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Volume 15 (2); March, 2025

Research Paper

Evaluation of metabolic status in Holstein cow under short-term cold stress

Oliynyk V, Zacharenko M, Shevchenko L, Mykhalska V, Poliakovskyi V, Slobodyanyuk N, Ivaniuta A, Pylypchuk O, Omelian A, and Gruntkovskiy M.

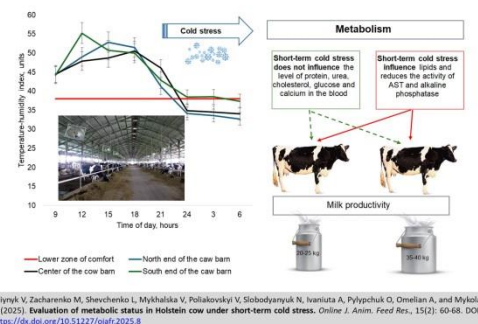
Online J. Anim. Feed Res., 15(2): 60-68, 2025; pii: S222877012500008-15
DOI: <https://dx.doi.org/10.51227/ojafr.2025.8>

Abstract

The research investigates the influence of short-term cold stress on the metabolic status of Holstein cows, when they are kept in large cowsheds in the Ukrainian climate. In the winter (cold season), the air temperature in such cowsheds depends on the ambient air temperature. The temperature-humidity index of the cowshed air is less than 38 at night, which is estimated as mild cold stress. Short-term cold stress has no effect on the level of total protein, urea, cholesterol, glucose, and calcium, but it increases the total lipids in the blood plasma of second-lactation cows with a daily milk yield of 20-25 kg by 32.3%, and in those with a daily milk yield of 35-40 kg, by 1.6-fold. For third-lactation cows with a daily milk yield of 20-25 kg total lipids increase by 1.5-fold compared with the data for first-lactation cows with a daily milk yield of 20-25 kg. Cold stress has no significant effect on the activity of alanine aminotransferase (ALT) and amylase, but it significantly reduced the activity of aspartate aminotransferase (AST) in the blood plasma of second- and third-lactation cows with a daily milk yield of 20-25 kg by 14.3% and 17.8%, respectively, compared with first-lactation cows with a daily milk yield of 35-40 kg. Under short-term cold stress, the activity of plasma alkaline phosphatase decreases by 36% in second-lactation cows with a milk yield of 35-40 kg, by 44% in third-lactation cows with a milk yield of 20-25 kg, and by 38% in cows with a milk yield of 35-40 kg compared to first-lactation cows with a milk yield of 20-25 kg. It can be concluded that short-term cold stress causes changes in the metabolic profile of high-yielding Holstein cows, which can provide valuable information about the health of the animals during acclimatization and help develop corrective measures to prevent diseases and reduce milk productivity in the coldest period of the year.

Keywords: Acclimatization, Cattle, Climate, Enzymes, Lactation.

[Full text-PDF]



Oliynyk V, Zacharenko M, Shevchenko L, Mykhalska V, Poliakovskyi V, Slobodyanyuk N, Ivaniuta A, Pylypchuk O, Omelian A, and Mykhalska G (2025). Evaluation of metabolic status in Holstein cow under short-term cold stress. Online J. Anim. Feed Res., 15(2): 60-68. DOI: <https://dx.doi.org/10.51227/ojafr.2025.8>

Research Paper

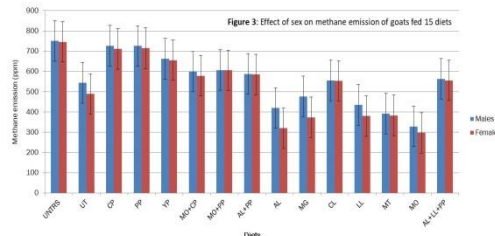
Methane emission of goats fed fifteen diets: on-farm observations

Sarkwa FO, Timpong-Jones EC, Adogla-Bessa T and Antwi V.

Online J. Anim. Feed Res., 15(2): 69-78, 2025; pii: S222877012500009-15
DOI: <https://dx.doi.org/10.51227/ojafr.2025.9>

Abstract

It is suggested that the measurement of methane production from enteric fermentation must be done under situations similar to that of typical farming methods. It is against this background that this study measured methane emission from goats on a farm to ascertain the real situation on most farms. The objective of this study was to measure performance and methane emission from goats fed Ghanaian ruminant diets comprising of basal diets supplemented with browse leaves and to determine the effects of temperature and humidity on methane emission. Ten West African dwarf goats (5 males and 5 females; average weight 14 kg \pm 1.01) were fed fifteen Ghanaian ruminant diets for four months. Each diet was randomly fed twice in 24 hours for 2 days in a month. Methane emission, temperature and humidity were measured using handheld gas methane detector. Completely randomized design was used. Dry matter intake (DMI) was lowest ($P < 0.05$) when cassava (*Manihot esculenta*) peels were fed and highest ($P < 0.05$) when plantain peels were supplemented with *Moringa oleifera*. Weight gain, DMI and methane emission from manure increased with time. The highest enteric methane emission was recorded ($P < 0.05$) when untreated rice straw (749 ppm) was fed and the lowest was recorded ($P < 0.05$) when *Moringa oleifera* leaves (313 ppm) were fed. High environmental temperature favored low methane emission and high humidity was associated with high methane emission. In conclusion, feeding browse leaves alone and browse supplementation with basal diets resulted in lower methane emission than feeding basal diets alone. Moderate weight gains were recorded. High environmental temperature was inversely related to methane emission and high environmental humidity was directly related to methane emission. It is recommended that, browse leaves be



Sarkwa FO, Timpong-Jones EC, Adogla-Bessa T and Antwi V (2025). Methane emission of goats fed fifteen diets: on-farm observations. Online J. Anim. Feed Res., 15(2): 69-78. DOI: <https://dx.doi.org/10.51227/ojafr.2025.9>

incorporated in the feed of ruminants, especially when environmental temperatures are low and humidity is high.

