnu Buon chickens (Table 4) gradually increased with age, averaging 45.4 g for roosters and 39.7 g for hens throughout the rearing period. Feed intake of Mnu Buon chickens was lower than that of Ri Lac Son chickens (50.2g/chicken/day; Thinh et al., 2020), Long Cam chickens (51.9 g/chicken/day; Mui et al., 2012), and Van Linh chickens (60.9 g/chicken/day) (Huong et al., 2023). The feed intake per kg of weight gain in Mnu Buon chickens was 4.9 kg in roosters and 5.4 kg in hens. This result was higher than that of Van Linh chickens from 1 to 18 feed intakes was 3.98 kg (Huong et al., 2023). Feed consumption of Mnu Buon chickens is lower than that of Mong chickens (Son et al., 2022). Feed intake increases with age, averaging 45.4g for roosters and 39.7g for hens. This is lower than Ri Lac Son, Long Cam, and Van Linh chickens. The carcass yield of Mnu Buon chickens (Table 5) shows that roosters have higher slaughter weight, carcass weight, thigh weight, breast weight, breast meat ratio, and thigh - breast meat ratio are higher than hens, with statistically significant differences ($P < 0.05$). Mnu Buon chickens have lower meat yield than some Vietnamese native chicken breeds. Tham et al. (2016) showed that 24-week-old Dong Tao chickens have a slaughter weight of 2,716.55 g for roosters and 2,266.67g for hens. The carcass ration was 69.55% and 64.16%, the thigh meat yield was 23.24% and 23.57%, and the breast meat yield was 13.92% and 16.85%. Mui et al. (2012) reported that 15-week-old Long Cam chickens had a slaughter weight of 1,930.33g, carcass ration of 69.60%, thigh meat ratio of 22.25%, and breast meat ratio of 14.36% in males, and correspondingly in females of 1,430g, 68.4%, 22.34%, and 15.27%. Thinh et al. (2020) showed that 24-week-old Lac Thuy chickens had a carcass ratio of 81.29% in males and 75.48% in females; Carcass ratio in roosters and hens was 72.65% and 65.59%, respectively; thigh meat ratio was 23.71% in roosters and 20.19% in hens. Mnu Buon chickens have carcass yield, thigh meat yield, and breast meat yield comparable to Ri Dabaco chickens and yellow-legged fighting chickens (Dung et al., 2021). Slaughter weight, carcass yield, thigh meat yield, and breast meat yield in Mnu Buon chickens are comparable to fighting chickens (Giang et al., 2023).

Meat quality

The results in Table 6 show that the meat quality indicators (sensory quality and processing quality) did not differ significantly between Mnu Buon roosters and hens ($P > 0.05$). A similar trend was also found in studies on chickens with the indicators of light color L^* , red color a^* , yellow color b^* , storage and processing water loss percentage, pH15 and pH24 values (N'dri et al., 2007).

The L* value of Mnu Buon chicken breast meat is equivalent to that of Vạn Linh chicken (60.89), while the L* value of Mnu Buon chicken thigh meat (54.77) is higher than that of Vạn Linh chicken (52.64) (Huong et al., 2023). The L* values of Mnu Buon chicken breast and thigh meat are higher than those of Lạc Thủy chicken breast and thigh meat (56.43; 46.96) (Thinh and Giang, 2020). The light color of Thai local chicken is 54.8 (Wattanachant et al., 2004). When comparing Vietnamese Mnu Buon chicken with Thai local chicken, the light color of Mnu Buon chicken in this experiment is higher. According to the classification of Qiao et al. (2002), Mnu Buon chicken meat belongs to the light-colored meat category ($L^* > 53$). Afrin et al. (2024) suggest that the values of brightness (L^*), yellowness (b^*), and yellowness (a^*) are influenced by different chicken breeds, and rapid growth rate is also related to the yellowness value (b^*). The meat color values in this study are consistent with studies on Ross, JA757, and ISA Dual chicken breeds, where the L^* , b^* , and a^* values are significantly influenced by breed quality (Chodová et al., 2021). The rate of water loss during storage in thigh and breast meat was 0.89% and 1.52%, respectively ($P < 0.05$); there was no difference between roosters and hens ($P > 0.05$). Compared to other studies, Mnu Buon chicken meat had lower water loss than Lạc Thủy chicken (2.45% and 2.33%) (Thinh et al., 2020) and Ri Ninh Hoa chicken (2.85% and 2.48%). The processing water loss rate in thigh and breast meat was 21.07% and 18.87%, respectively ($P > 0.05$). The processing water loss rate in breast meat of Mnu Buon chicken was comparable to that of Vạn Linh chicken (19.35%) (Huong et al., 2023). The storage and processing water loss rates of Mnu Buon chicken were within the limits of native chicken meat.

CONCLUSION

Mnu Buon chickens are a breed that adapts well to the farming methods of ethnic minorities in Dak Lak province. They have a high survival rate of 91.5%. Mnu Buon chickens belong to the low-weight group; at 24 weeks old, roosters weigh 1572.8g/chicken, and hens weigh 1233.8g/chicken. The feed consumption per kg of weight gain of Mnu Buon chickens at 24 weeks of age for roosters and hens was 4.9 and 5.4 kg, respectively. Mnu Buon chickens have lower meat yield than some Vietnamese native chicken breeds. The meat quality of Mnu Buon chickens is within the limits of Vietnamese native chicken breeds.

DECLARATION

Corresponding author

Correspondence and requests for materials should be addressed to Van Tien Dung, 567 Le Duan Street, Ea Kao Ward, Dak Lak province, Viet Nam. E-mail: vtdung@ttn.edu.vn; ORCID: <https://orcid.org/0009-0004-1827-1383>

Acknowledgements

The authors sincerely thank the Dak Lak Provincial Department of Science and Technology for its financial support under grant number 365-HĐ-SKHCN.

Authors' contributions

All authors had similar participation in designing, experiment, analysis and drafting the manuscript.

Ethical statement

All process of experiments was in according to animal welfare rules under monitoring of Tay Nguyen university ethical team.

Availability of data and material

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

SDGs addressed

Zero Hunger, Decent Work and Economic Growth.

Funding

The authors declare that no funds, grants, or other support were received during the preparation or publication of this manuscript.

Competing interests

The authors declare no competing interests.

REFERENCES

Adebayo MO, Abiona JA, Uyanga VA, Onagbesan OM, and Oke OE (2023). Growth and reproductive performance of broiler breeders reared to puberty under the open-sided housing in a tropical environment. *Animal Production Science*, 64(1): AN23183. DOI: <https://doi.org/10.1071/AN23183>

- Afrin A, Ahmed T, Lahiry A, Rahman S, Dey B, Hashem MA, et al. (2024). Profitability and meat quality of fast-, medium-and slow-growing meat-type chicken genotypes as affected by growth and length of rearing. *Saudi Journal of Biological Sciences*, 31(8):104025. DOI: <https://doi.org/10.1016/j.sjbs.2024.104025>
- Baublits RT, Pohlman FW, Brown Jr AH and Johnson ZB (2006). Effects of enhancement with differing phosphate types, concentrations, and pump rates, without sodium chloride, on beef biceps femoris instrumental color characteristics. *Meat science*, 72(3):503-512. DOI: <https://doi.org/10.1016/j.meatsci.2005.08.018>
- Berthouly-Salazar C, Rognon X, Nhu Van T, Gély M, Vu Chi C, Tixier-Boichard M, et al. (2010). Vietnamese chickens: a gate towards Asian genetic diversity. *BMC genetics*, 11(1):53. DOI: <https://doi.org/10.1186/1471-2156-11-53>
- Brondum J, Munck L, Henckel P, Karlsson A, Tornberg E and Engelsen SB (2000). Prediction of water-holding capacity and composition of porcine meat by comparative spectroscopy. *Meat Science*, 55(2):177-185. DOI: [https://doi.org/10.1016/S0309-1740\(99\)00141-2](https://doi.org/10.1016/S0309-1740(99)00141-2)
- Doan BH, Mai NT, Son NT and Dat NT (2011). Indicators used in poultry farming research, Agricultural Publishing House. <https://www.studocu.vn/vn/document/dai-hoc-nong-lam-thanh-pho-ho-chi-minh/phuong-phap-nghien-cuu-khoa-hoc/cac-chi-tieu-nghien-cuu-trong-chan-nuoi-gia-cam-pgsts/138243111>
- Chebo C, Betsha S, and Melesse A (2022). Chicken genetic diversity, improvement strategies and impacts on egg productivity in Ethiopia: a review. *World's Poultry Science Journal*, 78(3):803-821. DOI: <https://doi.org/10.1080/00439339.2022.2067020>
- Chodová D, Tůmová E, Ketta M, and Skřivanová V (2021). Breast meat quality in males and females of fast-, medium-and slow-growing chickens fed diets of 2 protein levels. *Poultry Science*, 100(4):100997. DOI: <https://doi.org/10.1016/j.psj.2021.01.020>
- Choo YK, Kwon HJ, Oh ST, Um JS, Kim BG, Kang CW, et al. (2014). Comparison of growth performance, carcass characteristics and meat quality of Korean local chickens and silky fowl. *Asian-Australasian journal of animal sciences*, 27(3):398. DOI: <https://doi.org/10.5713/ajas.2013.13638>
- Dung NT, Tuyet L and Diu BT (2021). Growth and meat yield potential of Ri Da Ba Co chickens and yellow-legged fighting chickens raised semi-free-range on industrial feed in Thanh Hoa. *Journal of Science*, Hong Duc University, 55.2021: 28-35. <https://vjol.info.vn/index.php/DHHD/article/view/64761/54574>
- Giang NT, Hang LT, Khoa DV, Chau MH, Loan PP, Loan LT, et al (2023). Effect of the herb mixture as phytogenic feed additive on growth performance and carcass traits of Noi chickens. *Livestock Research for Rural Development*, 35:44. <http://www.lrrd.org/lrrd35/5/3544ntgi.html>
- Honikel KO (1998). Reference methods for the assessment of physical characteristics of meat. *Meat science*, 49(4):447-457. DOI: [https://doi.org/10.1016/S0309-1740\(98\)00034-5](https://doi.org/10.1016/S0309-1740(98)00034-5)
- Huong DT, Giang N, Thong NV, Toan NK, Nhung DT, and Thanh NH (2023). Appearance characteristics, growth ability and meat yield of Van Linh chickens. *Vietnam Journal of Agricultural Sciences*, 21(5): 560-569. <https://tapchi.vnua.edu.vn/wp-content/uploads/2023/05/tap-chi-5.4.pdf>
- Kanyama CM, Moss AF, and Crowley TM (2024). Strategies for promoting sustainable use and conservation of indigenous chicken breeds in Sub-Saharan Africa: Lessons from low-income countries. *F1000Research*, 11: 251. DOI: <https://doi.org/10.12688/f1000research.75478.6>
- Kpomasse CC, Kouame YA, N'nanle O, Houndonougbo FM, Tona K, and Oke OE (2023). The productivity and resilience of the indigenous chickens in the tropical environments: improvement and future perspectives. *Journal of Applied Animal Research*, 51(1): 456-469. DOI: <https://doi.org/10.1080/09712119.2023.2228374>
- Minh L, Quang NH, DTH and Truong ND (2022). Preliminary results of conservation of native chickens of the Mong ethnic group in Thai Nguyen province. *Journal of Animal Science and Technology*, 133: 2-12. <https://scholar.dlu.edu.vn/thuvienso/bitstream/DLU123456789/267666/1/CVv25S1332022002.pdf>
- Mogano RR, Mpofu TJ, Mtileni B, and Hadebe K (2025). South African indigenous chickens' genetic diversity, and the adoption of ecological niche modelling and landscape genomics as strategic conservation techniques. *Poultry science*, 104(1):1-12. DOI: <https://doi.org/10.1016/j.psj.2024.104508>
- Mui NB, Nguyen CT, Duc LA, Hieu NB (2012). Appearance characteristics and meat yield of the local Long Chin chicken in Luc Ngan, Bac Giang. *Journal of Science and Development*, 10(7): 978-985. <https://tapchi.vnua.edu.vn/wp-content/uploads/old/122013-tapchi7-5.pdf>
- N'dri AL, Mignon-Grasteau S, Sellier N, Beaumont C, and Tixier-Boichard M (2007). Interactions between the naked neck gene, sex, and fluctuating ambient temperature on heat tolerance, growth, body composition, meat quality, and sensory analysis of slow growing meat-type broilers. *Livestock Science*, 110(1-2):33-45. DOI: <https://doi.org/10.1016/j.livsci.2006.09.025>
- Ngo TKC, Nguyen VT, Dang NG and Pham CT (2021). Productivity and meat quality of broiler polydactyl chicken. *Journal of Animal Science and Technology*, 129: 38-48. <https://tapchivcn.vn/khcn/article/view/244>
- Qiao M, Fletcher DL, Northcutt JK, and Smith DP (2002). The relationship between raw broiler breast meat color and composition. *Poultry science*, 81(3):422-427. DOI: <https://doi.org/10.1093/ps/81.3.422>
- Quentin M, Bouvarel I, Berri C, Le Bihan-Duval E, Baéza E, Jégo Y, and Picard M (2003). Growth, carcass composition and meat quality response to dietary concentrations in fast-, medium-and slow-growing commercial broilers. *Animal Research*, 52(1):65-77. DOI: <https://doi.org/10.1051/animres:2003005>
- Tham LT, Thai NX, Thang VV, Hiep ĐT, Soan DV, Ton VD and Binh DV (2016). Growth, yield and meat quality of Dong Tao chickens. *Vietnam Journal of Agricultural Science* No. 11 (14): 16-25. https://tapchi.vnua.edu.vn/wp-content/uploads/old/512017-so%2011%20ban%20bong%205.7_03.pdf

- Thinh N, Dang P, Hang VTT, Hoang AT, Doan BH. (2016). Some morphological characteristics and production capabilities of the Multi-toed chicken raised in Xuan Son National Forest, Tan Son District, Phu Tho Province. *Journal of Science and Development*, 14(1): 9-20. 10-20. https://tapchi.vnua.edu.vn/wp-content/uploads/old/1132016-TC%20so%201.2016%20ban%20bong4_02.pdf
- Thinh NT, Doan B and Giang NTP (2020). Growth potential and meat quality of Ri Lac Son chicken. *Science and Technology of Animal Husbandry*, 256: 14-18. [in Vietnamese]. <https://www.researchgate.net/publication/342064862>
- Wattanachant S, Benjakul S, and Ledward DA (2004). Composition, color, and texture of Thai indigenous and broiler chicken muscles. *Poultry science*, 83(1):123-128. DOI: <https://doi.org/10.1093/ps/83.1.123>

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

THE EFFECTS OF PEN DESIGN FOR FARROWING AND BOAR BREED ON REPRODUCTIVE TRAITS OF SOWS

Sergiy HLUKHENKYI¹, Vadym LYKHACH¹, Anna LYKHACH²✉, Yevhen BARKAR³, Olena IZHBOLDINA⁴, and Roman MYLOSTYVYI⁵

¹Department of Animal Technology, Faculty of Animal Science and Water Bioresources, National University of Life and Environmental Sciences of Ukraine, Horikhuvatskyi shliakh Street, 19, Kyiv, 03041, Ukraine

²Department of Applied Biology, Animal Breeding and Genetics, Faculty of Animal Science and Water Bioresources, National University of Life and Environmental Sciences of Ukraine, Horikhuvatskyi shliakh Street, 19, Kyiv, 03041, Ukraine

³Department of Biotechnology and Bioengineering, Faculty of Technology of Production and Processing of Livestock Products, Standardization and Biotechnology, Mykolayiv National Agrarian University, Georgiy Gongadze Street, 9, Mykolayiv, 54020, Ukraine

⁴Department of Animal Feeding and Breeding Technology, Faculty of Biotechnology, Dnipro State Agrarian and Economic University, S. Efremov Street, 25, Dnipro, 49600, Ukraine

⁵Department of Animal Products Processing Technology, Faculty of Biotechnology, Dnipro State Agrarian and Economic University, S. Efremov Street, 25, Dnipro, 49600, Ukraine

✉ Email: avlykhach@nubip.edu.ua

✉ Supporting Information



ABSTRACT: Modern pig production increasingly requires compliance with welfare-oriented housing standards, prompting farms to modernize farrowing systems while maintaining productive performance. This study aimed to evaluate the effects of farrowing pen design during the suckling period and boar line on the reproductive performance of sows, piglet growth, and survival under industrial conditions aligned with contemporary animal welfare requirements. The experiment was conducted using a 2 × 2 factorial design involving 192 sows (Large White × Landrace) of second to fourth parity and 2,443 piglets obtained through terminal crossbreeding with Maxter and PIC 337 boars. Animals were fed complete diets and housed under controlled microclimate conditions. Reproductive traits, piglet survival, and sow backfat thickness were assessed using standardized methods and subjected to statistical analysis. Sows housed in fixed traditional farrowing pens exhibited superior reproductive performance compared with those kept in improved welfare-oriented pens. The boar line significantly affected the total number of piglets born and the number born alive, with the highest total number of piglets born (14.1 piglets) recorded in sows inseminated with PIC 337 semen. Farrowing pen design had a significant effect on litter size, litter uniformity, stillbirth rate, litter weight at weaning, sow backfat thickness, and piglet weaning weight. The highest average daily gain and piglet live weight at weaning were observed in Group 1, whereas the lowest values were recorded in Group 4. Piglet survival to weaning was the highest in Group 3 (97.3%). A combined effect of farrowing pen design and boar line was identified for milk performance (defined as total milk output estimated from litter weight gain), litter uniformity index (reflecting within-litter variation in piglet body weight), and piglet survival. The practical significance of these findings lies in providing science-based recommendations for selecting farrowing pen configurations and boar lines that support welfare-compliant and efficient pig production systems in Ukraine. The study demonstrates a performance–welfare trade-off, whereby traditional pens improved piglet growth parameters, while welfare-oriented improved pens enhanced sow mobility. This balance should be considered when implementing welfare-compliant farrowing systems.

Keywords: Breeding systems, Fertility, Performance, Pig, Welfare.

INTRODUCTION

Modern pig production increasingly emphasizes the implementation of welfare-oriented housing systems that comply with international standards for the humane treatment of animals (Nevrkla et al., 2024; Vandresen et al., 2024). According to current scientific protocols, farrowing pen design must meet not only technological and sanitary-hygienic requirements but also the biological needs of animals at different physiological states (Dourmad et al., 2022). Properly organized pen designs reduce stress, minimize injuries to sows and piglets, and facilitate effective control of feeding, microclimate and sanitary conditions, thereby positively influencing productive performance and overall pork production efficiency (Leonard et al., 2020; Pacheco et al., 2024). The most critical periods in sow production are gestation, farrowing, lactation and weaning, during which animals are particularly sensitive to external stimuli and changes in housing and feeding conditions (Robbins et al., 2021; Do et al., 2023; Seddon and Moustsen, 2023). The use of specialized farrowing pen design protects piglets from crushing, ensures convenient access to the udder, provides designated areas for rest and activity, and allows farrowing operators to monitor and manage animals effectively while minimizing interference with natural behavioral patterns (Jang and Oh, 2022).

In many pig-producing regions, farms face economic constraints, limited access to modern equipment, and the need to balance production efficiency with rising welfare requirements (EFSA AHAW Panel, 2022; Vandresen et al., 2024).

RESEARCH ARTICLE
 PII: S222877012600008-16
 Received: September 30, 2025
 Revised: March 19, 2026
 Accepted: March 20, 2026

These challenges encourage for the development of cost-effective technological solutions, including the reconstruction of existing facilities and the gradual modernization of farrowing pen designs. Such approaches enable producers to optimize housing conditions, maintain or enhance animal welfare, and adapt to contemporary industry standards without compromising economic viability. Consequently, pork producers increasingly prioritize engineering flexibility, practical innovation, and strategic decision-making to meet both welfare expectations and economic demands (Lykhach et al., 2025).

In addition to technological and economic considerations, ethical aspects play an increasingly important role in the design and improvement of sow housing systems, particularly in the context of the European animal welfare movement and the adoption of advanced production technologies (Mahfuz et al., 2022; Turner et al., 2024). Growing attention to humane treatment promotes the implementation of housing designs that allow sows to partially express natural behavioral, including standing, lying down, changing body position, and interacting with piglets and the surrounding environment (Choi et al., 2020). Practical experience indicates that even under limited financial resources, farms can achieve substantial welfare improvements through rational pen redesign and the replacement or modification of individual structural elements (Ludwiczak et al., 2021; Malak-Rawlikowska et al., 2024).

At the same time, the use of adaptive farrowing pens – such as convertible structures that allow spatial adjustment according to piglet age and sow condition - represents a promising direction in housing system development. combining functional efficiency with animal welfare requirements and facilitating the expression of natural behaviors (Nevrkla et al., 2024; Mackinnon, 2024; Klassen et al., 2025). Such systems contribute to reduced injury risk, improved hygiene, simplified management, and, consequently, increased piglet survival and improved reproductive performance of sows (Glencorse et al., 2019). As a result, pig producers not only achieve better animal health and higher production efficacy, but also gain increased consumer trust, as public awareness of the ethical origin of animal products continues to grow.

Accordingly, even under current challenging economic conditions, investment in the reconstruction and functional improvement of farrowing pens represents strategic decision that enables national pig farms to remain competitive in both domestic and foreign markets, while supporting alignment with European integration goals and the sustainable development of the sector (Sossidou et al., 2025). At the same time, increasing attention is being paid to compliance with European regulatory standards in pig production, particularly the implementation of Council Directive 2008/120/EU, which restricts the long-term use of fixed farrowing crates (Council Directive 2008/120/EU). This regulatory framework presents both a challenge and an incentive for scientific research aimed at identifying optimal, adaptable housing solutions suitable for commercial farm conditions. The present study not only identifies farrowing pen design features that positively influence sow reproductive performance but also provides practical recommendations for the reconstruction and improvement of existing housing systems.

In view of the above, the motivation for this experiment was to ensure stable pork production under conditions of economic uncertainty, enhance the reproductive traits of sows and harmonize housing systems with European animal welfare. Therefore, this research is both timely and of direct practical relevance.

MATERIALS AND METHODS

Experimental design

The practical experiment was conducted on a commercial pig farm belonging to Viktoria Private Lease Enterprise (PLE) located in the Bashtanskiy district, Mykolaiv region, Ukraine. A total of 192 litters from suckling sows housed in the farrowing unit, comprising 2443 suckling piglets, were included in the study. The maternal genotype consisted of Large White × Landrace crossbred sows breeds, while the paternal genotypes were boars of the Maxter terminal line (France Hybrides, France) and the PIC 337 terminal line (PIC, United Kingdom). Artificial insemination of sows in the experimental groups was performed using the standard vaginal insemination technique, in accordance with the farm's approved reproductive protocol. Disposable insemination catheters manufactured by MS Schippers (Netherlands) were used to ensure a high level of biosecurity and ease of application. Freshly diluted semen obtained from boars housed at the farm's artificial insemination station was used, thereby minimizing ejaculate quality loss and improving fertilization efficiency. All insemination procedures were conducted in compliance with established technological, veterinary, and sanitary standards and were synchronized with the optimal estrus period of the sows.

The experiment was carried out in the reproduction and farrowing units, and the housing and management conditions were organized in accordance with the Departmental Norms of Technological Design: Pig Enterprises (Complexes, Farms, Small Farms-2005) and the recommendations of genetic companies, with due consideration of animal welfare principles.

According to the experimental scheme (Table 1), four experimental groups of sows were formed based on the principle of analogues (Ladyka and Khmelnychiy, 2023). A total of 192 clinically healthy sows of similar parity (second to fourth farrowing), live weight, body condition, and expected farrowing date were selected. Group formation followed the principle of analogues to ensure uniformity before allocation. After matching, sows were randomly assigned to one of four experimental groups using simple randomization within analogue blocks based on individual animal identification

numbers. All groups were balanced for parity, body condition, and previous reproductive performance, and no significant differences were detected among groups prior to the start of the experiment.

During the non-productive (idle) and gestation periods, sows from all four groups were housed in group pens (Figure 1) manufactured by AgroDana LLC (Ukraine). Five days prior to the expected farrowing date, sows were transferred to the farrowing unit and allocated to housing treatments as follows: sows in Groups 1 and 2 were housed in traditional fixed farrowing pens with a total area of 4.32 m² throughout the suckling period (Figure 2), whereas sows in Groups 3 and 4 were housed in improved farrowing pens with an increased total area of 7.20 m², allowing free movement from day 7 postpartum until weaning (Figure 3). All farrowing pens were manufactured by AgroDana LLC (Ukraine). Two types of farrowing pen designs were evaluated in the experiment, differing in construction, available space, and degree of sow mobility. Traditional pens (groups 1 and 2; total area 4.32 m²) were equipped with a standard fixed farrowing crate that restricted sow movement throughout the entire lactation period. The crate prevented the sow from turning around and limited forward and backward movement. A piglet creep area with a heated floor and protective anti-crushing rails was positioned along one side of the crate. This housing system represents the conventional farrowing design commonly used in intensive pig production. Improved pens (groups 3 and 4; total area 7.20 m²) were designed as partially free farrowing systems. During the first seven days postpartum, sows were temporarily restrained in a fixation crate to reduce the risk of piglet crushing during the critical neonatal period. After day 7, the crate was opened, allowing the sow free movement within the pen. The increased pen area enabled the sow to turn around, freely change body position, and interact with the surrounding environment. Improved pens were additionally equipped with an enlarged piglet creep area, anti-crushing rails, and a fully slatted plastic floor.

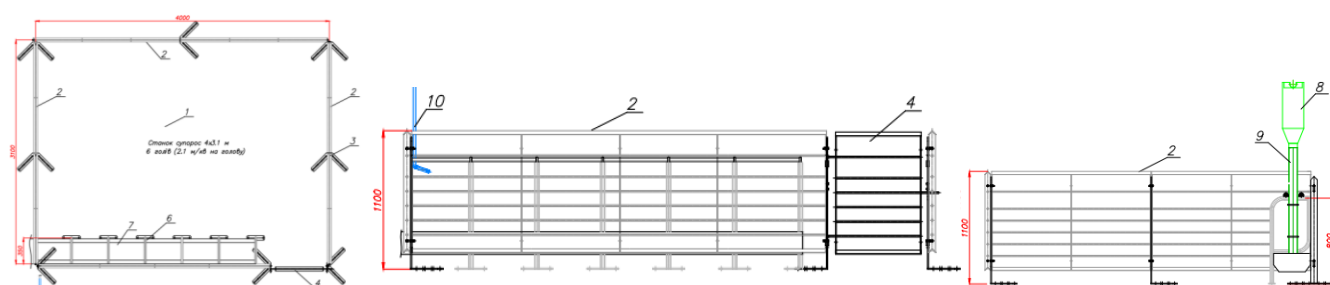


Figure 1 - Group pen for keeping sows during farrowing.

1 = Pen (top view); 2 = Side of the pen; 3 = Rack of fasteners to the concrete slatted floor; 4 = Pen's door; 6 = Delimiter; 7 = Group feeder; 8 = Individual feeding dispenser; 9 = Dispenser outlet pipe; 10 = Nipple drinker.

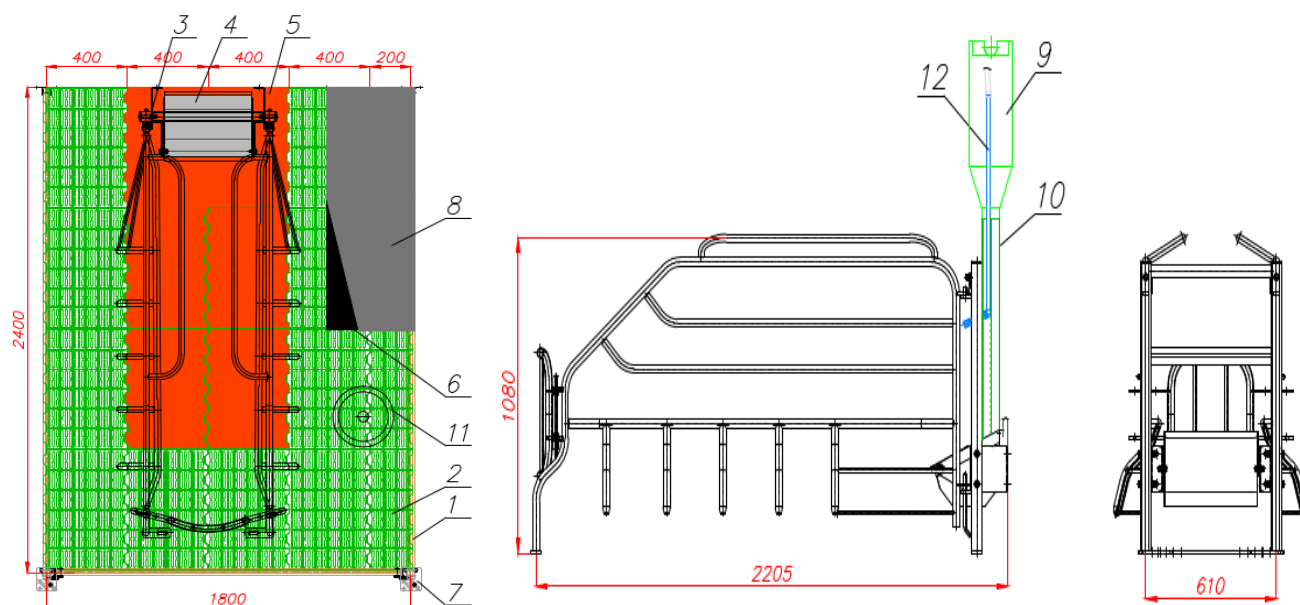


Figure 2 - Pen for keeping lactation sows and suckling piglets (traditional).

1 = Fence of the pen of polyvinyl chloride; 2 = Slatted plastic floor (50% open); 3 = Fixing pen; 4 = Sow feeder; 5 = Plastic floor (100% solid); 6 = heating carpet; 7 = Mounting rack; 8 = Piglet rest and heating space; 9 = Feeding dispenser; 10 = Dispenser lowering pipe; 11 = Suckling piglet feeder; 12 = Mounting and drinking bowl for the sow.

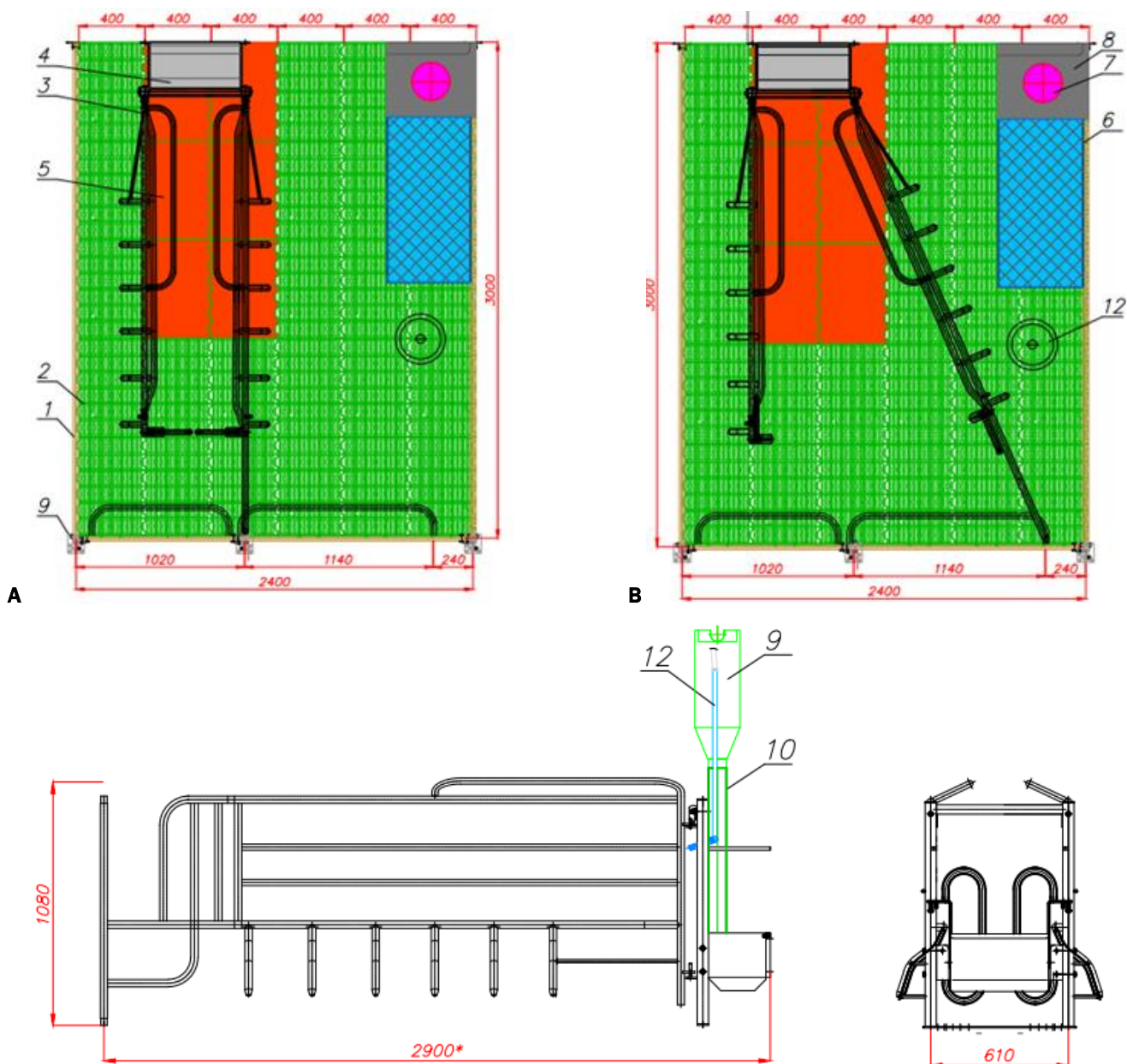


Figure 3 - Pen for free keeping of sows from 7 days after farrowing to weaning (improved).

A: Location of the fixing pen until 7 days after farrowing; B: Free keeping of sows after 7 days from the date of farrowing; 1 = Fence of the pen of polyvinyl chloride; 2 = Slatted plastic floor (50% open); 3 = Fixing pen; 4 = Sow feeder; 5 = Plastic floor (100% solid); 6 = Heating carpet; 7 = mounting rack; 8 = Piglet rest and heating space; 9 = Feeding dispenser; 10 = Dispenser lowering pipe; 11 = Suckling piglet feeder; 12 = mounting and drinking bowl for the sow.

Feeding

Sows were fed specialized complete compound diets formulated according to their physiological state, with separate rations for gestating and lactating sows. All feeds were produced at the farm's feed mill and formulated in accordance with internationally recognized nutritional standards for swine (NRC, 2012). To ensure adequate balancing of protein, minerals, and vitamins in the basal diet, protein-mineral-vitamin supplements and premixes manufactured by Cehavae Korm LLC (Ukraine) were incorporated into the feed. This approach ensured high nutritional value and compliance with the nutrient requirements of animals at different stages of the reproductive cycle. Feeding management was adjusted according to physiological status. During the non-productive (idle) and gestation periods, sows were fed restrictively at 2.8–3.1 kg per head per day. During the 28-day lactation period, sows received feed ad libitum, except on the day of farrowing, when feed allowance was limited to 1.0 kg per head. Suckling piglets were offered a commercial pelleted prestarter feed from day 7 postpartum via automated self-feeders. The ingredient composition and calculated nutritional values of all experimental diets are presented in Table 1. Water was supplied to sows through nipple drinkers to ensure continuous access and hygienic conditions. Suckling piglets were provided water via cup drinkers, positioned 7 cm above the floor, allowing easy access even for the smallest piglets.

Table 1 – Feed ingredients and nutritional value of diets for sows and piglets

Ingredients, %	Gestating sows	Lactating sows	Piglets Prestarter
Wheat	34.0	43.5	-
Barley	45.0	30.0	-
Sunflower meal	14.5	10.0	-
Soybean meal	3.0	11.5	-
Premix*	3.5	5.0	-
Complete pelleted feed**	-	-	100.0
Total	100.0	100.0	100.0
Nutritional value			
Metabolizable energy, MJ	12.20	12.40	13.60
Crude protein, g/kg	144.91	165.61	190.0
Crude fat, g/kg	16.62	16.09	58.0
Fiber, g/kg	57.68	50.82	27.0
Calcium, g/kg	7.67	9.88	7.40
Total phosphorus, g/kg	4.71	5.35	6.00
Digestible phosphorus, g/kg	3.02	3.67	5.50
Sodium, g/kg	2.14	2.50	2.50
Lysine, g/kg	6.30	9.33	15.60
Digestible lysine, g/kg	4.95	7.88	14.00
Methionine, g/kg	2.62	3.49	6.30
Digestible methionine, g/kg	2.18	3.06	5.90
Methionine+cystine, g/kg	5.42	6.49	9.20
Digestible methionine+cystine, g/kg	4.29	5.37	8.10
Threonine, g/kg	4.93	6.79	10.40
Digestible threonine, g/kg	3.50	5.30	8.80
Tryptophan, g/kg	1.73	1.98	3.50
Digestible tryptophan, g/kg	1.31	1.55	3.10
Vitamin A, IU/g	13149	13000	15000
Vitamin D3, IU/g	2023	2000	2500
Vitamin E, mg/kg	101.15	100.00	100.00

* - Cehavit Sow Gestation, Cehavit Sow Lactation; ** - Prestarter Cehavit Pig Lux C12.

Microclimate conditions

To ensure optimal microclimate conditions in piglet nests, a combined local heating system was used, including infrared incandescent lamps, electric heating mats, and brooders. This approach enabled the creation of comfortable thermal conditions for newborn piglets, thereby reducing stress and mobility and improving piglet survival. Ambient air temperature in the sow area was maintained at 20–22°C, while temperatures in piglet nests were maintained at 37–34°C during the first week postpartum and gradually reduced to 26–28°C by weaning. Relative humidity was kept within the range of 60–70%, and air velocity did not exceed 0.2 m/s to prevent draughts.

Ventilation in the pig housing was provided by a combined system of exhaust fans and aerodynamic air inlets operating under negative pressure (Deschenko et al., 2024). This system ensured effective removal of contaminated air and a continuous supply of fresh air, maintaining microclimatic parameters in accordance with technological requirements. Ventilation rates ranged from 5–10 m³/kg live weight per hour during the cold season and increased to 80–100 m³/kg live weight per hour during warm periods, ensuring efficient dilution and removal of ammonia, moisture, and dust. Manure removal was achieved using a periodically operated vacuum self-flowing system. Manure was collected in channels located beneath the slatted floor, allowing effective separation of animals from waste and contributing to reduced ammonia concentration and humidity levels in the housing. Veterinary preventive and therapeutic procedures were carried out identically for all experimental groups in accordance with the farm's approved veterinary health program. All procedures complied with biosafety requirements and included vaccination, deworming, and routine health monitoring. Housing and management conditions for experimental animals complied with the Departmental Norms of Technological Design: «Pig Enterprises (Complexes, Farms, and Small Farms)» (2005) and the recommendations of genetic companies.