Keywords: Basal diets, Browse leaves, Dry matter intake, Humidity, Temperature, Weight gain.

[Full text-PDF]

Research Paper

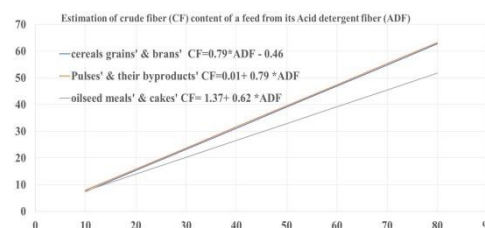
Estimation of crude fiber content of a feed from its ADF value where there is no laboratory service

Assefa Woldemariam G

Online J. Anim. Feed Res., 15(2): 79-88, 2025; pii: S222877012500010-15
DOI: <https://dx.doi.org/10.51227/ojafr.2025.10>

Abstract

Because of the cost and inaccessibility of laboratory facilities, animal feed formulation at the farm level, in many parts of Ethiopia, is based on feed database information. However, nowadays many laboratories are phasing out the Weende crude fiber (CF) method of analysis. The fiber content of feeds available in most feed databases (including the sub-Saharan Africa feeds composition database) are a result of detergent method analysis (NDF, ADF and lignin). However, CF is still used in poultry feed formulation and forage analysis for horses, in addition to the neutral detergent fiber (NDF) fraction for determining fiber in different countries. Since there is a statistically ($P < 0.01$) difference between the CF and acid detergent fiber (ADF) value of a feed, ADF can't be used directly in place of CF. Therefore, this work aims to formulate a regression equation that could roughly estimate the CF level of a feed from its NDF and ADF values. Considering the strong multicollinearity between NDF and ADF, this study developed separate models for ADF and NDF and compared them based on R^2 and Akaike Information Criterion (AIC), and the ADF-based model provided a better fit. The equations $0.79 \times \text{ADF} - 0.46$, $0.01 + 0.79 \times \text{ADF}$, and $1.37 + 0.62 \times \text{ADF}$ have effectively predicted CF for cereal grains and beans, pulses and byproducts, and also oilseed meals and cakes, respectively. For grass forages, the equation $3.38 + 0.76 \times \text{ADF}$, tested on 10 forages, showed potential but remains unreliable due to its R^2 value below 0.8. Finally, it is concluded that this approach provides a practical alternative for estimating CF where laboratory services or database information are unavailable.



Assefa Woldemariam G (2025). Estimation of crude fibre content of a feed from its ADF value where there is no laboratory service. Online J. Anim. Feed Res., 15(2): 79-88. DOI: <https://dx.doi.org/10.51227/ojafr.2025.10>

Keywords: Crude Fiber, Estimation, Feed database information, Prediction, Regression.

[Full text-PDF] [Supplementary materials]

Research Paper

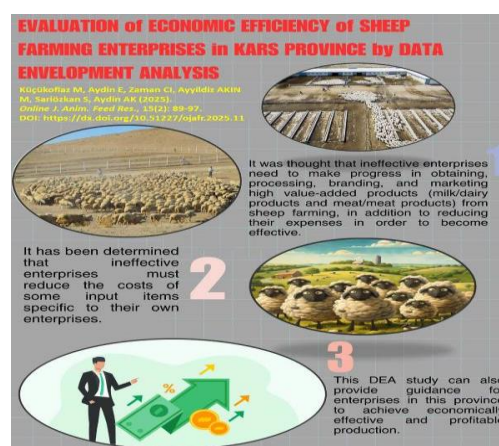
Evaluation of economic efficiency of sheep farming enterprises in Kars province by data envelopment analysis

Küçükoflaz M, Aydın E, Zaman CI, Ayyildiz AKIN M, Sariözkan S, Aydın AK.

Online J. Anim. Feed Res., 15(2): 89-97, 2025; pii: S222877012500011-15
DOI: <https://dx.doi.org/10.51227/ojafr.2025.11>

Abstract

In this study, it was aimed to determine the economic activities of sheep farming enterprises in Kars province, Turkey by Data Envelopment Analysis (DEA). For this purpose, data obtained from face-to-face surveys conducted on 99 sheep farming enterprises in Kars Province were used. In the DEA applied to determine the economic efficiency of sheep enterprises, the Charnes Cooper Rhodes (CCR) was used according to the input-oriented scale. According to the study findings, the average age of the owners of the enterprises, all of whom were male, was 46 years, and their experience was average 9 years. It was determined that the majority of the farm owners (76.8%) were primary and secondary school graduates. It was determined that 67.7% of the enterprises were farming only Akkaraman, 3% were farming only Morkaraman, and 29.3% were farming both of the breeds. According to the DEA results used to determine the economic efficiency of enterprises, 41 enterprises (41.4%) were determined to be effective and 58 (58.6%) were determined to be inefficient. Consequently, it was concluded that inefficient enterprises need to reduce their input costs to become economically effective. In addition, it has been considered that it is very important for enterprises to make progress in the stages of obtaining, processing, branding and marketing high value-added products (milk/dairy products and meat/meat products, wool) from sheep farming to increase their income and



profitability.

Keywords: Data envelopment, Economic efficiency, Kars, Sheep farming.

[Full text-PDF]

Research Paper

Filleting attributes, length-weight relationship and condition factor of some local fish species collected from Yanbu fish market (Red Sea coast, Saudi Arabia)

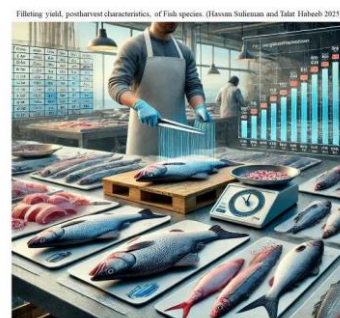
Adam Sulieman HM and Habeeb TH.

Online J. Anim. Feed Res., 15(2): 98-107, 2025; pii: S222877012500012-15
DOI: <https://dx.doi.org/10.51227/ojafr.2025.12>

Abstract

Analyzing the filleting attributes of fish is essential for evaluating the commercial viability of fish products. This study assesses the filleting attributes, length-weight relationships, and condition factors of three commercially important fish species (*Lethrinus nebulosus*, *Epinephelus tauvina*, and *Plectorhinchus gaterinus*) from the Yanbu fish market in Saudi Arabian Red Sea coast. Fillet production results indicated a decreasing trend in edible portions among these species, with *Lethrinus nebulosus* yielding the most, followed by *Plectorhinchus gaterinus* and *Epinephelus tauvina*. Fish with smaller heads and medium-sized skeletons produced higher edible fillet yields. Linear regression analysis revealed no significant differences, establishing a linear correlation between net edible weight and fillet yield. The length-weight relationship analyses for *Lethrinus nebulosus*, *Plectorhinchus gaterinus*, and *Epinephelus tauvina* indicated positive allometric growth. Condition factor analysis showed that *Lethrinus nebulosus* had the lowest mean condition factor (1.05 ± 0.05), while *Epinephelus tauvina* had the highest mean condition (1.67 ± 0.15). A robust association between weight and fillet yield components was also observed. These findings enhance our understanding of the biological and economic characteristics of these species along the Yanbu coastline, supporting fisheries management and postharvest research in line with conservation and restoration efforts.

Keywords: Condition factor, Edible weight, Filleting yield, Fish products, Postharvest characteristics.



Adam Sulieman HM and Habeeb TH (2025). Filleting attributes, length-weight relationship and condition factor of some local fish species collected from Yanbu fish market (Red Sea coast, Saudi Arabia). Online J. Anim. Feed Res., 15(2): 98-107. DOI: <https://dx.doi.org/10.51227/ojafr.2025.12>

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Review

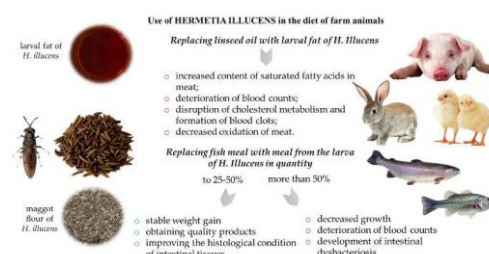
Prospects for using *Hermetia illucens* larvae in the diet of farm animals: a review

Maltseva T, Rudoy D, Olshevskaya A, Odabashyan M, and Shevchenko V.

Online J. Anim. Feed Res., 15(2): 108-116, 2025; pii: S222877012500013-15
DOI: <https://dx.doi.org/10.51227/ojafr.2025.13>

Abstract

Hermetia illucens larvae is a promising raw material as an alternative ecological raw material for obtaining feed ingredients. The aim of this review is to gain a comprehensive understanding of the current state of research in this topic by critically analyzing existing studies. Based on the review, recommended doses of defatted *Hermetia illucens* larval meal in the diet were identified. Replacing fish meal with *Hermetia illucens* larval meal in the amount of 25 and 50% ensures stable weight gain and high-quality fish products. When feeding largemouth bass and red hybrid tilapia, the recommended proportion of replacing fish meal with insect meal is no more than 30%. Substitution of vegetable protein with *Hermetia illucens* protein in the diet of sea bass in the amount of 40% improves the histological condition of intestinal tissue. Replacing linseed fat in the amount of 30 and 60 g/kg of feed with fat from *Hermetia illucens* larvae in feeding rabbits revealed a negative effect on meat quality: a high content of saturated fatty acids is observed. As a positive effect of *Hermetia illucens* fat, a decrease in meat oxidation can be noted. The use of full-fat *Hermetia illucens* meal in the diet of piglets should be limited to 2%. However, the protein of the *Hermetia illucens* larvae has great potential and can be partially replaced in combination with the protein of other insects. A number of studies presented in this review have proven the economic efficiency of using *Hermetia illucens* larval meal in feed production: the cost of *Hermetia illucens* larval meal is lower than the cost of fish meal by 0.35 USD/kg, which increases the profitability of using this type of raw material by 25%. The problems of the widespread use of *Hermetia illucens* larval meal in animal feeding have been identified, which consist in the low attractiveness of meat and fish products grown on



Maltseva T, Rudoy D, Olshevskaya A, Odabashyan M, and Shevchenko V (2025). Prospects for using *Hermetia illucens* larvae in the diet of farm animals: a review. Online J. Anim. Feed Res., 15(2): 108-116. DOI: <https://dx.doi.org/10.51227/ojafr.2025.13>

feed using insects. In order to reduce the negative attitude of consumers to such food products, it is necessary to increase public awareness of the environmental friendliness and safety of using such components in animal feeding. Keywords: Fat sources, Feed components, *Hermetia illucens*, Insect flour, Protein sources.

[Full text-PDF]

Research Paper

Effectiveness of *Lactobacillus fermentum* CMUL-54 and *Lactobacillus fermentum* B978 as probiotic candidates producing mannanase, cellulase and protease activities for poultry

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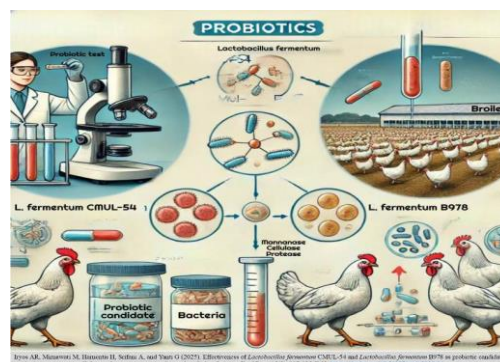
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Abstract