Performance parameters

Reproductive performance of sows in the experimental groups (Table 2) was evaluated using the following indicators: total number of piglets born (TNB, head), number of piglets born alive (NBA, head), proportion of stillborn piglets (PSB, %), litter weight at birth (LWB, kg) and at weaning (28 days; LWW, kg), average piglet weight at birth (AWPB, kg) and at weaning (28 days; APWW, kg), number of piglets at weaning (NW28d, head), average daily gain of suckling piglets (ADG, g), and piglet survival rate (%). All measurements were performed according to generally accepted methodologies

(Ladyka and Khmelnychiy, 2023). To obtain an integrated assessment of sow reproductive performance, a composite reproductive index was calculated based on a limited number of key parameters (Ladyka and Khmelnychiy, 2023):

$$I=B+2W+35G \quad (1)$$

Where I is the reproductive performance index; B is the number of piglets at birth (head); W is the number of piglets at 28 days of age (head) G is the average daily weight gain of piglets until weaning (kg).

Sow body condition was assessed by measuring backfat thickness at the P2 anatomical point, located 65 mm lateral to the dorsal midline at the level of the head of the last rib. Measurements were taken twice: immediately before farrowing and on the day of piglet weaning, allowing assessment of changes in sow energy reserves during lactation. Backfat thickness was measured using a Renco ultrasonic scanner, which provides a rapid, non-invasive, and reliable estimation of subcutaneous fat depth. These data enabled an objective evaluation of sow condition and facilitated adjustments to feeding or housing management when necessary to maintain optimal physiological status throughout the reproductive cycle (Ladyka and Khmelnychiy, 2023).

Table 2 – The scheme of the experiment researching reproductive traits of sows

Group			
1 n = 48	2 n = 48	3 n = 48	4 n = 48
Breeding			
♀(LW ^a × L ^b) × ♂Maxter ^c	♀(LW × L) × ♂PIC 337 ^d	♀(LW × L) × ♂Maxter	♀(LW × L) × ♂PIC 337
Housing idle and gestation sows of group pens keeping on the farrowing unit			
Traditional pen of fixation of the sow during the suckling period		Improved pen for free-range sows from 7 days after farrowing until weaning	
a – Large White breed; b – Landrace breed; c – terminal line of boars «Maxter»; d – terminal line of boars PIC 337.			

Statistical analysis

Experimental data were processed using standard biometrical methods (Kramarenko et al., 2019) and analyzed using Statistica 12.0 (StatSoft Inc., 2014; www.statsoft.com) and Microsoft Excel 2019. Performances that met the assumptions of parametric analysis were evaluated using two-way analysis of variance (ANOVA). The fixed effects included: A: type of farrowing pen (traditional vs. improved), B: boar line (Maxter vs. PIC 337), and A × B: the interaction between pen design and boar line. When significant main or interaction effects were detected identified, pairwise comparisons among group means were performed using Tukey's multiple comparison test implemented in Statistica. Quantitative data are presented as means ± standard error ($M \pm SE$). Categorical variables, including piglet survival, were analyzed using Pearson's chi-square (χ^2) test. Statistical significance was accepted at $P < 0.05$, with $P < 0.01$ and $P < 0.001$ considered highly significant.

Ethical regulation

All experimental procedures complied with European legislation governing the protection and welfare of farm animals, including Council Directive 95/58/EC on the protection of animals kept for farming purposes (as amended by Regulation No. 806/2003) and Council Directive 91/630/EEC on minimum standards for the protection of pigs. The study was also conducted in accordance with Ukrainian legislation on animal welfare during farming practices (Law of Ukraine "On Veterinary Medicine", 2021; Order of the Ministry for Development of Economy, 2021). The experimental protocol was reviewed and approved by the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine (June 26, 2025; Protocol No. 039).

RESULTS

Table 3 presents the results of the two-way analysis of variance assessing the effects of farrowing pen type, boar line, and their interaction on sow reproductive performance at farrowing.

A significant effect of boar line was observed for the total number of piglets born (TNB; $F = 19.95$; $P < 0.001$). For the number of piglets born alive (NBA), significant effects were identified for both boar line ($F = 12.46$; $P < 0.001$) and farrowing pen type ($F = 12.46$; $P < 0.001$). The highest TNB values (14.1 piglets) were recorded in sows from Groups 2 and 4 inseminated with semen from PIC 337 boars, irrespective of pen type. In contrast, sows inseminated with Maxter boar semen (Groups 1 and 3) produced 0.5–1.1 fewer piglets (Figure 4).

The highest NBA values were observed in groups 1, 2 and 4, whereas sows in group 3 exhibited significantly lower values by 0.9–1.1 piglets. A similar pattern was noted for litter weight at birth (LWB), with Group 3 sows (improved pens × Maxter boars) exhibiting the lowest values. Sows in the remaining groups exceeded these values by 0.7–1.1 kg.

The proportion of stillborn piglets (PSB) was significantly influenced by pen type ($F = 4.11$; $P = 0.044$). The lowest PSB (5.1%) was observed in Group 1, while the highest value (8.4%) occurred in Group 4 ($P < 0.05$). Group 2 and 3 showed intermediate values. No significant effects of pen type or boar line were detected for average piglet weight at birth (AWPB). Although piglets in Groups 2 and 4 exhibited slightly lower AWPB values, these differences were not statistically significant (Figure 5).

Litter uniformity was significantly affected by both pen type ($F = 17.50$; $P < 0.001$) and boar line ($F = 4.82$; $P = 0.029$). The highest uniformity index was recorded in Group 2 (71.9 points), while the lowest was observed in Group 3 (61.1 points) (Figure 6). Although improved pens enhance piglet safety by reducing crushing risk, unrestricted sow movement on fully slatted floors may increase injury risk and negatively affect piglet growth uniformity, emphasizing the need for careful management and technological optimization.

Table 4 summarizes the effects of pen type, boar line, and their interaction on reproductive traits at weaning and piglet growth performance. For most traits, pen type exerted a significant influence, whereas for NW28d only a tendency was observed. Significant interaction effects were detected for milk performance and piglet survival to weaning.

The highest litter uniformity index and milk performance values were observed in Group 2 (44.1 points and 73.8 kg, respectively), while the lowest values were recorded in Groups 3 and 4. Sows in Group 1 showed intermediate values but differed significantly from the other groups, particularly in milk performance (Figure 7).

A similar pattern was observed for the number of piglets at weaning (NW28d), with a significant difference between Groups 2 and 3, while Groups 1 and 4 occupied intermediate positions. Litter weight at weaning (LWW) was strongly influenced by pen type ($F = 20.34$; $P < 0.001$), with sows housed in traditional pens (Groups 1 and 2) outperforming those housed in improved pens, regardless of boar line.

The highest average piglet weight at weaning (APWW) was recorded in Group 1 (7.34 kg), while the lowest value was observed in Group 4 (6.81 kg). Piglets from Groups 2 and 3 showed intermediate values. A similar trend was observed for average daily gain (ADG), with the highest gains recorded in Groups 1 and 2 and the lowest in Groups 3 and 4 (Figure 8).

Piglet survival to weaning was highest in Group 3 (97.3%), whereas significantly lower survival rates were observed in the remaining groups (Figure 9). Analysis of piglet mortality at the sow level revealed significant differences among all groups ($\chi^2 = 29.05$; $P < 0.001$). The probability of at least one piglet death before weaning was 6.36 times higher in Groups 1, 2, and 4 compared with Group 3 ($P < 0.001$).

Table 5 presents the effects of pen type, boar line, and their interaction on sow backfat thickness. Backfat thickness before farrowing was significantly influenced by pen type ($F = 24.81$; $P < 0.001$), with sows housed in traditional pens (Groups 1 and 2) exhibiting higher values than those in improved pens. At weaning, backfat thickness remained significantly affected by pen type ($F = 33.18$; $P < 0.001$) and, to a lesser extent, by boar line ($F = 4.05$; $P = 0.046$).

The highest backfat thickness at weaning was observed in Group 1 (14.1 mm), while the lowest values (12.4–12.6 mm) were recorded in sows housed in improved pens. Significant differences were also detected between Groups 1 and 2 ($P < 0.05$). The greatest backfat losses during lactation were observed in Groups 2, 3, and 4 (4.92–5.27 mm), significantly exceeding losses in Group 1 (Figure 10). Although backfat loss remained within acceptable physiological limits across all groups, sows housed in improved pens exhibited greater energy depletion, likely due to increased physical activity associated with free movement.

Table 3 - Variability indicators ($M \pm SE$) and results of two-factor analysis of variance (F ; P) of the influence of the type of pens (farrowing) (A), breed of boars (B) and their combined effect ($A \times B$) on the reproductive traits of sows during farrowing

Parameter	Group				Factor		
	1	2	3	4	Type of pen (farrowing) (A)	Boar's breed (B)	$A \times B$
TNB, head	13.6 ^b ±0.18	14.1 ^a ±0.22	13.0 ^c ±0.14	14.1 ^a ±0.18	2.95 (ns)	19.95 (< 0.001)	2.22 (ns)
NBA, head	12.9±0.16	13.1±0.16	12.0±0.14	12.9±0.15	12.46 (< 0.001)	12.46 (< 0.001)	3.49 (0.063)
PSB, head	5.1 ^b ±0.87	6.8 ^{ab} ±0.97	7.2 ^{ab} ±0.83	8.4 ^a ±1.00	4.11 (0.044)	2.33 (ns)	0.07 (ns)
LWB, kg	18.7 ^a ±0.29	18.6 ^a ±0.22	17.6 ^b ±0.25	18.3 ^a ±0.20	7.10 (0.008)	1.72 (ns)	2.92 (ns)
AWPB, kg	1.45±0.016	1.42±0.015	1.46±0.014	1.43±0.015	0.41 (ns)	4.56 (ns)	0.01 (ns)
Litter uniformity, points	66.5 ^b ±1.42	71.9 ^a ±1.76	62.1 ^c ±1.61	63.4 ^{bc} ±1.29	17.50 (< 0.001)	4.82 (0.029)	1.77 (ns)

n = number of records; $M \pm SE$ = estimate of the arithmetic means and statistical error; F = estimate of the Fisher-Snedecor test; P = significant level; ns = $P > 0.05$. Significance differences between the means of individual groups ($P < 0.05$) based on Fisher's LSD multiple comparison test are indicated by different letters; TNB = total number of piglets at born; NBA = number born alive; PSB = proportion of stillborn piglets; LWB = litter weight at birth; AWPB = a live weight of piglet at birth.

Table 4 - Variability indicators ($M \pm SE$) and results of two-factor analysis of variance (F; P) of the influence of the type of pens (farrowing) (A), breed of boars (B) and their combined effect (A×B) on the reproductive traits of sows at weaning and the growth of piglets

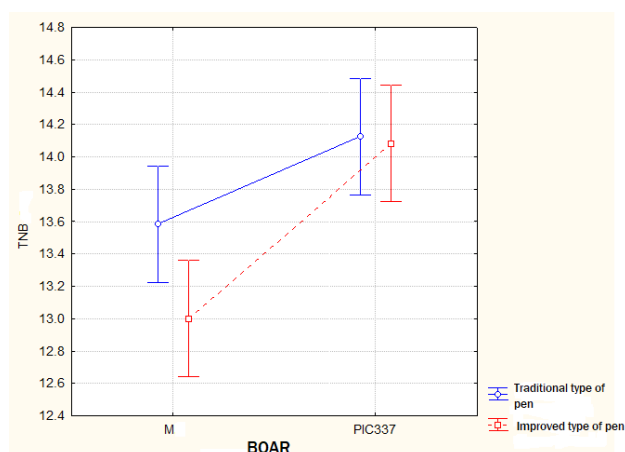
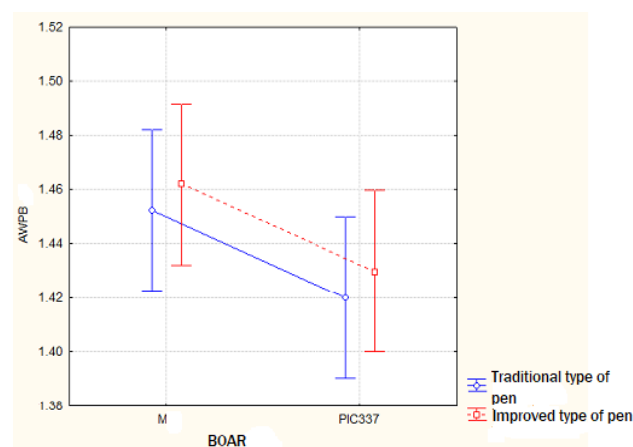
Parameter	Group				Factor		
	1	2	3	4	Type of pen (farrowing) (A)	Boar's breed (B)	A × B
Index, points	43.5 ^{ab} ±0.39	44.1 ^a ±0.41	42.0 ^c ±0.41	42.7 ^{bc} ±0.35	13.94 (< 0.001)	3.15 (ns)	0.03 (ns)
Milk performance, kg	70.7 ^b ±0.79	73.8 ^a ±1.01	67.6 ^c ±1.03	65.5 ^c ±0.78	39.14 (< 0.001)	0.31 (ns)	8.26 (0.004)
NW28d, head	11.9 ^{ab} ±0.14	12.1 ^a ±0.12	11.7 ^b ±0.13	11.8 ^{ab} ±0.12	3.56 (0.060)	1.51 (ns)	0.33 (ns)
LWW, kg	87.0 ^a ±0.99	87.6 ^a ±1.68	82.3 ^b ±1.61	80.2 ^b ±0.91	20.34 (< 0.001)	0.28 (ns)	1.04 (ns)
APWW, kg	7.34 ^a ±0.074	7.24 ^{ab} ±0.115	7.03 ^{bc} ±0.113	6.81 ^c ±0.057	15.83 (< 0.001)	3.05 (ns)	0.36 (ns)
ADG, g	196.4 ^a ±2.49	193.9 ^{ab} ±3.80	185.5 ^{bc} ±3.82	179.3 ^c ±1.81	16.75 (< 0.001)	1.97 (ns)	0.37 (ns)
Piglet survival, %	92.6 ^b ±0.81	92.6 ^b ±0.78	97.3 ^a ±0.60	91.9 ^b ±0.76	7.46 (0.007)	13.05 (< 0.001)	13.29 (< 0.001)

n = number of records; $M \pm SE$ = estimate of the arithmetic means and statistical error; F = estimate of the Fisher-Snedecor test; P = significant level; ns= $P > 0.05$. Significance differences between the means of individual groups ($P < 0.05$) based on Fisher's LSD multiple comparison test are indicated by different letters; NW28d = number of piglets at weaning (28 days); LWW = litter weight at weaning (28 days); APWW = a live weight of piglet at weaning (28 days); ADG = average daily gain.

Table 5 - Variability indicators ($M \pm SE$) and results of two-factor analysis of variance (F; P) of the influence of the type of pens (farrowing) (A), breed of boars (B) and their combined effect (A×B) on the thickness of the fat of sows

Parameter	Group				Factor		
	1	2	3	4	Type of pen (farrowing) (A)	Boar's breed (B)	A × B
Fat thickness before farrowing, mm	18.3 ^a ±0.19	18.8 ^a ±0.20	17.6 ^b ±0.22	17.3 ^b ±0.26	24.81 (< 0.001)	0.06 (ns)	2.76 (ns)
Fat thickness at weaning, mm	14.1 ^a ±0.17	13.5 ^b ±0.19	12.6 ^c ±0.30	12.4 ^c ±0.23	33.18 (< 0.001)	4.05 (0.046)	0.84 (ns)
Fat thickness losses per lactation, mm	4.19 ^b ±0.212	5.27 ^a ±0.214	4.98 ^a ±0.243	4.92 ^a ±0.254	0.89 (ns)	4.86 (0.029)	6.13 (0.014)

n = number of records; $M \pm SE$ = estimate of the arithmetic means and statistical error; F = estimate of the Fisher-Snedecor test; P = significant level; ns= $P > 0.05$. Significance differences between the means of individual groups ($P < 0.05$) based on Fisher's LSD multiple comparison test are indicated by different letters.

**Figure 4** - Estimates of arithmetic means ($\pm 95\%$ CI) of the total number of piglets at birth (TNB) depending on the type of pen (farrowing) and breed of boar**Figure 5** - Estimates of arithmetic mean ($\pm 95\%$ CI) a live weight of piglet at birth (AWPB) depending on the type of pen (farrowing) and breed of boar

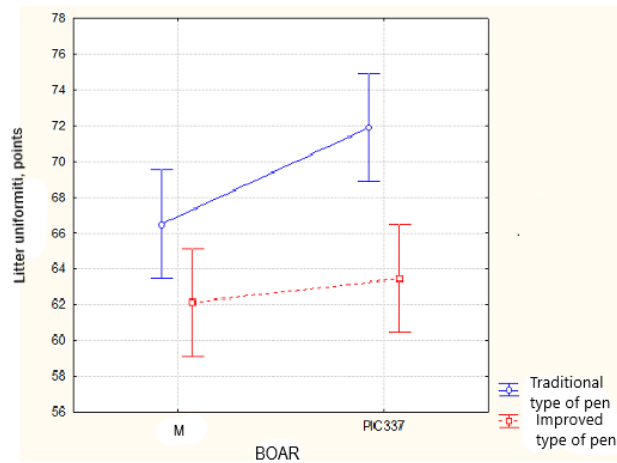


Figure 6 - Estimates of arithmetic mean ($\pm$ 95% CI) a litter uniformity depending on the type of pen (farrowing) and breed of boar

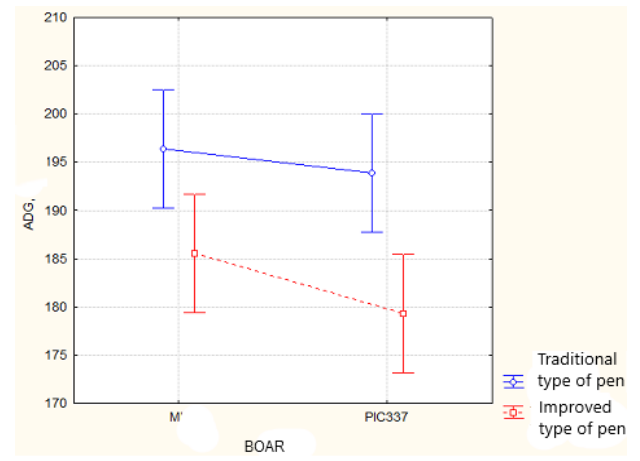


Figure 8 - Estimates of arithmetic mean ($\pm$ 95% CI) an average daily gain of piglets depending on the type of pen (farrowing) and breed of boar

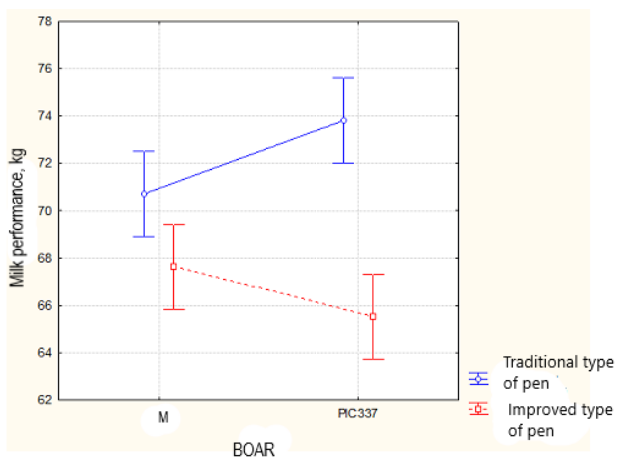


Figure 7 - Estimates of arithmetic mean ($\pm$ 95% CI) a milk performance depending on the type of pen (farrowing) and breed of boar

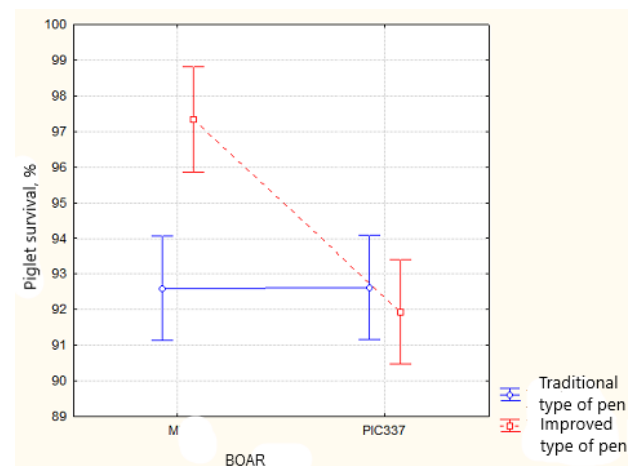


Figure 9 - Estimates of arithmetic mean ($\pm$ 95% CI) a piglet survival depending on the type of pen (farrowing) and breed of boar

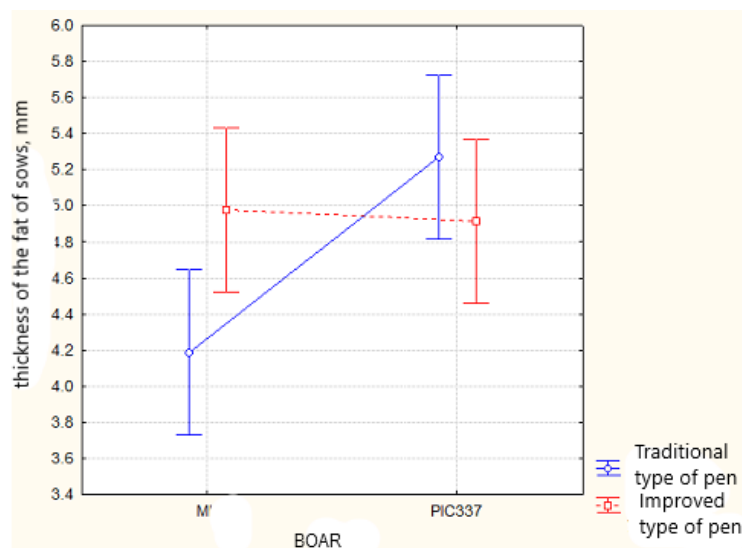


Figure 10 - Estimates of arithmetic mean ($\pm$ 95% CI) a loss of backfat thickness in sows during the lactation period depending on the type of pen (farrowing) and breed of boar

DISCUSSION

In modern pig breeding, which aims to improve a reproductive efficiency and piglet survival, it is essential to consider a complex set of factors that shape sow reproductive performance and piglet viability. The results of the present study are consistent with findings recorded by Ukrainian and international researchers who emphasize the interaction between the genetic characteristics of boars and sow housing conditions in determining reproductive traits (King et al., 2020; Shvachka et al., 2022). The present research demonstrated that the total number of piglets born is significantly influenced by boar breed, highlighting the important genetic contribution of the paternal component. These findings are consistent with findings reported by Knap et al. (2023).

In particular, Sá et al. (2025) reported that trait transformation significantly affects the assessment of sperm trait repeatability, indicating that boar breed has a substantial influence on both quantitative and qualitative traits of offspring, including litter size, litter weight, and piglet uniformity.

At the same time, the present experiments showed that litter size was significantly affected not only by boar breed, but also by the type of farrowing pens in which sows and suckling piglets were housed. This confirms the need for a comprehensive approach to pig housing and insemination management. Similar results were reported by Pacheco et al. (2024), who demonstrated that farrowing pen design influences piglet performance, taking into account seasonal effects and parity number. These findings underscore the importance of appropriate housing management in pig production systems.

Regarding technological factors, particularly pen design, there is consensus among researchers that the design features of individual and group farrowing pens determine not only sow comfort but also piglet survival and the proportion of piglets weaned. Baxter and Edwards (2020), in «Optimizing sow and piglet welfare during farrowing and lactation», reported that improved pen designs reduce piglet injuries, enhance thermoregulation, and improve access to teats, thereby increasing piglet survival to weaning, and improving overall welfare.

Roy et al. (2022) also indicated that pen type, especially the degree of movement restriction, as well as the availability of environmental enrichment, affects stress levels in sows. These stress responses may influence milk production, farrowing behavior, and litter uniformity. Furthermore, Kinane et al. (2022) demonstrated that free-lactation pens improve sow welfare by increasing locomotor activity and reducing tear-stain scores, with lower stress levels compared to conventional farrowing crates.

The present experiments further showed that total litter weight at birth was influenced by the combined effects of boar breed and pen type, suggesting an interaction between genotype and housing conditions. Wiechers et al. (2022) reported that unrestricted sows spent more time nursing piglets, resulting in higher litter weights compared with sows housed in fixed systems. This can be considered a valuable technological strategy for improving piglet growth and weight uniformity at weaning.

In contrast, no significant effects of boar breed or pen type were observed for piglet fertility, likely due to greater individual variability in the expression of sow reproductive potential. Alexopoulos et al. (2018) noted that optimal reproductive outcomes are achieved through a specific combination of boar genotype and sow housing conditions. This observation aligns with the present findings on the combined effects of boar breed and pen design on litter weight at birth, milk performance, and piglet survival. At the same time, Kremez et al. (2022) suggested that large litter size is less sensitive to environmental factors than traits such as total number born or litter uniformity.

Both boar breed and farrowing pen design significantly influence litter uniformity, emphasizing the importance of uniform intrauterine development for providing newborn piglets with an equal start. Milk performance and piglet survival to weaning were affected by the combined action of genetic and technological factors, highlighting the need to consider both when optimizing farrowing management. Total litter weight at weaning was primarily influenced by pen type, irrespective of boar breed, underscoring the dominant role of housing conditions in piglet survival and growth. Conversely, piglet live weight at weaning was significantly affected by boar breed, confirming its decisive role in the realization of growth potential. Earnhardt-San et al. (2023) demonstrated genetic relationships between sow milk performance and the number of piglets weaned and emphasized the importance of incorporating these traits into breeding programs to enhance piglet survival and sustainable pork production. Accordingly, genetic selection programs should consider sow milk yield and the number of functional teats at farrowing to improve both productivity and animal welfare. In addition, the backfat thickness in sows before farrowing was significantly influenced by pen type, suggesting that this parameter may serve as an indicator of adaptive responses to housing conditions. Similar findings were reported by Farmer and Edwards (2021), who demonstrated that appropriate sow body condition and targeted feeding strategies enhance piglet survival and support effective litter size management.

The results indicate a complex interaction between genetic factors (boar breed) and technological factors (pen design) that jointly shape reproductive traits, growth performance, and productivity in pigs. The rational integration of these factors represents a key strategy for improving modern pig production systems and emphasizes the necessity for an integrated approach to reproductive planning that combines genetic selection with optimized housing conditions.

CONCLUSION

This study clearly demonstrates a trade-off between sow productivity and welfare when transitioning from traditional fixed farrowing pens to welfare-improved loose-housing systems. Sows housed in traditional fixed pens consistently showed superior reproductive performance, whereas improved pens allowed greater freedom of movement and enhanced welfare outcomes.

Boars breed of boars was a determining factor influencing reproductive output. Sows inseminated with PIC 337 boars achieved the highest total number of piglets born (TNB = 14.1), regardless of housing system, whereas sows inseminated with Maxter boars produced 0.5–1.1 fewer piglets. Pen design exerted a strong and statistically significant effect on litter uniformity, stillbirth proportion, litter weight at weaning (LWW), milk performance, and the composite reproductive index. Traditional pens resulted in lower stillbirth rates (5.1%) compared with improved pens (8.4%) and yielded higher values for number born alive, litter weight at birth, litter uniformity, and milk yield. The lowest overall performance was observed in Group III (improved pen + Maxter boar).

Litter weight at weaning and average piglet live weight at weaning (APWW) were significantly higher in traditional pens, independent of boar line. The highest APWW (7.34 kg) was recorded in Group 1, whereas the lowest value (6.81 kg) occurred in Group 4. Similarly, piglet daily gain and weaning weight followed similar trends.

Piglet survival to weaning reached its highest level in Group 3 (97.3%), indicating that despite lower maternal productivity, provide measurable welfare benefits that enhance piglet safety. The probability of losing at least one piglet before weaning was 6.36 times higher in Groups 1, 2, and 4 compared with Group 3 ($\chi^2 = 29.05$; $P < 0.001$), confirming the welfare advantage of partially loose-housing systems. Backfat thickness before farrowing and at weaning was significantly influenced by both pen type and boar line. Sows housed in traditional pens (Group 1) maintained the highest backfat levels (14.1 mm) and experienced lower mobilization during lactation, whereas sows in improved pens exhibited thinner backfat and greater body reserve depletion.

The combined effects of pen design and boar breed were particularly pronounced for milk performance, litter uniformity, and piglet survival, confirming the importance of integrating genetic and housing decisions in farrowing management.

Suggestion

Further research should focus on elucidating the behavioral mechanisms of sows and piglets in loose-housing systems and on evaluating the cost-effectiveness economic feasibility and cost-effectiveness of implementing improved farrowing technologies under commercial production conditions.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Anna LYKHACH; E-mail: avlykhach@nubip.edu.ua; ORCID: <https://orcid.org/0000-0002-0472-6162>

Data availability

The data generated and/or analyzed in this study can be obtained from the corresponding author upon reasonable request.

Authors' contribution

H.Sergiy made the primary contribution to the study. He was responsible for organizing and conducting the experimental work on the farm, coordinating data collection, validating the obtained results, and preparing the initial version of the manuscript. His role included ensuring accurate implementation of the experimental protocol and performing the first-stage interpretation of the results. L.Vadym acted as the scientific supervisor of the project. He coordinated the overall study design, developed the methodological framework, oversaw the statistical analysis and interpretation of the outcomes, and provided essential scientific guidance throughout all stages of the research. He also contributed substantially to refining the manuscript and ensuring its scientific integrity. L.Anna contributed to the conceptual development of the study, assisted in the organization and monitoring of experimental procedures, structured the manuscript, and performed major critical revisions and editions required for the final version. B.Yevhen, I.Olena, and M.Roman participated in data collection, data arrangement, and calculations, supported the implementation of the experimental work, and contributed to preparation of supplementary materials. All authors reviewed the manuscript, approved its final version, and agreed to its publication.

Funding

The experimental studies performed within the Initiative topic of the National University of Life and Environmental Sciences of Ukraine «Improvement of technology in industrial pig breeding according to the principles of welfare» (state registration number 0122U201294, 2023-2027).

Acknowledgements

The authors would like to thank the owner, employees of the commercial farm where the experimental study was conducted.

Competing Interests

The authors have not declared any competing interests.

REFERENCES

- Alexopoulos JG, Lines DS, Hallett S, and Plush KJ (2018). A Review of success factors for piglet fostering in lactation. *Animals*, 8 (3): 38. <https://doi.org/10.3390/ani8030038>
- Baxter EM, and Edwards S (2020). Understanding the behaviour and improving the welfare of pigs : book. London: Taylor & Francis. <https://doi.org/10.1201/9781003048220>
- Choi Y, Min Y, Kim Y, Jeong Y, Kim D, Kim J, and Jung H (2020). Effects of loose farrowing facilities on reproductive performance in primiparous sows. *Journal of Animal Science and Technology*, 62(2):218-226. <http://dx.doi.org/10.5187/jast.2020.62.2.218>
- Council Directive 2008/120/EU of 18 December 2008 laying down minimum standards for the protection of pigs (Codified version). Official Journal of the European Union. L 47. 18.2.2009, 5-13. <https://eur-lex.europa.eu/eli/dir/2008/120/oj/eng>
- Departmental norms of technological design: Pig enterprises (complexes, farms, small farms). (2005). Retrieved from. https://lugdpss.gov.ua/images/bezpechnist_veterynariya/Svynarski-pidpryemstva-VNTP-APK-02.05.pdf
- Deschenko A, Lykhach A, Lykhach V, Lenkov L, Barkar Y, and Shpetny M (2024). the impact of ventilation system type on the microclimate of boar's pen and their clinical triad parameters. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(3): 420-434. <https://doi.org/10.29133/yyutbd.1424785>
- Do S, Jang J-C, Lee G-I, and Kim Y-Y (2023). The role of dietary fiber in improving pig welfare. *Animals*, 13(5): 879. <https://doi.org/10.3390/ani13050879>
- Dourmad JY, Velly VL, Gourdiere JL, and Renaudeau D (2022). Effect of ambient temperature in lactating sows, a meta-analysis and simulation approach in the context of climate change. *Animal-Open Space*, 1:100025 <https://doi.org/10.1016/j.anopes.2022.100025>
- Earnhardt-San AL, Gray KA, and Knauer MT (2023). Genetic parameter estimates for teat and mammary traits in commercial sows. *Animals*, 13 (15): 2400. <https://doi.org/10.3390/ani13152400>
- EFSA AHAW Panel (2022). Welfare of pigs on farm. *EFSA Journal*, 20(8):7421. <https://doi.org/10.2903/j.efsa.2022.7421>
- Farmer C, and Edwards SA (2021). Improving the performance of neonatal piglets. *Animal*, 16 (2): 100350. <http://dx.doi.org/10.1016/j.animal.2021.100350>
- Glencorse D, Plush K, Hazel S, D'Souza D, and Hebart M (2019). Impact of non-confinement accommodation on farrowing performance: A systematic review and meta-analysis of farrowing crates versus pens. *Animals*, 9(11): 957. <https://doi.org/10.3390/ani9110957>
- Jang JC, and Oh SH (2022). Management factors affecting gestating sows' welfare in group housing systems – A review. *Animal Bioscience*, 35:1817–1826. <https://doi.org/10.5713/ab.22.0289>
- Kinane O, Butler F, and O'Driscoll K (2022). Freedom to Move: Free Lactation Pens Improve Sow Welfare. *Animals*, 12(14):1762. <https://doi.org/10.3390/ani12141762>
- King RL, Matheson SM, Baxter EM, and Edwards SA (2020). Sow behaviour and piglet weight gain after late cross-fostering in farrowing crates and pens. *Animal*, 14: 1923–1933. <http://dx.doi.org/10.1017/S1751731120000580>
- Klassen KE, Pempek JA, Richert BT, Horback KM, Stewart KR, Marchant JN, et al. (2025). Impact of farrowing crate enrichment strategies on the welfare and performance of sows, suckling piglets, and post-weaned piglets. *Journal of Animal Science*, 103: skaf196. <https://doi.org/10.1093/jas/skaf196>
- Knap PW, Knol EF, Sørensen AC, Huisman AE, van der Spek D, and Zak LJ (2023). Genetic and phenotypic time trends of litter size, piglet mortality, and birth weight in pigs. *Frontiers in Animal Science*, 4: 1218175. <http://dx.doi.org/10.3389/fanim.2023.1218175>
- Kramarenko SS, Lugovoy SI, Lykhach AV, Kramarenko OS. (2019) Analysis of biometric data in animal breeding and selection: A textbook. Mykolaiv: MNAU, p. 211. <https://dspace.mnau.edu.ua/jspui/bitstream/123456789/6208/1/Analiz%20biometrychnykh%20danykh%20u%20rozvelenni%20ta%20selektii%20tvaryn.pdf>
- Kremetz M, Povod M, Mykhalko O, Susol R, Trybrat R, Onishenko L, et al. (2022). Reproductive characteristics of pigs of Irish selection and manifestation of different forms of heterosis by different methods of breeding in modern conditions of industrial pork production. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Agricultural Sciences*, 24 (96): 78-88. <https://doi.org/10.32718/nlvvet-a9610>
- Ladyka VI, and Khmelnychiy LM. (Eds.) Technology of production and processing of livestock products: A textbook for graduate students. Odesa: Oldi+, 2023. p.244
- Leonard SM, Xin H, Brown-Brandl TM, Ramirez BC, Dutta S, and Rohrer GA (2020). Effects of farrowing stall layout and number of heat lamps on sow and piglet production performance. *Animals*, 10(2):348. <https://doi.org/10.3390/ani10020348>
- Lykhach V, Lenkov L, Lykhach A, Reznichenko V, and Faustov R (2025). Technological solutions for improving the welfare of gestating sows in group housing. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(2): 61-74. <https://doi.org/10.31548/dopovidi/2.2025.61>
- Ludwiczak A, Skrzypczak E, Składanowska-Baryza J, Stanisław M, Ślósarz P, and Racewicz P (2021). How housing conditions determine the welfare of pigs. *Animals*, 11(12):3484. <https://doi.org/10.3390/ani11123484>
- Mahfuz S, Mun H-S, Dilawar MA, and Yang C-J (2022). Applications of smart technology as a sustainable strategy in modern swine farming. *Sustainability*, 14(5): 2607. <https://doi.org/10.3390/su14052607>

- Mackinnon JD (2024). Adaptive farrowing systems – a commercial review of adaptive farrowing systems in indoor pig production. National Pig Association Report, UK. p. 41 <https://nationalpigassociation.co.uk/wp-content/uploads/2024/09/A-Commercial-Review-of-Adaptive-Farrowing-Systems-NPA-Part-I-FINAL-Descriptive-findings.pdf>
- Malak-Rawlikowska A, Majewski E, Potori N, Hamulczuk M, Szili V, and Egri E (2024). Unlocking sows' welfare: the farm-level economic impact of phasing out farrowing crates for sows in the European Union's pig breeding industry. *Agriculture*, 14(2):187. <https://doi.org/10.3390/agriculture14020187>
- Nevrkla P, Sečkář J, Weisbauerová E, Horký P, Knitlová D Č, Lustyková A, Hadaš Z, and Rozkot M (2024). The effect of different farrowing housing systems on the reproductive performance of sows and the losses and growth of piglets. *Agriculture*, 14(7):1084. <https://doi.org/10.3390/agriculture14071084>
- NRC. (2012). Nutrient Requirements of Swine (11th Revised Edition). National Research Council, National Academies Press. <https://doi.org/10.17226/13298>
- Pacheco MV, Brown-Brandl TM, Rohrer GA, Vieira de Sousa R, and Silva Martello L (2024). Impacts of farrowing pen design, season, and sow parity on litter performance and piglet mortality. *Animals*, 14(2):325. <https://doi.org/10.3390/ani14020325>
- Reznichenko V, and Lykhach V (2023). Sow and piglet productivity improvement in the farrowing department using milk substitutes. *Animal Science and Food Technology*, 14(4):74-86. <https://doi.org/10.31548/animal.4.2023.74>
- Robbins LA, Green-Miller AR, Lay Jr DC., Schinckel A.P, Johnson JS, and Gaskill BN (2021). Evaluation of sow thermal preference across three stages of reproduction. *Journal of Animal Science*, 99:1–10. <https://doi.org/10.1093/jas/skab202>
- Roy C, Kyeiwaa V, Mancera KF, Seddon YM., Connor LM, and Brown JA (2022). Effects of enrichment type, presentation and social status on enrichment use and behavior of sows-Part 2: free access stall feeding. *Animals*, 12(14):1768. <https://doi.org/10.3390/ani12141768>
- Sá P, Godinho RM, Gòdia M, Sevillano CA, Harlizius B, Madsen O, et al. (2025). Genetic parameters and parental and early-life effects of boar semen traits. *Genetics Selection Evolution*, 57:4. <https://doi.org/10.1186/s12711-025-00954-6>
- Seddon YM, and Moustsen VA (2023). Design of the farrowing pen 64. The farrowing environment sets the conditions for sow and piglet productivity as well as their welfare. *Animal-science proceedings*, 14:707–811. <http://dx.doi.org/10.1016/j.anscip.2023.08.065>
- Shvachka R, Povod M, Mykhalko O, Shpetnyi M, Korzh O, Verbelchuk T, Shcherbyna O (2022). Reproductive qualities of sows at different durations of previous lactation. *Scientific Papers. Series «Management, Economic Engineering in Agriculture and rural development»*, 22, 1, 579-584. Link: PRINT ISSN 2284-7995. <https://managementjournal.usamv.ro/index.php/scientific-papers/2806-reproductive-qualities-of-sows-at-different-durations-of-previous-lactation>
- Sossidou EN, Baniass GF, Batsioulas M, Termatzidou S-A, Simitzis P, Patsios SI, et al. (2025). Modern pig production: aspects of animal welfare, sustainability and circular bioeconomy. *Sustainability*, 17(11): 5184. <https://doi.org/10.3390/su17115184>
- StatSoft Inc. (2014). STATISTICA (data analysis software system), version 12.0. Available at: <http://www.statsoft.com>
- Turner S P, Camerlink I, Baxter EM, D'Eath RB, Desire S, and Roehe R (2024). 19 - Breeding for pig welfare: Opportunities and challenges. In *Woodhead Publishing Series in Food Science, Technology and Nutrition, Advances in Pig Welfare (Second Edition)*, Woodhead Publishing, P.429-447. <https://doi.org/10.1016/B978-0-323-85676-8.00003-1>
- Vandresen B, Chou J-Y, and Hötzel M J (2024). How is pig welfare assessed in studies on farrowing housing systems? A systematic review. *Applied Animal Behaviour Science*, 275:106298. <https://doi.org/10.1016/j.applanim.2024.106298>
- Wiechers D-H, Herbrandt S, Kemper N, and Fels M (2022). Does nursing behavior of sows in loose-housing pens differ from that of sows in farrowing pens with crates? *Animals*, 12(2): 137. <https://doi.org/10.3390/ani12020137>

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

PHYSICAL PROPERTIES AND NUTRITIONAL QUALITY OF WAFER MADE FROM SUGARCANE BAGASSE-INDIGOFERA MIXTURE AS FEED FOR RUMINANT

Arsyadi ALI¹✉, Muhammad RODIALLAH¹, Restu MISRIANTI¹, Anwar Efendi HARAHAPE¹,
Jepri JULIANTONI¹, and Denis Herian Miswanto Saputra LASE²

¹Department of Animal Science, Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau, Pekanbaru, Indonesia

²Graduated of Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau, Pekanbaru, Indonesia

✉Email: arsyadi.ali@uin-suska.ac.id

✉Supporting Information



ABSTRACT: The purpose of this study was to determine the best composition of sugarcane bagasse and Indigofera (legume plant) as main ingredients for wafer formulation for ruminant feed based on physical properties, nutritional quality and palatability. The research was conducted using a complete randomized design (CRD) consisting of 3 treatments with 5 replicates. The treatment was a ration formulation consisting of 6 feed ingredients arranged by the trials and error method with estimated crude protein content (CP) of 12.1-14.2%. The use of Indigofera from 30% to 40% was significantly ($P<0.05$) improved wafer color score. The use of 35% and 40% Indigofera was significantly ($P<0.05$) increased the wafer texture score from 3.06 to 3.35. An increase in Indigofera used from 30% to 40% was significantly ($P<0.05$) improved wafer odor scores. It was found that the difference in the composition of bagasse and Indigofera in wafer formulation gave a significant ($P<0.05$) difference to the value of wafer density. The difference in the percentage of bagasse and Indigofera in the formulation was not significantly ($P>0.05$) affecting the water absorption of wafers. The increase in the percentage of Indigofera was significantly ($P<0.05$) increased the CP and ash content and decreased crude fibre (CF) content of wafer. The increasing in the percentage of Indigofera from 30% to 35% and 40% was significantly ($P<0.05$) increased in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD), ammonia nitrogen ($\text{NH}_3\text{-N}$) and volatile fatty acid (VFA) total of wafer. The increase in the use of Indigofera from 30% to 35% and 40% in wafer formulations was significantly ($P<0.05$) lowering wafer palatability in goats. Based on data from physical properties, nutrient content, and in vitro digestibility, it can be concluded that the best wafer formulation was 25% bagasse + 40% Indigofera.

Keywords: Legume, Nutritional quality, Physical treatment, Physical quality, Sugarcane by-product, Wafer formulation.

INTRODUCTION

Sugarcane bagasse (bagasse) is one of energy source for ruminant that is relatively inexpensive (Kaewhom, 2020; Zafeer et al., 2023). Bagasse is a by-product of the sugarcane milling (extraction) proses that contains high crude fibre. Paroha et al. (2020) reported that bagasse's crude fiber and lignin content was 57.55% and 27.22%, respectively. So that bagasse is categorized as roughage feed ingredients. Nevertheless, it is a rich source of cellulose (32–45%) and hemicellulose (20–32%) (Kumar et al., 2021).

Bagasse can be a rich supply of nutritional fibre but its most crucial problem is its low digestibility that is due to association of lignin with cellulose and hemicellulose (lignocellulose). Lignin reduces the digestibility of cellulose and hemicellulose. This limits its use and inhibits its conversion into a source of ruminant animal feed by means of physically protective them against enzyme degradation. Lignocellulose needs to be treated to stretch or break its bond with lignin (delignification). There are several treatments that can be given including physical, chemical and biological treatment. Suryaningrum and Samsudin (2021) found that the use of crude enzyme extract from *B. subtilis* increase protein, dissolved protein, reducing sugars and decrease ash, crude fibre and lignin content in bagasse. Flouring, wafering and fermentation treatments are expected to reduce the crude fibre content of bagasse.

Bagasse cannot be stored for long periods of time nor can it be given directly to beef cattle. Therefore, it is necessary to have feed processing and preservation technology so that bagasse is durable, easy to store, and easy to fed to livestock. One of the feed processing and preservation technologies that can be applied is physical treatment in the form of flour and processed into wafers. Wafer is a form of processed feed that is made in such a way through a pressing process using a felting machine through heating with high temperatures, so that this wafer can be stored for a long time, and is used to overcome the scarcity of forage for ruminant livestock such as goats, sheep and cattle. The advantages of pressed dry feed such as wafer are long shelf life under normal conditions, easy to carry on the way and easily stored (Retnani et al., 2009).

RESEARCH ARTICLE
 PII: S222877012600009-16
 Received: November 12, 2025
 Revised: January 18, 2026
 Accepted: January 20, 2026

Improving the quality of complete feed can be conducted by adding protein source feed ingredients to the formulation. Feed ingredients for protein sources that are easily available and cheap are *Indigofera zollingeriana* (indigofera). Indigofera is one type of legume plant that has high protein quality and leaf production. Indigofera biomass contains 20.47-21.39% of crude protein (CP) (Abdullah, 2010) and in vitro digestibility of dry matter and organic matter of Indigofera silage pellet was 70.90% and 70.20%, respectively (Ali et al., 2023). Barokah et al. (2017) reported that the addition of Indigofera biomass to the ensilage process by up to 60% can reduce the crude fibre (CF) content of oil palm fronds silage by 4.67% (from 32.60% to 27.93%). Meanwhile Ali et al. (2023) found that increasing of Indigofera biomass percentage in the formulation can increase the digestibility value and fermentation characteristics of pellets made from palm frond silage and Indigofera biomass. The effect of adding Indigofera on the nutritional quality of bagasse has also been reported by several researchers. Rodiallah et al. (2023) reported that the addition of Indigofera to the complete ration silage made from sugarcane bagasse up to 50% can increase the CP content from 4.27% to 20.85% and decrease CF content from 33.40% to 15.30%. Meanwhile, different results were reported by Ali et al. (2019) the addition of Indigofera biomass by up to 45% did not decrease the fiber fraction (NDF and ADF) content of bagasse silage flour.

The purpose of this study was to determine the best composition of bagasse and Indigofera as main ingredients for wafer formulation for ruminant feed based on physical properties, nutritional quality and palatability.

MATERIALS AND METHODS

Research places

Wafer manufacturing process, physical properties tests, and palatability tests of the wafer were carried out at the Laboratory of Nutrition and chemistry, Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau (UIN Suska Riau). Proximate analysis and In vitro digestibility tests were carried out at the PAU Laboratory and Dairy Nutrition Laboratory, IPB University.

Materials

The material used was sugarcane bagasse, which was obtained from sugarcane juice traders around Tampan Pekanbaru sub-district of Indonesia. According to Ali et al. (2019), each sugarcane juice trader will produce 3-5 kg/day of bagasse, so that in 100 traders, 300-500 kg of bagasse per day will be produced. Indigofera biomass comes from the experimental field of the Laboratory of Agronomy and Agrostology UIN Suska Riau (Indonesia). Rice bran as a component of rations was obtained from animal feed traders in Pekanbaru City of Indonesia. The tools used for the wafer manufacturing process are choppers, flour machines, squeezing machines, plastics, scales, basins, stirring spoons and wafer machine (Figure 1).



Figure 1 - Wafer machines

Livestock

The livestock used for the palatability test were 3 goats weighing 18-20 kg and $\pm$ 2 years old. The goats were intensively raised in the stage cage of the Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau.

Research methods

The research was conducted using a complete randomized design (CRD) consisting of 3 treatments with 5 replicates. The treatment was a ration formulation consisting of 6 feed ingredients arranged by the trial and errors method with an estimated CP of 12.1-14.2%. The composition of the ration which is the treatment is presented in Table 1.

Table 1 - Ingredient of wafer treatment formulation

Feed Ingredients (%)	Treatment	1	2	3
Bagasse		35	30	25
Concentrate		9	9	9
Rice bran		20	20	20
Indigofera		30	35	40
Molasses		5	5	5
CaCO ₃		1	1	1
Sum		100	100	100
Estimated CP (%)		12.1	13.15	14.2

Table 2 - Physical properties score of wafers

Physical Properties	Characteristic	Score	Description
Color	Dark brown	3-3.9	Very good
	Light brown	2-2.9	Good
	Brownish yellow	1-1.9	Fair
Texture	Has a firm, dense texture (not easily broken)	3-3.9	Very good
	Has a firm texture, easy to break	2-2.9	Good
	Has a wet texture, easily broken and slimy	1-1.9	Fair
Odor	Smells	3-3.9	Very good
	Odourless	2-2.9	Good
	Rancid	1-1.9	Fair

Source: Solihin and Sutrisna (2015)

Parameters measured**Physical properties**

The physical properties measured consisted of the general state of the wafer. The Odor, color and texture of wafer were measured using scores based on Solihin and Sutrisna (2015) (Table 2). Testing of these parameters was carried out by 25 untrained panelists who had an even level of sensitivity and did not receive formal training. Score of density and water absorption of wafer were measured according to Retnani et al. (2010).

The wafer density was calculated by the formula: $D = W / (L \times W_i \times T)$ (1)

Where, D = Density ($g\ cm^{-3}$); W = Weight of test sample (g); L = Length of test sample (cm); W_i = Width of test sample (cm); T = Thickness of test sample (cm).

The water absorption of wafer was calculated by the formula: $WA = ((W_2 - W_1) / W_1) \times 100\%$ (2)

Where, WA = Water absorption; W_1 = weight before immersion; W_2 = weight after immersion

Nutritional content

The nutrient content in the form of crude protein (CP), crude fiber (CF), ether extract (EE) and ash was measured according to the method of AOAC (2016).

In vitro digestibility and rumen characteristics

In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were measured according to Ali et al. (2023). Supernatants from in vitro digestibility tests were collected for the analysis of volatile fatty acid (VFA) total and ammonia-nitrogen (NH_3-N) based according to Ali et al. (2023).

Palatability

The palatability test of the three wafer feed treatments was carried out by looking at the level of feed consumption from 3 goats. The palatability test was carried out by giving each treatment wafer to each goat for one hour of observation every day, namely at 08.00 - 09.00 AM. This observation was carried out for 6 days. The level of palatability of wafers was obtained from how many wafers (g) were consumed by the goats per day.

Wafer manufacturing procedure

Wafer manufacturing was carried out by processing as follows: 1) Collection of bagasse and Indigofera which will be used as raw materials for wafers; 2) Bagasse and Indigofera were cut using *forage chopper* machine with a size of 2-3 cm. It was then dried until its weight is constant; 4) Bagasse and Indigofera were finely ground using a flour mill; 5) Then the

two ingredients are weighed and mixed with other feed ingredients (according to the treatment formulation) until they were smooth (homogeneous); 6) The feed material that has been mixed was then molded into a wafer shape using an extrusion machine (wafer machine) which had a wafer printer size of 5 x 5 x 2 cm. Pressing was carried out for 10 minutes at a temperature of 120 °C (Figure 1); 7) Wafer conditioning was carried out by leaving it in the open air (room temperature) until the moisture content and weight of wafer constant.

Statistical analysis

The data from the study results were analyzed using analysis of variance (ANOVA) based on a CRD. Duncan's Multiple Range Test (DMRT) at 5% level was used to determine the difference between treatments.

RESULTS AND DISCUSSION

Physical properties of wafer

The general overview of the wafer results of the study was the weight of one piece of wafer 33 g, with a length of 5 cm, a width of 5 cm, and a thickness of 1.5 (5 cm x 5 cm x 1 cm) so that the volume of 1 piece of wafer is 25 cm³. With the size of this wafer piece, it was very efficient because it can reduce storage space, make it easier to handle and serve feed. The height of the wafer was lower than the size of the printer. This was because the materials that make up the wafer have particles that can be pressed well. The wafer color score was in the range of 3.04 - 3.25 with very good description (Table 3). The use of Indigofera from 30% to 40% was significantly ($P<0.05$) improved wafer color score. As illustrated in Figure 2, it can be seen that the composition and percentage of Indigofera affect the color of bagasse wafers. These findings indicate that legume flour can increase the rate of color development. The reason seems to be an increase in the Maillard reaction with an increase in the amino acid content of legume flour (Tufan et al., 2020).

The texture score of the wafer was affected by the level of Indigofera. The use of 35% and 40% Indigofera was significantly ($P<0.05$) increased the wafer texture score from 3.06 to 3.35 (Table 3). The more portions of Indigofera flour, the smoother the texture of the wafer produced. This was due to the fact that Indigofera flour particles are finer compared to bagasse flour particles. The increase in the use of Indigofera flour in wafer formulation will have an impact on improving the quality of wafers. The fine particles will improve the retention and release of nutrients during digestion due to their larger surface area, which allows for more efficient enzymatic action and better absorption of nutrients (Jima et al., 2025). The texture description based on treatment was illustrated in Figure 3.

The wafer odor produced in this study was in the typical description of wafer smell, with a score range of 3.16-3.35 (Table 3). Good pressure and heating will cause a Maillard reaction so that the resulting wafer was fragrant (typical wafer). An increase in Indigofera used from 30% to 40% was significantly ($P<0.05$) improved wafer odor scores. This shows that the aroma of wafers was also influenced by the type of ingredients it makes up.

Wafer Density is one important factor in the physical characteristic of wafers that determine wafer strength (Retnani et al., 2010). In this study, it was found that the difference in the composition of bagasse and Indigofera in wafer formulation gave a significant difference ($P<0.05$) to the value of wafer density (Table 4). The highest density was found in wafer contained of 30% bagasse + 35% Indigofera. This finding indicates that wafer density was not only affected by the particle size of the material but was also affected by the suitability percentage of the material. Meanwhile, Retnani et al. (2009) stated that the density of wafers was also influenced by the density of the constituent materials.

Water absorption is used to gauge the wafer feed's capacity to draw in ambient water (air humidity), which adheres to the material particles or is retained in the pores between the material particles (Sukaryana et al., 2023). The results of study showed that the difference in the percentage of bagasse and Indigofera in the formulation was not significantly ($P>0.05$) affecting the water absorption of wafers (Table 4). However, the percentage of water absorption tends to decrease with an increase in the percentage of Indigofera. This was due to the lower percentage of Indigofera, resulting in higher wafer fiber content. Retnani et al. (2010) reported that materials with more fibre content that have more air cavity to absorb more water.

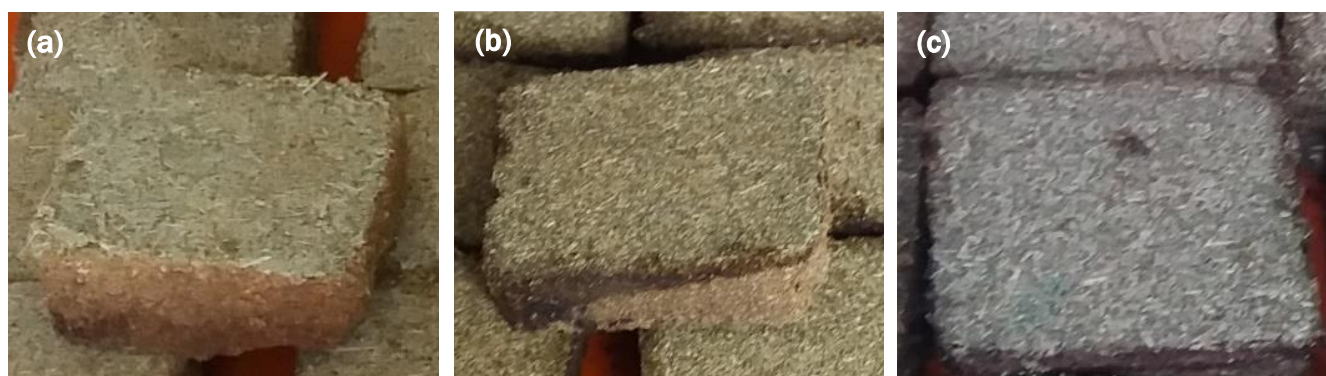
Table 3 - Color, Texture, and odor score of wafer

Score	Color	Texture	Odor	Description
Wafer treatments				
T1 (35% B + 30% I)	3.04 ^a ±0.37	3.06 ^a ±0.33	3.16 ^a ±0.33	Very good
T2 (30% B + 35% I)	3.20 ^{ab} ±0.40	3.35 ^b ±0.30	3.32 ^{ab} ±0.30	Very good
T3 (25% B + 40% I)	3.25 ^b ±0.34	3.35 ^b ±0.43	3.35 ^b ±0.33	Very good
Note: Means in the same column with different superscripts differ significantly at 5% level; T1= Treatment 1; T2= Treatment 2; T3= Treatment 3; B= bagasse; I= Indigofera				

Table 4 - Value of density and water absorption of wafer

Wafer treatments	Density (g/cm ³)	Water absorption (%)
T1 (35% B + 30% I)	0.39±0.03 ^a	156.42±8.69
T2 (30% B + 35% I)	0.44±0.04 ^b	154.11±7.42
T3 (25% B + 40% I)	0.38±0.03 ^a	144.89±10.99

Note: Means in the same column with different superscripts differ significantly at 5% level; T1= Treatment 1; T2= Treatment 2; T3= Treatment 3; B=bagasse; I=Indigofera

**Figure 2 - Color of wafer treatments: (a) T1; (b) T2; (c) T3****Figure 3 - Texture of wafer treatments: a) T1; b) T2; c) T3**

Nutritional Content

The nutritional content of the main constituent ingredients of wafers and treatment wafers is presented in Table 5. Based on Table 5, it can be seen that the moisture content of the wafer in each treatment was less than 11%. This indicated that the process of drying the wafer has been performed effectively. Drying process is a method that effectively reduces the moisture content and play a role in maintaining the stability of product quality (Boateng, 2023; Xu et al., 2024; Zhao et al., 2025). The moisture content of animal feed ingredients that was less than 15% will be able to maintain the quality of the wafer and be safe to store for a long period of time. Low moisture content will limit the reach of microorganisms in the process of spoilage of feed materials (Zambrano et al., 2019; Katu et al., 2025).

Crude protein is a very important nutrient for ruminant livestock, where it supports rumen microbes in degrading feed and that is needed in the growth of livestock body tissues (Ramaiyulis et al., 2019; Sari et al., 2022; Dai et al., 2023; Riaz et al., 2025). The data in Table 5 shows that the increase in the percentage of Indigofera from 30 to 35 and 40% significantly ($P<0.05$) increased the CP content of the wafer from 10.41% to 12.20% and 13.52%. These results show that every 5% increase in Indigofera can increase the CP content of 1.79% and 1.32%. And the increased use of 10% Indigofera will be able to increase the CP content of wafers by 3.11%. This increase was due to Indigofera containing much higher CP than bagasse. This study corroborates previous research, that to increase the CP content of roughage and forage low CP content can be added or mixed with forage from protein sources such as legume (Hawu et al., 2022; Castro-montoya et al., 2023; Ebro et al., 2024).

The increasing in the percentage of Indigofera used also significantly ($P<0.05$) reduced the content of CF Wafer (Table 5). The increasing the percentage of Indigofera from 30 to 35 and 40% can reduce the CF content of treatment wafers from 18.47% to 16.63% and 15.52%. The decrease in CF wafer content for every 5% increase in Indigofera was 1.84% and 1.11% or decreased by 9.96% and 6.67%. The decrease in the percentage of CF wafers with the use of Indigofera from 30 to 40 was 2.95% or significantly ($P<0.05$) decreased by 15.97%. This shows that the use of bagasse in wafer formulations must be balanced with feed ingredients that have a low CF content such as biomass legume (Indigofera). The low CF content and high CP content of Indigofera make it an excellent material for improving the

nutritional quality of animal feed, especially those from agricultural by-products (Ali et al., 2023; Antari et al., 2022; Termizi et al., 2024). The variation in the use of Indigofera in formulations (30%, 35% and 40%) did not have a significant effect on the ether extract content of the wafer, which was in the range of 2.39%-2.62% (Table 5). However, this increase in variation also provides a significant increased ($P<0.05$) in wafer ash content. This increase was strongly influenced by the mineral content of Indigofera. Ernawati et al., (2021) stated that *Indigofera zollingeriana* could serve as a local legume that provides protein and minerals for livestock.

Table 5 - Nutrient content of bagasse, Indigofera and treatment wafer (%)

Wafer Ingredient and treatment	Nutrient content				
	Moisture	CP	CF	EE	Ash
Wafer main ingredient formulation					
Bagasse	7.96	4.32	23.67	1.51	4.59
Indigofera	10.5	19.76	11.38	4.3	8.83
Treatments					
T1 (35% B + 30% I)	8.69±1.15	10.41 ^a ±0.67	18.47 ^b ±1.22	2.40±0.13	6.32 ^a ±0.22
T2 (30% B + 35% I)	8.22±0.48	12.20 ^b ±0.24	16.63 ^{ab} ±0.77	2.39±0.46	6.73 ^b ±0.16
T3 (25% B + 40% I)	8.18±0.29	13.52 ^c ±0.75	15.52 ^a ±0.47	2.62±0.39	7.23 ^c ±0.06

Not: Means in the same column with different superscripts on treatments differ significantly at 5% level; T1= Treatment 1; T2= Treatment 2; T3= Treatment 3; B=bagasse; I=Indigofera; CP=crude protein; CF=crude fibre; EE=ether extract

In vitro digestibility

In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) of bagasse and indigofera wafer and treatment wafer are presented in Table 6. When reviewed from the main materials that make up wafers, Indigofera wafers have higher IVDDM and IVDOM than Bagasse wafers. These results proved that the digestibility of legume was higher than fibre source feed materials such as grass and agricultural industry by-products. Castro-montoya et al. (2023) reported generally, legumes have a higher organic matter (OM) digestibility than grasses. The results of this study showed that the increase in the percentage of Indigofera from 30% to 35% and 40% significantly increased ($P<0.05$) IVDMD wafer treatment from 67.83% to 70.03% and 72.04%, respectively. The increase in IVDMD wafers at every 5% increase in Indigofera was 2.20% and 2.01% or increased by 3.24% and 2.87%. The increase in the percentage of IVDMD wafers in the use of Indigofera from 30 to 40 was 4.21% or an increase of 6.21%.

Meanwhile, the increase in IVOMD wafers at every 5% increase of Indigofera was 2.23% and 2.00% or an increase of 3.32% and 2.88%, respectively. The increase in the percentage of IVOMD wafer in the use of Indigofera from 30 to 40 is 4.23% or an increase of 6.30%. These results were in line with the reported by Ratnawati et al. (2018), Roca-fernández et al. (2020) and Herliatika et al. (2025) that the use of legumes in the diet can improve the digestibility.

Table 6 - The in vitro digestibility of wafer and characteristics of rumen fluid

Main Ingredient and treatments	In vitro digestibility		In vitro characteristics of rumen fluid		
	DM (%)	OM (%)	pH	NH ₃ -N (mM/L)	VFA (mM/L)
Wafer main ingredient formulation					
Bagasse	54.83	53.62	6.88	5.87	74.78
Indigofera	76.11	75.47	6.78	9.39	115.12
Treatments					
T1 (35% B + 30% I)	67.83 ^a ±0.48	67.17 ^a ±0.60	6.87±0.008	6.13 ^a ±0.14	69.45 ^a ±9.86
T2 (30% B + 35% I)	70.03 ^b ±0.53	69.40 ^b ±0.66	6.80±0.016	7.11 ^b ±0.17	94.53 ^b ±4.53
T3 (25% B + 40% I)	72.04 ^c ±0.32	71.40 ^c ±0.54	6.75±0.012	8.35 ^c ±0.32	114.65 ^c ±10.41

Note: Means in the same column with different superscripts on treatments differ significantly at 5% level. 1=treatment 1; T2= treatment 2; t3= treatment 3; B=bagasse; I=Indigofera; DM=dry matter; OM=organik matter; pH= potential of hydrogen; NH₃-N=ammonia nitrogen; VFA=volatile fatty acid

Characteristics of rumen fermentation

One factor that determines whether the rumen conditions are appropriate for the fermentation process is the pH of the rumen fluid (Zain et al., 2023). The pH value on incubation of all wafer treatments in the present study was in the normal range, namely 6.75-6.88 (Table 6). Mao and Wang (2025) reported that in the rumen, microbial populations flourish within an ideal pH range, usually between 6.0 and 7.0, maintaining a stable and balanced ecology. Therefore, all of wafer treatments provide a balanced pH during the fermentation process thus supporting the ecosystem of microorganisms in the rumen. The pH range should be kept within the normal range. When the pH of the rumen falls

below 6, it will result in changes in the environment for rumen microorganisms. This results in a decrease in the growth of fiber-degrading bacteria (Mao and Wang, 2025). When the pH of the rumen rises above 7.5 is referred to ruminal alkalosis, there is decrease in the number of ruminal microflora (Kumbhar et al., 2018).

The total concentrations of $\text{NH}_3\text{-N}$ and VFA total of bagasse and Indigofera wafers, and treatment wafers are presented in Table 6. The data in Table 6 show that Indigofera wafers have higher concentrations of $\text{NH}_3\text{-N}$ and VFA total than bagasse wafers. This result was thought to be related to the CP content and carbohydrate structure, where Indigofera has a higher CP content and a lower ADF content than bagasse. Tolera and Sundstøl (2000), and Hristov and Ropp (2003) explained that the high concentration of $\text{NH}_3\text{-N}$ in the rumen is a consequence of the high protein content in the ration. Meanwhile, the higher ADF content of the cell wall causes carbohydrates to be not digested optimally, which has an impact on the lower concentration of VFA total produced in the carbohydrate overhaul process.

The VFA total of bagasse and Indigofera wafers ranges from 74.78-115.12 mM/L (Table 6) was comparable to the range of VFA total production in the rumen which can reach concentrations of 70-150 mM/L (McDonald et al., 2010). The results also showed that for every 5% increase of Indigofera in wafer formulation was significantly ($P<0.05$) increased the concentration of $\text{NH}_3\text{-N}$ and VFA total. The percentage increase of $\text{NH}_3\text{-N}$ concentration in Indigofera used from 30% to 35% and from 35% to 40% was 0.98% and 1.24%, respectively or increased by 15.99% and 17.44%, respectively. And the cumulative increase in the percentage of $\text{NH}_3\text{-N}$ on the use of Indigofera in ration formulations from 30% to 40% was 36.22%. Meanwhile, the increase in the VFA total concentration at each 5% increase of Indigofera was 25.08% and 20.12% or significant ($P<0.05$) increased by 36.11% and 21.28%. The cumulative increase in the total VFA concentration of wafers on the use of Indigofera in rations from 30% to 40% was 45.20%. Increased use of Indigofera will increase the protein content of the wafer, which leads to increased protein degradation, thereby increasing the concentration of $\text{NH}_3\text{-N}$ (Zain et al., 2023).

Palatability of wafer

The palatability test in this study was carried out to find out how much the goats liked wafers made of bagasse with the addition of Indigofera as a protein source. The results of this study showed that increasing the use of Indigofera in wafer formulations from 30% to 35% and 40% was significantly ($P<0.05$) decreased wafer palatability in goats (Table 7). The decrease in the level of palatability reached 30.66 g/day. This may be related to the particle size and texture of the wafer. The higher the percentage of Indigofera, the finer the particle size and texture of the wafer. The study results of (Pujaningsih et al., 2016) indicated that goats prefer forage with larger sizes, especially particle sizes similar to their basal feed. However, goats are one of the livestock that have a high selective nature to feed.

Table 7 - The value of wafer palatability

Wafer treatments	The palatability value
T1 (35% B + 30% I)	54.83 ^b ±6.82
T2 (30% B + 35% I)	24.17 ^a ±1.62
T3 (25% B + 40% I)	24.17 ^a ±7.49
Note: Means in the same column with different superscripts differ significantly at 5% level; T1= Treatment 1; T2= Treatment 2; T3= Treatment 3; B=bagasse; I=Indigofera	

CONCLUSION

Based on data from physical properties, nutrient content, and in vitro digestibility, it can be concluded that the best wafer formulation was 25% sugarcane bagasse + 40% Indigofera. The recommendation for further research is to determine the effect of the use of wafers made from bagasse and Indigofera as feed on the intake and weight of goats.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Arsyadi Ali; E-mail: arsyadi.ali@uin-suska.ac.id; ORCID: <https://orcid.org/0009-0004-3483-3913>

Authors' contribution

A. Ali contributed to performed the experimental and exploring work, literature review, drafted the manuscript; M. Rodiallah contributed to supervised all experiments, preparation and checked the analysis result and manuscript preparation; R. Misrianti contributed to supervised all experiments and manuscript drafting and graphically abstract; A.E.

Harahap contributed to all experiment and manuscript preparation; J. Juliantoni contributed in manuscript drafting and critical revisions; DHMS Lase contributed to all experiment and data analysis. Generally, all authors of the current manuscript are main contributors and have an equal primary role in conducting research according to their fields of expertise and publishing this article in highly reputable and globally indexed journals.

Data availability

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

Ethical considerations

This experiment followed research ethics guidelines pertaining to livestock, in accordance with the Animal Science and Health regulations outlined in Government Law No. 41/2014 issued by the Republic of Indonesia.

Acknowledgements

The author thanks to Lembaga Penelitian dan Pengadain Masyarakat (LP2M) and the Faculty of Agriculture dan Animal Science at Universitas Islam Negeri Sultan Syarif Kasim Riau for their support and resources in completing this research.

Funding

The authors declare that no funds, grants, or other support were received during the preparation or publication of this manuscript.

Competing interests

We declare that there are no competing interests with any financial organization regarding the material discussed in this paper.

REFERENCES

- Abdullah L (2010). Herbage production and quality of *Indigofera* shrubs treated with different concentrations of foliar fertilizer. *Media Peternakan*, 33(3): 169–176. <https://doi.org/10.5398/medpet.2010.33.3.169>
- Ali A, Kuntoro B, and Misrianti R (2019). Fiber fraction content of sugarcane bagasse silage meal supplemented with *Indigofera* biomass as feed (Kandungan fraksi serat silase ampas tebu yang disuplementasi biomassa *Indigofera* sebagai pakan). *Jurnal Peternakan*, 16(1):10–17. <https://ejournal.uin-suska.ac.id/index.php/peternakan/article/view/6213>
- Ali A, Misrianti R, Roflimas, and Irawati E (2023). In vitro digestibility and gas production of pellets made from oil palm frond and *Indigofera zollingeriana* silage with different compositions. *Livestock Research for Rural Development*, 35(3): 27. <https://www.lrrd.cipav.org.co/lrrd35/3/3527arsy.html>
- AOAC (2016). Official methods of analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Virginia. <https://www.aoac.org/?s=aoac+2016>
- Barokah Y, Ali A, and Erwan E (2017). Nutrient content of oil palm frond silage added with *Indigofera zollingeriana* (Nutrisi silase pelepah kelapa sawit yang ditambah biomassa *Indigofera zollingeriana*). *Jurnal Ilmu-Ilmu Peternakan*, 20(2):59–68. <https://doi.org/10.22437/jiip.v20i2.4772>
- Boateng ID (2023). A review of solar and solar-assisted drying of fresh produce: State of the art, drying kinetics, and product qualities. *Journal of the Science of Food and Agriculture*, 103(13): 6137–6149. <https://doi.org/10.1002/jsfa.12660>
- Castro-Montoya JM Albarrán-PortilloB, Posgrado, E Agronómicas FDC, Salvador UE, Salvador S, and Salvador E (2023). Digestibility of additional crude protein from tropical legumes in mixed grass–legume rations for ruminants. *Chilean Journal of Agricultural and Animal Sciences (Ex Agro-Ciencia)*, 39(1): 65–74. <https://doi.org/10.29393/CHJAA39-6DAJB20006>
- Dai R, Ma X, Dingkao R, Huang C, La Y, Li X, et al. (2023). Effects of dietary crude protein levels in concentrate supplementation after grazing on rumen microbiota and metabolites using metagenomics and metabolomics in Jersey–yak. *Frontiers in Microbiology*, 14: 1124917. <https://doi.org/10.3389/fmicb.2023.1124917>
- Ebro A, Alvarez A, Namera F, Bijdevaate L, Addis H, Demise H, et al. (2024). Inclusion levels of tree and herbaceous legumes on the nutritive quality of grass silage: Results from on-farm trials. *Agroforestry Systems*, 98(1): 103–113. <https://doi.org/10.1007/s10457-023-00893-5>
- Ernawati A, Abdullah L, and Permana G (2021). Mineral content and uptake of *Indigofera zollingeriana* at different planting densities (Kandungan dan Serapan Mineral Pucuk *Indigofera zollingeriana* dari Tanaman dengan Kerapatan Tanam Berbeda). *Jurnal Ilmu Nutrisi dan Teknologi Pakan*, 19(2):49–58. <https://doi.org/10.29244/jintp.19.2.49-58>
- Hawu O, Ravhuhali KE, Mokoboki HK, Lebopa CK, and Sipango N (2022). Sustainable use of legume residues: Effect on nutritive value and ensiling characteristics of maize straw silage. *Sustainability*, 14(11): 67–43. <https://doi.org/10.3390/su14116743>
- Herliatika A, Yulistiani D, Puastuti W, Pujiawati Y, Haryati T, Sinurat AP, et al. (2025). Evaluation of feed supplementation using legume leaf meal and spent substrate fermented by *Eupenicillium javanicum* as a potential feed for ruminants: An in vitro study. *Journal of Animal and Feed Sciences*, 34(1): 78–86. <https://doi.org/10.22358/jafs/189327/2024>

- Hristov AN, and Ropp JK (2003). Effect of dietary carbohydrate composition and availability on utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. *Journal of Dairy Science*, 86(7):2416–2427. [https://doi.org/10.3168/jds.S0022-0302\(03\)73836-3](https://doi.org/10.3168/jds.S0022-0302(03)73836-3)
- Jima BR, Abera AA, and Kuyu C G (2025). Effect of particle size on compositional, functional, pasting, and rheological properties of teff (*Eragrostis teff* [Zucc.] Trotter) flour. *Applied Food Research*, 5: 100986. <https://doi.org/10.1016/j.afres.2025.100986>
- Kaewhom P (2020). Nutritive value and methods of improving sugarcane bagasse quality for application in animal feeds. *Journal of Mahanakorn Veterinary Medicine*, 15(2): 131–140. <https://li01.tci-thaijo.org/index.php/jmvm/article/view/243669>
- Katu J K, Toth T and Varga L (2025). Enhancing the nutritional quality of low-grade poultry feed ingredients through fermentation: A review. *Agriculture* 15: 476. <https://doi.org/10.3390/agriculture15050476>
- Kumar A, Kumar V, and Singh B (2021). Cellulosic and hemicellulosic fractions of sugarcane bagasse: Potential, challenges and future perspective. *International Journal of Biological Macromolecules*, 169: 564–582. <https://doi.org/10.1016/j.ijbiomac.2020.12.175>
- Kumbhar N, Borikar S, Digaskar S, Shaikh S, and Ajabe J (2018). Occurrence, etiological studies, and clinical findings of ruminal alkalosis in cattle of Parbhani and adjoining areas. *Journal of Entomology and Zoology Studies*, 6(6): 680–683. <https://www.entomoljournal.com/archives/2018/vol6issue6/PartL/6-6-12-851.pdf>
- Mao J, and Wang L (2025). Rumen acidosis in ruminants: A review of the effects of high-concentrate diets and the potential modulatory role of rumen foam. *Frontiers in Veterinary Science*, 12: 1595615. <https://doi.org/10.3389/fvets.2025.1595615>
- McDonald P, Edwards RA, Greenhalgh JF D, Morgan CA, Sinclair L A, and Wilkinson R (2010). *Animal nutrition* (7th ed., 714 pp.). Prentice Hall, New Jersey. https://www.up.lublin.pl/files/animal/ANIM%20NUTR/animal-nutrition_mcdonald_et_al.pdf
- Paroha S, Srivastava V P, and Chaturvedi N (2020). Sugarcane bagasse as dietary fibre. *Indian Journal of Pure and Applied Biosciences*, 8(6): 590–597. <https://doi.org/10.18782/2582-2845.8442>
- Pujaningsih R I, Fani F, and Pambudi AW (2016). Effect of particle size of forage processing technology application on consumption efficiency, palatability, and digestibility of local goats (Pengaruh ukuran partikel pada penerapan teknologi pengolahan hijauan terhadap efisiensi konsumsi, palatabilitas, dan pencernaan kambing lokal). *Animal Production*, 18(1): 8–13. <https://doi.org/10.20884/1.anprod.2016.18.1.529>
- Ramailulis ZM, Ningrat RWS, and Warly L (2019). Protection of Protein in Cattle Feed Supplement from Rumen Microbial Degradation with Addition of Gambier Leaf Residue. *International Journal of Zoological Research*, 15(1): 6–12. <https://doi.org/10.3923/ijzr.2019.6.12>
- Ratnawati S, Hartutik, and Chuzaemi S (2018). The effect of herbaceous legumes in feed on in vitro digestibility. *IOP Conference Series: Earth and Environmental Science*, 119: 012012. <https://doi.org/10.1088/1755-1315/119/1/012012>
- Retnani Y, Basymeh S, and Herawati L (2009). Effect of forage type and storage duration on the physical properties of feed wafers (Pengaruh jenis hijauan dan lama penyimpanan terhadap sifat fisik wafer pakan). *Jurnal Ilmiah Ilmu-Ilmu Peternakan*, 12(4): 196–202. <https://doi.org/10.22437/jiip.v0i0.169>
- Retnani Y, Syananta FP, Herawati L, Widiarti W, and Saenab A (2010). Physical characteristics and palatability of market vegetable waste wafers for sheep (Karakteristik fisik dan palatabilitas wafer limbah sayuran pasar untuk domba). *Animal Production*, 12(1): 29–33. <https://www.animalproduction.net/index.php/JAP/article/view/275>
- Riaz R, Bilal R M, Hassan M U, Todaro M, Gannuscio R, Inal F, et al. (2025). Crude protein degradation kinetics of selected tropical forages in buffalo using NorFor in situ standards. *Animals*, 15(4): 585. <https://doi.org/10.3390/ani15040585>
- Roca-Fernández AI, Dillard SL, and Soder KJ (2020). Ruminal fermentation and enteric methane production of legumes containing condensed tannins fed in continuous culture. *Journal of dairy science*, 103(8):7028-7038. <https://doi.org/10.3168/jds.2019-17627>
- Rodiallah M, Harahap AE, Ali A, Adelina T, Mucra DA, Solfan B, et al. (2023). Nutritional profile and fiber fractions of complete silage feed based on sugarcane bagasse with *Indigofera* legume and molasses supplementation (Profil nutrisi dan fraksi serat pakan silase lengkap berbasis ampas tebu dengan leguminosa *Indigofera* dan suplementasi molases). *Jurnal Triton*, 14(1): 18–28. <https://doi.org/10.47687/jt.v14i1.377>
- Sari RM, Zain M, Jamarun N, Ningrat RWS, Elihasridas, and Putri E M (2022). Improving rumen fermentation characteristics and nutrient digestibility by increasing rumen degradable protein in ruminant feed using *Tithonia diversifolia* and *Leucaena leucocephala*. *International Journal of Veterinary Science*, 11(3): 353–360. <https://doi.org/10.47278/journal.ijvs/2021.121>
- Solihin M and Sutrisna R (2015). Effect of storage duration on moisture content, physical quality, and fungal distribution of vegetable- and tuber-waste-based wafers (Pengaruh lama penyimpanan terhadap kadar air, kualitas fisik, dan distribusi jamur wafer berbasis limbah sayuran dan umbi-umbian). *Jurnal Ilmiah Peternakan Terpadu*, 3(2): 77–83. <https://jurnal.fp.unila.ac.id/index.php/JIPT/article/view/767/0>
- Sukaryana Y, Zairiful, and Panjaitan I (2023). Identification and digestibility of wafer feed based on palm kernel cake in cattle. *Proceeding of ABEC*, September 21, Bengkalis, Indonesia. <https://doi.org/10.4108/FAI.21-9-2023.2342926>
- Suryaningrum LH and Samsudin R (2021). Improvement of sugarcane bagasse quality as a fish feed ingredient. *IOP Conference Series: Earth and Environmental Science*, 679: 012003. <https://doi.org/10.1088/1755-1315/679/1/012003>
- Termizi AZAM, Razak ASA, Nasarudin MAS, Hairolnizam NFA, Amalina F, Sulaiman S, et al. (2024). A review of the utilization of *Indigofera zollingeriana* as an animal feed supplement. *Construction*, 4(2): 118–123. <https://doi.org/10.15282/construction.v4i2.118>
- Tolera A, and Sundstøl F (2000). Supplementation of graded levels of *Desmodium intortum* hay to sheep feeding on maize stover harvested at three stages of maturity: 1. Feed intake, digestibility and body weight change. *Animal Feed Science and Technology*, 85(3-4):239-257. [https://doi.org/10.1016/S0377-8401\(00\)00205-4](https://doi.org/10.1016/S0377-8401(00)00205-4)
- Tufan B, Sahin S, and Sumnu G (2020). Utilization of legume flours in wafer sheets. *Legume Science*, 2(1): 1–9. <https://doi.org/10.1002/leg3.12>
- Xu P, Yang Z, Li X, Zhang Z, and Yang J (2024). Effects of different drying methods on drying characteristics and quality of silage *Broussonetia papyrifera* L. *LWT – Food Science and Technology*, 210: 116872. <https://doi.org/10.1016/j.lwt.2024.116872>
- Zafeer MK, Prabhu R, Rao S, Mahesha GT, and Bhat K S (2023). Mechanical characteristics of sugarcane bagasse fibre reinforced polymer composites: A review. *Cogent Engineering*, 10(1): Article 2200903. <https://doi.org/10.1080/23311916.2023.2200903>
- Zambrano MV, Duttaa B, Mercerb DG, MacLeana H L, and Touchie MF (2019). Assessment of moisture content measurement methods of dried food products in small-scale operations in developing countries: A review. *Trends in Food Science & Technology*, 88: 484–496. <https://doi.org/10.1016/j.tifs.2019.04.006>