The present research investigated the potential of *Lactobacillus fermentum* strains CMUL-54 and B978 as probiotic candidates with mannanase, cellulase, and protease activities. The materials used in this research included *L. fermentum* CMUL-54, *L. fermentum* B978, MRS Broth containing oxgall, and various equipment and chemicals for analyzing probiotic candidates, mannanase, cellulase, and protease activities. This study utilized quantitative analysis conducted using a paired two-sample t-test with ten replications. The results revealed that *L. fermentum* CMUL-54 could be significantly ($P < 0.01$) used as a probiotic candidate, showing resistance to temperatures of 42°C ($9.9 \times 10^9 \pm 0.71$ CFU/ml), gastric pH ($72.35 \pm 0.80\%$), bile salt resistance ($87.69 \pm 3.66\%$), and hydrophobicity test to the intestine ($92.40 \pm 0.30\%$). *Lactobacillus fermentum* CMUL-54 also exhibited significant inhibitory zones against lactic acid bacteria (LAB) and pathogenic bacteria such as *Escherichia coli* (13.27 ± 0.13 mm), *Salmonella enteritidis* (13.91 ± 0.13 mm), *Staphylococcus aureus* (17.75 ± 0.24 mm), high activity mannanase (12.36 ± 0.61 U/ml), cellulase (12.42 ± 0.24 U/ml) and protease (11.30 ± 0.08 U/ml). It is concluded that *L. fermentum* CMUL-54 exhibited superior probiotic properties compared to *L. fermentum* B978, thus positioning it as a more promising candidate for improving broiler performance through enhanced digestion and overall health.

Keywords: Enzyme activity, *Lactobacillus fermentum* CMUL-54, *L. fermentum* B978, Probiotics

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EVALUATION OF METABOLIC STATUS IN HOLSTEIN COW UNDER SHORT-TERM COLD STRESS

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Supporting Information

ABSTRACT: The research investigates the influence of short-term cold stress on the metabolic status of Holstein cows, when they are kept in large cowsheds in the Ukrainian climate. In the winter (cold season), the air temperature in such cowsheds depends on the ambient air temperature. The temperature-humidity index of the cowshed air is less than 38 at night, which is estimated as mild cold stress. Short-term cold stress has no effect on the level of total protein, urea, cholesterol, glucose, and calcium, but it increases the total lipids in the blood plasma of second-lactation cows with a daily milk yield of 20-25 kg by 32.3%, and in those with a daily milk yield of 35-40 kg, by 1.6-fold. For third-lactation cows with a daily milk yield of 20-25 kg total lipids increase by 1.5-fold compared with the data for first-lactation cows with a daily milk yield of 20-25 kg. Cold stress has no significant effect on the activity of alanine aminotransferase (ALT) and amylase, but it significantly reduced the activity of aspartate aminotransferase (AST) in the blood plasma of second- and third-lactation cows with a daily milk yield of 20-25 kg by 14.3% and 17.8%, respectively, compared with first-lactation cows with a daily milk yield of 35-40 kg. Under short-term cold stress, the activity of plasma alkaline phosphatase decreases by 36% in second-lactation cows with a milk yield of 35-40 kg, by 44% in third-lactation cows with a milk yield of 20-25 kg, and by 38% in cows with a milk yield of 35-40 kg compared to first-lactation cows with a milk yield of 20-25 kg. It can be concluded that short-term cold stress causes changes in the metabolic profile of high-yielding Holstein cows, which can provide valuable information about the health of the animals during acclimatization and help develop corrective measures to prevent diseases and reduce milk productivity in the coldest period of the year.

Keywords: Acclimatization, Cattle, Climate, Enzymes, Lactation.

INTRODUCTION

The cattle's ability for acclimatization is of great importance, especially during importation to Ukraine. The cattle are capable of feeling cold stress in the coldest season of the year, which falls on January-February in this climatic zone (Borshch et al., 2021).

Cold stress may have an adverse effect on the metabolic and immunological status of cows (Hu et al., 2021), which in turn reduces milk production of the animals, while increasing the economic losses. These changes may be associated with differences in the low-temperature adaptation of high-producing cows, when the organism needs additional energy for maintaining body temperature and reducing heat losses. Studies conducted on the meat-type cattle have shown that a long stay in a cold environment may cause a stress reaction based on metabolic regulation of heat generation. It has been proven (Wang et al., 2023) that the behavior, digestive functions, enzyme activity, and hormone levels in the tissues of Simmental cattle changed during the winter, which increased heat generation by the cattle for maintaining a constant body temperature and eventually led to impaired growth and development.

Studies by Kim et al. (2023) have shown that when cattle experience cold stress, the metabolic rate increases, which causes an increase in the heart rate, rectal temperature, deeper breathing, and muscle tremors. In such a state, the level of cortisol and unesterified fatty acids increases in young cattle against the background of decreased blood glucose levels, which has a significant influence on the fodder intake due to changes in energy metabolism of the organism. Against the background of cold stress, the behavior pattern of cattle also changes, including the time spent standing and lying down, in particular, there has been a report of increased (Kaygusuz and Akdağ, 2021) duration of a standing and decreased duration of a lying down in the Simmental cows under cold stress. This indicates that, due to a combination of low temperatures and high humidity, the wet floor of the cowshed affects the behavior of cattle, and they prefer to stand rather than lie down in order to balance their body's heat loss.

It is noted that when the ambient air temperature decreases from -8.0 to -22.6 °C within 24 hours, the air temperature in the frame-type cowshed dropped from 9.7 to -0.39 °C and during the entire period depended on the daily

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fluctuations of the ambient air temperature (Zakharenko et al., 2020). Under such conditions, the temperature of the skin on the head, neck, thoracic and hind limbs, trunk and mammary gland drops in lactating cows. It is observed that when the air temperature in the cowshed drops within 24 hours, the number of cows standing or lying in the boxes increases, but the number of cows moving, consuming fodder and water decreases.

Different stages of growth, physiological state, milk productivity and breed characteristics of cattle give a mixed response to energy needs and metabolic reactions, which leads to different levels of resistance to cold stress (Kang et al., 2020). However, the question of cow's metabolic reaction to the effects of cold stress, depending on daily milk yield and age, remain unclear. The adaptation of productive animals, particularly cattle, to environmental conditions is largely based on ensuring thermal comfort, which significantly influences the realization of their genetic productive and reproductive potential (Polli et al., 2020; Silva et al., 2021).