- Zain M, Despal, Hidayat U, Tanuwiria Pazla R, Putri EM, and Amanah U (2023). Evaluation of legumes, roughages, and concentrates based on chemical composition and rumen degradable and undegradable proteins using an in vitro method. *International Journal of Veterinary Science*, 12(4): 528–538. <https://doi.org/10.47278/journal.ijvs/2022.218>
- Zhao C, He Z, Song X, Zhang X, Xiao Y, Yu J, et al. (2025). Evaluation of different drying methods on the quality parameters of *Acanthopanax senticosus* fruits. *Foods*, 14(7): 1100. <https://doi.org/10.3390/foods14071100>

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

IN SITU DEGRADATION OF HEAT-TREATED PLANT PROTEIN LEAF MEALS TO EXTRAPOLATE RUMEN BYPASS POTENTIAL IN RUMEN - FISTULATED BRAHMAN CATTLE

Cindy Nazario CORTEZ¹ , Lolito Casane BESTIL² , Elmar Mantuhac PATIGA³  , Manuel Datig GACUTAN⁴ , and Marisel LEORNA⁵ 

¹Biliran Province State University – Biliran Campus, Biliran, Biliran 6566, Philippines

²Adjunct Professor, Visayas State University, Visca, Baybay City, Leyte 6521-A, Philippines

³Agribusiness Program, Samal Island City College, Island Garden City of Samal, Davao del Norte 8119, Philippines

⁴Department of Animal Science, Visayas State University, Visca, Baybay City, Leyte 6521-A, Philippines

⁵Visayas State University, Visca, Baybay City, Leyte 6521-A, Philippines

✉ Email: elmar.patiga@bisu.edu.ph

✉ Supporting Information



ABSTRACT: Many plant protein leaf meals (LM) remain unassessed for their rumen bypass potential following heat treatment. This study aimed to evaluate whether heat treatment could reduce ruminal degradability and improve bypass protein availability in five LMs—ipil-ipil (ILM), kakawate (KaLM), moringa (MLM), kudzu (KuLM), and gumamela (GLM). A total of 60 nylon bags (3 replicates per treatment) containing untreated or heat-treated LMs were incubated for 24 h in a rumen-fistulated Brahman bull using a completely randomized design. Heat treatment reduced mean crude protein degradability (CPD) by 7.6% (81.41% untreated vs. 73.77% treated; $P = 0.006$), with the largest reductions recorded in ILM (−23.9%), KuLM (−14.7%), and GLM (−6.8%). In contrast, dry matter degradability (DMD: 69.76% vs. 68.65%) and organic matter degradability (OMD: 74.44% vs. 70.56%) were not significantly affected. Among the LMs, moringa consistently exhibited the highest degradability (CPD = 98.27%, DMD = 94.98%, OMD = 97.00%), whereas kudzu had the lowest (CPD = 68.69%, DMD = 48.52%, OMD = 54.21%). Degradability trends were influenced by drought-resistance, botanical classification (legume vs. non-legume), and growth form (tree, shrub, creeping). Legume LMs were 16% less digestible in protein content than non-legumes, and creeping types were 19% less digestible than non-creeping types. Heat treatment further lowered CPD in both categories, indicating its capacity to enhance bypass protein potential. These findings suggest that targeted application of heat treatment, especially for ipil-ipil, kudzu, and gumamela, offers a practical strategy to improve protein utilization efficiency in ruminant feeding systems by increasing post-ruminal protein supply.

Keywords: Brahman cattle, Heat treatment, In situ degradation, Leaf meal, Rumen.

INTRODUCTION

The rumen is a complex environment inhabited by different microbial species having different nutrient requirements and fermentative processes. In the process of fermentation, the majority of the dietary protein that the rumen microorganisms consume must be transformed into peptides that release amino acids and ammonia (NH_3) required for microbial protein synthesis (Owens and Basalan 2016). Protozoal species, which require 100% amino acids (Niepes et al., 2023) and bacteria, which require both amino acids (approximately 25%) and ammonia (approximately 75%), both use amino acids to synthesize microbial cells (Patiga et al., 2020b). As digesta flows down, these microbes will eventually give their lives to the host animal as a source of amino acids through enzymatic digestion in the abomasum and intestines. Stern et al., (2006) reported that microbial protein synthesized in the rumen accounts for 50 to 80 % of the total absorbable proteins supplied in the small intestine of the ruminants. As protozoa and bacteria are being digested in the abomasum, the amino acids produced are absorbed through the intestinal wall and carried by the blood to the different body cells, which are then synthesized into new proteins in the body (e.g., muscles, hormones, and others).

Microbial cells become the source of amino acids digested and absorbed in the abomasum and intestines, but this may be inadequate when dealing with highly productive animals such as growers, fatteners, and dairy. High-yielding animals require a higher proportion of nutrients in the diet that bypass the rumen without degradation to supply the 20 different amino acids at the intestinal level (Singh et al., 2019). Some methods of protecting the dietary protein to escape rumen fermentation have been tested to reduce the microbial degradation of protein supplements in the rumen, such as chemical treatment with tannic acid, chemical treatment with formaldehyde and heat treatment (Arisya et al., 2019; Thakur et al., 2024). In addition, the use of spent sulphite liquor and pelleting with calcium lignosulfonate has been found

RESEARCH ARTICLE
 PII: S222877012600010-16
 Received: July 14, 2025
 Revised: March 21, 2026
 Accepted: March 22, 2026

by others to be effective (Atole, 2011). However, the most common and safest way is heat treatment in the light of practical application among farmers in the humid tropics.

The high cost of protein supplements necessitates treatment methods that increase the amount of protein (Patiga et al., 2020b and 2024) undegraded in the rumen and, therefore, increase the amount of protein of high post-ruminal degradability. However, many protein sources of plant-origin grains have not been tested for their bypass potential and whether heat treatment is effective enough to protect their protein from rumen degradation. Hence, this study will assess the effectiveness of heat treatment in reducing protein degradation in the rumen of plant protein concentrates and leaf meals as a basis for extrapolating their potential as bypass protein sources using rumen-fistulated Brahman cattle.

MATERIALS AND METHODS

Preparation of the experimental animal and area

A Brahman bull fitted with a rumen cannula (Bar Diamond Lane, Parma, ID, U.S.A.) from the Beef Cattle Project of the Department of Animal Science (DAS) was used in this experiment. The experimental animal was confined and observed for its health condition, appetite, behavior and live weight prior to the experiment. The experimental animal was injected with Ivermectin at 1 mL/50 kg BW subcutaneously to prevent internal and external parasites. The experimental area was thoroughly cleaned and disinfected a week prior to the conduct of the experiment. Likewise, the feed bin and water troughs were checked, repaired, and disinfected to secure the animal's cleanliness, comfort, health, and safety.

Preparation of the basal diet and test leaf meals

The basal diet for the experimental animal was fresh Napier grass that was chopped at about 3-4 inches (7.62 – 10.16 cm) long before feeding (Patiga et al., 2020a and Patiga et al., 2024). The test plant protein leaf meals consisted of ipil-ipil leaf meal, kakawate leaf meal, moringa leaf meal, kudzu leaf meal, and gumamela leaf meal and were collected fresh at Brgy. Canila Biliran, Biliran, sun-dried until it became crispy and was processed into leaf meal using the hammer milled at the Department of Animal Science, Visayas State University, Visca, Baybay City, Leyte. The experiment followed a completely randomized design (CRD) with three replicates and a one-way analysis of variance treatment design in one uniform rumen environment of a rumen-fistulated Brahman bull for 24 hours as shown in Table 1.

Table 1 - Treatment design for the basal diet and test leaf meals.

T ₁	Without heat treatment, Ipil-ipil (<i>Leucaena leucocephala</i> Lam.) leaf meal
T ₂	Without heat treatment, Kakawate (<i>Gliricidia sepium</i> Jacq.) leaf meal
T ₃	Without heat treatment, Moringa (<i>Moringa oleifera</i> L.) leaf meal
T ₄	Without heat treatment, Kudzu (<i>Pueraria phaseoloides</i> Roxb.) leaf meal
T ₅	Without heat treatment, Gumamela (<i>Hibiscus</i> sp.) leaf meal
T ₆	Heat-treated Ipil-ipil (<i>Leucaena leucocephala</i> Lam.) leaf meal
T ₇	Heat-treated Kakawate (<i>Gliricidia sepium</i> Jacq.) leaf meal
T ₈	Heat-treated Moringa (<i>Moringa oleifera</i> L.) leaf meal
T ₉	Heat-treated Kudzu (<i>Pueraria phaseoloides</i> Roxb.) leaf meal
T ₁₀	Heat-treated Gumamela (<i>Hibiscus</i> sp.) leaf meal

Heat treatment of plant protein concentrates

Heat treatment of plant protein concentrates was accomplished by spreading samples in a tray at about 1-inch-thick and was placed in a forced-draft oven set at 149 °C for four hours (Glimp et al., 1967). The concentrates were then cooled down and consequently placed in the nylon bags.

Feeding of experimental cattle

The experimental animal was fed twice a day (8:00 AM and 4:00 PM) of a ration comprising 70% basal diet of Napier grass (*Pennisetum purpureum*) and 30% of the test protein concentrates in equal proportion (6% each) based on DM requirement of the cattle. Feeding was done *ad libitum*. Drinking water was also offered free choice.

Rumen incubation of unheated and heat-treated protein concentrates

The nylon bag technique estimates ruminal degradation of the plant protein concentrates. The nylon bags measuring 5 x 10 cm each were oven-dried at 65 °C for 30 minutes and their empty weights were measured immediately (Osuji et al., 1993). The nylon bags used had a pore size of ±53 microns, enough for rumen microbes to enter but small enough for

feed samples to escape from the inside (Ørskov et al., 1979; Wirayudha et al., 2022). Approximately 5g of dry weight for each dietary treatment (untreated and heat-treated concentrates) was placed inside the nylon bags and heat-sealed. These were then placed inside a lingerie bag with stainless steel pieces to prevent the nylon bags from floating on top of the rumen fluid once placed as presented in Figure 1, which may give variable degradation rates (Preston, 1986; Patiga et al., 2020).

After 24 hours of incubation, all the nylon bags were harvested and washed immediately under running water with gentle rubbing of the thumb and fingers until the water runs clear. Washed nylon bags were oven-dried at 60 °C for 48 hours and then weighed immediately using a digital weighing scale (Atole, 2011). The dry matter (DM) content of the residue was determined by subtracting the weight of the nylon bag from the gross weight of the oven-dried residue plus the nylon. The DM degradation was calculated by using this formula:

$$\text{DMD \%} = \frac{(W1 - W2)}{W1} \times 100$$

Where W1 = weight of dried sample before incubation, g; W2 = weight of dried sample after incubation, g

In situ degradation trial

Day 1 – 8 adjustment period

Feeding the cattle with a 70:30 ratio diet of basal and test leaf meals was done at an amount equivalent to about 2 % body weight dry matter (DM) intake. Feeding was done twice a day with access to clean drinking water at all times.

Day 9 – 10 feed reduction period

Two days before incubation of the test diets, the amount of feed offered was reduced by half to provide space to accommodate the nylon bags with the test concentrates without adversely affecting the normal rumen function and make the recovery of bags easier (Bestil et al., 2014).

Day 11 incubation period

The bags containing feed samples of approximately 5 grams, with three samples per bag, were incubated for 24 hours. A total of 30 bags were placed inside the rumen for this study.

Day 12 recovery period

After incubation of feed samples, harvesting of the nylon bags was done. The nylon bags with feed samples were then washed under running tap water with gentle rubbing with the thumb and fingers until the water runs clear.

Data gathered

1. Percent dry matter degradation (DMD)

$$\text{DMD (\%)} = \frac{(DM1 - DM2)}{DM1} \times 100$$

Where DM1 = Dry matter weight of dried sample before incubation, g; DM2 = Dry matter weight of dried sample after incubation, g.

2. Percent organic matter degradation (OMD)

$$\text{OMD (\%)} = \frac{(OM1 - OM2)}{(OM2)} \times 100$$

Where OM1 = Organic matter weight of feed sample before incubation, g; OM2 = Organic matter weight of the residue (after incubation), g.

3. Percent crude protein degradation (CPD)

$$\text{CPD (\%)} = \frac{(CP1 - CP2)}{(CP1)} \times 100$$

Where CP1 = CP of feed sample before incubation (g); CP2 = CP of feed sample after incubation (g)

Statistical analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) using the PROC MIXED procedures of Statistical Analysis System (SAS) Version 9. Differences among treatment means were compared using group comparisons and declared significant when $P < 0.05$ using adjusted Tukey's Honest Significant Difference (HSD).

RESULTS AND DISCUSSION

Nutrient analysis of plant protein leaf meals

Plant protein leaf meals untreated or heat-treated were measured to assess their potential as a bypass protein source by assessing the extent of protection from rumen degradation in fistulated cattle. Contents of dry matter (DM), organic matter, ash (minerals), and crude protein in leaf meals tested were analyzed and the results are presented in Table 2. Dry matter content among feedstuffs shows that ipil-ipil leaf meal has a high percentage of DM at 93%, and the lowest was gumamela leaf meals at 87%. For percent organic matter, ipil-ipil leaf meals at 91 % and has reverse results in ash, showing that ipil-ipil leaf meals have the lowest value of 9%. Moreover, Table 2 presents high CP in kudzu leaf

meal 27% and lowest CP in gumamela leaf meal at 12%. While the difference in crude protein content between untreated treatment and heat treatment may not be significant, there seemed to be a pattern of a slight reduction in crude protein content among the heat treatment than those untreated.

Table 2 - Nutrient analysis of the test leaf meal diets before incubation.

Treatment	Nutrient analysis	%DM	%OM	%ASH	%CP
T ₁		93.01	85.32	14.68	24.84
T ₂		89.30	87.82	12.18	20.98
T ₃		89.86	89.09	10.91	26.43
T ₄		88.36	86.59	13.41	27.39
T ₅		87.44	83.98	16.02	14.00
T ₆		92.13	91.19	8.81	23.22
T ₇		90.37	89.58	10.42	20.78
T ₈		91.97	88.82	11.18	26.07
T ₉		90.66	89.94	10.06	27.18
T ₁₀		91.02	85.05	14.95	12.30

***In situ* degradation of plant protein leaf meals**

The degradation of dry matter, organic matter and crude protein of leaf meals are presented in Table 3. Analysis of variance across the plant protein leaf meals untreated or heat-treated showed significant differences in dry matter degradation (DMD), organic matter degradation (OMD) and crude protein degradation (CPD). Moringa leaf meal is highest in DMD and the lowest is ipil-ipil leaf meal and kudzu leaf meal significant at $P < 0.0001$. Organic matter degradation (OMD) has the highest result in moringa leaf meal and lowest in kudzu leaf meal significant at $P < 0.0001$. Crude protein degradation (CPD) has the highest result in moringa leaf meal, lowest in ipil-ipil leaf meal and kudzu leaf meal significant at $P < 0.0001$. Among plant protein leaf meals tested, heat treatment appeared to be effective in protecting the protein in ipil-ipil leaf meal, kudzu leaf meal and gumamela leaf meal. Other mechanisms, such as a Browning reaction in the presence of reducing sugars in the leaf material, may also be in play. Further, heat application might increasingly protect forage leaf meals as apparently occurs when some meal is dehydrated at a temperature increasing from 65 °C to 160 °C (Zahari et al., 2006).

Table 3 - *In situ* degradation of dry matter, organic matter and crude protein of leaf meals.

Treatment	Chemical analysis	DMD,%	OMD,%	CPD,%
T ₁		53.25 ^f	57.40 ^g	75.30 ^e
T ₂		63.76 ^e	68.38 ^e	70.51 ^f
T ₃		94.98 ^a	97.00 ^a	98.27 ^a
T ₄		48.52 ^g	54.21 ^h	68.69 ^g
T ₅		88.55 ^c	95.23 ^c	94.28 ^{bc}
T ₆		52.68 ^f	54.90 ^h	57.35 ⁱ
T ₇		63.36 ^{ef}	65.01 ^f	70.18 ^f
T ₈		93.88 ^b	96.61 ^b	94.80 ^b
T ₉		46.31 ^h	51.96 ⁱ	58.60 ^h
T ₁₀		87.04 ^d	84.34 ^d	87.91 ^d
SEM		1.7880	2.7631	2.6194
P-Value		0.0001***	0.0001***	0.0001***

Legend: DMD = Dry Matter Degradation; OMD = Organic Matter Degradation; CPD = Crude Protein Degradation

Comparison of protein leaf meals on dry matter degradation

Group comparison of protein leaf meals on DMD is presented in Table 4. Dry matter degradation was significantly different across groupings for leaf meal samples except for L₁ (untreated leaf meal vs heat treated leaf meal). For L₂ and L₃ groupings on non-drought resistant leaf meal (ipil-ipil, moringa, kudzu and gumamela) shows a higher percentage of dry matter degradation (DMD) than drought-resistant leaf meal (kakawate) in both untreated and heat-treated leaf meals samples ranging 70% to 71% resulting for drought-resistant leaf meal as a potential in reducing degradability in the rumen at 63 % -64%. On the other hand, L₄ and L₅ legume leaf meal (ipil-ipil, kakawate, moringa and kudzu) for both

unheated and heated leaf meal samples have a lower percent DMD ranging from 64% - 65% had, reduce the outflow of dry matter from the rumen compared to non-legume leaf meal (gumamela) ranging at 87% - 89% DMD. Furthermore, L₆ and L₇ groupings for both untreated and treated leaf meals shown that shrub type leaf meal (moringa and gumamela) has a higher DMD at 90% - 92%, while tree type leaf meal (ipil-ipil and kakawate) a lower DMD ranging at 58% - 59% and therefore could bypass the dry matter at ruminal level. Lastly L₈ and L₉ for both untreated and treated leaf meals non-creeping type of leaf meal (ipil-ipil, kakawate, moringa, and gumamela) has higher DMD ranging at 74%-75% compared to creeping type of leaf meal (kudzu) at 46% - 48% DMD. Therefore, results with lower DMD could be a potential source of bypass.

Table 4 - Group comparison of plant protein leaf meals on *in situ* dry matter degradability.

Contrast	Group 1	Mean %	Group 2	Mean %	p-value	SEM
L ₁ - UT Vs HT	T ₁ , T ₂ , T ₃ , T ₄ , T ₅	69.76	T ₆ , T ₇ , T ₈ , T ₉ , T ₁₀	68.65	0.6619 ^{ns}	0.68
L ₂ - UT Drought Resistant Leaf Meal VS UT Non Drought Resistant Leaf Meals	T ₂	63.76	T ₁ , T ₃ , T ₄ , T ₅	71.26	0.0001***	0.74
L ₃ - HT Drought Resistant Leaf Meal VS HT Non-Drought Resistant Leaf Meal	T ₇	63.36	T ₆ , T ₈ , T ₉ , T ₁₀	69.98	0.0001***	1.23
L ₄ - UT Legume Leaf Meal VS UT Non Legume Leaf Meal	T ₁ , T ₂ , T ₃ , T ₄	65.06	T ₅	88.55	0.0001***	0.58
L ₅ - HT Legume Leaf Meal VS HT Non Legume Leaf Meal	T ₆ , T ₇ , T ₈ , T ₉	64.06	T ₁₀	87.04	0.0001***	0.73
L ₆ - UT Tree Type Leaf Meal VS UT Shrub Type Leaf Meal	T ₁ , T ₂	58.51	T ₃ , T ₅	91.77	0.0001***	1.23
L ₇ - HT Tree Type Leaf Meal VS HT Shrub Type Leaf Meal	T ₆ , T ₇	58.02	T ₈ , T ₁₀	90.46	0.0001***	0.59
L ₈ - UT Creeping Type Leaf Meal VS UT Non-Creeping Type Leaf Meal	T ₄	48.25	T ₁ , T ₂ , T ₃ , T ₅	75.14	0.0001***	0.74
L ₉ - HT Creeping Type Leaf Meal VS HT Non-Creeping Type Leaf Meal	T ₉	46.31	T ₆ , T ₇ , T ₈ , T ₁₀	74.24	0.0001***	1.23

UT = Untreated; HT = Heat treated

Group comparison of protein leaf meals on organic matter degradation

Group comparison of protein leaf meals on OMD is presented in Table 5. Organic matter degradation (OMD) is significantly different across groupings for leaf meal samples except for L₁ (untreated leaf meal vs heat treated leaf meal). For L₂ and L₃ groupings on non-drought resistant leaf meal (ipil-ipil, moringa, kudzu and gumamela) showing high percentage of OMD than drought-resistant leaf meal (kakawate) in both untreated and heat-treated leaf meals samples ranging 72% to 76% resulting for drought-resistant leaf meal as a potential in reducing organic matter degradability in the rumen at 65% - 68%. L₄ untreated legume leaf meal (ipil-ipil, kakawate, moringa and kudzu) samples have a lower percent OMD at 69% had reduced organic matter's outflow from the rumen compared to untreated non-legume leaf meal (gumamela) at 95% OMD. Also, L₅ groupings for treated legume leaf meal (ipil-ipil, kakawate, moringa and kudzu) samples still have a lower percent OMD at 67% had, reduced the outflow of samples from the rumen compared to treated non- legume leaf meal (gumamela) at 84% OMD. Furthermore, L₆ and L₇ groupings for both untreated and treated leaf meals shown that shrub type leaf meal (moringa and gumamela) has a higher OMD resulting 90% - 96% while tree type leaf meal (ipil-ipil and kakawate) a lower OMD ranging at 60% - 63% and therefore could be a good bypass of organic matter at the ruminal level. Moreover, L₈ and L₉ for both untreated and treated leaf meals non-creeping type of leaf meal (ipil-ipil, kakawate, moringa, and gumamela) has higher OMD ranging at 75%-80% compared to creeping type of leaf meal (kudzu) at 52% - 54% OMD. Therefore, OMD with a lower value could be a potential source of the bypass from the ruminal level and may increase the organic matter to the intestinal level.

Group comparison of protein leaf meals on crude protein degradation

Group comparison of protein leaf meals on CPD is presented in Table 6. The table presented that CPD was significantly different across all groupings for leaf meal samples. L₁ group for untreated leaf meals had high CPD at 81% compared to heat-treated leaf meals at 74%. This confirms the report of [Aguilera et al. \(1992\)](#) that heating decreases protein solubility and renders it more resistant to ruminal degradation. For L₂ and L₃ groupings on non-drought resistant leaf meals (ipil-ipil, moringa, kudzu and gumamela) showing high percentage of CPD in both untreated and heat-treated leaf meals samples ranging 75% to 84% than drought-resistant leaf meal (kakawate) ranging at 70% - 71% CPD this result shows potential in reducing protein degradability in the rumen. Some range plants, which are drought-tolerant environmental, can be used as protein supplements to straws for ruminants ([Al-masri, 2007](#)). L₄ untreated legume leaf meal samples (ipil-ipil, kakawate, moringa and kudzu) have a lower percent CPD at 78% reduced organic matter's outflow

from the rumen compared to untreated non-legume leaf meal (gumamela) at 94% CPD. Also, L₅ groupings for treated legume leaf meal (ipil-ipil, kakawate, moringa and kudzu) samples still have a lower percent CPD at 70% had reduce the outflow of samples in the ruminal level compared to treated non- legume leaf meal (gumamela) at 88% CPD. Furthermore, L₆ and L₇ groupings for both untreated and treated leaf meals showed that shrub type leaf meal (moringa and gumamela) has a higher CPD resulting in 91% - 96%, while tree type leaf meal (ipil-ipil and kakawate) a lower CPD ranging at 64% - 73% and therefore could be a good bypass of crude protein at the ruminal level. Moreover, L₈ and L₉ for both untreated and treated leaf meals non-creeping type of leaf meal (ipil-ipil, kakawate, moringa, and gumamela) have higher CPD ranging at 78%-85% compared to creeping type of leaf meal (kudzu) at 59% - 69% CPD. Therefore, CPD with lower value could be a potential source of the bypass from the ruminal level and may increase the crude protein to the intestinal level.

Table 5 - Group comparison of plant protein leaf meals on *in situ* organic matter degradability.

Contrast	Group 1	Mean %	Group 2	Mean %	P-value	SEM
L ₁ - UT Vs HT	T ₁ , T ₂ , T ₃ , T ₄ , T ₅	74.44	T ₆ , T ₇ , T ₈ , T ₉ , T ₁₀	70.56	0.7881 ^{ns}	0.07
L ₂ - UT Drought Resistant Leaf Meal Vs UT Non Drought Resistant Leaf Meals	T ₂	68.38	T ₁ , T ₃ , T ₄ , T ₅	75.96	0.0001***	0.68
L ₃ - HT Drought Resistant Leaf Meal Vs HT Non-Drought Resistant Leaf Meal	T ₇	65.01	T ₆ , T ₈ , T ₉ , T ₁₀	71.95	0.0001***	0.74
L ₄ - UT Legume Leaf Meal Vs UT Non Legume Leaf Meal	T ₁ , T ₂ , T ₃ , T ₄	69.25	T ₅	95.23	0.0001***	1.23
L ₅ - HT Legume Leaf Meal Vs HT Non Legume Leaf Meal	T ₆ , T ₇ , T ₈ , T ₉	67.12	T ₁₀	84.34	0.0001***	0.58
L ₆ - UT Tree Type Leaf Meal Vs UT Shrub Type Leaf Meal	T ₁ , T ₂	62.89	T ₃ , T ₅	96.12	0.0024**	0.73
L ₇ - HT Tree Type Leaf Meal Vs HT Shrub Type Leaf Meal	T ₆ , T ₇	59.96	T ₈ , T ₁₀	90.48	0.0001***	1.23
L ₈ - UT Creeping Type Leaf Meal Vs UT Non-Creeping Type Leaf Meal	T ₄	54.21	T ₁ , T ₂ , T ₃ , T ₅	79.50	0.0001***	0.59
L ₉ - HT Creeping Type Leaf Meal Vs HT Non-Creeping Type Leaf Meal	T ₉	51.96	T ₆ , T ₇ , T ₈ , T ₁₀	75.22	0.0001***	0.74

UT = Untreated; HT = Heat treated

Table 6 - Group comparison of plant protein leaf meals on *in situ* crude protein degradability.

Contrast	Group 1	Mean %	Group 2	Mean %	P-value	SEM
L ₁ - UT Vs HT	T ₁ , T ₂ , T ₃ , T ₄ , T ₅	81.41	T ₆ , T ₇ , T ₈ , T ₉ , T ₁₀	73.77	0.0060 ^{ns}	0.45
L ₂ - UT Drought Resistant Leaf Meal Vs UT Non Drought Resistant Leaf Meals	T ₂	70.51	T ₁ , T ₃ , T ₄ , T ₅	84.14	0.0034**	1.02
L ₃ - HT Drought Resistant Leaf Meal Vs HT Non-Drought Resistant Leaf Meal	T ₇	70.18	T ₆ , T ₈ , T ₉ , T ₁₀	74.67	0.0001***	2.05
L ₄ - UT Legume Leaf Meal Vs UT Non Legume Leaf Meal	T ₁ , T ₂ , T ₃ , T ₄	78.19	T ₅	94.28	0.0001***	3.86
L ₅ - HT Legume Leaf Meal Vs HT Non Legume Leaf Meal	T ₆ , T ₇ , T ₈ , T ₉	70.23	T ₁₀	87.91	0.0001***	2.24
L ₆ - UT Tree Type Leaf Meal Vs UT Shrub Type Leaf Meal	T ₁ , T ₂	72.91	T ₃ , T ₅	96.28	0.0002***	1.20
L ₇ - HT Tree Type Leaf Meal Vs HT Shrub Type Leaf Meal	T ₆ , T ₇	63.77	T ₈ , T ₁₀	91.36	0.0003***	1.01
L ₈ - UT Creeping Type Leaf Meal Vs UT Non-Creeping Type Leaf Meal	T ₄	68.69	T ₁ , T ₂ , T ₃ , T ₅	84.59	0.0001***	0.69
L ₉ - HT Creeping Type Leaf Meal Vs HT Non-Creeping Type Leaf Meal	T ₉	58.60	T ₆ , T ₇ , T ₈ , T ₁₀	77.56	0.0001***	1.13

UT = Untreated; HT = Heat treated

CONCLUSION AND RECOMMENDATION

While heat treatment had no effect on dry matter and organic matter degradability, it did considerably lower crude protein degradability—by up to 24% in ipil-ipil and 15% in kudzu—implying increased potential for post-ruminal protein supply. The inherent poorer digestibility of legume and creeping-type leaf meals suggests a structural or chemical resistance to rumen decomposition, which heat treatment accentuates. These studies demonstrate the combined effect of botanical kind and processing method on optimizing bypass protein sources for bovine diets.

Among protein-rich leaf meals, heat treatment has been observed to reduce protein digestibility, particularly in ipil-ipil (*Leucaena leucocephala*), kudzu, and gumamela leaf meals. Therefore, further studies on the potential of these plant-based protein sources as bypass protein are recommended. Specifically, different categories of leaf meals such as drought-resistant species, legumes, and those classified as tree, shrub, or creeping types should be thoroughly investigated. Evaluating these factors in relation to rumen bypass characteristics and their effects on cattle performance will contribute valuable knowledge to the scientific community and support the development of more efficient feeding strategies.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Elmar Mantuhac Patiga E-mail: elmar.patiga@bisu.edu.ph; ORCID: <https://orcid.org/0009-0002-0207-4495>

Data availability

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

Ethical considerations

This study adhered to the Philippines' Animal Welfare Act, which ensured the humane treatment of rumen-fistulated Brahman cattle through suitable housing, feeding, veterinary care, and gentle handling. Approval was obtained on March 19, 2019, under the IACUC code MHAM-0219-119-01.

Authors' contribution

Cindy Cortez managed fund acquisition, experimentation, data collection, conceptualization, analysis, and drafting; Lolito C. Bestil supervised methodology and revisions; Manuel D. Gacutan Jr. oversaw data collection, analysis, and writing assistance; Marisel A. Leorna handled literature review, interpretation, and proofreading; Elmar Patiga conducted experimentation, data collection, figure preparation, writing, editing, and proofreading of the manuscript.

Funding

The authors declare that no funds, grants, or other support were received during the preparation or publication of this manuscript.

Competing Interests

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

REFERENCES

- Aguilera JF, Bustos M, and Molina E (1992). The degradability of legume seed meals in the rumen: Effect of heat treatment. *Animal Feed Science and Technology*, 36(1-2):101-12. DOI: [https://doi.org/10.1016/0377-8401\(92\)90090-S](https://doi.org/10.1016/0377-8401(92)90090-S)
- Al-Masri MR (2007). An in vitro evaluation of some drought-tolerant native range plants in terms of ruminal microbial nitrogen, microbial biomass and their fermentation characteristics utilising a gas-production technique. *Tropical Grasslands*. 41(4):292. DOI: https://www.tropicalgrasslands.info/public/journals/4/Historic/Tropical%20Grasslands%20Journal%20archive/PDFs/Vol_41_2007/Vol_41_04_2007_pp292_300.pdf
- Arisya W, Ridwan R, Ridla M, and Jayanegara A (2019). Tannin treatment for protecting feed protein degradation in the rumen in vitro. *Journal of Physics: Conference Series*, 1360(1): 012022. DOI: <https://doi.org/10.1088/1742-6596/1360/1/012022>
- Atole AFF (2011). In situ degradation of selected protein source for rumen bypass potential in Brahman cattle (*Bos indicus*, Linn.). Department of Animal Science, College of Agriculture and Food Science (DAS - CAFS), Visayas State University (VSU, Visca, Baybay, City Leyte). DOI: <https://library.ched.gov.ph/cgi-bin/koha/opac-detail.pl?biblionumber=4372>
- Bestil LC, Atole AFF and Rama JM (2014). Chemical composition and digestibility of in situ common feed resources for ruminants in marginal uplands. *Annals of Tropical Research*, 36(Supplement): 179-190. DOI: <https://doi.org/10.32945/atr36s12.2014>

- Glimp HA, Karr MR, Little CO, Woolfolx PG, Mitchell GE, Jr. and Hudson LW (1967). Effect of reducing soybean protein solubility by dry heat on the protein utilization of young lambs. *Journal of Animal Science*, 26(4): 858-861. DOI: <https://doi.org/10.2527/jas1967.264858x>
- Niepes RA and Bestil LC (2023). Nutrient digestibility of fibrous feedstuffs in high-concentrate diet with sodium-bicarbonate (NaHCO₃) addition in rumen - fistulated Brahman bull. *Online Journal of Animal and Feed Research*, 13(4): 234-241. DOI: <https://dx.doi.org/10.51227/ojafr.2023.35>
- Ørskov ER. (1979). Recent information on processing of grain for ruminants. The Rowett Research Institute, Bucksburn, Aberdeen. *Livestock Production Science*, 6 (1979): 335–347. DOI: [https://doi.org/10.1016/0301-6226\(79\)90002-2](https://doi.org/10.1016/0301-6226(79)90002-2)
- Osuji PO, Nsahlai IV, and Khalili H (1993). ILCA Manual 5. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia. pp. 40. DOI: <https://cgspace.cgiar.org/server/api/core/bitstreams/25b5d9e5-0bc2-40e4-9e36-a4681bccbc91/content>
- Owens FN, and Basalan M (2016). Ruminant fermentation. In *Rumenology 2016 Sep 23* (pp. 63-102). Cham: Springer International Publishing. DOI: https://doi.org/10.1007/978-3-319-30533-2_3
- Patiga E, Bestil LC, Mondejar HP, and Intong AJ (2024). Effects of urea-molasses, maize bran, and ipil-ipil leaf meal on the quality of cogon grass silage at different harvesting intervals. *Mindanao Journal of Science and Technology*, 22 (2): 87-99. DOI: <https://doi.org/10.61310/mjst.v22i2.2183>
- Patiga EM, Bestil LC, and Mondejar HP (2020a). Utilization of cogon (*Imperata cylindrica* L.) silage and urea-treated corn (*Zea mays* L.) Stover as Affected by Supplementation. *CMU Journal of Science*, 24(1), 11-18. DOI: <https://doi.org/10.52751/DZSG8945>
- Patiga E, Bestil, LC, and Mondejar HP (2020b). In situ digestibility of cogon grass (*Imperata cylindrica* L.) in various forms and harvesting intervals in rumen fistulated Brahman cattle. *Mindanao Journal of Science and Technology*, 18(2): 73-83. DOI: <https://doi.org/10.61310/mndjstea.0941.20>
- Preston TR (1986). Better utilization of crop residues and by-products in animal feeding: Research Guidelines 2. A practical manual for research workers. Food and Agriculture Organization (FAO), 00100 Rome, Italy. ISBN 92-5-102422-7. DOI: <https://www.fao.org/4/x6554e/X6554E00.htm>
- Singh A, Sidhu S, and Singh P (2019). Bypass protein technology: A review. *The Pharma Innovation Journal*, 8(8): 150-153. DOI: <https://www.thepharmajournal.com/archives/2019/vol8issue8/PartC/8-7-144-808.pdf>
- Stern MD, Bach A, and Calsamiglia S. (2006). New concepts in protein nutrition of ruminants. Department of Animal Science, University of Minnesota, St. Paul, USA. 21st Annual Southwest Nutrition & Management Conference. DOI: <https://www.icrea.cat/cvs/17413/alex-bach-ariza/>
- Thakur S, Dey A, and Kumar S (2024). Rumen bypass protein: An effective technology for enhancing performance of ruminants. *Indian Journal of Animal Health*, 63(2)- Special Issue): 43-52. DOI: <https://doi.org/10.36062/ijah.2024.spl.01124>
- Wang Z, Yin L, Liu L, Lan X, He J, Wan F, Shen W, Tang S, Tan Z, and Yang Y (2022). Tannic acid reduced apparent protein digestibility and induced oxidative stress and inflammatory response without altering growth performance and ruminal microbiota diversity of Xiangdong black goats. *Frontiers in Veterinary Science*, 9: 1004841. DOI: <https://doi.org/10.3389/fvets.2022.1004841>
- Wirayudha P, Setyono W, Budisatria I, Rahmawati, and Kustantinah (2022). Effects of dietary supplementation with jackfruit leaves and soybean meal on nutrient intake and digestibility in sheep. *Atlantis Press International B.V. Proceedings of the 6th International Seminar of Animal Nutrition and Feed Science (ISANFS 2021)*. DOI: <https://www.atlantis-press.com/proceedings/isanfs-21/125972754> ; <https://doi.org/10.2991/absr.k.220401.018>
- Zahari MW, Nordin MSY, Najib MA, Suparjo NM, Abu Bakar C, Aminah A, et al. (2006). Improving animal productivity by supplementary feeding of multinutrient blocks, controlling internal parasites and enhancing utilization of alternate feed resources. *Animal Production and Health Section Joint FAO/IAEA Division International Atomic Energy Agency Pages 153*. DOI: https://www.pub.iaea.org/MTCD/Publications/PDF/te_1495_web.pdf

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

INFLUENCE OF DIFFERENT LEVELS OF RUMEN CONTENT AS A REPLACEMENT FOR GRASS MEAL IN COMPOUND FEED ON THE PRODUCTIVITY OF KAZAKH FINE-WOOL SHEEP

Gulmira KENENBAY , Urishbay CHOMANOV , and Marzhan IDAYATOVA  

LLP "Kazakh Research Institute of Processing and Food Industry", Serkebayev street, 62, Almaty, 050000, Kazakhstan

Email: idayatova_m@mail.ru

Supporting Information



ABSTRACT: This study evaluated the effects of replacing grass meal with different inclusion levels of dried rumen content on the growth performance of Kazakh fine-wool sheep. Twenty five-month-old intact male sheep (initial body weight 32.1 ± 0.20 kg) were randomly allocated to four dietary treatment groups for 60 days following a fifteen-day adaptation period. The control diet contained no rumen content, while experimental diets included 10%, 20%, and 30% dried rumen content (a mixture of cattle and sheep rumen content at a ratio of 60:40). Inclusion of rumen content at 10–20% significantly improved average daily gain and feed conversion ratio compared to the control diet ($P < 0.05$), whereas a 30% inclusion level reduced feed palatability and productive performance. No adverse effects on animal health were observed during the experiment. The results indicate that dried rumen content can be safely used as a partial replacement for grass meal in sheep diets at inclusion levels up to 20%.

Keywords: Alternative feedstuffs; Feed palatability; Sheep; Rumen content; Ruminant nutrition

INTRODUCTION

Global demand for meat and livestock products has increased in recent decades, driving a rapid expansion of animal production. Global meat output now exceeds 340 million tonnes per year, and abattoirs generate vast quantities of byproducts. Roughly half of a slaughtered animal's weight remains as offal and inedible by-products (blood, organs, rumen contents, etc.). Efficient valorization of these by-products is critical: by-product reuse can alleviate feed shortages, decrease production costs, and reduce environmental pollution (Alibekov et al., 2024). Rumen content is readily available in slaughterhouses and is rich in nutrients: it typically contains crude protein along with microbial biomass, volatile fatty acids, minerals and B vitamins (Alao et al., 2017; Cherdthong, 2020; Soressa et al., 2025).

Experimental evidence supports the potential of rumen content as a feed ingredient (Cherdthong, 2020; Lazarus et al., 2025). In various trials, animals fed diets containing moderate levels of dried rumen content indicated no reduction in productive performance. Previous studies indicated that dried rumen content can be incorporated into poultry diets without adverse effects on growth performance or feed efficiency (Okorie, 2006; Yitbarek et al., 2020; Mishra et al., 2015, Adeniji et al., 2021). According to T. F. Mbahi, cane can be included in the diet of weaned rabbits in quantities of up to 40% without negative effects on growth rates, blood composition, carcass characteristics and internal organs (Mbahi et al., 2020).

Despite these promising results, research on the use of rumen contents in sheep diets remains limited, particularly regarding the conditions of Central Asia. Only a small number of studies have investigated the application of the slaughter by-product in ruminant feeding; therefore, data regarding the use of such by-products in Kazakh fine-wool sheep are virtually rare. This gap is especially relevant to the scale and ongoing growth of sheep production in Kazakhstan. Currently, the country produces approximately 1.0 million tons of meat annually (Bureau of National Statistics of Kazakhstan, 2025) while a considerable proportion of slaughter by-products, including rumen contents, remains underutilized (Alibekov et al., 2024). With the continuing increase in livestock numbers and the corresponding rise in slaughter waste volumes, the evaluation of feed applications for such by-products demands a timely and practically important research direction.

The aim of the present study was to evaluate the effects of using different levels of cattle rumen contents in compound feeds on the productive performance of Kazakh fine-wool sheep. Determining optimal inclusion levels and their effects on growth and production parameters may contribute to the existing literature and provide practical recommendations to reduce feed costs and promote the rational utilization of waste products in the meat-processing industry.

RESEARCH ARTICLE
 PII: S222877012600011-16
 Received: September 03, 2025
 Revised: March 25, 2026
 Accepted: March 25, 2026

MATERIALS AND METHODS

Ethical approval

All animal procedures were conducted in accordance with the Law of the Republic of Kazakhstan “On Responsible Treatment of Animals” (2021) and international guidelines for the welfare of farm animals. The experimental protocol was approved by the Ethics Committee of the Kazakh Research Institute of Processing and Food Industry LLP.

Preparation of ingredients

Rumen contents from clinically healthy cattle and sheep were collected at the slaughterhouse of LLP “Rural Industrial Complex DALA FOOD” following veterinary inspection. In total, approximately 100 kg of cattle rumen content and 50 kg of sheep rumen content were collected, pooled, and transported to the feed mill of the Kazakh Research Institute of Processing and Food Industry LLP. Animals were raised under typical local production systems: the cattle received a roughage-based fattening diet with concentrates, while the sheep were fed pasture grass and hay. The rumen contents were dried at 48 °C to a moisture content of 9.8% and ground into grits. Other feed ingredients (triticale, wheat bran, brewer's grain, grass meal, and fillers) were purchased locally (Almaty, Kazakhstan) and subjected to standard quality control procedures. Table 1 shows the chemical composition of the main components of the diet (triticale, wheat bran, brewer's grain, and grass meal (alfalfa)).

Table 1 - Chemical composition of the main components of the diet,%

Variable	Triticale	Wheat bran	Brewer's grain	Grass meal
Dry matter (DM, %)	89.89	88.90	91.33	88.86
Crude protein (CP, %)	16.81	14.88	23.44	14.13
Fat (%)	1.32	3.16	7.75	3.85
Crude fiber (CF, %)	2.99	8.76	14.3	29.09
Nitrogen-free extract (NFE, %)	66.79	57.57	43.44	33.67
Sugar (%)	1.63	2.06	-	-
Starch (%)	51.51	12.69	-	-
Ash (%)	1.97	4.53	2.5	8.11
Calcium (Ca, %)	0.09	0.22	0.37	1.59
Phosphorus (P, %)	0.30	0.71	0.5	0.18

Feed formulation and production

Four groups of compound feeds were formulated: a control diet group without rumen content (A) and three experimental diet groups containing 10% (B), 20% (C), and 30% (D) rumen content. The three levels experimental diet were selected to represent low, moderate, and high replacement rates to determine the optimal diet level for Kazakh fine-wool sheep. Grass meal accounted for 30% in the control diet was progressively replaced by rumen content in the experimental diets; in the 30% rumen diet, grass meal was completely excluded. A mixture of cattle and sheep rumen contents (60:40) was used in all experimental feeds. Table 2 shows the formula for combined feed for sheep with the addition of rumen content. Feed production contained mixing rumen content with fillers and zeolite (adsorbent), moistening to ~20% moisture, granulation, and subsequent cooling and drying.

Animals and experimental design

Twenty healthy Kazakh fine-wool sheep (intact males), five-months old, with an initial body weight of 32.10 ± 0.09 kg, were randomly allocated to four groups (n = 5) using a completely randomized design balanced by body weight. Animals were housed individually under identical zoo-hygienic conditions with free access to water.

Feeding regimen and data collection

Sheep were fed once daily at 08:00 h for 75 days: a fifteen-day adaptation period and a sixty-day experimental period. Experimental diets were introduced gradually during adaptation. Daily feed intake was recorded by weighing feed offered and refusals. Feed palatability was expressed as the percentage of feed was consumed. Animals were weighed weekly before feeding to determine total and average daily weight gain. Feed conversion ratio (FCR) was calculated as total dry matter intake divided by live weight gain. Animal health was monitored daily during the experiment. Feed palatability (acceptability) was measured as the percentage of feed consumed based on the amount offered, following the formula: $\text{Palatability (\%)} = (\text{Feed intake} / \text{Feed offered}) \times 100$. Animal health was monitored daily during the experiment. Health status was evaluated daily by visual observation and clinical examination. The health indicators monitored during the experiment were appetite, general behavior, locomotor activity, and fecal consistency. Fecal consistency was scored using a five-point scale as: 1 = watery diarrhea, 2 = loose feces, 3 = normal well-formed feces, 4 = firm feces, and 5 = very dry

feces. During the experimental period, none of the animals showed clinical signs of digestive disturbances (diarrhea, bloating, lethargy, or reduced feed intake), and no clinically relevant abnormalities were detected.

Table 2 - Formula for combined feed for sheep with the addition of rumen content, %

Variable	A	B	C	D
Rumen content mix (60% cattle + 40% sheep)	0	10	20	30
Triticale	27	27	27	27
Wheat bran	19	19	19	19
Brewer's grains	19	19	19	19
Grass meal	30	20	10	-
Premix*	1	1	1	1
Salt	1	1	1	1
Zeolite	3	3	3	3
Dry matter (DM, %)	91.60	91.00	91.10	94.20
Crude protein (CP, %)	14.07	14.38	14.74	14.54
Fat (%)	2.33	2.42	2.64	1.82
Crude fiber (CF, %)	12.19	12.67	13.55	14.75
Nitrogen-free extract (NFE, %)	55.85	53.76	51.48	55.66
Sugar (%)	1.17	1.23	1.32	1.92
Starch (%)	20.63	19.71	20.89	22.86
Ash (%)	7.16	7.76	8.68	7.42

A: diet without the addition of rumen content; B: diet with 10% of rumen content; C: diet with 20% of rumen content; D: diet with 30% of rumen content. The premix contains the following per kilogram of diet: calcium 90 g/kg, phosphorus 10 g/kg, sodium 17 g/kg, cobalt 12 mg/kg, iron 250 mg/kg, copper 215 mg/kg, zinc 1100 mg/kg, manganese 900 mg/kg, iodine 15 mg/kg, selenium 1.50 mg/kg, Vitamin A 54,000 IU/kg, vitamin B 3,000 IU/kg.

Chemical and physicochemical analysis

Sample preparation and weighing

Prior to the analysis, feed ingredients were ground using an electronic grain mill (IKA A 11, Germany) designed for cereals, legumes, and forage crops with a moisture content $\leq 18\%$. Weighing of samples, reagents, and materials was performed using Shimadzu laboratory electronic scales (Japan) with high precision.

Nutrient composition analysis

Crude fiber, fat, protein, sugar, and starch contents were determined using the NIR DS 2500 feed analyzer (Sweden). The instrument performs near-infrared (IR) analysis of crushed, uncrushed, and granulated feed samples over a spectral range of 850–2500 nm that provides high analytical accuracy for nutrient determination.

Quality control procedures

To ensure the reliability of analytical results, all measurements were performed in triplicate. Instruments were calibrated according to the manufacturers' specifications prior to analysis. The MX-50 Moisture Analyzer (Japan) and Shimadzu electronic scales (Japan) were calibrated daily before usage. Ash content was measured in accordance with GOST 13979.6–69 using a SNOL muffle electric furnace (Lithuania). Samples were turned to ash at 650 °C until a constant mass was achieved. The NIR DS 2500 (Sweden) analyzer was validated against certified reference samples to guarantee spectral accuracy across the full wavelength range. For ash determination, the muffle furnace temperature was cross-checked using a certified thermometer prior to experimental series. Macro- and microelement concentrations were determined in accordance with GOST 32343–2013. Vitamin content was determined using chromatographic methods specified in GOST 31483–2012. Organoleptic properties of the finished compound feed containing different levels of rumen content (color, odor, and structure) were assessed based on standard sensory evaluation practices.

Statistical analysis

Data were analyzed using one-way ANOVA with dietary treatment as the main factor. Individual animals served as experimental samples ($n = 5$). Differences among means were assessed using Tukey's HSD test, with significance level of $P < 0.05$. Statistical analyses were performed using OriginLab PRO.

RESULTS AND DISCUSSION

Physico-chemical parameters of cattle and sheep rumen content

The physico-chemical composition of the cattle and the sheep rumen content is presented in Table 3. No significant differences were observed in dry matter (DM) content between the cattle and the sheep rumen samples (92.10% vs. 92.19%, NS) that indicate a comparable overall solids content in both species. In contrast, crude protein (CP) content was significantly higher in the sheep rumen content than in the cattle (9.37% vs. 3.59%, $P < 0.05$). A similar trend was observed for the fat content, which was markedly greater in the sheep rumen content (2.21%) compared to the cattle (0.10%, $P < 0.01$).

Table 3 - Physico-chemical parameters of cattle and sheep rumen content

Variable	Cattle rumen content	Sheep rumen content	P-value
Dry matter (DM, %)	92.10	92.19	NS
Crude protein (CP, %)	3.59 ^b	9.37 ^a	<0.05
Fat (%)	0.10 ^b	2.21 ^a	<0.01
Crude fiber (CF, %)	36.93 ^b	37.75 ^a	<0.05
Nitrogen-free extract (NFE, %)	42.22 ^a	33.67 ^b	<0.05
Sugar (%)	0.67	-	-
Starch (%)	-	-	-
Ash (%)	9.26 ^a	9.19 ^b	<0.05
Calcium (Ca, %)	-	0.53	-
Phosphorus (P, %)	-	0.23	-
Amino acid composition			
Arginine	0.262 ^b	0.347 ^a	<0.05
Lysine	0.375 ^b	0.596 ^a	<0.05
Tyrosine	0.194 ^b	0.244 ^a	<0.05
Phenylalanine	0.300 ^b	0.439 ^a	<0.05
Histidine	0.094	0.087	NS
Leucine + isoleucine	0.387 ^b	0.596 ^a	<0.05
Methionine	0.081 ^b	0.146 ^a	<0.05
Valine + Proline	0.456 ^b	0.596 ^a	<0.05
Threonine	0.362 ^b	0.509 ^a	<0.05
Serine	0.350 ^b	0.504 ^a	<0.05
Alanine	0.293 ^b	0.482 ^a	<0.05
Glycine	0.368 ^b	0.542 ^a	<0.05
Vitamins			
A, mg/100g	-	-	-
E, mg/100g	22.13 ^b	212.0 ^a	<0.05
B1, mg/100g	0.024 ^a	0.010 ^b	<0.05
B2, mg/100g	0.030 ^a	0.013 ^b	<0.05
B3, mg/100g	0.094 ^a	0.057 ^b	<0.05
B5, mg/100g	0.051	0.044	NS
B6, mg/100g	0.017 ^a	0.009 ^b	<0.05
Mineral elements			
Ca, mg/100g	103.25	96.42	NS
P, mg/100g	118.61	115.63	NS
Fe, mg/100g	8.79 ^a	5.06 ^b	<0.05
Zn, mg/100g	3.16	3.22	NS
Mg, mg/100g	24.18 ^a	21.5 ^b	<0.05
Na, mg/100g	132.44 ^a	109.74 ^b	<0.05
K, mg/100g	208.96 ^b	213.16 ^a	<0.05
Cu, mg/100g	3.71 ^a	1.95 ^b	<0.05

Values within a row with different superscript letters (a, b) differ significantly at $P < 0.05$. NS — not significant; “-” — not determined.

Crude fiber (CF), nitrogen-free extract (NFE), and ash content differed significantly between species ($P < 0.05$). Sugar and starch were not determined in either sample. The sheep rumen content was characterized by higher crude fiber levels, whereas the cattle rumen content contained a significantly higher proportion of nitrogen-free extract that suggests a greater amount of residual soluble carbohydrates. The analysis of the amino acid profile showed that most essential and non-essential amino acids (arginine, lysine, tyrosine, phenylalanine, leucine + isoleucine, methionine, valine + proline, threonine, serine, alanine, and glycine) were present in significantly higher concentrations in the sheep rumen content compared to the cattle ($P < 0.05$). Histidine content did not differ significantly between the two species ($P > 0.05$). For instance, lysine content was 0.596% in the sheep versus 0.375% in the cattle, while leucine + isoleucine accounted for 0.596% and 0.387%, respectively.

In case of vitamin composition, vitamin E and all analyzed B-group vitamins, except B5, were significantly higher in the sheep rumen content ($P < 0.05$). The evaluation of pantothenic acid (vitamin B5) showed that there is no significant difference between species. Among mineral elements, iron (Fe), magnesium (Mg), sodium (Na), potassium (K), and copper (Cu) differed significantly ($P < 0.05$), whereas calcium (Ca), phosphorus (P), and zinc (Zn) exhibited no significant variation.

Overall, the physico-chemical characteristics of the cattle and the sheep rumen content indicate their potential value as unconventional feed resources, which are consistent with earlier studies (Esonu et al., 2006; Agbabiaka et al., 2012;

Elfaki and Abdelatti, 2015; Mamudu et al., 2020). The higher crude protein and fat contents observed in the sheep rumen content are mainly attributed to the differences in feeding regimes, rumen microbial activity, and digesta retention time between small and large ruminants, as previously reported by Mamudu et al. (2020).

The high crude fiber content observed in both cattle and sheep rumen contents (36.93–37.75%) confirms that rumen digesta is a rich source of structural carbohydrates that supports earlier findings by Sakaba et al. (2017). Furthermore, the increased concentrations of important amino acids nutrition such as lysine, methionine, and threonine in the sheep rumen content suggest a higher contribution of microbial protein synthesized in the rumen, which has also been noted in previous reviews on rumen content utilization (Feedipedia, 2010).

The inclusion of rumen content

The inclusion of rumen content in the compound feed affected its organoleptic properties (Figure 1). The control diet (A, 0%) and the diet containing 10% of rumen content (B) showed a uniform color, homogeneous structure, and neutral odor. At 20% inclusion (C), a slight darkening of color and a minor increase in moisture were observed, while the feed remained organoleptically acceptable. However, the diet containing 30% of rumen content (D) exhibited a darker burgundy-brown color, reduced homogeneity with the appearance of small lumps, and a more pronounced odor.

Similar changes in odor and physical appearance of feeds containing rumen content have been reported in previous studies. Esonu et al. (2006) in their study on poultry, noted that dried rumen digesta imparts a characteristic odor to compound feeds, which may influence feed acceptance at higher inclusion levels. In addition, Okorie (2006) reported that increasing levels of rumen content in animal diets can affect sensory properties, particularly odor intensity that potentially reduces palatability. Furthermore, Yitbarek et al. (2020) found that moderate inclusion levels of rumen content can be used without adverse effects on feed quality, whereas excessive inclusion may negatively affect feed structure and acceptance. Organoleptic properties such as color, texture, and odor play a crucial role in determining feed palatability and voluntary intake in ruminants (Forbes, 2007).

Feed		Color	Structure of the feed mixture	Odor
A		Uniform, light brown	Homogeneous, without lumps 	Neutral, without pronounced foreign impurities
B		Practically unchanged	Homogeneous, without lumps 	No significant changes
C		Slight lightening	Fairly homogeneous, with a slight increase in moisture 	Slightly more pronounced, but within acceptable limits
D		Light brown burgundy-brown	Less homogeneous, small lumps appear 	More pronounced, which may affect feed perception

Figure 1 - Comparative analysis of external characteristics of the feed mixture with different percentages of rumen content (Author's own illustration). A — diet without the addition of rumen content; B — diet with 10% rumen content; C — diet with 20% rumen content; D — diet with 30% rumen content.

Growth performance

Table 4 presents the growth performance of young Kazakh fine-wool sheep fed diets containing different levels of rumen content. Incorporation of rumen content into livestock diets allows recycling of valuable microbial protein and fermentation products; however, the high moisture content and unpleasant odor of such diets are recognized limitations. Rumen content contains microbial protein, volatile fatty acids, amino acids, and non-protein nitrogen, which can positively influence rumen fermentation and nutrient utilization.

Table 4 - Effect of different levels of rumen content in the diet on growth performance of Kazakh fine-wool sheep.

Variable	A	B	C	D	SEM	P-value
IBW (kg)	32.0	31.9	32.2	32.3	0.15	0.617
FBW (kg)	39.3 ^b	39.6 ^b	41.0 ^a	39.0 ^b	0.30	<0.001
TWG (kg)	7.3 ^b	7.7 ^b	8.8 ^a	6.7 ^c	0.20	<0.001
DWG (g/day)	122 ^b	128 ^b	147 ^a	112 ^c	4.1	<0.001
DFI (g)	680	690	670	650	20	0.094
FCR (F/G)	5.58 ^b	5.37 ^b	4.56 ^c	5.80 ^a	0.29	<0.001

A: diet without the addition of rumen content; B: diet with 10% rumen content; C: diet with 20% rumen content; D: diet with 30% rumen content. IBW: Initial body weight, FBW: Final body weight, TWG: Total weight gain, DWG: Daily weight gain, DFI: Daily feed intake, FCR: feed conversion ratio (kg of feed consumed per 1 kg of body weight gain). a-c: Means with different superscripts within a row differ ($P < 0.05$). SEM: Standard error of means.

In the present study, the inclusion of rumen content at 20% of the diet (Group C) resulted in the highest final body weight, total weight gain, and average daily gain, as well as the most favorable feed conversion ratio. In contrast, increasing the inclusion level to 30% (Group D) had a negative effect on growth performance.

Initial body weight did not differ significantly among treatments ($P = 0.617$) that indicates uniform starting conditions. Final body weight, total weight gain, and daily weight gain were significantly influenced by dietary treatment ($P < 0.001$). Sheep in Group C achieved the highest final body weight (41.0 kg), total gain (8.8 kg), and daily gain (147 g/day), whereas the lowest values were observed in Group D. These results suggest a nonlinear response of growth performance to increasing rumen content inclusion.

The superior performance of sheep fed in the 20% rumen content diet agrees with previous studies. [Sadeghi et al. \(2024\)](#) reported that inclusion of 20% biologically treated rumen content in calf diets significantly improved dry-matter and organic-matter digestibility, average daily gain, and feed efficiency. Similar positive responses to moderate rumen content inclusion (10–20%) are reported in lambs and cattle, suggesting that the microbial protein and fermentation products present in rumen content can support animal growth.

In contrast, the reduced performance observed in sheep fed of the 30% rumen content diet is consistent with earlier findings. [Al-Wazeer \(2016\)](#) reported that while 10–20% dried rumen content had no adverse effects on growth in Awassi lambs, a 30% inclusion level significantly reduced weight gain and feed efficiency. Similarly, higher inclusion levels were associated with reduced digestibility and increased fiber intake, which may limit nutrient availability. Collectively, the results indicate that rumen content inclusion above approximately 20% can impair growth performance in sheep.

CONCLUSIONS

In summary, findings of the study suggest that fine-wool sheep can consist up to ~20% slaughterhouse rumen content in the diet, the extent that is without negative effects and even gains slightly more weight to the sheep due to the extra microbial nutrients. However, inclusion above this level (30% in the present study) reduces feed intake and growth. The sensory issue (unfavorable sensory characteristics) and high fiber of rumen content likely caused the decline in Group D with 30% of rumen content. For future studies, it is recommended to optimize processing methods of rumen content to improve its palatability, as well as to evaluate its effects on other animal species.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Idayatova M.A. E-mail: idayatova_m@mail.ru; ORCID: <https://orcid.org/0000-0001-8521-681X>

Authors' contribution

Gulmira Kenenbay: development of the concept and design of the study, analysis of the obtained data, writing the original text of the manuscript. Urishbay Chomanov: statistical processing of data, literature review, editing of the text. Marzhan Idayatova: participation in the experiment, sample collection and laboratory analysis, correction of the manuscript. All authors approved the final version of the article and are responsible for the accuracy of the presented data.

Acknowledgements

The authors express their gratitude to the Ministry of Agriculture of the Republic of Kazakhstan for financial support of this study.

Data availability

The authors will provide the analysed datasets on reasonable request to the corresponding author.

Ethical regulation

All animal procedures were conducted in accordance with the Law of the Republic of Kazakhstan “On Responsible Treatment of Animals” (2021) and international guidelines for the welfare of farm animals. The experimental protocol was approved by the Ethics Committee of the Kazakh Research Institute of Processing and Food Industry LLP. The study was conducted and reported in accordance with the ARRIVE guidelines.

Funding

This research was funded by the Ministry of Agriculture of the Republic of Kazakhstan grant number BR24892775. The presented research was carried out within the framework of the program “Developing technology for deep processing of agricultural raw materials to ensure high-quality, safe food production”), under the project “Development of technology for compound feed using non-traditional raw materials (rumen content)”.

Competing interests

The authors declare no competing interests.

REFERENCES

- Al-Wazeer AA (2016). Effect of different levels of dried rumen content on nutrient intake, digestibility and growth performance of Awassi lambs. *International Journal of Advanced Research*, 4(9):2106-2113. <https://dx.doi.org/10.21474/IJAR01/1696>
- Adeniji AA, and Balogun OO (2002). Utilisation of flavour treated blood-rumen content mixture in the diets of laying hens. *Nigerian Journal of Animal Production*, 29(1):34-9. <https://doi.org/10.21474/IJAR01/1696>
- Agbabiaka LA, Madubuike FN, and Amadi SA (2012). Studies on nutrients and anti-nutrients of rumen digesta from three most domesticated ruminants in Nigeria. *Pakistan Journal of Nutrition*, 11(7):678-80. <https://doi.org/10.3923/pjn.2012.678.680>
- Alao BO, Falowo AB, Chulayo A and Muchenje V (2017). The potential of animal by-products in food systems: Production, prospects and challenges. *Sustainability*, 9(7):1089. <https://doi.org/10.3390/su9071089>
- Alibekov RS, Alibekova ZI, Bakhtybekova AR, Taip FS, Urabayeva KA and Kobzhasarova, ZI (2024). Review of the slaughter wastes and the meat by-products recycling opportunities. *Frontiers in Sustainable Food Systems*, 8, 1410640. <https://doi.org/10.3389/fsufs.2024.1410640>
- Bureau of National Statistics of Kazakhstan, (2025). Key indicators of livestock development in the Republic of Kazakhstan: January–June 2025, [in Kazakh]. <https://stat.gov.kz/>
- Cherdthong A (2020). Potential use of rumen digesta as ruminant diet—a review. *Tropical animal health and production*, 52(1):1-6. DOI: <https://doi.org/10.1007/s11250-019-02018-6>
- Elfaki M and Abdelatti K (2015). Nutritive evaluation of rumen content from cattle, camel, sheep and goat. *Global Journal of Animal Scientific Research*, 3(3):617–621. https://www.researchgate.net/publication/334173126_Nutritive_Evaluation_of_Rumen_Content_from_Cattle_Camel_Sheep_and_Goat
- Esonu BO, Ogbonna UD, Anyanwu GA, Emenalom OO, Uchegbu MC, Etuk EB, et al.(2006). Evaluation of performance, organ characteristics and economic analysis of broiler finisher fed dried rumen digesta. *International Journal of Poultry Science*, 5(12):1116–1118. DOI: <http://doi.org/10.3923/ijps.2006.1116.1118>
- Feedipedia (2010). Rumen contents. Datasheet under construction. <https://www.feedipedia.org/node/218>
- Forbes JM (2007). *Voluntary food intake and diet selection in farm animals* (2nd ed.). CABI Publishing. <https://doi.org/10.1079/9781845932794.0000>
- Lazarus EJ, Wole BY, Hilakore MA, and Lawa ED (2025). Growth and economic performance of Kacang goats fed concentrates containing cattle rumen content silage. *Journal of Advanced Veterinary & Animal Research*, 12(4):1363-1369. Doi: <http://doi.org/10.5455/javar.2025.1996>
- Mamudu HA, Ayimbire A and Jacob A (2020). Nutritional value of dried rumen digesta from cattle, sheep and goat: A case of Bolgatanga abattoir, Ghana. *Journal of Livestock and Animal Health*, 2(1):8–15. <http://doi.org/10.47941/ahj.445>
- Mbahi TF, Adi A, Yakubu B and Wafar RJ (2020). Effect of yeast-treated bovine blood–rumen content mixture on performance and blood profile of weaner rabbits. *Nigerian Journal of Animal Production*, 45(2):106–113. <https://doi.org/10.51791/njap.v45i2.485>
- Mishra J, Abraham RJ, Rao VA, Rajini RA, Mishra BP and Sarangi NR (2015). Chemical composition of solar dried blood and ruminal content and its effect on performance of Japanese quails. *Veterinary World*, 8:82–87. <https://doi.org/10.14202/vetworld.2015.82-87>
- Okorie KC (2006). Effects of dried pulverized rumen contents on the performance, carcass and organ characteristics of finisher broilers. *Animal Production Research Advances*, 1(2). <http://doi.org/10.4314/apra.v1i2.36299>

- Sadeghi K, Parnian-Khajehezaj F, Ganjkhanlou M et al. (2024). Feeding biotreated rumen digesta affects nutrient digestion, ruminal fermentation, and blood parameters in calves. *Scientific Reports*, 14:31114. <https://doi.org/10.1038/s41598-024-82410-x>
- Sakaba AM, Hassan AU, Harande I, Isgogo MS, Maiyama FA and Danbare M (2017). Proximate composition of rumen digesta from sheep slaughtered in Zuru abattoir, Kebbi State, Nigeria. *Journal of Agricultural Science and Practice*, 2(5):86–89. <http://doi.org/10.31248/JASP2017.057>
- Soressa S, Demissu HS, Malatji DP (2025) Dried Blood–Rumen Content Mixtures as Sustainable Poultry Feed: A Review on Nutritional, Economic, and Environmental Potential. *Food Science & Nutrition*. 13 (9): e70796. <https://doi.org/10.1002/fsn3.70796>
- Yitbarek MB, Tamir B and Mengistu A (2016). Effect of dried blood rumen content mixture on carcass characteristics of SASSO C44 broiler chicks. *European Scientific Journal*, 12(12):166–181. <https://doi.org/10.19044/esj.2016.v12n12p166>

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

BIOCONVERSION OF CEREAL BY-PRODUCTS THROUGH *Pleurotus ostreatus* MEDIATED SOLID-STATE FERMENTATION OF THREE AGRO BY-PRODUCTS

Dennis AFRAM¹✉, Emmanuella Ekua Tweneboah GYAMFI¹, Jennifer VAN-ESS¹, Nene WILLIAMS¹, Victoria ATTOH-KOTOKU¹, Agyemang Felix ABOAGYE¹, Kenneth Gyimah OWUSU¹, Prince SASU¹, Benjamin ADJEI-MENSAH², and Armstrong DONKOH¹

¹Department of Animal Science, Faculty of Agriculture, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

²Department of Animal Science, University of Ghana, Legon, Accra, Ghana

✉Email: aframdennis1@gmail.com

Supporting Information



ABSTRACT: Solid-state fermentation (SSF) offers a promising approach to enhance the nutritional value of abundant but underutilized agro-industrial by-products in sub-Saharan Africa. The study investigated the effect of SSF using *Pleurotus ostreatus* on rice bran (RB), rice husk (RH) and maize cob (MC). The design used was a 3 × 3 factorial arrangement of treatments in a completely randomized design (CRD). The factors were three substrates (RB: rice bran, RH: rice husk, and MC: maize cob) and three fermentation periods (0, 30, 45 days). Unfermented and fermented samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash, crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). *In vitro* gas production (3-96 h) was measured using rumen liquor. *In vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), net energy (NE), short-chain fatty acids (SCFAs), and microbial protein (MP) were estimated using established models. Significant differences ($P < 0.05$) in CP and EE contents were observed among RB, RH, and MC within fermentation periods. The CP contents at days 30 and 45 were 9.44%, 8.10% for RB, 3.07%, 4.69% for RH, and 5.32%, 12.19% for MC, respectively. Fibre fractions varied among substrates at each fermentation period, with NDF differing among RB, RH, and MC, and ADL showing significant differences among substrates at day 30. Potential gas production (*b*) rose in MC (52.7 ml) and RH (53.9 ml) after 45 days. The rate of gas production (*c*) ranged from 0.03-0.08 h⁻¹ at day 0 and 30, but after 45 days, RH reached 0.08 h⁻¹, while RB and MC slowed to 0.01 h⁻¹. IVOMD, ME, NE, SCFA and MP were all higher in fermented RH and MC, with peak improvements at day 30. The study results indicated that SSF for 30-45 days could improve the nutritional value of agro-industrial by-products.

Keywords: Chemical composition, *In vitro* gas production, Maize cob, Rice bran, Rice husk, Oyster mushroom, Solid-state fermentation.

INTRODUCTION

The rise in global demand for sustainable livestock production has made researchers focus on developing nutritional feed resources from agricultural by-products. Multiple agricultural by-products, including rice bran, rice husk and maize cob, are abundant feed resources, but their use in ruminant diets is limited by low nutritional value and high lignocellulosic content (Omarini et al., 2019; Fidriyanto et al., 2020). The high fibre and lignin content of these by-products limits nutrient absorption and reduces digestibility, posing a challenge to ruminant production, particularly in regions where feed is scarce and conventional ingredients are expensive (Sufyan et al., 2021).

Rice bran, due to its fat and protein content, is prone to rancidity, whereas rice husk and maize cob are rich in cellulose, hemicellulose and lignin, resulting in low protein and energy availability (Omarini et al., 2019; Fidriyanto et al., 2020). The high crude protein content of rice bran makes it optimum for use, whereas the fibrous nature reduces the overall feed value of these by-products (Wang et al., 2012; Liu et al., 2023). In developing countries, limited resources constrain ruminant production, creating a need for cost-effective technologies to convert agricultural residues into high-quality animal feeds (Wang et al., 2023). To address this need, enhancing the nutritional value of agro-industrial wastes through microbial bioconversion has emerged as a promising approach, particularly via the biotechnological process of solid-state fermentation (SSF).

White-rot fungi, specifically *Pleurotus* species, exhibit high potential in biological transformations due to their filamentous growth (Eliopoulos et al., 2022). Their lignocellulolytic enzymes selectively degrade lignin, thereby releasing cellulose and hemicellulose for improved digestibility (Eliopoulos et al., 2022). Additionally, these fungi reduce anti-nutritional factors in raw substrates while contributing enzymes, mycelial biomass and bioactive compounds that enhance rumen fermentation (Wang et al., 2023).

RESEARCH ARTICLE
 PII: S222877012600012-16
 Received: November 06, 2025
 Revised: March 27, 2026
 Accepted: March 28, 2026

Previous research has demonstrated that solid-state fermentation (SSF) using *Pleurotus* species can significantly enhance the nutritional value of agricultural by-products. Fermentation of rice bran with *Pleurotus sapidus* significantly increased protein and reduced fiber fractions, enhancing ruminal digestibility (Omarini et al., 2019). Sufyan et al. (2021) demonstrated that applying solid-state fermentation (SSF) to cereal straws and corn by-products significantly enhanced crude protein concentration and improved *in vitro* digestibility characteristics. Solid-state fermentation (SSF) using *Pleurotus* species has been shown to degrade lignin and enhance protein content, improving digestibility and energy availability of agro-industrial residues (Eliopoulos et al., 2022). Although SSF has been applied to various cereal and corn by-products, limited information exists regarding its effects on rice bran, rice husk, and maize cob, particularly using *Pleurotus ostreatus*. Therefore, this study aims to evaluate the effect of SSF using *Pleurotus ostreatus* mycelia on the chemical composition and *in vitro* fermentation characteristics of these by-products.

MATERIALS AND METHODS

Location and climate of the experimental site

The study was carried out at the Nutrition and Pathology Laboratories of the Departments of Animal Science and Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The site lies in South Eastern Kumasi at 6° 43'N latitude, 1° 36'W longitude and an elevation of 261 m above sea level. The area experiences bimodal rainfall, with an annual mean of 1194 mm and average temperatures ranging from 22.3 °C to 31.9 °C.

Source of materials

The samples were obtained and randomly collected from vendors at the Zongo local market within the Ejisu community. The oyster mushroom for the solid-state fermentation was purchased from the Fresh Logistic Greenhouse, Mango Road, Kwame Nkrumah University of Science and Technology (KNUST).

Preparation of materials

Rice bran, rice husk and maize cob were air-dried under shade for two weeks to reduce moisture content and prevent nutrient loss. The rice bran, which had the 2mm particle size was packed after drying. The rice husk and maize cob after drying were milled to 2 mm sieve sizes using a laboratory Hammer Mill. Fresh oyster mushrooms (*Pleurotus ostreatus*) were surface sterilized with cotton wool moistened in 70% ethanol. The gills were gently opened to expose internal tissue, and a small piece of pileus was aseptically transferred to sterile potato dextrose agar (PDA) plates using sterile forceps. The plates were incubated at room temperature (25–28 °C) for 14 days until full mycelial growth was observed. This procedure ensured the establishment of a pure culture for subsequent solid-state fermentation experiments. Figures 1 and 2 show the experimental image used in the study.

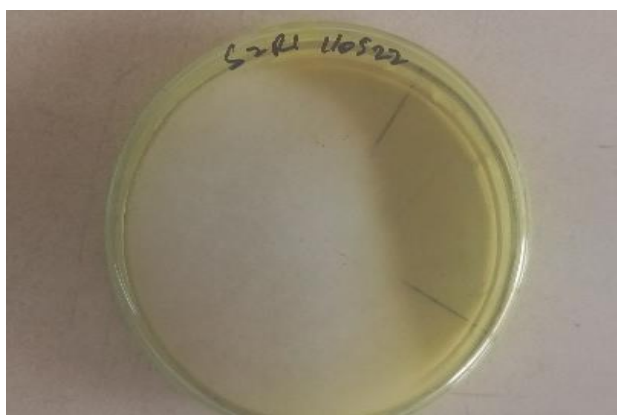


Figure 1 - Oyster mushroom culture



Figure 2 - Oyster mushroom

Experimental design

A 3×3 factorial arrangement in a completely randomised design (CRD) was used. The factors were three fermentation periods 0, 30 and 45 days and three by-products Rice husk, maize cob and rice bran. There were 9 treatments with three replications each. The treatments were Rice bran at day zero, day 30 and day 45 after fermentation; Rice husk at day zero, days 30 and 45 after fermentation and Maize cob at day zero, day 30 and day 45 after fermentation.

Chemical analyses

The samples underwent proximate analyses using the procedures of AOAC (1990) to determine dry matter (DM), crude protein (CP), ash, CF and ether extract (EE). The NDF, ADF and ADL contents were determined using the Sulfite method of Van Soest et al. (1991).

In vitro gas production

Rumen liquor was collected from three ewes (*Ovis aries*) slaughtered at the Kumasi Abattoir, Ghana. The animals were maintained on a grass diet and starved for 12 hours before slaughter. Ten minutes after slaughter and removal of the visceral organs, rumen liquor was collected into pre-warmed flasks, tightly sealed and promptly transported to the laboratory for analysis. *In vitro* gas production measurements were carried out using the method of [Menke and Steingass \(1988\)](#) in which 200 mg dried samples were incubated three times in a water bath at a temperature of 39 °C, with 20 ml of artificial saliva, bicarbonate buffer, macro- and micro- minerals, resazurin, a reducing solution and 10 ml of rumen liquor. The volume of the gas was measured as the displacement of the syringe plunger at 0, 3, 6, 12, 24, 48, 72, and 96 h of incubation. SigmaPlot for Windows (version 15.0) software ([Systat Software Inc., 2017](#)) was used to fit each gas reading into the model $Y = b(1 - e^{-ct})$.