It had been proven that even long-term exposure to mild cold conditions in animals fosters the development of an adaptive response, which includes increased heat generation, fodder consumption, and metabolic activity in tissues, which in turn leads to changes in digestive system function (Hu et al., 2022). Studies on Sanhe and Holstein heifers exposed to -25°C for an hour have shown that the latter exhibited a more pronounced metabolic response to cold, but both breeds responded to acute cold exposure by altering volatile fatty acid and glucose metabolism (Hu et al., 2022).

Cold stress also has an influence on the hormone levels of the thyroid, pancreas, and adrenal glands (Fu et al., 2022; Lezama-García et al., 2022), which leads to adaptive changes and intensification of metabolic mechanisms aimed at generating or conserving heat. It is considered that the elevated cortisol level in cattle blood indicates the activation of the immune defense system in response to critically low ambient temperatures.

The main response of cattle to the cold stress is a change in feeding behavior (Méndez et al., 2020; He et al., 2022). The ability of dairy cows to acclimatize to environment conditions is essential for finding the best management strategy for cattle breeding, as the animals may react differently depending on the characteristics of each region. Thus, appropriate adjustments to production practices are made based on determining the effects of stress caused by seasonal environmental fluctuations (Summer et al., 2018).

Cold stress often causes reduced growth rates and increased mortality, which leads to significant economic losses in cattle breeding throughout the world (Hu et al., 2021). According to the intensity and duration of its effects on the organism, cold stress is classified as acute (short-term) or chronic (long-term) (Zhao., 2020).

Currently, the problem of restoring and increasing of the cattle population, especially dairy cows, is particularly acute in Ukraine. This can be achieved by importing heifers from Western European countries which involves their adaptation to the climatic zone of Ukraine, which is characterized by significant temperature fluctuations in the warm and cold seasons of the year (Borshch et al., 2021). Moreover, if dairy cows are kept in frame-type cowsheds made of easy-to-assemble metal structures, they are not always sufficiently protected them from winter wind and snow, which may lead to a decrease in air temperature to sub-zero levels and an increase in cold stress of cattle (Zakharenko et al., 2019).

The ambient temperature has the greatest influence on animal physiology. Therefore, the limits of the comfort zone, which are within the range of temperatures that provides a relatively stable and balanced functioning of the thermoregulatory system, have been established for each species and age-sex group (Krishnan et al., 2023). At the same time, the digestive function is inhibited, which leads to increased consumption of feed dry matter that is unable to meet heat generation needs and results in decreased productivity and growth intensity of the meat cattle.

Reports by Nakajima et al. (2019) and Abbas et al. (2020) also mention that there are physiological, metabolic and immune changes in cattle influenced by cold stress, which are aimed at maintaining homeostasis but lead to decreased productivity in cattle. At the same time, it is pointed out that the range of heat stress zone for cattle has been determined and experimentally confirmed in accordance with the temperature-humidity index (THI), while the range of cold stress and its effects on the metabolic state of dairy cows have not yet been determined.

The purpose of the study was to assess the temperature and humidity parameters of a frame-type cowshed in the coldest season of the year in Ukraine and the the metabolic response to short-term cold stress in lactating cows of the German-bred black-and-white Holstein breed, which will allow for the development of preventive measures and the reduction of possible economic losses.

MATERIALS AND METHODS

All experiments were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes dated 1986, as well as the Law of Ukraine "On the Protection of Animals from Cruelty Treatment" dated 21.02.2006 No. 3447-IV and amended on 04.08.2017. The study was approved by the bioethics commission of the National University of Bioresources and Nature Management of Ukraine in November, 2018.

The study was carried out on the lactating cows of the black-and-white Holstein breed imported to Ukraine from Germany. It was carried out at Ukrainian Milk Company LLC in February 2021. The cows with an average weight of 550 to

600 kg were selected for the experiment. For this, 6 groups of 12 cows each, including first, second and third-lactation cows, were formed according to the scheme indicated in Table 1.

Table 1 - Distribution scheme of lactating cows into groups to study the effect of short-term cold stress, n=12

Cows (daily-average milk yield, kg)					
First lactation		Second lactation		Third lactation	
n = 12	n = 12	n = 12	n = 12	n = 12	n = 12
20-25	35-40	20-25	35-40	20-25	35-40
Sectional housing of cows in cowshed of 250 cows each					

After reaching the minimum air temperature within 10 days of -15 °C or lower, in the morning before the feeding of all cows in all groups, the blood samples were taken from the tail vein in 5 ml syringes with lyophilized heparin for analysis. The cows were kept in the frame-type cowshed made of easy-to-assemble metal structures, designed for the simultaneous housing of 1,000 cows, which was divided into 4 sections of 250 cows each. The plenum ventilation of this facility is provided by the regulation of the side curtains, the exhaust ventilation is achieved through slotted holes on the roof ridge.

The cow housing system is a confinement system with a loose cubicle housing method. The cows are milked three times a day in a milking house equipped with the "Parallel" milking machine for simultaneous milking of 50 cows. Animal manure is mechanically removed from the cowshed using a tractor with a bulldozer attachment.

The physical properties of the ambient air and the cowshed air were determined on a daily basis at intervals of 3 hours: at 9:00 a.m., 12:00 p.m., 3:00 p.m., 6:00 p.m., 9:00 p.m., 12:00 a.m. and 3:00 a.m. The temperature and relative humidity of the cowshed were measured at three points: at the ends (northern section (part 1) and southern section (part 2)) and in the center at the level of the midline of the cows' trunks.

The temperature and relative humidity of the air were determined using of the electronic weather meter Kestrel-3000 (USA).

The temperature-humidity index was calculated using the following formula:

$$THI = 0.8 \times AT + (RH (\%)/100) \times [(AT - 14.4) + 46.4] \text{ (Mader et al., 2006).}$$

where AT is the air temperature in degree Celsius (°C) and RH represents the percentage of the relative humidity.