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

In vitro organic matter digestibility (%), metabolisable (ME MJ/kg DM), net energy (NE, MJ/kg DM), short-chain fatty acids (SCFA), and microbial protein (MP) were also calculated using the stoichiometric equations presented by [Menke and Steingass \(1988\)](#) and [Close and Menke \(1986\)](#):

$$\text{ME (MJ/kg DM)} = (0.136 \text{ IVGP24}) + 0.057 \times \text{CP} + 2.20, \dots\dots\dots (1)$$

$$\text{NE (MJ/kg DM)} = [(0.149 \times \text{EE}) + (0.057 \times \text{CP}) + (0.0272 \times \text{IVGP24}) + 2.20] \div 14.64, \dots\dots\dots (2)$$

$$\text{MP} = \text{IVOMD} \times 1.93 \div 10, \dots\dots\dots (3)$$

$$\text{SCFA} = [-0.0222 \times \text{IVGP24 (ml/0.5g DM)}] \times 100, \dots\dots\dots (4)$$

$$\text{IVOMD (\%)} = [0.889 \text{ IVGP24} + 0.0651 \text{ Ash} + 0.45 \text{CP (\%DM)}] + 14.88, \dots\dots\dots (5)$$

Note: IVGP24 is the volume of gas produced after 24 hours of incubation.

Statistical analyses

The data were analyzed using a completely randomized design and subjected to analysis of variance (ANOVA). The Statistical Package for Social Scientists (SPSS), version 20 ([IBM Corp., 2011](#)), was used, and mean separation was performed using Tukey Pairwise comparison at a 5% threshold for declaring significance. SigmaPlot for Windows (version 15.0) software ([Systat Software Inc., 2017](#)) was used to fit each gas reading into the model $Y = b(1 - e^{-ct})$. Gas kinetics were fitted to an exponential model to estimate potential gas production (b) and rate (c). Graphs were generated using Microsoft Excel 2021 ([Microsoft Corporation, Redmond, WA, USA](#)).

Although the experimental design followed a 3 × 3 factorial arrangement (feed type × fermentation period), the data were analyzed separately for each fermentation time using one-way analysis of variance (ANOVA). This analytical approach was adopted to assess the effect of feed type within each fermentation period independently. Therefore, interactions between feed type and fermentation period were not statistically evaluated, and the results should be interpreted accordingly.

RESULTS AND DISCUSSION

Proximate and fibre composition of the three feed substrates before and after fermentation

Table 1 shows the proximate and fibre composition of rice bran, rice husk, and maize cob at different fermentation periods.

Significant differences ($P < 0.05$) in chemical composition were observed among substrates within fermentation periods, except for acid detergent lignin (ADL) at day 0, crude fibre (CF) at day 30, and ADL at day 45, where differences were not significant. Crude protein (CP) and ether extract (EE) contents differed significantly among substrates at days 30 and 45. Dry matter (DM), ash, CF, neutral detergent fibre (NDF), acid detergent fibre (ADF), and ADL also showed significant variation ($P < 0.05$) among substrates within fermentation periods, indicating differential modification of these components among RB, RH, and MC during fermentation.

The CP content of RB increased from 7.07% at day 0 to 9.44% at day 30, before slightly declining to 8.10% at day 45. This aligns with [Christ-Ribeiro et al. \(2017\)](#), who reported increased CP in rice bran after fermentation with *Rhizopus oryzae*, though differences in fungal strains and fermentation duration may explain variations. Maize cob showed a significant decrease in CF after fermentation, consistent with findings of [Akinfemi \(2010\)](#), [Abdel-Aziz et al. \(2015\)](#), and [Ibhaze et al. \(2022\)](#). The CP content of Rice husk increased from 2.25% to 4.69% by day 45, with concomitant decreases in CF, NDF, ADF and ADL, reflecting trends reported by [Dairo et al. \(2017\)](#) and [Sjofjan et al. \(2020\)](#). Maize cob CP content increased from 4.45% at day 0 to 12.19% at day 45, in agreement with [Ibhaze et al. \(2022\)](#). Variations in literature are attributed to differences in fermentation period, microbial strains, and initial substrate composition ([Zaid and Oyedokun Ganiyat, 2009](#); [Oliveira et al., 2010](#); [Omarini et al., 2019](#)). Overall, the fermentation process enhanced the nutritional quality of all three substrates, particularly rice husk and maize cob, by increasing CP and EE and reducing fiber fractions. These changes suggest improved digestibility and potential suitability as ruminant feed, consistent with previous studies ([Belewu & Babalola, 2009](#); [Adamafio et al., 2010](#); [Akinfemi, 2010](#); [Yafetto et al., 2023](#)).

Table 1 - Proximate and fibre composition of the three feed substrates at different fermentation periods.

PARAMETERS	DAY 0			SEM	P-value	DAY 30			SEM	P-value	DAY 45			SEM	P-value
	CRB	CRH	CMC			RB:30	RH:30	MC:30			RB:45	RH:45	MC:45		
DM (%)	85.30 ^{ab}	90.06 ^a	80.76 ^b	1.82	0.01	81.81 ^a	80.98 ^a	71.25 ^b	2.19	0.01	78.94 ^a	73.55 ^b	67.47 ^c	2.01	<0.001
EE (%)	6.90 ^{ab}	8.39 ^a	5.21 ^b	0.62	0.02	8.55 ^b	11.25 ^a	8.70 ^b	0.59	0.04	15.39 ^a	13.76 ^a	7.65 ^b	1.47	<0.001
ASH (%)	12.82 ^b	18.39 ^a	2.11 ^c	3.01	0.00	11.74 ^b	16.29 ^a	1.81 ^c	2.71	0.00	12.25 ^b	17.14 ^a	1.16 ^c	3.02	<0.001
CF (%)	25.26 ^a	27.04 ^a	30.78 ^b	1.07	0.01	20.51	21.25	20.21	0.35	0.60	9.06 ^c	10.08 ^b	12.66 ^a	0.67	<0.001
CP (%)	7.07 ^a	2.25 ^c	4.45 ^b	0.89	0.01	9.44 ^a	3.07 ^c	5.32 ^b	1.18	0	8.10 ^b	4.69 ^c	12.19 ^a	1.01	<0.001
NDF (%)	48.07 ^b	49.49 ^b	84.72 ^a	7.57	0	38.26 ^b	41.40 ^b	61.53 ^a	4.61	0	46.26 ^a	30.74 ^b	26.30 ^c	3.87	<0.001
ADF (%)	32.01 ^b	31.43 ^b	42.55 ^a	2.32	0	20.23 ^b	21.84 ^b	39.81 ^a	4.01	0	32.00 ^b	41.76 ^a	22.28 ^c	3.55	<0.001
ADL (%)	4.63	4.28	4.00	0.23	0.61	2.38 ^b	2.22 ^b	3.60 ^a	0.28	0.01	1.40	1.60	1.80	0.14	0.62

^{abcd}: Means in the same row with different superscripts are significantly different (P<0.05); NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; ADL = Acid Detergent Lignin, CRB: Rice bran at day zero; CRH: Rice husk at day zero; CMC: Maize cob at day zero; 30RB: Rice bran at 30 days fermentation; 30RH: Rice husk at 30 days fermentation; 30MC: Maize cob at 30 days fermentation; 45RB: Rice bran at 45 days fermentation ; 45RH: Rice husk at 45 days fermentation; 45MC: Maize cob at 45 fermentation

Effects of fermentation periods on the gas production profile of the substrates

Figure 1 illustrates the cumulative gas production for the substrates at 0, 30, and 45 days of fermentation. Rice bran consistently produced the highest gas volumes at 0 days, indicating higher digestibility of unfermented material. Rice husk showed the lowest gas production, reflecting its high lignin and silica content. Maize cob demonstrated intermediate values.

After 30 days of fermentation, gas production patterns shifted: maize cob and rice husk showed improved cumulative gas production, while rice bran decreased slightly, likely due to depletion of fermentable carbohydrates by fungal activity. The high rate of gas production within 60 to 96 hours, from 4.75 ml to 17.5 ml, indicates that the structure of rice husk might have changed during SSF treatment with enhanced accessibility to the microbes present in the rumen. This improvement corresponded with the results of [Tuyen et al. \(2013\)](#), who established that fungal treatment of rice by-products enhances the ability to cleave lignocellulosic bonds, thereby increasing fermentable carbohydrate yield. The improvements in the cumulative gas production of maize cob are consistent with the work done by [Akinfemi et al. \(2010\)](#), who reported enhanced *in vitro* digestibility of maize cob after fungal pre-treatment. By 45 days, maize cob exhibited the largest increase in gas production (up to 53.03%), suggesting substantial lignocellulosic breakdown and improved fermentability. Rice husk showed moderate improvement (16.10%), and rice bran a smaller increase (21.45%).

Compared with the 30-day fermentation, the 45-day fermentation of rice husk exhibited a completely different pattern. The gas production started slowly at the beginning, but gradually increased over the 96 hours. The increase in gas production from 12 hours to 24 hours ranged from 2.75 ml to 5.25 ml, and this indicates that the longer fermentation time has produced a substrate that is highly fermentable and the rumen microbes have adapted to. The sustained high rate of gas production up to 96 hours suggests that the 45 days of fermentation have converted rice husk into a slowly degradable yet sustained fermentable substrate, which is in support of the findings by [Tuyen et al. \(2013\)](#), who stated that increased fungal treatment of rice straw resulted in enhanced degradation of cell wall components.

The results clearly indicate that a 45-day fermentation period enhanced the digestibility of maize cob, as evidenced by higher gas production compared to both the control and the 30-day fermentation. Gas production at 6 hours (3.25 ml) was equivalent to that of the control at 12 hours, suggesting that fermentation made nutrients more readily available for rapid microbial utilization. This observation is consistent with [Akinfemi et al. \(2010\)](#), who reported higher soluble carbohydrate content in fungal-treated maize cob.

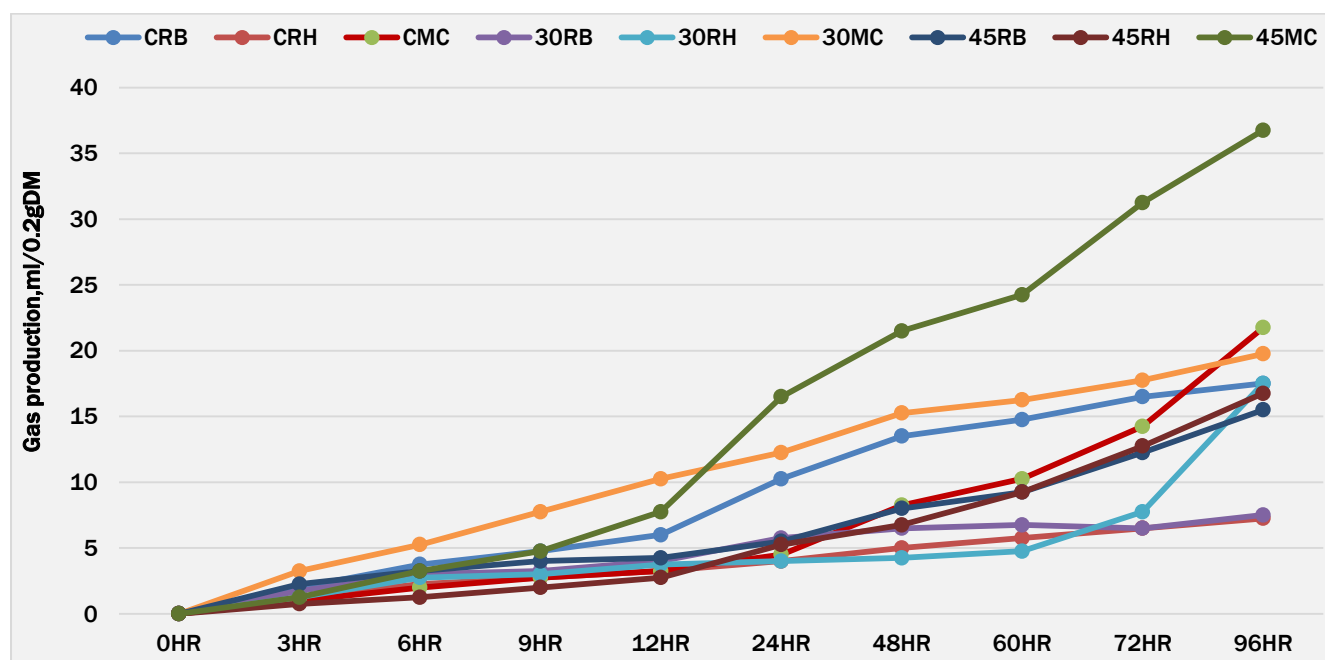


Figure 3 - In vitro gas production of the rice bran, rice husk and maize cob at 0, 30 and 45 days of fermentation. Data points represent mean volumes (ml) of triplicate samples per 0.2 g DM. CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation; CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation; 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

Gas production kinetics of the substrates at different fermentation periods

Results of gas production kinetics of the feed types over the fermentation days are shown in Table 2. Kinetics of gas production describe the amount of gas that a material produces (b), and 'c' is the rate at which these gases are produced. These parameters show how digestible a feeding material is and how long it would remain in the gut ([Osafo et al., 2023](#)). The results indicate that at day 0, Maize cob (MC) had the highest gas production (19.55%), suggesting it contains a higher proportion of fermentable carbohydrates. Rice husk (RH) had the lowest (6.44%), likely due to its high

lignin and silica content, which limits microbial degradability. After the 30 days of fermentation, maize cob recorded 17.81%, and rice husk value was 16.21%, showing significant improvement in gas production, likely due to the breakdown of lignocellulosic structures by fungi. After 45 days of fermentation, the Maize cob recorded a value of 53.03%, which had an extremely high increase, indicating enhanced delignification and availability of fermentable substrates. Rice husk also recorded 16.10%, showing improvement, but remained lower, probably due to persistent high fiber content. Thus, gas production potential (b) significantly increased ($P < 0.05$) for all substrates after 45 days of fermentation, particularly for maize cob, which showed a marked increase from 19.55% to 53.03%. Such an enhancement suggests that the fermentative treatment substantially improves the availability of fermentable carbohydrates and overall substrate digestibility.

This observation is consistent with reports demonstrating improved substrate breakdown through microbial action during extended fermentation periods (Asiegbu et al., 1994). In the case of rice bran, a moderate improvement from 17.68% to 21.44% in gas potential was observed, which aligns with studies showing that fermentation can enhance the nutritional quality and digestibility of rice bran by modifying its carbohydrate profile and reducing anti-nutritional factors (Widiyastuti et al., 2019). Conversely, rice husk, which initially had a lower gas production potential (6.44%), exhibited an increase to 16.10% following longer fermentation. This improvement is attributable to the microbial degradation of lignocellulosic components, rendering the substrate more accessible to ruminal microbes (Fapohunda et al., 2013).

The rate of gas production ($c \text{ hr}^{-1}$) showed no significant differences among most treatments, indicating that the fungal fermentation primarily influenced the extent rather than the speed of fermentation. From Table 2, it can be observed that rice bran's rate decreased from 0.03 hr^{-1} to 0.01 hr^{-1} after extended fermentation. This reduction likely reflects a shift from rapid fermentation of readily available carbohydrates to a slower fermentation phase as the easily fermentable components are depleted, a phenomenon noted in kinetic studies of ruminal fermentation (Widiyastuti et al., 2019). For rice husk, an increase in the rate from 0.03 hr^{-1} to 0.08 hr^{-1} was observed, indicating that fermentation may have enhanced the accessibility of fiber constituents, thereby accelerating initial microbial action (Fapohunda et al., 2013). In contrast, maize cob's rate decreased significantly from 0.08 hr^{-1} to 0.01 hr^{-1} , suggesting that while the overall extent of fermentation was improved (as shown by the increased gas production potential), the fermentation kinetics shifted toward a slower phase once the rapidly fermentable fractions were utilized (Asiegbu et al., 1994).

Estimated digestibility and energy parameters of the substrates at different fermentation periods

Table 3 shows the results of the *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), net energy (NE), short-chain fatty acids (SCFA) and microbial protein (MP) values for the substrates. Rice bran (RB) at day zero had the highest digestibility and metabolizable energy values (IVOMD, ME) as well as SCFA and MP yield. Rice husk (RH) and maize cob (MC) had similar values for IVOMD, ME, NE and MP. After solid-state fermentation (SSF) with *Pleurotus ostreatus*, maize cob showed the largest gains: IVOMD significantly increased from 21.01 at day zero to 34.74 g/kg DM by day 45 and the energy values (ME and NE) also showed a substantial improvement. In contrast, RB's digestibility and energy slightly decline with SSF, while RH shows modest improvement over longer fermentation.

A study by Espinosa-Páez et al. (2017) confirms that SSF using *Pleurotus ostreatus* tends to increase crude protein and metabolizable energy but can reduce fiber and even lower digestibility if excessive. The present study was consistent with the study by Nasehi et al. (2016), which found that SSF of crop by-products (including rice husk) raised CP, ash, ME, and NEL in all substrates; however, IVOMD and SCFA gains were lower for rice husk (there was no significant change in husk digestibility or SCFA). Conversely, according to Ikwunze et al. (2024) *Pleurotus*-treated maize cob significantly improved OMD and SCFA, with OMD and fermentable fraction increasing as the number of days increased. These findings align with the present study, which showed that the digestibility (IVOMD) and energy contents of MC improved significantly after SSF using *Pleurotus ostreatus*. The MP values increased from 4.15 at day zero to 6.79 at day 45.

Rice bran at day zero showed the highest IVOMD and ME values of 27.82 g/kg and 3.98 MJ/kg, respectively, but these declined to 24.4 g/kg and 3.57 MJ/kg at day 45. This reduction aligns with the study of Sánchez et al. (2002) who explained that prolonged fermentation periods can result in a reduction of fat and fiber fractions, alongside notable increases in protein and ash content. Thus, the RB fermented in the current study had more fungal protein but lost some soluble nutrients, lowering its IVOMD and ME (Ibarruri et al., 2021). Rice bran had the lowest IVOMD value of 20.74 % at day zero due to the presence of lignin and silica. The IVOMD value increased to 22.89 at day 45. The SCFA content also followed the same trend. Studies by Nasehi et al. (2016) using *Pleurotus florida* for solid-state fermentation on rice husk led to similar results with no substantial improvement in rice husk digestibility and SCFA production. The total metabolic energy of rice husk increased slightly from 2.91 to 3.32 MJ/kg during the fermentation process.

The ME content 2.99 MJ/kg of MC at day zero increased to 5.03 MJ/kg at day 45. Sufyan et al. (2021) screened *P. ostreatus*, *Pleurotus eryngii* and *Pleurotus florida* using corn cob and found that *Pleurotus florida* gave the largest lignin degradation and digestibility. Ikwunze et al. (2024) in their research found that *Pleurotus tuber-regium*-treated maize cob significantly increased *in vitro* digestibility, gas production, SCFA and metabolizable energy as fungal content increased. Akinfemi (2010) similarly reported *Pleurotus ostreatus* raised maize cob CP (3.89 to 10.11%) and OMD (control 48.32% to 60.75%), with ME up to 8.59 MJ/kg.

Table 2a - Gas production kinetics of the substrates at different fermentation periods.

Parameters	Feed substrate (Day 0)			SEM	P-values	30 days fermentation			SEM	P-Values
	CRB	CRH	CMC			RB:30	RH:30	MC:30		
<i>b</i> (%)	17.68 ^a	6.44 ^b	19.55 ^a	2.60	<0.001	6.87 ^b	16.21 ^a	17.81 ^a	2.16	<0.001
<i>c</i> (hr ⁻¹)	0.03	0.03	0.08	0.029	0.07	0.05	0.07	0.06	0.01	0.78

^{abcd} Means found in the same row with different superscripts are significantly different (P<0.05); CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation, CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation); 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

Table 2b - Gas production kinetics of the substrates at different fermentation periods.

Variables	45 days fermentation			SEM	P-Values	30 and 45 fermentations (Interaction)						SEM	P-Values
	RB:45	RH:45	MC:45			RB:30	RH:30	MC:30	RB:45	RH:45	MC:45		
<i>b</i> (%)	21.45 ^b	16.10 ^b	53.03 ^a	7.38	0.004	6.87 ^c	16.21 ^b	17.81 ^b	21.45 ^b	16.10 ^b	53.03 ^a	4.43	<0.001
<i>c</i> (hr ⁻¹)	0.01 ^b	0.08 ^a	0.01 ^b	0.01	0.001	0.05	0.07	0.06	0.01	0.08	0.01	0.01	0.07

^{abcd} Means found in the same row with different superscripts are significantly different (P<0.05); CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation, CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation); 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

Table 3a - Estimated digestibility and energy parameters of the substrates at different fermentation periods.

Parameters	Feed substrate (Day 0)			SEM	P-values	30 days fermentation			SEM	P-Values
	CRB	CRH	CMC			RB:30	RH:30	MC:30		
IVOMD- (%)	27.82 ^a	20.74 ^b	21.01 ^b	1.47	<0.001	25.15 ^a	20.94 ^c	22.98 ^b	0.77	<0.001
ME (MJ/kg DM)	3.98 ^a	2.91 ^b	2.99 ^b	0.22	0.001	3.61	2.99	3.51	0.13	0.055
NE (MJ/kg DM)	3.00 ^a	2.62 ^a	2.57 ^a	0.09	0.047	3.05	2.70	2.83	0.07	1.117
SCFA	21.85 ^a	8.55 ^b	9.79 ^b	2.68	<0.001	12.38 ^a	8.59 ^b	13.60 ^a	0.96	<0.001
MP	5.49 ^a	4.10 ^b	4.15 ^b	0.29	0.004	4.92 ^a	4.13 ^{ab}	4.58 ^a	0.15	0.035

^{abcd} Means found in the same row with different superscripts are significantly different (P<0.05); IVOMD: *In vitro* organic matter digestibility, MP: Microbial protein, ME: metabolizable energy, SCFA: Short chain fatty acids, NE: Net energy; CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation, CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation; 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

Table 3b - Estimated digestibility and energy parameters of the substrates at different fermentation periods.

Variables	45 days fermentation			SEM	P-Values	30 and 45 fermentations (Interaction)						SEM	P-Values
	RB:45	RH:45	MC:45			RB:30	RH:30	MC:30	RB:45	RH:45	MC:45		
IVOMD (g/kg DM)	24.34 ^b	22.89 ^b	34.74 ^a	2.36	<0.001	25.15 ^b	20.9 ^e	22.98 ^d	24.3 ^c	22.89 ^d	34.74 ^a	1.35	<0.001
ME (MJ/kg DM)	3.57 ^b	3.32 ^b	5.03 ^a	0.34	0.004	3.61 ^b	2.99 ^b	3.51 ^b	3.57	3.32 ^b	5.03 ^a	0.19	<0.001
NE (MJ/kg DM)	3.15	2.84	3.60	0.16	0.091	3.05 ^a	2.70 ^b	2.83 ^b	3.15 ^a	2.84 ^b	3.60 ^a	0.09	0.019
SCFA	11.87 ^b	11.37 ^b	35.25 ^a	4.98	<0.001	12.38 ^c	8.59 ^e	13.60 ^b	11.87 ^{cd}	11.37 ^d	35.25 ^a	2.70	<0.001
MP	4.77 ^b	4.55 ^b	6.79 ^a	0.45	0.002	4.92 ^b	4.13 ^c	4.58 ^b	4.77 ^b	4.55 ^b	6.79 ^a	0.26	<0.001

^{abcd} Means found in the same row with different superscripts are significantly different (P<0.05); IVOMD: *In vitro* organic matter digestibility, MP: Microbial protein, ME: metabolizable energy, SCFA: Short chain fatty acids, NE: Net energy; CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation, CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation; 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

CONCLUSION AND RECOMMENDATION

Solid-state fermentation (SSF) using *Pleurotus ostreatus* improved the nutritional quality and *in vitro* fermentation characteristics of rice bran, rice husk, and maize cob. The process improved crude protein content and reduced the content of the detergent fibre fractions, resulting in enhanced fermentability and *in vitro* digestibility, especially in maize cob and rice husk. Upon treatment, *In vitro* organic matter digestibility, metabolizable energy, short-chain fatty acids, Net energy, and Microbial protein were also improved. The study confirms that SSF using *Pleurotus ostreatus* is a promising technique for upgrading low-quality agro-by-products into more digestible and nutritionally valuable ruminant feed resources.

The findings of this study should be interpreted considering the analytical limitations. Improvements observed in nutritional and fermentation characteristics should be specified according to the particular substrate and fermentation period in which they occurred. Future research should employ a factorial analysis to investigate potential interactions between feed type and fermentation duration, which would provide a more comprehensive understanding of their combined effects. In addition, *in vivo* trials with ruminant animals are recommended to validate the *in vitro* results and to evaluate animal performance indicators such as feed intake, weight gain, milk yield, and feed conversion efficiency.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dennis Afram; E-mail: aframdennis1@gmail.com; ORCID: 0009-0000-4421-4877

Data availability

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

Authors' contribution

D. AFRAM contributed to the design of the experiment, data collection, statistical analyses and interpretations, and the writing of the manuscript. V. ATTOH-KOTOKU made significant contributions to the conceptualization and experimental design, was involved in the interpretation of results, contributed to both drafting and revision of the manuscript, and supervised the study. E.E.T.GYAMFI participated in data collection, interpretation of results and manuscript drafting. J.VAN-ESS participated in data collection, interpretation of results and manuscript drafting. N.WILLIAMS participated in data collection, interpretation of results and manuscript drafting. A.F.ABOAGYE participated in data collection, interpretation of results and manuscript drafting. N.WILLIAMS participated in data collection, interpretation of results and manuscript drafting. A. DONKOH was involved in the interpretation of results and the manuscript preparation. P. SASU participated in the interpretation of results and facilitated the drafting and revision of the manuscript. K.G. OWUSU engaged in data collection, statistical analysis, and manuscript drafting. B.ADJEI-MENSAH participated in the interpretation of results and the drafting of the manuscript.

Funding

The authors declare that no funds, grants, or other support were received during the preparation or publication of this manuscript.

Competing interests

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

REFERENCES

- Abdel-Aziz NA, Salem AZ, El-Adawy MM, Camacho LM, Kholif AE, Elghandour MM, et al. (2015). Biological treatments as a mean to improve feed utilization in agriculture animals—An overview. *Journal of Integrative Agriculture*, 14(3):534-543. [https://doi.org/10.1016/S2095-3119\(14\)60829-7](https://doi.org/10.1016/S2095-3119(14)60829-7)
- Adamafio N, Obodai M, and Brimpong B (2010). Solid state fermentation of maize (*Zea mays*) cob by *Pleurotus ostreatus* strain EM-1: Biopolymer profiles and cellulose degradability. *International Journal of Biological and Chemical Sciences*, 3(6). <https://doi.org/10.4314/ijbcs.v3i6.53169>
- Akinfemi A (2010). Nutritive value and *in vitro* gas production of fungal-treated maize cobs. *African Journal of Food, Agriculture, Nutrition and Development*, 10(8). <https://doi.org/10.4314/ajfand.v10i8.60878>
- Animal Research Ethics Committee (AREC) (2018). Standard operating procedures (pp. 21–31). Quality Assurance and Planning Unit, Kwame Nkrumah University of Science and Technology
- AOAC (1990). Official methods of analysis of the Association of Official Analytical Chemists (16th ed.). AOAC.

- Asiegbu F, Morrison I, Paterson A, and Smith J (1994). Analyses of fungal fermentation of lignocellulosic substrates using continuous culture rumen simulation. *Journal of General and Applied Microbiology*, 40(4):305-318. <https://doi.org/10.2323/jgam.40.305>
- Belew MA, and Babalola FT (2009). Nutrient enrichment of some waste agricultural residues after solid-state fermentation using *Rhizopus oligosporus*. *Journal of Applied Biosciences*, 13:695-699. <https://www.cabidigitallibrary.org/doi/full/10.5555/20093214461>
- Christ-Ribeiro A, Graça CDS, Chiattoni LM, Massarolo KC, Duarte FA, Mellado MDLS, et al. (2017). Fermentation process in the availability of nutrients in rice bran. *Research & Reviews: Journal of Microbiology and Biotechnology*, 6(2):45-52. <https://www.academia.edu/download/93875135/fermentation-process-in-the-availability-of-nutrients-in-rice-bran.pdf>
- Close WH, and Menke KH (1986). Evaluation on the basis of metabolizable energy: a manual prepared for the 3rd Hohenheim course on animal nutrition in the tropics and semi-tropics (2nd ed., pp. 60-63). Feldafing, Germany.
- Dairo AY, Ogunlade SW, and Oluwasola T (2017). Proximate composition and amino acid profile of rice husk biodegraded with *Pleurotus ostreatus* for different periods. *African Journal of Food, Agriculture, Nutrition and Development*, 17(3):12243-12255. <https://doi.org/10.18697/ajfand.79.16235>
- Eliopoulos C, Márkou G, Chorianopoulos N, Haroutounian S, and Arapoglou D (2022). Preliminary research concerning the enrichment of industrial hemp extract residues via solid-state fermentation with *Pleurotus ostreatus*. *Applied Sciences*, 12(5):2376. <https://doi.org/10.3390/app12052376>
- Espinosa-Páez E, Alanis-Guzmán M, Hernández-Luna C, Báez-González J, Amaya-Guerra C, and Andrés-Grau A (2017). Increasing antioxidant activity and protein digestibility in *Phaseolus vulgaris* and *Avena sativa* by fermentation with the *Pleurotus ostreatus* fungus. *Molecules*, 22(12):2275. <https://doi.org/10.3390/molecules22122275>
- Fapohunda SO, Ganiyu OT, Fapohunda T, Ajose A, and Murtala M (2013). Comparative responses of food-borne *Aspergillus niger* JX 442527 and *Penicillium minioluteum* JX 442528 to selected plant extracts. *Greener Journal of Biological Sciences*, 3(5):192-201. <https://doi.org/10.15580/gjbs.2013.5.052813644>
- Fidriyanto R, Ridwan R, Astuti W, Rohmatussolihat R, Sari N, Watman M, et al. (2020). *In vitro* ruminal fermentation and degradability of rice husk on rice bran substitution. *Annales Bogorienses*, 24(1):50-58. <https://doi.org/10.14203/ann.bogor.2020.v24.n1.50-58>
- Ibarruri J, Goiri I, Cebrián M, and García-Rodríguez A (2021). Solid-state fermentation as a tool to stabilize and improve nutritive value of fruit and vegetable discards: Effect on nutritional composition, *in vitro* ruminal fermentation, and organic matter digestibility. *Animals*, 11(6):1653. <https://doi.org/10.3390/ani11061653>
- Ibhaze GA, Ogumjemite G, Fajemisin AN, and Usif B (2022). Nutritional quality of treated maize cob and husk in a complete diet by West African dwarf goats. *Journal of Global Agriculture and Ecology*, 14(3):1-7. <https://doi.org/10.56557/jogae/2022/v14i37628>
- IBM Corp. (2011). IBM SPSS Statistics for Windows (Version 20.0) [Computer software]. IBM Corp.
- Ikwunze K, Jiwuba PDC, Ekanem NJ, Ahamefule FO, and Okah U (2024). Evaluation of nutritive value of *Pleurotus tuber-regium* biodegraded maize cob using *in vitro* gas production and fermentation characteristics. *International Journal of Life Science and Agriculture Research*, 3(7):614-619. <https://doi.org/10.5281/zenodo.12799199>
- Liu Z, Liu H, Ma Z, and Guan T (2023). Phytosterols in rice bran and their health benefits. *Frontiers in Nutrition*, 10:1287405. <https://doi.org/10.3389/fnut.2023.1287405>
- Menke KH, and Steingass H (1988). Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Animal Research and Development*, 28:7-55.
- Microsoft Corporation (2021). Microsoft Excel (Version 2021) [Computer software]. Microsoft Corporation.
- Nasehi M, Torbatinejad NM, Zerehdaran S, and Safaie AR (2016). Effect of solid-state fermentation by oyster mushroom (*Pleurotus florida*) on nutritive value of some agro-by-products. *Journal of Applied Animal Research*, 45(1):221-226. <https://doi.org/10.1080/09712119.2016.1150850>
- Oliveira MS, Feddern V, Kupski L, Cipolatti EP, Badiale-Furlong E, and de Souza-Soares LA (2010). Physico-chemical characterization of fermented rice bran biomass. *CyTA - Journal of Food*, 8(3):229-236. <https://doi.org/10.1080/19476330903450274>
- Omarini AB, Labuckas D, Zunino MP, Pizzolitto R, Fernández-Lahore M, Barrionuevo D, et al. (2019). Upgrading the nutritional value of rice bran by solid-state fermentation with *Pleurotus sapidus*. *Fermentation*, 5(2):44. <https://doi.org/10.3390/fermentation5020044>
- Osafo ELK, Osman A, Attoh-Kotoku V, Antwi C, Abdul Aziz Y, and Idan F (2023). Chemical composition and *in vitro* gas production of *Brachiaria decumbens* harvested at different stages of growth in the hot humid region. *Online Journal of Animal and Feed Research*, 13(4):302-312. <https://doi.org/10.51227/ojafr.2023.45>
- Sánchez A, Ysunza F, Beltrán-García MJ, and Esqueda M (2002). Biodegradation of viticulture wastes by *Pleurotus*: a source of microbial and human food and its potential use in animal feeding. *Journal of Agricultural and Food Chemistry*, 50(9):2537-2542. <https://doi.org/10.1021/jf011308s>
- Sjofjan O, Nur Adli D, Djunaidi I, and Kuncoro K (2020). Utilization of biogas liquid waste for starter in the fermentation of rice husk as a potential feed for poultry. *Animal Production*, 22(1):24-30. <https://doi.org/10.20884/1.jap.2020.22.1.38>
- Sufyan A, Ahmad N, Shahzad F, Embaby MG, AbuGhazaleh A, and Khan NA (2021). Improving the nutritional value and digestibility of wheat straw, rice straw, and corn cob through solid-state fermentation using different *Pleurotus* species. *Journal of the Science of Food and Agriculture*, 102(6):2445-2453. <https://doi.org/10.1002/jsfa.11584>

Systat Software Inc. (2017). SigmaPlot for Windows (Version 15) [Computer software].

Tuyen DV, Phuong HN, Cone JW, Baars JJ, Sonnenberg AS, and Hendriks WH (2013). Effect of fungal treatments of fibrous agricultural by-products on chemical composition and *in vitro* rumen fermentation and methane production. *Bioresource Technology*, 129:256-263. <https://doi.org/10.1016/j.biortech.2012.10.128>

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

Wang Y, Gou C, Chen L, Liao Y, Zhang H, Luo L, et al. (2023). Solid-state fermentation with white rot fungi (*Pleurotus* species) improves the chemical composition of highland barley straw as a ruminant feed and enhances *in vitro* rumen digestibility. *Journal of Fungi*, 9(12):1156. <https://doi.org/10.3390/jof9121156>

Wang Y, Xin HS, Li Y, Zhang WW, Xia K, Wang ZB, et al. (2012). The effects of different processing methods on the estimated nutritional value of rice bran according to the NRC-2001 model or DVE/OEB system. *Journal of Animal and Feed Sciences*, 21(3):503-520. <https://doi.org/10.22358/jafs/66125/2012>

Widiyastuti T, Prayitno C, Munasik M, Rahayu S, and Muatip K (2019). Characteristics, digestibility and rumen fermentation products of rice bran from various varieties of rice. *IOP Conference Series: Earth and Environmental Science*, 372(1):012065. <https://doi.org/10.1088/1755-1315/372/1/012065>

Yafetto L, Odamtten GT, and Wiafe-Kwagyan M (2023). Valorization of agro-industrial wastes into animal feed through microbial fermentation: A review of the global and Ghanaian case. *Heliyon*, 9(4): e14814. <https://doi.org/10.1016/j.heliyon.2023.e14814>

Zaid AA, and Oyedokun G (2009). Comparative utilization of biodegraded and undegraded rice husk in *Clarias gariepinus* diet. *African Journal of Biotechnology*, 8(7):1358-1362. <https://www.ajol.info/index.php/ajb/article/download/60120/48376/0>

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

EFFECT OF *Nigella sativa* OIL ON LIVER FUNCTION AND LIPID PROFILE IN BROWN, ALBINO AND LOCAL RABBITS: A COMPARATIVE STUDY

Warvin Idrees HASAN¹ , Hoger Mardan HIDAYET² , Omar Hasan AZEEZ² , Sherwan Mostafa SEFDEEN³  , Cheya Ageed YATEEM⁴ , Kamal Noaman MUSTAFA¹ 

¹Department of Animal Production, College of Agricultural engineering Sciences, University of Duhok, Duhok, IRAQ

²Department of Pathology and Microbiology, College of Veterinary Medicine, University of Duhok, Duhok, IRAQ

³Department of Food Production and Technology, Technical College of Duhok, Duhok Polytechnic University, Duhok, IRAQ

⁴Ministry of Agricultural & Water Resources, General Directorate of Agriculture, Duhok, IRAQ

✉ Email: sherwan.seif@dpu.edu.krd

➤ Supporting Information



ABSTRACT: This study investigated the effects of *Nigella sativa* oil (NSO) on the metabolism of three rabbit breeds: Albino, Brown, and Local. A total of 48 rabbits were assigned to six groups and fed either a control diet or an NSO-supplemented diet (5 g/kg DM). Diets were alternated between groups every 15 days. Blood samples were analyzed for liver enzymes, kidney function parameters, lipid profile, thyroid hormones, and blood proteins. The results showed that NSO had no significant effect ($P > 0.05$) on blood glucose, liver enzymes, or lipid profile. However, numerical differences were observed among breeds. Albino rabbits showed higher total cholesterol 83.6% and low-density lipoprotein (LDL) cholesterol (177.6%) compared with the Local breed ($P \leq 0.01$). Brown rabbits exhibited higher aspartate aminotransferase (AST) activity (60-64%) than Albino rabbits ($P \leq 0.02$). Additionally, brown rabbits showed higher (51.2%) creatinine levels compared with Albinos ($P \leq 0.0001$). Uric acid levels slightly increased (15-18%) during the second treatment period. Protein levels remained relatively constant among groups; however, the brown breed had higher ($P \leq 0.02$) albumin levels (9%) than other breeds. In conclusion, rabbit breed had a greater influence on the measured parameters than dietary treatments which indicates that breed-specific genetic variations play an important role in such studies.

Keywords: Breeds, Liver function, Lipid profile, *Neglia sativa*, Rabbits.

INTRODUCTION

In recent years, there has been growing interest worldwide in the use of black seed oil (*N. sativa*) as a therapeutic agent due to its reported health benefits. These effects are attributed to its bioactive compounds, particularly thymoquinone and nigellone. These compounds may possess anti-inflammatory properties, reduce cellular oxidative damage, and support immune system function (Adam et al., 2022).