In the blood plasma, the concentration of glucose, total protein, urea, total lipids, cholesterol, calcium, inorganic phosphorus, as well as the activity of ALT, AST, α -amylase, alkaline phosphatase, gamma-glutamyl transpeptidase was determined using the reagent sets manufactured by Pointe Scientific Inc. (USA) and the semi-automatic analyzer Pointe 180 (Poland). The study results were statistically processed using ANOVA program, and the tabulated data are presented as $\bar{x} \pm SD$ (mean \pm standard deviation). The difference between the groups was considered significant at $P < 0.05$ according to the Tukey test (taking into account the Bonferroni correction).

RESULTS AND DISCUSSION

The determination of the ambient air temperature in the coldest period of the year has shown that its lowest value was recorded between 9:00 p.m. and 9:00 a.m., while after 9:00 a.m. it reached its maximum at 6:00 p.m., and after sunset it decreased again (Figure 1). Such daily fluctuations in the ambient air temperature significantly affected the air temperature in different parts of the large cowshed where the lactating cows were housed. Thus, the air temperature in the southern and northern parts and in the center of the cowshed from 9:00 a.m. to 9:00 p.m. was within the range of positive temperatures, and after 9:00 p.m. it decreased and by 3:00 a.m. was within the range of negative temperatures.

As for the dynamics of the temperatures in different parts of the premise, it was higher in the southern end at 12:00 p.m., as well as in the nighttime - from 12:00 a.m. to 3:00 a.m., than in the northern end and in the center of the premise, which is associated with a slightly greater heating of this zone due to the solar energy in the daytime. It should also be stated that in the southern end of the cowshed, the daily fluctuations of the air temperature were within the range of positive temperatures, while in the northern end and the center of the cowshed the air temperature decreased starting at 9:00 p.m., reaching the minimum negative temperatures from 12:00 a.m. to 3:00 a.m., which caused freezing of the fodder mixture on the fodder table and the animal manure in the manure channels.

The fluctuations of the relative air humidity in the cowshed within 24 hours depended to some extent on the ambient air humidity. The level of the relative humidity in the center, northern and southern ends of the cowshed decreased starting from 03:00 P.M. and was minimal from 06:00 p.m. to 09:00 p.m.

After 9:00 p.m. during rest, as well as in the daytime at 12:00 p.m., in the cowshed air, especially in the northern end, the increased relative humidity was observed more than 87%, while in the southern end, the peak values of the relative humidity were observed at 3:00 P.M. and 12:00 A.M., but they did not exceed the mark of 82% (Figure 2).

An analysis of the daily dynamics of the temperature-humidity index in the coldest period of the year in the large frame-type cowshed has shown that in all parts of the premise it did not fall below the lower comfort limit from 9:00 a.m. to 9:00 p.m. It began to decrease from 09:00 p.m. and reached its minimum at 12:00 a.m., after which it stabilized somewhat, but the average values of the temperature-humidity index were outside the comfort zone for the cows in the northern end and in the center of the cowshed until 06:00 a.m., and in the southern end it was on the border of the lower comfort zone (Figure 3). In accordance with the above-mentioned classification, the level of the cold stress for the cows, which were kept in the large frame-type cowsheds for our study, is estimated as mild.

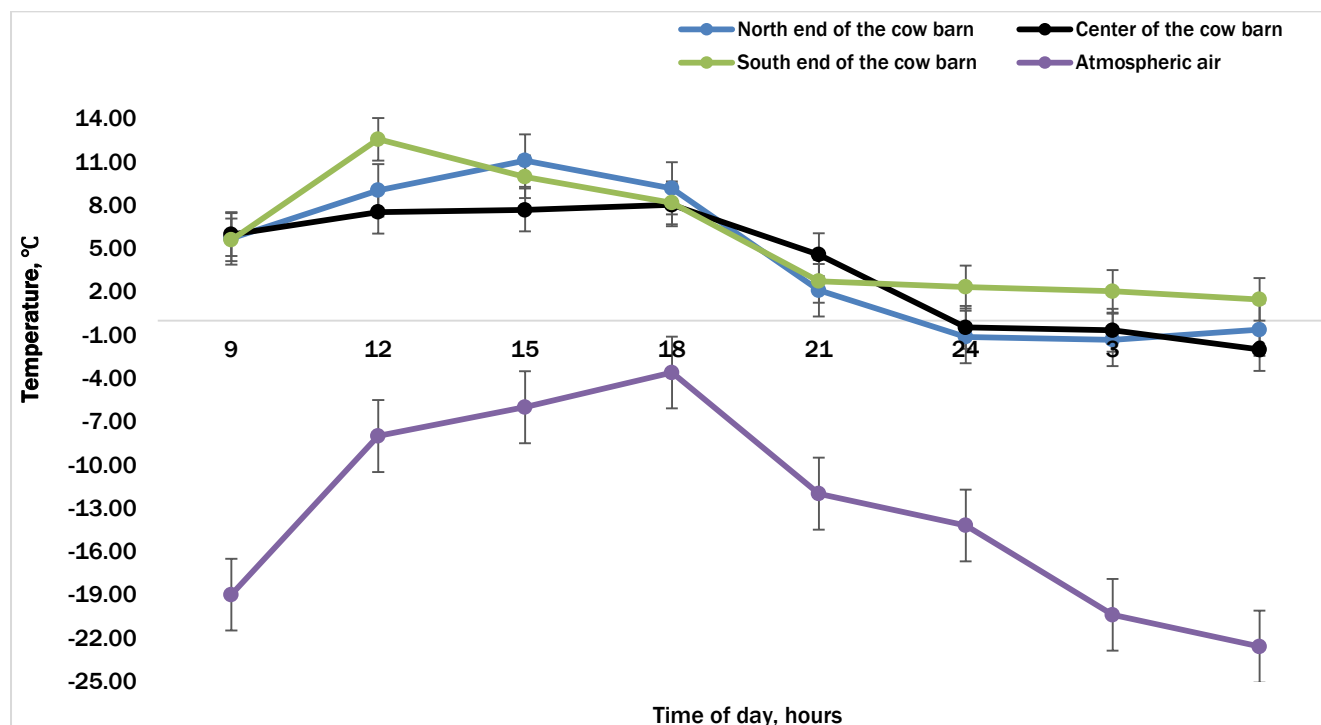


Figure 1 - Daily dynamics of air temperature in different cowshed parts in the coldest period of the year.

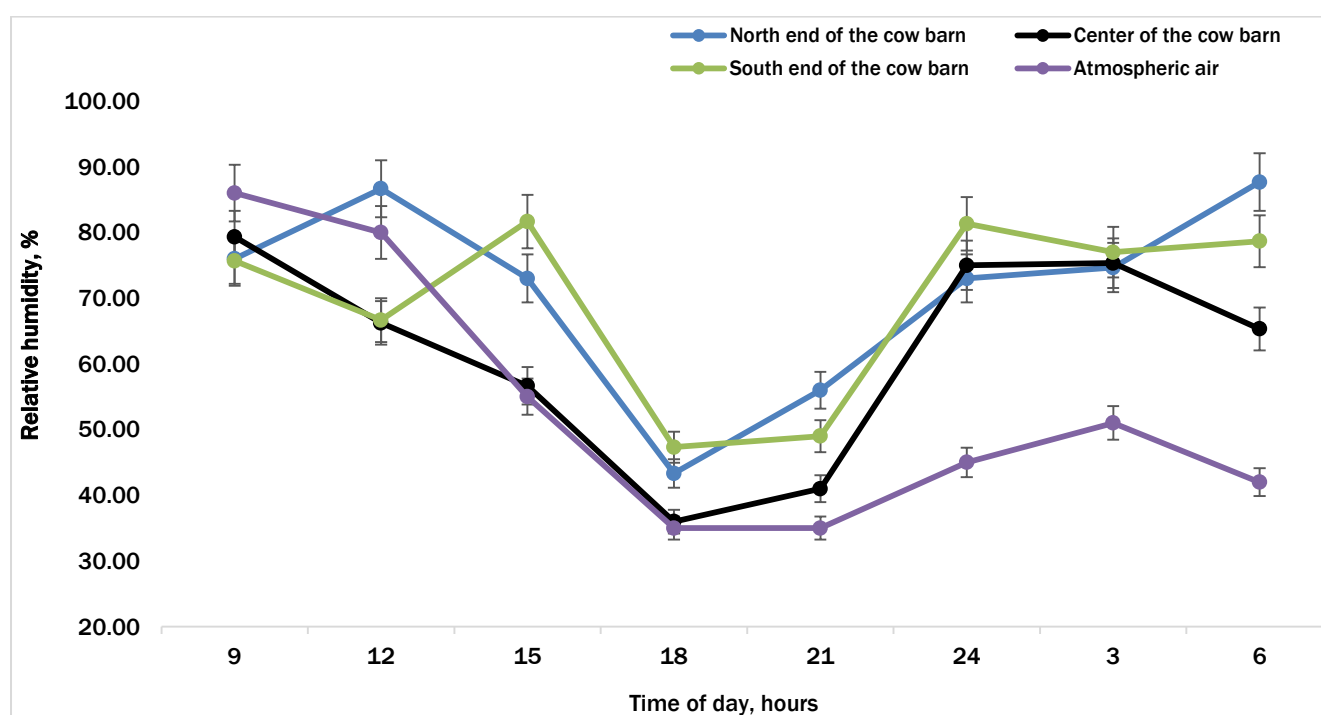


Figure 2 - Daily dynamics of relative air humidity in the coldest period of the year.

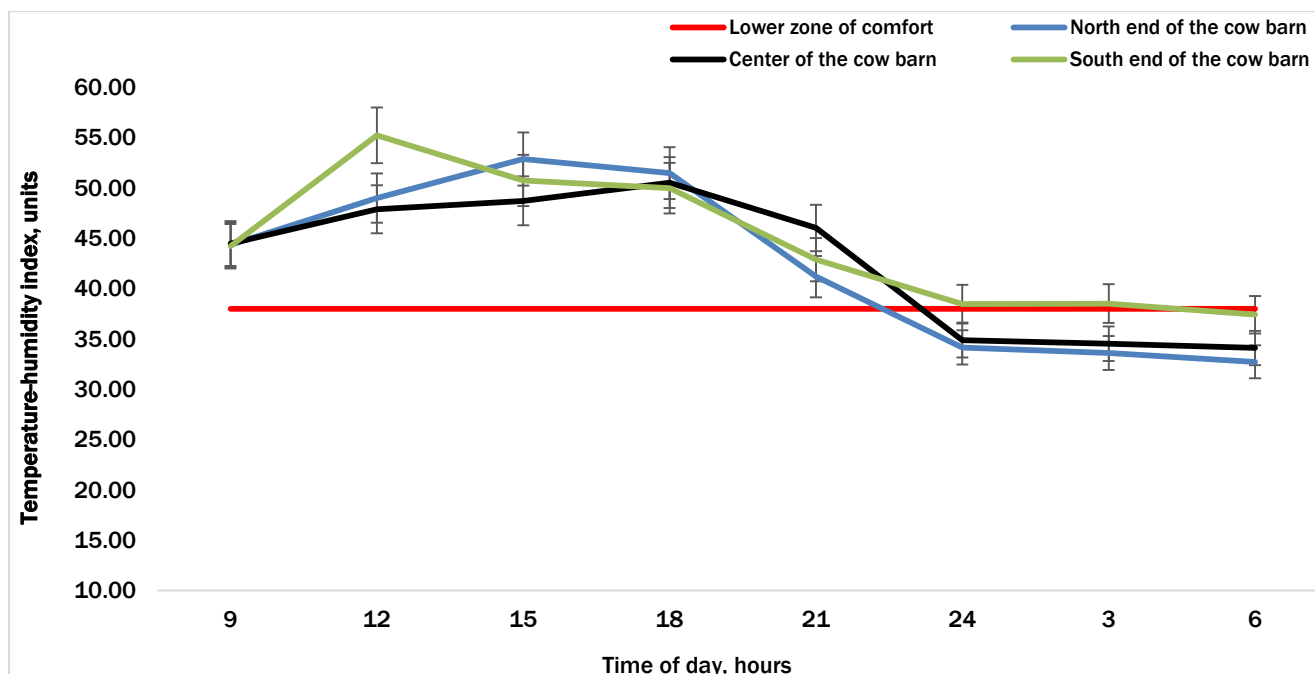


Figure 3 - Daily dynamics of air temperature-humidity index in different parts of cowshed.