Researchers have investigated the effects of *Nigella Sativa* (NS) on various organs, especially the liver heart (Mat et al., 2011 and Safithri et al., 2023). Evidence suggests that dietary supplementation with *N. Sativa* can lower blood glucose levels in animals, possibly by enhancing insulin secretion and improving pancreatic function (Tiware et al., 2022 and Shaukat et al., 2023). The liver is a vital organ responsible for metabolism, detoxification of harmful substances, and synthesis of essential proteins (Ozougwu, 2017). Liver damage caused by exposure to chemicals or infections typically results in elevated levels of specific enzymes in the blood, which serve as indicators of hepatic injury (Guo et al., 2025). Enzymes such as AST, and alkaline phosphatase (ALP) are commonly used biomarkers of liver dysfunction (Al-Razzuqi et al., 2011).

Several studies have reported that dietary supplemented with NSO reduces AST and ALP levels in rats with fatty liver disease or chemically induced liver damage caused by carbon tetrachloride (Hashim et al., 2025). These findings suggest that NSO may help protect liver cells and improve overall liver function (Rounagh et al., 2024; Derosa et al., 2024). Elevated cholesterol and triglyceride levels are well-known risk factors for cardiovascular disease. NSO has been reported to reduce these risks (Alu'datt et al., 2024). Studies in humans and animals have shown that *N. sativa* can decrease low-density lipoprotein (LDL) and triglyceride levels while increasing high-density lipoprotein (HDL), possibly by enhancing lipid metabolism and fat breakdown in the body (Zhang et al., 2021). Different rabbit breeds, such as albino, brown, and local, exhibit distinct physiological characteristics that may influence their response to dietary supplementation with NS (Ye et al., 2021). Breed-specific growth responses have been reported with *N. Sativa* was used as a feed additive in rabbit diets (Al-Shaar, 2020). In addition to its anti-inflammatory and immunomodulatory properties, *N. Sativa* also exhibits strong

RESEARCH ARTICLE
 PII: S222877012600013-16
 Received: February 02, 2026
 Revised: March 27, 2026
 Accepted: March 28, 2026

antioxidant activity (Alberts et al., 2024), which may help protect the body against conditions such as liver inflammation and cardiovascular complications (Bhavikatti et al., 2024).

Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), have been shown to increase in activity following NS supplementation (Hannan et al., 2021; Aslani et al., 2024). Evidence also suggests that NSO may protect kidney function, as reduced levels of creatinine, urea, and uric acid have been reported in animals receiving diets supplemented with different levels of NS (Algheshairy et al., 2025). In rats with kidney injury, NS oil improved both circulating biochemical parameters and renal tissue structure, likely due to its antioxidant properties (Naderi et al., 2022; Al-Hamdany and Raoof, 2023). In rabbit diets, NS supplementation has been associated with increased total protein, albumin, and globulin levels, indicating improved liver function and enhanced immune support. These changes occurred without evidence of liver or kidney damage, suggesting its safety as a dietary supplement (Al-Asadi et al., 2025). Several studies have also examined the effects of NS oil on thyroid hormones. In rats with thyroid dysfunction, NSO increased triiodothyronine (T3) and thyroxine (T4) levels while reducing thyroid-stimulating hormone (TSH), suggesting that NSO may help regulate thyroid hormone balance in both hypothyroid and hyperthyroid conditions, indicating a modulatory rather than stimulatory effect (Elghareeb et al., 2024).

Therefore, the present study aimed to evaluate the potential protective effects of NSO on liver enzyme activity and selected blood biochemical parameters in three rabbit breeds. Additionally, the effects of NSO on kidney function markers (creatinine, urea, and uric acid) and thyroid hormones (T3 and T4) were investigated.

MATERIALS AND METHODS

Experimental Design: A total of 48 rabbits (16 Albino, 16 Brown, and 16 Local) with an initial body weight ranging from 4 to 8 kg were used in the present study. The rabbits were acclimatized for one week in cages located at the Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok prior to the start of the experiment. Each breed was equally divided into two dietary groups (n = 8 per group): Control group, fed basal diet, and Treatment group, fed basal diet supplemented with 5 g NSO/kg dry matter (DM). The experimental period lasted five weeks including a five-day dietary adaptation period (basal diet only) followed by two experimental phases of 15 days each.

Housing Conditions: All rabbit groups were housed in clean dry cages bedded with soil and maintained under identical environmental conditions. Animals were provided with free access to water source and adequate lighting throughout the experimental period (Trocino and Tolini 2024; Wang et al., 2025).

Feeding Routine: Each young rabbit fed 30 g/d standardized feed mixture. Feed ingredients and the chemical composition of the diets are given in Table 1. Feed samples were chemically analyzed according to the methods described by AOAC (2000). Rabbits were fed twice a daily. Adult rabbits received 15 - 20 g of diet per kg of body weight per day. Feeding times and quantities were kept consistent throughout the study to prevent digestive disturbances and nutritional disorders.

Table 1 - Feed ingredients and the chemical composition of the diets.

Feed Ingredients, g/kg	Basal diet	Basal diet + 5 g NSO	Chemical composition of the diets	Basal diet	Basal diet + 5 g NSO
Barley	400	400	Moisture (%)	4.07	6.26
Wheat bran	200	200	Ash (g/kg)	61.1	59.1
Soybean meal	200	200	CP (g/kg)	130.4	130.9
Corn	150	150	EE (g/kg)	54.2	60.1
Vitamins-minerals premix	20	20	CF (g/kg)	35.6	36.6
Lysine	10	10	NFE (g/kg)	678.0	650.7
Methionine	10	10	-	-	-
Iodized salt	10	10	-	-	-
<i>Nigella sativa</i> oil (g/kg DM)	-	5	-	-	-

NSO: *Nigella sativa* oil

Blood sample collection

Blood samples were collected from the jugular vein into plain glass tubes without anticoagulants three times during the study period (days 0, 15, and 30) following the second round. Blood samples were allowed to clot at room temperature for approximately 30- 60 minutes and were then centrifuged at 3000 rpm for 15 minutes. The serum was subsequently separated into a 2-mL Eppendorf tubes and sorted at -20°C until further analysis. Blood sampling and processing procedures were conducted in accordance with standard rabbit blood collection and serum preparation techniques described by Nelson et al. (2010).

Biochemical analysis

Biochemical parameters were measured using Biolabo standard kits (France) through colorimetric methods. The evaluated parameters included liver enzymes (ALT, AST), creatine kinase (CK), lipid profile markers (total cholesterol, LDL, HDL, and triglycerides), kidney function markers (creatinine, urea, and uric acid), protein profile indicators (total protein, albumin, and globulin), and thyroid hormones (T3 and T4).

Statistical analysis

Data were analyzed as a change-over design procedure using SAS software (SAS Institute Inc., 2016) to assess the effects of treatment, breed, and round on the measured blood parameters. Least squares means were compared using the Tukey–Kramer test, and statistical significance was set at $P \leq 0.05$.

RESULTS

A comprehensive evaluation of NSO supplementation at a dose 5gm/kg DM across three rabbit breeds (Albino, Brown, and Local) revealed distinct patterns in biochemical parameters over two experimental rounds. Effect of NS Oil on Blood Glucose: Blood glucose levels remained stable across all breeds and treatments throughout the period of the study. No significant differences were observed due to dietary treatment, breed, or experimental round ($P > 0.05$). In all groups, blood glucose concentrations fluctuated by less than 5% from baseline, with Albino rabbits showing particularly stable levels (variation $< 3\%$ between rounds) (Table 2).

Effect of NS Oil on lipid profile

The lipid profile of the rabbit breeds included in the present study varied considerably (Table 3). Albino rabbits consistently exhibited the highest total cholesterol levels compared with the other breeds. In the first round, cholesterol levels in Albino rabbits were approximately 35% higher than those of Brown rabbits ($P \leq 0.05$) and 84% higher than those of local rabbits ($P \leq 0.007$). The same pattern persisted in the second round with cholesterol concentrations remaining 42% and 45% higher than those of Local and Brown rabbits, respectively. Albino rabbits also showed markedly higher LDL cholesterol levels than the other two breeds. In round 1, LDL levels were 70% higher than those of Brown rabbits ($P \leq 0.05$) and approximately 178% higher than those of the Local rabbits ($P \leq 0.005$). Elevated LDL concentration in Albino rabbits remained persisted across rounds, remaining 60% and 65% higher than those of local rabbits and 21% and 69% higher than those of brown rabbit, respectively. However, neither breeds nor treatments produced statistically significant differences in HDL or triglyceride levels. Although a modest increase in HDL levels ($P \leq 0.08$) and a slight reduction in triglycerides ($P \leq 0.07$) were observed in the treatment group, these changes did not reach statistical significance. Furthermore, no significant interaction effects were detected overall.

Effect of NS Oil on liver enzymes

As shown in Table 4, Brown rabbits exhibited 60% higher AST activity in round 1 and 64% higher activity in round 2 compared with Albino rabbits ($P \leq 0.02$). Additionally, AST activity in Brown rabbits was 70.67% higher than those of local rabbits in round 1 ($P \leq 0.05$). A consistent pattern was observed across rounds, with local rabbits exhibiting AST values 50% higher in round 2 and 40% higher in round 2 compared with Albino rabbits ($P \leq 0.05$). When data from both rounds were combined, AST levels in Brown rabbits were 174.07% higher than those in Albino rabbits. A round effect was also observed, with AST values in round 1 being 24.38% higher than those in round 2 ($P = 0.045$). Although ALT showed no significant breed effect ($P = 0.06$) and no treatment or interaction effects, creatine kinase (CK) and LDH activities varied among breeds by up to 59%. However, no statistically significant differences attributable to treatment or breed were detected ($P > 0.05$).

Effect of NS Oil on kidney function

Analysis of renal parameters revealed clear breed differences (Table 5). In round 1, the creatinine levels in brown rabbits were approximately 51.16% higher than those of albino rabbits ($P \leq 0.0001$). Although the difference was not statistically significant, Brown rabbits also appeared to have higher creatinine levels than Local rabbits during round 1. In round 2, Brown rabbits exhibited significantly higher creatinine concentrations ($P \leq 0.05$) than both Albino and Local rabbits, exceeding their levels by 41.57% and 57.36%, respectively. Local rabbits also showed significantly higher creatinine levels than Albino rabbits in both rounds (25.33% in round 1 and 21.56% in round 2; $P \leq 0.05$), indicating a consistent inter-breed trend in creatinine clearance. A continuous inter-breed trend in creatinine clearance was observed when local rabbit's creatinine was significantly higher ($P \leq 0.05$) than those of Albino rabbits in both rounds (25.33% and 21.56% in round 1 and 2, respectively). Urea concentrations in Local rabbits were significantly higher than those in Albino rabbits during round 1 by 15.6% ($P \leq 0.05$). In round 2, both Brown and Local rabbits showed higher urea levels compared with Albino rabbits (25.37% and 14.73%, respectively; $P \leq 0.05$). No significant differences were observed between Brown and Local rabbits in either round. Uric acid levels demonstrated a significant round effect, with values in round 2 being 46.43% higher than those in round 1 ($P \leq 0.042$). However, no treatment- or breed-related differences were detected for this parameter.

Table 2 - Effect of NSO on blood glucose (mg/dl) in different rabbit breeds

Breeds	Round 1		Round 2	
	Control	Treatment	Control	Treatment
Albino	113.75 ± 11.69	111 ± 12.86	102.75 ± 2.39	109 ± 7.78
Brown	115 ± 10.54	107.33 ± 7.88	109 ± 3.71	101 ± 4.73
Local	92.50 ± 6.12	109.33 ± 6.17	116.50 ± 2.36	92 ± 6

Data are presented as mean ± standard error (SE). Significant differences between groups are indicated by P (P ≤ 0.05).

Table 3 - Effect of NSO on lipid profile (mg/dl) in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
Total Cholesterol	Albino	105.25 ± 9.78 ^a	110.25 ± 10.50 ^a	128.50 ± 12.8 ^a	101.25 ± 15.35 ^a
	Brown	97 ± 13.97 ^b	77.33 ± 17.70 ^b	74 ± 9.29 ^b	89 ± 23.43 ^b
	Local	88 ± 30.35 ^b	69.67 ± 12.03 ^b	70 ± 6 ^b	65.67 ± 13.30 ^b
HDL	Albino	20.75 ± 1.25	23 ± 1.15	27.25 ± 1.97	25.75 ± 2.46
	Brown	22.25 ± 4.61	20 ± 2	21 ± 2.52	26 ± 6.08
	Local	15.50 ± 1.55	22.33 ± 2.19	26.33 ± 0.88	21.67 ± 2.40
LDL	Albino	78.50 ± 10.74 ^a	81.75 ± 9.57 ^a	105.50 ± 9.53 ^a	79.50 ± 18.54 ^a
	Brown	65.50 ± 13.57 ^b	47.33 ± 22.82 ^b	39 ± 12.00 ^b	47 ± 14.53 ^b
	Local	67 ± 35.77 ^b	39 ± 12.42 ^b	38 ± 6.56 ^b	39.33 ± 16.18 ^b
Triglycerides	Albino	94.25 ± 7.66	100.25 ± 22.97	82.25 ± 20.03	70.75 ± 5.59
	Brown	103.75 ± 10.42	103.33 ± 4.48	123 ± 19.08	86.67 ± 15.01
	Local	82.25 ± 17.99	79 ± 5.03	81.33 ± 6.69	68.67 ± 2.60

*Data are Mean ± SE. Superscript letters (^a, ^b) indicate significant breed differences for that parameter are indicated by (P ≤ 0.05). Significant breed effect was found in Total Cholesterol (P ≤ 0.007) and LDL (P ≤ 0.005).

Table 4 - Effect of NSO on liver enzymes (IU/L) in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
AST (IU/L)	Albino	22.50 ± 4.73 ^b	26.50 ± 3.52 ^b	13.50 ± 1.85 ^b	17.25 ± 1.55 ^b
	Brown	32.75 ± 5.78 ^a	34 ± 160.1 ^a	37 ± 11.00 ^a	26.33 ± 9.84 ^a
	Local	38.25 ± 8.93 ^a	44.33 ± 9.33 ^a	26.67 ± 4.91 ^a	21 ± 4.16 ^a
ALT (IU/L)	Albino	41.25 ± 9.51	60 ± 8.53	55.75 ± 10.24	56.50 ± 3.97
	Brown	54.50 ± 8.70	60.33 ± 6.12	52.50 ± 15.50	39.67 ± 14.89
	Local	57 ± 14.91	79.33 ± 10.84	92.33 ± 13.04	59.67 ± 18.70
CK (IU/L)	Albino	1934.50 ± 763	1101 ± 82.12	875.50 ± 162.73	1129.50 ± 197
	Brown	941.75 ± 121	704.33 ± 96.44	2302 ± 87	2338.33 ± 364
	Local	3605.50 ± 175	1712.33 ± 139	1403.50 ± 43.28	1615 ± 210
LDH (IU/L)	Albino	461.25 ± 158.39	429.50 ± 21.30	257.25 ± 38.21	279.50 ± 49.16
	Brown	485 ± 153.57	237.67 ± 50.85	682.67 ± 108.51	1047.67 ± 74.16
	Local	710.75 ± 178.32	579 ± 29.54	467.25 ± 43.91	471.33 ± 99.78

*Data are Mean ± Standard Error (SE). Superscript letters (^a, ^b) denote significant differences between breeds (P ≤ 0.05). Significant breed effect was detected for AST (P ≤ 0.02).

Table 5 - Effect of NSO on kidney function parameters in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
Creatinine (mg/dl)	Albino	0.86 ± 0.02 ^c	0.80 ± 0.07 ^c	0.76 ± 0.07 ^c	0.82 ± 0.09 ^c
	Brown	1.30 ± 0.03 ^a	1.04 ± 0.02 ^{ab}	1.25 ± 0.04 ^a	1.21 ± 0.03 ^a
	Local	0.96 ± 0.08 ^b	1.08 ± 0.02 ^b	1.07 ± 0.01 ^b	0.89 ± 0.08 ^b
Urea (mg/dl)	Albino	45.00 ± 4.88 ^b	40.50 ± 2.18 ^b	46.25 ± 2.56 ^b	47.25 ± 5.48 ^b
	Brown	59.00 ± 2.86 ^a	42.67 ± 4.84 ^{ab}	56.00 ± 1.73 ^a	53.33 ± 1.33 ^a
	Local	51.75 ± 5.02 ^a	51.67 ± 3.18 ^a	46.50 ± 3.20 ^b	55.00 ± 3.46 ^a
Uric Acid (mg/dl)	Albino	0.28 ± 0.07	0.31 ± 0.04	0.32 ± 0.04	0.33 ± 0.04
	Brown	0.35 ± 0.07	0.15 ± 0.02	0.58 ± 0.18	0.41 ± 0.18
	Local	0.36 ± 0.07	0.21 ± 0.02	0.41 ± 0.03	0.36 ± 0.06

Data are Mean ± SE. Superscript letters (^a, ^b, ^c) indicate significant breed differences ($P \leq 0.05$). Significant differences found in Creatinine ($P \leq 0.0001$) and Urea ($P \leq 0.01$). Uric acid showed a significant round effect only ($P \leq 0.042$), but no breed/treatment significance.

Effect of NS Oil on protein profile

Protein metabolism remained generally stable across all groups, with albumin being the most responsive parameter (Table 6). During round 1, albumin levels of Brown rabbits were 4.85% and 7.27% higher than those in Albino and local rabbits, respectively ($P \leq 0.05$). This pattern persisted in round 2, with Brown rabbits maintaining significantly higher albumin concentrations than Albino and Local rabbits by 6.65% and 0.66%, respectively ($P \leq 0.05$).

The consistently higher albumin levels observed in Brown rabbits may indicate breed-related variation in protein synthesis or absorption capacity. Across both rounds, albumin concentrations in Brown rabbits were approximately 8–9% higher than those in Albino rabbits. Breed and dietary treatment did not significantly affect total protein or globulin concentrations throughout the experimental period ($P > 0.05$). Although mild breed-related variation in globulin levels was observed ($P \leq 0.047$), this difference did not reach statistical significance. Total protein concentrations fluctuated within 3% of baseline values, suggesting maintained protein homeostasis across all groups.

Effect of NS Oil on thyroid hormones

Endocrine assessment revealed no significant differences ($P > 0.05$) in T3 or T4 concentrations attributed to treatment, breed, or experimental round (Table 7). Thyroid hormone levels remained stable, with T3 and T4 varying by less than 22% across all groups. T4 concentration in Brown rabbits remained particularly stable, deviating by less than 5% from baseline values.

Table 6 - Effect of NSO on serum protein profile (g/dl) in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
Total Protein	Albino	6.25 ± 0.07	6.20 ± 0.16	6.29 ± 0.19	6.19 ± 0.07
	Brown	6.96 ± 0.22	6.73 ± 0.29	6.59 ± 0.41	6.71 ± 0.48
	Local	6.93 ± 0.64	6.47 ± 0.23	6.62 ± 0.17	6.19 ± 0.18
Albumin	Albino	5.77 ± 0.09 ^b	5.71 ± 0.13 ^b	5.71 ± 0.11 ^b	5.74 ± 0.04 ^b
	Brown	6.05 ± 0.22 ^a	6.28 ± 0.29 ^a	6.09 ± 0.01 ^a	5.76 ± 0.18 ^a
	Local	5.64 ± 0.23 ^b	5.90 ± 0.23 ^b	6.05 ± 0.11 ^b	5.62 ± 0.16 ^b
Globulin	Albino	0.48 ± 0.14	0.49 ± 0.12	0.58 ± 0.27	0.44 ± 0.07
	Brown	0.91 ± 0.20	0.75 ± 0.66	0.50 ± 0.40	0.95 ± 0.32
	Local	1.30 ± 0.46	0.56 ± 0.04	0.57 ± 0.14	0.57 ± 0.16

Data are presented as mean ± standard error (SE). Superscript letters (^a, ^b) indicate significant breed differences ($P \leq 0.05$). Breed effect was significant for Albumin ($P \leq 0.02$).

Table 7 - Effect of NSO on thyroid hormones (nmol/L) in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
T3	Albino	2.74 ± 1.83	0.91 ± 0.04	0.79 ± 0.01	1.27 ± 0.34
	Brown	0.74 ± 0.06	0.78 ± 0.04	0.81 ± 0.07	0.84 ± 0.16
	Local	1.21 ± 0.17	1.00 ± 0.07	0.71 ± 0.10	0.99 ± 0.12
T4	Albino	38.52 ± 7.53	34.39 ± 5.35	36.49 ± 3.02	39.27 ± 2.46
	Brown	31.80 ± 1.70	30.97 ± 4.96	32.11 ± 3.20	33.33 ± 6.19
	Local	40.74 ± 4.15	41.36 ± 0.75	32.18 ± 4.72	32.69 ± 1.75
Data are presented as mean ± standard error (SE). Significant differences between groups are indicated by P (P ≤ 0.05).					

Data are presented as mean ± standard error (SE). Significant differences between groups are indicated by P (P ≤ 0.05).

DISCUSSION

This study investigated the effect of dietary NSO supplementation on selected biochemical markers in three rabbit breeds: Albino, Brown, and Local. The findings indicate that several observed differences were primarily breed-dependent, reflecting inherent metabolic variation rather than treatment effects. Overall, NSO supplementation did not produce large or consistent alterations across the measured biochemical parameters.

Blood glucose concentrations remained stable across breeds and dietary treatments. These findings are consistent with [Mahomoodally et al. \(2022\)](#), who found that NS reduces blood glucose primarily in diabetic animals rather than in healthy individuals with normal insulin regulation. Similarly, [Wang et al. \(2023\)](#) suggested that metabolic differences among rabbit breeds do not necessarily affect glucose homeostasis in healthy animals.

Significant breed-related variation was observed in total cholesterol and LDL cholesterol levels, with Albino rabbits exhibiting higher concentrations than Brown and Local breeds. These findings are consistent with the study of [Funes et al. \(2024\)](#), who attributed elevated lipid profiles in Albino rabbits to differences in hepatic cholesterol synthesis and lipoprotein metabolism. Although NSO has been widely reported to exert hypolipidemic effects largely attributed to thymoquinone-mediated inhibition of HMG-CoA reductase ([Kareem et al., 2022](#)) no significant treatment-related lipid changes were detected in the present study. This may be explained by the absence of experimentally induced hyperlipidemia, a condition under which NSO effects are typically more pronounced. Comparable improvements in lipid and antioxidant status have been reported primarily under oxidative or metabolic stress conditions ([Ahmed and Abdul-Rahman, 2023](#); [Fatima et al., 2024](#)). Nevertheless, a slight reduction in triglycerides and a modest round-related increase in HDL were observed, suggesting minor improvements in lipid turnover over time, consistent with previous experimental observations ([Atufe et al., 2022](#)).

Breed-related differences were also evident in liver enzyme activity. Specifically, AST levels were significantly elevated in Brown rabbits, whereas ALT exhibited a borderline trend among breeds. [Abdel-Hamid and Dawod \(2020\)](#) documented natural inter-breed variations in baseline liver enzyme activity, supporting the interpretation that the observed differences likely reflect physiological characteristics rather than dietary effects. Previous studies have demonstrated hepatoprotective properties of *Nigella sativa* under toxic stress conditions ([Hassan et al., 2012](#); [Hatipoğlu et al., 2023](#)). The generally normal liver enzyme profiles observed here may therefore indicate limited scope for antioxidant intervention under physiologically stable conditions ([Abdelsalam and Fathi, 2023](#)).

Higher creatinine and urea concentrations observed in Brown rabbits may reflect breed-related differences in renal clearance efficiency or muscle mass. Similar baseline variation among breeds has been reported by [Sze-Yu et al. \(2025\)](#), who noted that inherent physiological differences may obscure treatment effects in healthy animals. Conversely, [Dollah et al. \(2013\)](#) demonstrated nephroprotective effects of *Nigella sativa* through reductions in serum creatinine without evidence of nephrotoxicity. The round-related increase in uric acid observed in the present study, particularly during round 2, may reflect time-dependent metabolic or handling stress, as previously suggested by [De la Fuente et al. \(2004\)](#).

Albumin concentrations were consistently higher in Brown rabbits, potentially reflecting enhanced hepatic synthetic protein synthesis or improved protein assimilation. Differences in total protein and globulin among breeds were minimal, consistent with findings by [Umar et al. \(2018\)](#) and [Cimrin et al. \(2023\)](#), who reported improved protein status in rabbits receiving *Nigella sativa* supplementation, possibly due to enhanced nitrogen utilization and liver function. These findings suggest a potential protein-sparing or anabolic role of NSO, particularly during prolonged supplementation or nutritional stress ([El-Gindy et al., 2020](#)). Similar improvements in protein metabolism have been observed with antioxidant-rich supplements such as coenzyme Q10 and wheat germ oil in stressed rabbits ([Al-Samarai and Al-Janabi, 2021](#)).

Thyroid hormones (T3 and T4) remained unaffected by treatment, breed, or experimental round, supporting findings by [Elfaki and Elkhair \(2023\)](#), who reported minimal endocrine disruption following phytogetic supplementation. [Avci et al. \(2021\)](#) further suggested that *Nigella sativa* may act as a hormonal modulator primarily under pathological thyroid conditions rather than altering euthyroid physiological states.

Biological variability should also be considered when interpreting the findings. High standard errors were observed for several parameters, particularly liver enzymes, lipid fractions, and thyroid hormones. Such variability is common in rabbit models and may arise from genetic diversity, limited sample size, or stress responses related to handling and environmental factors. Previous studies have similarly documented fluctuations in enzymes such as AST, ALT, CK, and LDH associated with breed and stress exposure (Washington and Van Hoosier, 2012). Hormonal and metabolic markers are likewise influenced by circadian rhythms, feed intake, and adaptive physiological responses (Liang et al., 2022). Despite this variability, statistically significant differences were detected in key parameters such as creatinine, cholesterol, and albumin, suggesting that the observed effects are biologically meaningful. Future studies incorporating larger sample sizes and refined sampling protocols may help reduce variability and strengthen these findings.

CONCLUSION

Dietary supplementation with N.S oil (5 g/kg DM) produced breed-dependent biochemical responses among the three rabbit breeds studied. Albino rabbits exhibited higher cholesterol and LDL concentrations, whereas Brown rabbits showed elevated creatinine and urea levels. AST activity decreased more markedly in Albino and Local rabbits compared with the Brown breed. Overall, NSO supplementation appeared to support liver stability while maintaining normal kidney function without inducing adverse biochemical effects. Future research should investigate the effects of different inclusion levels of *Nigella sativa* oil to determine the optimal dosage for each rabbit breed, particularly in relation to lipid profile and kidney function parameters.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Sherwan Mostafa Sefdeen; E-mail: sherwan.seif@dpu.edu.krd; ORCID: <https://orcid.org/0000-0002-6395-1933>

Ethical approval statement

The study received ethical approval from the Animal Ethics Committee at the College of Veterinary Medicine, University of Duhok, Iraq (Ethical code No. DR2996919CV, approved January 11, 2021).

Data availability

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

Funding statement

This research did not receive specific grants from funding agencies in public, commercial or not-for-profit sectors.

Authors' contribution

W.I. Hasan and H.M. Hidayet conceived and designed the study. O.H. Azeez, C.A. Yateem, and K.N. Mustafa executed the experiment and analyzed the sera and tissue samples. S.M. Sefdeen analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual content, and approved the final version.

Acknowledgements

The authors are grateful to the help given by the College of Agricultural Engineering Sciences, especially Department of Animal Production, University of Duhok, Duhok-IRAQ.

Competing interests

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

REFERENCES

- Abdel-Hamid TM, and Dawod A (2020). Breed effects on growth performance, blood parameters, and the levels of metabolic hormones in rabbits under heat stress in Egypt. *Zagazig Veterinary Journal*, 48(3): 284–295. <https://doi.org/10.21608/zvjz.2020.28446.1107>
- Alu'datt MH, Rababah T, Al-u'datt DGF, Gammoh S, Alkandari S, Allafi A, et al. (2024). Designing novel industrial and functional foods using the bioactive compounds from *Nigella sativa* L. (black cumin): Biochemical and biological prospects toward health implications. *Journal of Food Science*, 89(4): 1865–1893. <https://doi.org/10.1111/1750-3841.16981>
- Algheshairy RM, Alharbi HF, Almujaydil MS, Alhomaied RM, and Ali HA (2025). The protective effect of various forms of *Nigella sativa* against hepatorenal dysfunction: Underlying mechanisms comprise antioxidation, anti-inflammation, and anti-apoptosis. *Frontiers in Nutrition*, 12: 1553215. <https://doi.org/10.3389/fnut.2025.1553215>
- Abdelsalam M, and Fathi M (2023). Improving productivity in rabbits by using some natural feed additives under hot environmental conditions - A review. *Animal Bioscience*, 36(4): 540–554. <https://doi.org/10.5713/ab.22.0354>
- Adam SH, Nasri NM, Kashim MI, Abd Latib EH, Juhari MA, and Mokhtar MH (2022). Potential health benefits of *Nigella sativa* on diabetes

- mellitus and its complications: A review from laboratory studies to clinical trials. *Frontiers in Nutrition*, 9:1057825. <https://doi.org/10.3389/fnut.2022.1057825>
- Ahmed SJ, and Abdul-Rahman SY (2023). Effects of castration and sex hormones on antioxidant status and some biochemical parameters of male rabbits exposed to oxidative stress. *Mesopotamia Journal of Agriculture*, 51(1): 92–114. <https://doi.org/10.33899/magrj.2023.138538.1220>
- Al-Asadi EH, Edan BJ, and Hussein HH (2025). Effects of aqueous extract of black cumin on cholesterol and total protein levels (albumin and globulin) in male healthy rabbits. *Journal of University of Babylon for Pure and Applied Sciences*, 33(2): 239–248. <https://www.journalofbabylon.com/index.php/JUBPAS/article/view/5796/4348>
- Alberts A, Moldoveanu E-T, Niculescu A-G, and Grumezescu AM (2024). *Nigella sativa*: A comprehensive review of its therapeutic potential, pharmacological properties, and clinical applications. *International Journal of Molecular Sciences*, 25(24): 13410. <https://doi.org/10.3390/ijms252413410>
- Al-Hamdany MZ, and Raoof RM (2023). Protective effects of black seed oil (*Nigella sativa* oil) against pulmonary, renal, and cardiac toxicity induced by nefopam in albino rats. *The Egyptian Journal of Histology*, 46(2): 772–781. <https://doi.org/10.21608/ejh.2022.113034.1621>
- Al-Razuqi RAM, Al-Hussaini JA, and Al-Jeboori AA (2011). Protective effect of *Nigella sativa* against carbon tetrachloride-induced acute liver injury in experimental rabbit models. *International Journal of Green Pharmacy*, 5(3): 198–200. <https://www.greenpharmacy.info/index.php/ijgp/article/view/202>
- Al-Samarai EAM, and Al-Janabi AAF (2021). The effect of using coenzyme Q10 and wheat germ oil on some blood characteristics of stressed rabbits. *Mesopotamia Journal of Agriculture*, 49(4): 35–47. <https://rsci.uomosul.edu.iq/index.php/magrj/article/view/57026/55269>
- Al-Shaar NMB (2020). Impact of black seeds *Nigella sativa* L. meal substitution instead of soybean meal on some growth traits of local rabbit males. *Mesopotamia Journal of Agriculture*, 48(3): 59–67. <https://alaw.uomosul.edu.iq/index.php/magrj/article/view/58472>
- Association of Official Analytical Chemists - AOAC (2000). Official methods of analysis of the Association of Official Analytical Chemists: method 984.13 (17th ed.). Washington: AOAC
- Aslani MR, Saadat S, and Boskabady MH (2024). Comprehensive and updated review on anti-oxidant effects of *Nigella sativa* and its constituent, thymoquinone, in various disorders. *Iranian Journal of Basic Medical Sciences*, 27(8): 923–951. <https://doi.org/10.22038/ijbms.2024.75985.16453>
- Atufe G, Oweh OT, Avidime OM, Awhin EP, and Nurudeen QO (2022). Ameliorative activities and safety of Moringa oleifera oil and *Nigella sativa* oil on diet-induced hyperlipidemic male Wistar rats. *Nigerian Journal of Experimental and Clinical Biosciences*, 10(1): 24–28. https://doi.org/10.4103/njecp.njecp_1_22
- Avci G, Ulutas E, Ozdemir V, Kivrak I, and Bulbul A (2021). The positive effect of black seed (*Nigella sativa* L.) essential oil on thyroid hormones in rats with hypothyroidism and hyperthyroidism. *Journal of Food Biochemistry*, 46(4): e13801. <https://doi.org/10.1111/jfbc.13801>
- Bhavikatti SK, Zainuddin SLA, Ramli RB, Nadaf SJ, Dandge PB, Khalate M, et al. (2024). Insights into the antioxidant, anti-inflammatory, and antimicrobial potential of *Nigella sativa* essential oil against oral pathogens. *Scientific Reports*: 14(1): 11878. <https://doi.org/10.1038/s41598-024-62915-1>
- Cimrin T, Alasahan S, Kazak F, Kutlu T, and Kisacam MA (2023). Effects of black cumin (*Nigella sativa* L.) seed on growth performance, blood parameters, liver oxidant/antioxidant levels, and fatty liver syndrome in quails. *Veterinary Research Forum*, 14(3): 121–129. <https://doi.org/10.30466/vrf.2022.549517.3404>
- De la Fuente J, Salazar MI, Ibáñez M, and González de Chavarri E (2004). Effects of season and stocking density during transport on live weight and biochemical measurements of stress, dehydration, and injury of rabbits at time of slaughter. *Animal Science*, 78(2): 285–292. <https://doi.org/10.1017/S1357729800054072>
- Derosa G, D'Angelo A, Maffioli P, Cucinella L, and Nappi RE (2024). The use of *Nigella sativa* in cardiometabolic diseases. *Biomedicine*, 12(2): 405. <https://doi.org/10.3390/biomedicine12020405>
- Dollah MA, Parhizkar S, Latiff LA, and Bin Hassan MH (2013). Toxicity effect of *Nigella sativa* on liver function in rats. *Advanced Pharmaceutical Bulletin*, 3(1):97–102. <https://pubmed.ncbi.nlm.nih.gov/24312819/>
- Elfaki MOA, and Elkhair NM (2023). Effect of *Nigella sativa* seed supplementation on hematology, acid-base parameters, and serum biochemical parameters in Nubian goat fed an aflatoxin contaminated diet. *Cluj Veterinary Journal*, 28(2): 2–12. <https://doi.org/10.52331/cvj.v28i2.48>
- Elghareeb MM, Elshopakey GE, Rezk S, Ateya A, El-Ashry ES, Shukry M, Ghamry HI, Alotaibi BS, and Hashem NMA (2024). *Nigella sativa* oil restores hormonal levels and endocrine signals among thyroid, ovarian, and uterine tissues of female Wistar rats following sodium fluoride toxicity. *Biomedicine & Pharmacotherapy*, 170, 116080. <https://doi.org/10.1016/j.biopha.2023.116080>
- El-Gindy Y, Zewail H, Zahran S, Abd El-Rahman M, and Eisa F (2020). Hematologic, lipid profile, immunity, and antioxidant status of growing rabbits fed black seed as natural antioxidants. *Tropical Animal Health and Production*, 52(3): 999–1004. <https://doi.org/10.1007/s11250-019-02091-x>
- Fatima S, Naureen I, Naeem MS, Sadaf R, and Ullah R (2024). Effect of black cumin (*Nigella sativa*) and high-fat supplemented diet on lipid profile, C-reactive protein, and cardiac histopathology in rabbits. *Journal of the Hellenic Veterinary Medical Society*, 75(2): 304–312. <https://doi.org/10.12681/jhvms.34692>
- Funes AK, Avena V, Boarelli PV, Monclus MA, Fernández Zoppino D, Saez–Lancellotti TE, et al. (2024). Cholesterol dynamics in rabbit liver: High-fat diet, olive oil, and synergistic dietary effects. *Biochemical et Biophysical research communications*. 733: 150675. <https://doi.org/10.1016/j.bbrc.2024.150675>
- Guo Y, Guo W, Chen, H, Sun J, and Yin, Y (2025). Mechanisms of sepsis-induced acute liver injury: a comprehensive review. *Frontiers in Cellular and Infection Microbiology*, 15: 1504223. <https://doi.org/10.3389/fcimb.2025.1504223>
- Hannan MA, Zahan MS, Sarker PP, Moni A, Ha H, and Uddin MJ (2021). Protective effects of black cumin (*Nigella sativa*) and its bioactive constituent, thymoquinone, against kidney injury: An aspect on pharmacological insights. *International Journal of Molecular Sciences*, 22(16): 9078. <https://doi.org/10.3390/ijms22169078>
- Hashim M, Anjum S, Mujahid H, Alotaibi KS, Albattal SB, Ghamry HI, et al. (2025). Thymoquinone loaded zinc oxide nanoformulations synthesis, characterization, and evaluation of their efficacy against carbon tetrachloride induced hepatorenal toxicity in rats. *Toxicological Research*, 14(2): tfaf037. <https://doi.org/10.1093/toxres/tfaf037>

- Hassan AS, Ahmed JH, and Al-Haroon SS (2012). A study of the effect of *Nigella sativa* (black seeds) in isoniazid (INH)-induced hepatotoxicity in rabbits. *Indian Journal of Pharmacology*, 44(6): 678–682. <https://doi.org/10.4103/0253-7613.103239>
- Hatipoğlu D, Özsan M, Dönmez HH, and Dönmez N (2023). Hepatoprotective effects of *Nigella sativa* oil against acrylamide-induced liver injury in rats. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 70(4): 419–426. <https://doi.org/10.33988/auvfd.1096306>
- Kareem MA, Al-Azzawi AFS, and Baraaj AH (2022). Histological and biochemical study of *Nigella sativa* seeds effects on liver of male albino rats treated with rifampicin. *Iraqi Journal of Science*, 57(4C): 2829–2839. <https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/6257>
- Liang ZL, Chen F, Park S, Balasubramanian B, and Liu W-C (2022). Impacts of heat stress on rabbit immune function, endocrine, blood biochemical changes, antioxidant capacity, and production performance, and the potential mitigation strategies of nutritional intervention. *Frontiers in Veterinary Science*, 9: 906084. <https://doi.org/10.3389/fvets.2022.906084>
- Mahomoodally MF, Aumeeruddy MZ, Legoabe LJ, Montesano D, and Zengin G (2022). *Nigella sativa* L. and its active compound thymoquinone in the clinical management of diabetes: A systematic review. *International Journal of Molecular Sciences*, 23(20): 12111. <https://doi.org/10.3390/ijms232012111>
- Mat MC, Mohamed AS, and Hamid SS (2011). Primary human monocyte differentiation regulated by *Nigella sativa* pressed oil. *Lipid in Health and Disease*, 10: 216. <http://www.lipidworld.com/content/10/1/216>
- Naderi E, Rad AKh, Nazari S, Khazaei M, Shahraki S, and Hosseini S (2022). *Nigella sativa* and its main constituent, thymoquinone, protect against glycerol-induced acute kidney injury in rats. *Avicenna Journal of Phytomedicine*, 12(6): 638–648. <https://doi.org/10.22038/AJP.2022.20921>
- Nelson EA, Keller GL, Mitchell TW, Pennypacker B, Rebbeck P, and Rogers IT (2010). A jugular bleeding technique in rabbits. *Lab Animal*, 39(1): 17–22. <https://doi.org/10.1038/labon0110-17>
- Ozougwu J C (2017). Physiology of the liver. *International Journal of Research in Pharmacy and Biosciences*, 4(8): 13–24. https://www.academia.edu/38598619/Physiology_of_the_liver
- Rounagh M, Musazadeh V, Hosseini Mohebat A, Falahatzadeh M, Kavyani Z, Rostami RB, et al. (2024). Effects of *Nigella sativa* supplementation on lipid profiles in adults: An updated systematic review and meta-analysis of randomized controlled trials. *Clinical Nutrition ESPEN*, 61: 168–180. <https://doi.org/10.1016/j.clnesp.2024.03.020>
- Safithri F, Hermayanti D, Isbandiyah I, Putra PYP, Maulana FS, and Azizah AMN (2023). Liver-protectant and cardiovascular-protectant effects of *Nigella sativa*: A meta-analysis. *Digital Chinese Medicine*, 6(2): 112–120. <https://doi.org/10.1016/j.dcm.2023.07.002>
- SAS Institute Inc. (2016). SAS/STAT® 14.2 User's Guide: Shared Concepts and Topics. Cary, NC: SAS Institute Inc
- Shaukat A, Zaidi A, Anwar H, and Kizilbash N (2023). Mechanism of the antidiabetic action of *Nigella sativa* and thymoquinone: A review. *Frontiers in Nutrition*, 10: 1126272. <https://doi.org/10.3389/fnut.2023.1126272>
- Sze-Yu Y, Chi-Hsuan S, Pin-Chen L, Ching-Fen W, Tsai-Lu L, Tsung-Li C, et al. (2025). Urinary chemistry in healthy cross-bred pet rabbits (*Oryctolagus cuniculus*) and rabbits with suspected chronic kidney disease. *Translational Animal Science*, 9: txf002. <https://doi.org/10.1093/tas/txaf002>
- Tiwari A, Surendra G, Meka S, Varghese B, Vishwakarma G, and Adela R (2022). The effect of *Nigella sativa* on non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Human Nutrition & Metabolism*, 28: 200146. <https://doi.org/10.1016/j.hnm.2022.200146>
- Trocino A, and Tolini C (2024). Measuring on-farm welfare in rabbits: A review with emphasis on animal-based indicators. *World Rabbit Science*, 32(4): 225–240. <https://doi.org/10.4995/wrs.2024.22647>
- Umar Z, Qureshi AS, Usman M, Sarfraz A, Umar T, Hussain M, and Umar S (2018). Response of dietary supplementation of black seed (*Nigella sativa*) oil on hematological parameters, serum biochemistry, and reproductive hormones in male rabbits. *Academic Journal of Medical Plant Sciences*, 6(9), 276-280. <https://www.newacademiapublishing.org/journals/ajmp/pdf/2018/Sep/Umar%20et%20al.pdf>
- Wang H, Xie X, Duan Y, Chai L, Li K, Qiu J, and Cheng Z (2023). Rabbit systemic glucose metabolism map by total-body dynamic PET/CT technology. *Nuclear Medicine Communications*, 44(12), 1144-1150. <https://doi.org/10.1097/MNM.0000000000001767>
- Wang Z, Zhang R, Du G, Ni M, He H, Li Z, Yuan D, et al. (2025). Effects of the welfare housing systems based on environmental enrichment on growth performance, meat quality, and intestinal health in rabbits. *FASEB Journal*, 39(10): e70636. <https://doi.org/10.1096/fj.202500042R>
- Washington IM, and Van Hoosier G (2012). Clinical biochemistry and hematology. In Suckow, M. A., Stevens, K. A., & Wilson, R. P. (Eds.), *The laboratory rabbit, guinea pig, hamster, and other rodents* 2nd ed., Academic Press, pp. 57–116. <https://doi.org/10.1016/B978-0-12-380920-9.00003-1>
- Ye X, Zhou L, Zhang Y, Xue S, Fu Gan, Q, and Fang S (2021). Effect of host breeds on gut microbiome and serum metabolome in meat rabbits. *BMC Veterinary Research*, 17: 24. <https://doi.org/10.1186/s12917-020-02732-6>
- Zhang Y, Fatima M, Hou S, Bai L, Zhao S, and Liu E (2021). Research methods for animal models of atherosclerosis (Review). *Molecular Medicine Reports*, 24: 871. <https://doi.org/10.3892/mmr.2021.12511>

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

EFFECT OF EMULSIFIED HIGH-PUFA OILS ENRICHED WITH NATURAL ANTIOXIDANTS ON IN VITRO GOAT RUMEN FERMENTATION PARAMETERS AND NUTRIENT DEGRADABILITY

Dilla Mareistia FASSAH¹✉ , Manik Nurul ALFIYYAH¹, Safiena Maura AZKA¹, Shafira Pramesti DAMAYANTI¹, Yusti PUJIAWATI^{1,2}, Lilis KHOTIJAH¹, and Novia Amalia SHOLEHA³

¹Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia

²Research Center for Animal Husbandry, Research Organization for Agriculture and Food, National Research and Innovation Agency Indonesia, Cibinong Science Center, Jalan Raya Jakarta-Bogor, Cibinong, Bogor, 16915, Indonesia

³College of Vocational Studies, IPB University, Jalan Kumbang No. 14, Bogor, 16151, Indonesia

✉Email: dilla_mareistia@apps.ipb.ac.id

➤Supporting Information



ABSTRACT: This study aims to evaluate the effect of emulsified high-PUFA oils enriched with natural antioxidants derived from jackfruit leaf extract on in vitro goat rumen fermentability and degradability. The study used a completely randomized design with two treatments and 5 replications. The treatments were supplementation with high-PUFA oils + natural antioxidants (P1, as the control), and supplementation with emulsified high-PUFA oils + natural antioxidants (P2). The parameters observed were rumen pH, total volatile fatty acid (VFA) concentration, NH₃ concentration, bacterial population, protozoa population, dry matter digestibility (DMD), and organic matter digestibility (OMD). The results showed that supplementing goat feed ration with emulsified high-PUFA oils enriched with natural antioxidants did not alter ($P>0.05$) rumen pH, total VFA, NH₃ concentration, bacterial and protozoal population, DMD, or OMD. Overall, emulsified high-PUFA oils with natural antioxidants did not alter the examined rumen fermentation parameters and degradability. Thus, emulsified high-PUFA oils with natural antioxidants can be potentially used as an effective feed supplement to support goats' performance.

Keywords: Emulsification, Feed supplement, Fermentation characteristics, Jackfruit leaves, Polyunsaturated fatty acid.

INTRODUCTION

Previous studies have shown that polyunsaturated fatty acids (PUFA), particularly n-3 and n-6, have a positive effect on the reproductive function, health, and performance of goats (Almeida et al., 2019; Geng et al., 2020). However, ruminants cannot synthesize n-3 and n-6 PUFAs de novo, so the fatty acids must be supplied through their diet (Roque-Jiménez et al., 2021). Flaxseed and sunflower oils are notable sources of essential n-3 and n-6 PUFAs. Flaxseed oil is rich in n-3 fatty acids, contains about 50% α -linoleic acid (ALA), which can be metabolized into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and has anti-inflammatory and antiatherosclerosis effects (Liao et al., 2021; Romanić et al., 2021). Sunflower seed oil is predominantly composed of n-6 PUFAs, particularly linoleic acid (LA), which reaches 60%-75% (Akkaya, 2018). However, high-PUFA oil supplementation in ruminant diets may negatively affect rumen fermentation by inhibiting microbial activity, particularly fiber-degrading bacteria, thereby reducing fermentation efficiency when supplied at inappropriate levels or in unprotected forms (Mirzaei-Alamouti et al., 2021). A previous study has also reported that dietary lipid supplementation exceeding 4-6% of dry matter may negatively affect rumen fermentation (Ribeiro et al., 2025).

In addition, PUFA metabolism may induce oxidative stress in livestock, leading to increased reactive oxygen species (ROS) and altering PUFAs' biological functions. Exogenous antioxidants can be used to prevent the side effects of PUFA metabolism (Fassah and Khotijah, 2016). A natural antioxidant derived from Jackfruit leaf extract, containing 0.34% antioxidant compounds, has been proposed as a strategy to mitigate oxidative stress (Pujiawati et al., 2023). Ojwang et al. (2017) reported that jackfruit leaves contain various bioactive compounds, including flavonoids (5.02-6.70 mg/g), tannins (1.97-2.56 mg/g), and phenolic compounds (30.92 - 37.39 mg/g). Nevertheless, directly mixing PUFA oils with jackfruit leaf extract may pose problems, as differences in polarity between hydrophilic antioxidants and lipophilic PUFA oils result in poor mixing efficiency, ineffective stabilization, and limited protective effects (Roschel et al., 2019).

Emulsification has emerged as a promising approach to improve the physical stability and dispersion of PUFA oils and antioxidants. Emulsification allows for a more homogeneous mixture of PUFA oils and antioxidants by adding surfactants that reduce interfacial tension between two liquid phases (Saxena and Mandal, 2022). Tween 80 is a non-ionic surfactant commonly used in emulsions that generally do not alter the pH since it does not dissociate into ions that could affect pH levels (López-Martínez and Rocha-Urbe, 2018; Pernin et al., 2019). Therefore, emulsifying high-PUFA oils with natural antioxidants may improve ruminal fermentation efficiency by stabilizing high-PUFA oils and natural

RESEARCH ARTICLE
 PII: S222877012600014-16
 Received: September 11, 2025
 Revised: March 28, 2026
 Accepted: March 29, 2026

antioxidants without impairing nutrient degradability. However, studies on the impact of emulsified high-PUFA oils-antioxidants combinations on rumen fermentation remain limited. Therefore, this study aims to evaluate the effect of emulsified high-PUFA oils enriched with natural antioxidants on in vitro rumen fermentation and degradability in goats.

MATERIALS AND METHODS

The research was conducted from October to December 2024 at the Meat and Draught Animal Nutrition Laboratory, IPB University, Indonesia. The in vitro analysis using goat rumen fluid was approved by the Animal Ethics Committee of the Directorate of Research and Innovation Licensing Governance and Scientific Authority (No. 123/KE.02/SK/06/2023).

Antioxidant extraction

The antioxidant compounds were extracted from jackfruit leaves using a maceration method in 70% ethanol (Najini and Wahdaningsih, 2024). The jackfruit leaves were briefly oven-dried at 60°C, then ground into a fine powder. A total of 1 kg of jackfruit leaves powder was macerated in 70% ethanol, then kept at room temperature for 24 hours. The mixture was then filtered using filter paper and concentrated using a rotary evaporator to form a viscous extract.

Feed supplement preparation

The feed supplement was prepared in oil-in-water (O/W) emulsion containing 15% high-PUFA oils (flaxseed oil and sunflower oil), 10% Tween 80, and 75% distilled water with 15 mg of antioxidant compounds derived from jackfruit leaf extracts. The oil phase consisted of 62% sunflower oil and 38% flaxseed oil (w/w), chosen to balance n-6/n-3 PUFA profile. Then, the mixture was homogenized using a sonicator (Lab Ultrasonic Homogenizer, Tefic Biotech Co., China) at 60 °C, power 100W, and 30 minutes of sonication to ensure a uniform distribution of the oil phase in the water phase (oil-in-water) (Chen et al., 2023). For both treatments, the total amount of high-PUFA oils and antioxidant compounds was standardized to deliver 150 mg of total oil and 15 mg of antioxidant per 100 mL emulsion.

In vitro ruminal fermentation characteristics

A Completely Randomized Design with 2 treatments and 5 replications per treatment was applied in this study. The treatments consisted of adding high-PUFA oil and natural antioxidant compounds separately to rumen fluid (P1; the control), or adding an emulsified high-PUFA oil enriched with natural antioxidant compounds to rumen fluid (P2). The treatments were evaluated using the in vitro two-stage method described by Tilley and Terry (1963). The formula and nutrient composition of the basal diet, as well as the composition of the supplement, are provided in Table 1.

Table 1 - Formula and nutrient compositions of the treatment feed rations

Feedstuff	Composition (%)	P1	P2
<i>Pennisetum purpureum</i>		30.00	30.00
Cassava waste		15.51	15.51
Copra meal		20.68	20.68
Soybean meal		10.34	10.34
Pollard		17.17	17.17
CaCO ₃		0.72	0.72
Premix		0.72	0.72
Salt		0.72	0.72
Molasses		4.14	4.14
Total		100	100
Supplement			
High-PUFA oils and natural antioxidants		2.31	-
Emulsified high-PUFA oils and natural antioxidants		-	2.31
Nutrient composition (%DM)			
Dry matter (DM) ¹		89.22	89.22
Ash ¹		7.48	7.48
Crude protein (CP) ¹		15.02	15.02
Ether extract (EE) ¹		2.01	2.01
Crude fiber (CF) ¹		15.13	15.13
Nitrogen-free extract (NFE) ¹		60.38	60.38
Total digestible nutrient (TDN) ²		69.14	69.14

P1: High-PUFA oils and natural antioxidants supplementation; P2: Emulsified High-PUFA oils and natural antioxidants supplementation; ¹Analyzed using Near infrared spectroscopy (NIRS); ²TDN = 40.265 - 1.379 (CF) + 1.1903 (EE) + 1.363 (NFE) + 0.1969 (CP) (Wardeh, 1981)

Rumen fluid was collected from three healthy donor goats using a stomach tube method before morning feeding (Shen et al., 2012). The animals were fed a basal diet consisting of 70% concentrate and 30% elephant grass (Crude protein = 9.52%, TDN = 66.45%). Rumen fluid was filtered through six-layer gauze, pooled into a single homogenized sample to reduce individual variation, and placed in an insulated bottle at 39 °C. A total of 0.5 g of the sample was incubated under anaerobic conditions at 39 °C with 10 mL of rumen fluid and 40 mL of McDougall buffer solution. Both supplements were added at a concentration of 23 mg/g sample. After 48 hours of incubation, 2-3 drops of HgCl₂ were added, followed by centrifugation at 3000 rpm for 15 minutes. The samples were filtered and separated into residue and liquid samples. The residue sample was collected, dried at 105 °C for 24 hours, and combusted in a muffle furnace for 4 hours (AOAC, 2019) to determine dry matter (DMD) and organic matter degradability (OMD). Meanwhile, the liquid sample was collected to analyze rumen pH, total volatile fatty acid (VFA), ammonia (NH₃), and microbial population. Although the method primarily assessed nutrient degradability, the rumen fermentation parameters were also determined at the end of incubation as endpoint indicators of rumen fermentation conditions.

The liquid samples were tested for rumen pH using a pH meter. The concentration of ammonia (NH₃) was calculated using the method by Chaney and Marbach (1962) using UV-Vis spectrophotometry at 630 nm. The total VFA was measured using a steam distillation method (Despal et al., 2022). The total culturable bacterial population in the rumen was determined using the spread plate method with Brain Heart Infusion (BHI) medium, as described by Oliveira et al. (2021). The rumen protozoa population was analyzed following Ogimoto and Imai (1981). Briefly, 2 drops of the rumen fluid sample were mixed with a trypan blue formalin saline solution in a 1:1 ratio, and the mixture was observed under a microscope.

Data analysis

The data obtained were analyzed using an independent sample t-test in the statistical software SPSS 25.0. Data was considered significantly different between treatments at P<0.05.

RESULTS AND DISCUSSION

Ruminal fermentation characteristics

The results showed that adding emulsified high-PUFA oils enriched with natural antioxidants to the ration did not significantly affect (P>0.05) pH value, total VFA, ammonia concentration, and bacterial and protozoal population (Table 2), indicating the emulsion's lack of adverse effect on rumen fermentation. In this study, the emulsion containing high-PUFA oils and natural antioxidants was stabilized with Tween 80, which has been shown to remain stable during digestion (Lamothe et al., 2019). Therefore, Tween 80 may have contributed to maintaining stable rumen pH. The ruminal pH values in this study were within the normal range of 6-7 (McDonald et al., 2022), which supports the growth and activity of rumen microbes required for optimal feed degradation. Therefore, the emulsion of high-PUFA oils-enriched with antioxidants did not interfere with the rumen's buffering capacity.

Table 2 – Rumen fermentation characteristics due to Emulsified high-PUFA oil with natural antioxidant supplementation

Parameters	Treatments	P1	P2	P-value
pH		7.10 ± 0.24	6.95 ± 0.30	0.523
Bacteria population (log cfu/mL)		6.25 ± 0.85	6.95 ± 0.20	0.446
Protozoa population (log cell/mL)		6.21 ± 0.12	6.19 ± 0.03	0.767
NH ₃ (mM)		8.07 ± 1.91	8.12 ± 0.69	0.971
Total VFA (mM)		100.04 ± 4.23	111.76 ± 6.41	0.057

P1 = high-PUFA oils and natural antioxidants supplementation, P2 = emulsified high-PUFA oils and natural antioxidants supplementation. VFA = volatile fatty acid, NH₃ = ammonia

Furthermore, the emulsified high-PUFA oils with natural antioxidants had no significant effect (P>0.05) on both the total ruminal bacteria and protozoa population. The lack of significant changes in the rumen microbial population may be due to the protective role of emulsifiers, which prevent oils and antioxidants from directly interacting with bacteria and protozoa. Previous study has reported that nano-emulsified oils modulate the proportions of volatile fatty acids in ruminal cultures, but do not affect total bacterial counts (El-Sherbiny et al., 2016). In addition, caffeic acid, a phenolic antioxidant, did not significantly affect bacterial counts when administered in emulsified form (Sørensen et al., 2008). In this study, the bacterial population findings align with those of Suharti et al. (2019), who reported that the total ruminal bacterial population with the addition of canola and flaxseed oil calcium soap ranged from 6.00 to 6.68 log cfu/mL. Meanwhile, the protozoa population in this study aligns with Zahera et al. (2024), who reported that the proportion of unprotected to protected fat supplementation in dairy rations ranged from 5.96 to 6.27 log cells/mL.

Next, the emulsified high-PUFA oils enriched with natural antioxidants did not change ($P>0.05$) NH_3 concentration. This finding indicates that protein degradation and microbial nitrogen assimilation remained unaffected by the supplementation form. In this study, the overall rumen microbial population remained unchanged with the addition of emulsified high-PUFA oils enriched with natural antioxidants, which may have maintained the ammonia concentration. Rumen-ammonia-producing bacteria, such as *Clostridium aminophilum*, are primarily responsible for producing ammonia from protein degradation (Adeniyi et al., 2023; Chanu et al., 2024). Additionally, protozoa such as *Entodinium caudatum* engulf bacteria and release oligopeptides and amino acids, which are fermented to ammonia by amino acid-fermenting bacteria (Park and Yu, 2023). The NH_3 concentration in this study aligns with that reported by Riestanti et al. (2024), who found that unsaturated fat supplementation in the form of calcium soap and prill fat resulted in NH_3 concentrations ranging from 7.73 to 9.73 mM. Therefore, our research indicates that supplementing goat feed rations with emulsified high-PUFA oils enriched with natural antioxidants can maintain normal NH_3 concentrations, supporting a well-functioning rumen.

Moreover, the total VFA was not altered by the supplementation of high-PUFA oils enriched with natural antioxidants (Table 2). Emulsification reduces the tension between oil and water, allowing better dispersion of oil droplets (Losada - Barreiro et al., 2020). In addition, the antioxidants at the oil-water interface effectively enhance the oxidative stability of emulsions (Li et al., 2022). The absence of significant changes in total VFA concentration suggests that the emulsified high-PUFA oils enriched with natural antioxidants neither stimulated nor suppressed overall fermentation activity. This result also indicates that emulsification may have played a protective role, maintaining PUFA and antioxidant function without affecting microbial fermentation. The total VFA levels in this study were comparable with those reported by Mukhtiani et al. (2024), ranging from 87.52 to 164.38 mM. Thus, emulsified high-PUFA oils with natural antioxidants may enhance supplementation efficiency in goats. However, further in vivo studies are required to confirm the protective effect of emulsification.

Ruminal in vitro degradability

Adding emulsified high-PUFA oils enriched with natural antioxidants did not influence ($P>0.05$) DMD and OMD (Table 3). The lack of a significant difference in DMD and OMD levels with supplementation in the emulsified form may be caused by the content of active compounds within the emulsion that did not interfere with the rumen microbial population and rumen pH value. The microbial population and pH conditions in the rumen may facilitate the fermentation and feed degradation process (Mickdam et al., 2016). In our study, the DMD and OMD remain unchanged as the emulsified substrates may not be fully utilized by rumen microbes (Eftaxias et al., 2021). Additionally, the antioxidant components in the emulsion may have created a barrier that limits the nutrient degradability (Obando et al., 2015). The digestibility of DM and OM in this study aligns with those reported by Reproto (2025), which ranged from 42.01% to 76.90% and from 65.1% to 88.36%, respectively. Thus, adding emulsified high-PUFA oils enriched with natural antioxidants to the goat ration results in consistent DMD and OMD levels, reflecting its potency as a feed supplement without altering rumen fermentation or degradability.

Table 3 - Ruminal dry matter and organic matter degradability

Parameters	Treatments	P1	P2	P-value
Dry matter degradability (DMD)		76.58 ± 4.73	74.97 ± 2.98	0.643
Organic matter degradability (OMD)		77.28 ± 6.26	74.80 ± 2.65	0.562

P1 = high-PUFA oils and natural antioxidants supplementation, P2 = emulsified high-PUFA oils and natural antioxidants supplementation

CONCLUSION

This study found that emulsified high-PUFA oils enriched with natural antioxidants does not alter rumen pH, microbial population, total VFA, ammonia concentration, and the degradability of dry matter and organic matter. Thus, emulsified high-PUFA oils enriched with natural antioxidants may be used as an efficient and safe feed supplement for goats. Nevertheless, further in vivo studies are strongly required to assess the long-term effects of supplementation on rumen fermentation and animal performance.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dilla Mareistia Fassah. E-mail: dilla_mareistia@apps.ipb.ac.id; ORCID: 0000-0003-4309-9218.

Data availability

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

Authors' contribution

Dilla Mareistia Fassah: Conceptualization, methodology, data curation, data analysis, writing – original draft; Manik Nurul Alfiyyah; Methodology, data curation, data analysis; Safiena Maura Azka: Methodology, data curation, data analysis; S P Damayanti: Methodology, data curation, data analysis; Y Pujiawati: Conceptualization, methodology, writing – review and editing; Lilis Khotijah: Conceptualization, methodology, validation, writing – review and editing; N A Sholeha: Conceptualization, methodology.

Acknowledgements

The authors gratefully acknowledge the Directorate General of Higher Education, Science, and Technology, Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, which supports this study through the BIMA Program 2024 grant (Contract No. 027/E5/PG.02.00.PL/2024).

Ethical approval

All the experimental procedures were conducted by the Animal Ethics Committee of the Directorate of Research and Innovation Licensing Governance and Scientific Authority (No 123/KE.02/SK/06/2023).

Funding

Directorate General of Higher Education, Science, and Technology, Ministry of Higher Education, Science, and Technology of the Republic of Indonesia provided financial support for this study through the BIMA Program 2024 grant (Contract No. 027/E5/PG.02.00.PL/2024).

Consent to publish

All authors agree to the publication of this manuscript.

Competing interests

The authors have not declared any competing interests.

REFERENCES

- Adeniyi A, Bello I, Mukaila T, Monono E and Hammed A (2023). Developing rumen mimicry process for biological ammonia synthesis. *Bioprocess and Biosystems Engineering*, 46(7): 1011–1020. <https://doi.org/10.1007/s00449-023-02880-7>
- Akkaya MR (2018). Fatty acid compositions of sunflowers (*Helianthus annuus* L.) grown in east mediterranean region. *Rivista Italiana Delle Sostanze Grasse*, 95: 239–247. <https://openaccess.atu.edu.tr/items/f679c88b-2d97-40ee-951b-4e648b05af56/full>
- Almeida OC, Ferraz MVC, Susin I, Gentil RS, Polizel DM, Ferreira EM, et al. (2019). Plasma and milk fatty acid profiles in goats fed diets supplemented with oils from soybean, linseed or fish. *Small Ruminant Research*, 170: 125–130. <https://doi.org/10.1016/j.smallrumres.2018.11.002>
- AOAC (2019). Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International, 21st Edition. Association of Official Analytical Chemists International, Washington DC.
- Chaney AL and Marbach EP (1962). Modified reagents for determination of urea and ammonia. *Clinical Chemistry*, 8(2): 130–132. <https://doi.org/10.1093/clinchem/8.2.130>
- Chanu YM, Paul SS, Dey A and Andonissamy J (2024). Deciphering hyperammonia-producing bacteria (HAB) in the rumen of water buffaloes (*Bubalus bubalis*) and their inhibition through plant extracts and essential oils. *Microorganisms*, 12(10): 2040. <https://doi.org/10.3390/microorganisms12102040>
- Chen Y, Cheng H and Liang L (2023). Effect of oil type on spatial partition of resveratrol in the aqueous phase, the protein interface and the oil phase of O/W emulsions stabilized by whey protein and caseinate. *Antioxidants*, 12(3): 589. <https://doi.org/10.3390/antiox12030589>
- Despal D, Irmadani D, Permana IG, Zahera R and Nuraina N (2022). Effect of different unsaturated fatty acids sources on in-vitro fermentability and digestibility of ration in dairy cattle. *Online Journal of Animal and Feed Research*, 12(3): 154-159. <https://doi.org/10.51227/ojafr.2022.20>
- Eftaxias A, Diamantis V, Michailidis C, Stamatelatou K and Aivasidis A (2021). The role of emulsification as pre-treatment on the anaerobic digestion of oleic acid: process performance, modeling, and sludge metabolic properties. *Biomass Conversion and Biorefinery*, 11(2): 251–260. <https://doi.org/10.1007/s13399-019-00600-4>
- El-Sherbiny M, Cieślak A, Szczechowiak J, Kołodziejewski P, Szulc P and Szumacher-Strabel M (2016). Effect of nanoemulsified oils addition on rumen fermentation and fatty acid proportion in a rumen simulation technique. *Journal of Animal and Feed Sciences*, 25(2): 116–124. <https://doi.org/10.22358/jafs/65571/2016>
- Fassah DM and Khotijah L (2016). Vitamin-E supplementation on rich poly-unsaturated fatty acid ration to blood profile of lactation ewes [Pengimbuhan vitamin-E dalam ransum kaya asam lemak tidak jenuh terhadap profil darah induk domba laktasi]. *Jurnal Veteriner*, 17(3): 430–439. <https://doi.org/10.19087/jveteriner.2016.17.3.430>

- Geng Y, Lin G, Zhang K, Dai B, Weng Y, Wu J, et al. (2020). Effects of docosahexaenoic acid-enriched algae supplementation on growth performance, slaughter performance, muscle quality and fatty acid composition in male kids of dairy goats. *Chinese Journal of Animal Nutrition*, 32(4): 1734–1744. <https://doi.org/10.3969/j.issn.1006-267x.2020.04.032>
- Lamothe S, Desroches V and Britten M (2019). Effect of milk proteins and food-grade surfactants on oxidation of linseed oil-in-water emulsions during in vitro digestion. *Food Chemistry*, 294: 130–137. <https://doi.org/10.1016/j.foodchem.2019.04.107>
- Li H, Pan Y, Yang Z, Rao J and Chen B (2022). Modification of β -lactoglobulin by phenolic conjugations: Protein structural changes and physicochemical stabilities of stripped hemp oil-in-water emulsions stabilized by the conjugates. *Food Hydrocolloids*, 128:107578. <https://doi.org/10.1016/j.foodhyd.2022.107578>
- Liao Z, Li Q, Chen J, Du L, Wang J and Fang X (2021). Research progress on the functional activity of flaxseed oil components. *Modern Food Science and Technology*, 37(11): 379–389. <https://doi.org/10.13982/j.mfst.1673-9078.2021.11.0005>
- López-Martínez A and Rocha-Urbe A (2018). Antioxidant hydrophobicity and emulsifier type influences the partitioning of antioxidants in the interface improving oxidative stability in O/W emulsions rich in n-3 fatty acids. *European Journal of Lipid Science and Technology*, 120(1): 1700277. <https://doi.org/10.1002/ejlt.201700277>
- Losada-Barreiro S, Bravo-Díaz C and Paiva-Martins F (2020). Why encapsulate antioxidants in emulsion-based systems, where they are located, and how location affects their efficiency. In: *Emulsion-based Encapsulation of Antioxidants*. Abouzadeh MA, editor. Springer Nature, Cham. https://doi.org/10.1007/978-3-030-62052-3_1
- McDonald P, Edwards RA, Greenhalgh JD, Morgan CA, Sinclair LA and Wilkinson RG (2022). *Animal Nutrition*. 8th Edition. Pearson Education Limited, Harlow.
- Mickdam E, Khiaosa-ard R, Metzler-Zebeli BU, Klevenhusen F, Chizzola R and Zebeli Q (2016). Rumen microbial abundance and fermentation profile during severe subacute ruminal acidosis and its modulation by plant derived alkaloids in vitro. *Anaerobe*, 39: 4–13. <https://doi.org/10.1016/j.anaerobe.2016.02.002>
- Mirzaei-Alamouti H, Abdollahi A, Rahimi H, Moradi S, Vazirigohar M and Aschenbach JR. (2021). Effects of dietary oil sources (sunflower and fish) on fermentation characteristics, epithelial gene expression and microbial community in the rumen of lambs fed a high-concentrate diet. *Archives of Animal Nutrition*, 75(6): 405–421. <https://doi.org/10.1080/1745039X.2021.1997539>
- Muktiani A, Widiyanti W and Pandupuspitasari NS. (2024). Supplementation of zinc palm oil soap improves feed fermentability and unsaturated fatty acid profile in rumen liquid. *Tropical Animal Science Journal*, 47(3): 371-380. <https://doi.org/10.5398/tasj.2024.47.3.371>
- Najini R and Wahdaningsih S (2024). The effect of maceration and soxhletation method on total phenolic content and antioxidant activity of jackfruit leaves (*Artocarpus heterophyllus* L.) [Pengaruh metode maserasi dan metode soxhletasi terhadap kadar fenolik total dan aktivitas antioksidan daun Nangka (*Artocarpus heterophyllus* L.)] *Jurnal Farmasi IKIFA*, 3(1): 13–25. <https://epik.ikifa.ac.id/index.php/jfi/article/view/109>
- Obando M, Papastergiadis A, Li S and De Meulenaer B (2015). Impact of lipid and protein co-oxidation on digestibility of dairy proteins in oil-in-water (O/W) emulsions. *Journal of Agricultural and Food Chemistry*, 63(44): 9820–9830. <https://doi.org/10.1021/acs.jafc.5b03563>
- Ogimoto K and Imai S (1981). *Atlas of Rumen Microbiology*. Japan Scientific Societies, Tokyo.
- Ojwang RA, Muge EK, Mbatia B and Ogoyi DO (2017). Comparative analysis of phytochemical composition and antioxidant activities of methanolic extracts of leaves, roots and bark of jackfruit (*Artocarpus heterophyllus*) from selected regions in Kenya and Uganda. *Journal of Advances in Biology & Biotechnology*, 16(1): 1–13. <https://doi.org/10.9734/JABB/2017/37355>
- Oliveira RV, Amaral L da S, Milagres CA, Santos CTB, Pelli A and Paiva AD (2021). Assessment of temperature and acid tolerance of *Bacillus subtilis* isolated from a Brazilian fruit juice-added soy beverage. *Interação*, 21(2): 38–48. <https://doi.org/10.53660/inter-105-s103-p38-48>
- Park T and Yu Z (2022). Interactions between *Entodinium caudatum* and an amino acid-fermenting bacterial consortium: fermentation characteristics and protozoal population in vitro. *Journal of Animal Science and Technology*, 65(2): 387. <https://doi.org/10.5187/jast.2022.e111>
- Pernin A, Bosc V, Soto P, Le Roux E and Maillard M (2019). Lipid oxidation in oil-in-water emulsions rich in omega-3: effect of aqueous phase viscosity, emulsifiers, and antioxidants. *European Journal of Lipid Science and Technology*, 121(9): 1800462. <https://doi.org/10.1002/ejlt.201800462>
- Pujiawati Y, Khotijah L and Wiryawan I (2023). Screening of antioxidant activities and their bioavailability of tropical plants. *IOP Conference Series: Earth and Environmental Science*, 1182(1): 012083. <https://doi.org/10.1088/1755-1315/1182/1/012083>
- Reproto RO. (2025). Effects of lipid source supplementation on rumen microbial population dynamics and in vivo digestibility of napier grass in goats. *International Journal of Agriculture and Biosciences*, 14(3): 483–489. <https://doi.org/10.47278/journal.ijab/2025.028>
- Ribeiro MD, Cabral SLS, Fabino R, Calaça AMM, Silva CJ, Zaccaroni OF, et al. (2025). Inclusion of oils in the in vitro fermentation of elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon). *Revista Brasileira de Zootecnia*, 54. <https://doi.org/10.37496/rbz5420240160>
- Riestanti LU, Despal, Retnani Y and Andarwulan N (2024). Unsaturated fat supplemented in the form of Ca-soap and prill fat in dairy cattle ration: in vitro study. *BIO Web of Conferences*, 123: 01016. <https://doi.org/10.1051/bioconf/202412301016>
- Romanić RS, Lužaić TZ and Radić BD (2021). Enriched sunflower oil with omega 3 fatty acids from flaxseed oil: prediction of the nutritive characteristics. *LWT*, 150: 112064. <https://doi.org/10.1016/j.lwt.2021.112064>
- Roque-Jiménez JA, Rosa-Velázquez M, Pinos-Rodríguez JM, Vicente-Martínez JG, Mendoza-Cervantes G, Flores-Primo A, et al. (2021). Role of long chain fatty acids in developmental programming in ruminants. *Animals*, 11(3): 762. <https://doi.org/10.3390/ani11030762>
- Roschel GG, Silveira TFF, Cajaiba LM and Castro IA (2019). Combination of hydrophilic or lipophilic natural compounds to improve the oxidative stability of flaxseed oil. *European Journal of Lipid Science and Technology*, 121(5). <https://doi.org/10.1002/ejlt.201800459>
- Saxena N and Mandal A (2022). Surfactants in Petroleum Industry. In: *Natural Surfactants: Application in Enhanced Oil Recovery*. Springer International Publishing, Cham. pp. 11–17. https://doi.org/10.1007/978-3-030-78548-2_3
- Shen JS, Chai Z, Song LJ, Liu JX and Wu YM (2012). Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows. *Journal of Dairy Science*, 95(10): 5978–5984. <https://doi.org/10.3168/jds.2012-5499>
- Sørensen A-DM, Haahr A-M, Becker EM, Skibsted LH, Bergenstahl B, Nilsson L, et al. (2008). Interactions between iron, phenolic compounds, emulsifiers, and pH in omega-3-enriched oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 56(5): 1740–

1750. <https://doi.org/10.1021/jf072946z>

Suharti S, Aliyah D and Suryahadi S (2019). In vitro rumen fermentation characteristics with the addition of vegetable oil calcium soaps under different buffer systems [Karakteristik fermentasi rumen in vitro dengan penambahan sabun kalsium minyak nabati pada buffer yang berbeda] Jurnal Ilmu Nutrisi dan Teknologi Pakan, 16(3): 56-64. <https://journal.ipb.ac.id/index.php/jurnalintp/article/view/24939>

Tilley JMA and Terry RA (1963). A two-stage technique for the in vitro digestion of forage crops. Grass and Forage Science, 18(2): 104-111. <https://doi.org/10.1111/j.1365-2494.1963.tb00335.x>

Wardeh MF (1981). Models for Estimating Energy and Protein Utilization for Feeds. Dissertation. Utah State University, Utah, USA. <https://doi.org/10.26076/9026-5aad>

Zahera R, Pratiwi MI, Fitri A, Koike S, Permana IG and Despal (2024). Coconut fatty acid distillate Ca-soap with different calcium sources: Effects of varied proportions of protected and unprotected fat supplementation in dairy rations. Dairy, 5(3): 542-554. <https://doi.org/10.3390/dairy5030041>

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

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
- j. CONCLUSION

The sections "RESULTS AND DISCUSSION" can be presented jointly.

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, 1992b, 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 euphrasens*) 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: Αα - alpha, Ββ - beta, Γγ - gamma, Δδ - delta, Εε - epsilon, Ζζ - zeta, Ηη - eta, Θθ - theta, Ιι - iota, Κκ - kappa, Λλ - lambda, Μμ - mu, Νν - nu, Ξξ - xi, Οο - omicron, Ππ - pi, Ρρ - rho, Σσ - sigma, Ττ - tau, Υυ - 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

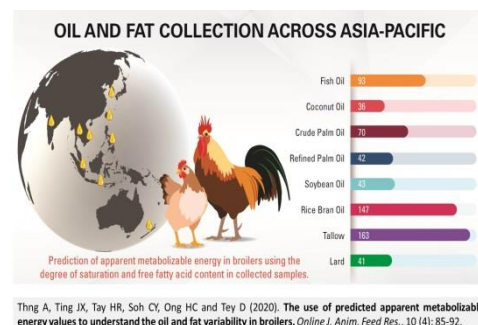
Abbreviations should be presented in one paragraph, in the format: "term: definition". Please separate the items by ";".
E.g. ANN: artificial neural network; CFS: closed form solution;

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](#).



Review/Decisions/Processing

Firstly, all manuscripts will be checked by one of the plagiarism finding tools ([iThenticate](#) and or [Turnitin](#)). A double-blind reviewing model is used by OJAfr for non-plagiarized papers. The manuscript is edited by the English language editor and checked by at least 2 reviewers at least 2 reviewers who are not part of the journal's editorial staff and mostly suggested by section editors. Manuscripts that are judged to be of insufficient quality or unlikely to be competitive enough for publication are returned to the authors at the initial stage.

We always try to avoid delays in the reviewing process, but it relies on the time and cooperation of the referees that works without any remuneration, hence, it may take 2 weeks to 2 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 [Turnitin](#) 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.



[ABOUT US](#)

| [CONTACT US](#)

| [PRIVACY POLICY](#)

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@ojafir.ir
Submit Online >>

Journal of Civil Engineering and Urbanism



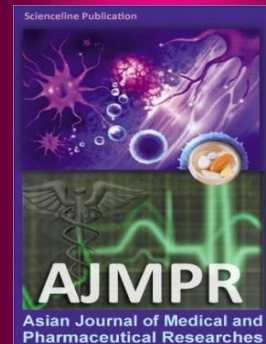
ISSN 2252-0430; Bi-monthly
View Journal | Editorial Board
Email: ojceu@ojceu.ir
Submit Online >>

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



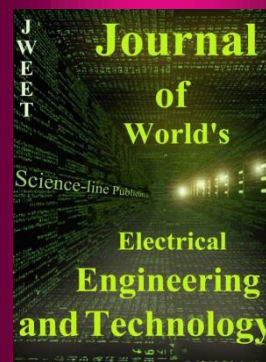
ISSN: 2322-4568; Quarterly
View Journal | Editorial Board
Email: editor@wj.science-line.com
Submit Online >>

Journal of Educational and Management Studies



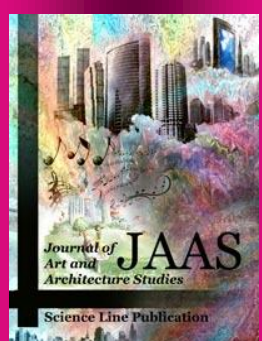
ISSN: 2322-4770; Quarterly
View Journal | Editorial Board
Email: info@jems.science-line.com
Submit Online >>

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 >>

ABOUT
LEADERSHIP
AIMS AND SCOPE
PUBLISHING ETHICS
POLICIES
TERMS AND CONDITIONS
CONTACT US

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