It has been established that the parameters of protein metabolism in the tissues of the lactating cows, in particular, the level of total protein and urea, influenced by short-term cold stress did not differ between the groups and did not depend on the level of milk productivity and the age of the cows (Table 2).

At the same time, in second-lactation cows with a daily milk yield of 20-25 kg, the glucose level in the blood plasma was higher ($P < 0.05$) by 16% compared to similar values in the first lactation cows with a daily milk yield of 35-40 kg. As for the parameters of lipid metabolism in the tissues of the cows affected by short-term cold stress, total lipids in the blood plasma of the second lactation cows with a daily milk yield of 20-25 kg increased by 32.3% ($P < 0.05$), with a daily milk yield of 35-40 kg – by 1.6-fold ($P < 0.05$), third-lactation cows with a daily milk yield of 20-25 kg increased by 1.5-fold ($P < 0.05$) compared to similar parameters in first lactation cows with a daily milk yield of 20-25 kg. At the same time, no significant difference in total lipids in the blood plasma of the cows was found within each lactation based on milk productivity. Cholesterol and calcium levels in the blood plasma of first-, second-, and third-lactation cows remained within physiological norms and showed no significant differences among the cattle groups.

The study of enzymatic activity in the blood plasma of lactating cows with different milk productivity levels across different lactations under short-term cold stress showed a slight but significant difference in aspartate aminotransferase activity. In older cows, namely second- and third-lactation cows with a daily milk yield of 20-25 kg, this activity was lower by 14.3% ($P < 0.05$) and 17.8% ($P < 0.05$), respectively, compared to first-lactation cows with a daily milk yield of 35-40 kg (Table 3). Alanine aminotransferase (ALT) activity in the blood plasma of lactating cows, regardless of productivity level or age, did not change under short-term cold stress. No significant difference in amylase activity in the blood plasma of cows exposed to cold stress was observed (Table 3).

Table 2 - Metabolism metabolites in blood serum of lactating cows under short-term cold stress, $\bar{x} \pm SD$, $n=12$

Parameter	Cows (daily-average milk yield, kg)					
	First lactation		Second lactation		Third lactation	
	20-25	35-40	20-25	35-40	20-25	35-40
Total protein, g/l	97.6 \pm 3.75	94.00 \pm 1.96	89.8 \pm 2.82	93.33 \pm 2.89	98.67 \pm 3.16	98.00 \pm 2.60
Urea, mmol/l	7.30 \pm 0.45	7.33 \pm 0.48	6.80 \pm 0.56	7.67 \pm 0.37	8.42 \pm 0.43	8.40 \pm 0.60
Glucose, mmol/l	3.40 \pm 0.11 ^{ab}	2.97 \pm 0.15 ^b	3.54 \pm 0.13 ^a	3.40 \pm 0.05 ^{ab}	3.15 \pm 0.12 ^{ab}	3.48 \pm 0.10 ^a
Calcium, mmol/l	2.05 \pm 0.08	2.23 \pm 0.10	2.27 \pm 0.19	2.11 \pm 0.09	1.99 \pm 0.07	2.07 \pm 0.11
Total lipids, g/l	4.60 \pm 0.57 ^c	5.73 \pm 0.39 ^{ab}	6.80 \pm 0.50 ^a	7.40 \pm 0.54 ^a	6.93 \pm 0.66 ^a	6.40 \pm 0.35 ^{ab}
Cholesterol, mmol/l	5.05 \pm 0.62	4.71 \pm 0.22	5.46 \pm 0.23	5.99 \pm 0.64	4.88 \pm 0.53	5.69 \pm 0.67

*Note: different letters of the superscripts ^{a, b, c} indicate the values that were significantly different in one table row ($p < 0.05$) according to the comparison results using the Tukey test.

Table 3 - Enzymatic activity of blood serum of lactating cows under short-term cold stress, $\bar{x} \pm \text{SD}$, $n=12$

Parameter	Cows (daily-average milk yield, kg)					
	First lactation		Second lactation		Third lactation	
	20-25	35-40	20-25	35-40	20-25	35-40
AST, $\mu\text{kat/L}$	0.25 \pm 0.01 ^{ab}	0.280 \pm 0.01 ^a	0.24 \pm 0.01 ^b	0.27 \pm 0.01 ^{ab}	0.23 \pm 0.01 ^b	0.25 \pm 0.01 ^{ab}
ALT, $\mu\text{kat/L}$	0.20 \pm 0.01	0.21 \pm 0.01	0.19 \pm 0.02	0.22 \pm 0.01	0.20 \pm 0.01	0.21 \pm 0.02
ALP, $\mu\text{mol/h/ml}$	12.86 \pm 0.78 ^a	9.84 \pm 1.10 ^{ab}	9.95 \pm 2.04 ^{ab}	8.22 \pm 1.23 ^b	7.17 \pm 0.89 ^b	8.00 \pm 1.28 ^b
Amylase, g/h/l	38.40 \pm 4.80	34.56 \pm 2.63	34.56 \pm 2.63	33.60 \pm 3.59	35.20 \pm 3.51	36.00 \pm 2.77

*Note: different letters of the superscripts a, b indicates the values that were significantly different in one table row ($p < 0.05$) according to the comparison results using the Tukey test. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase,

It has been established that the alkaline phosphatase activity of the blood plasma decreases in cows as their age and milk productivity increase. This is evidenced by the decreased alkaline phosphatase activity of the blood plasma in second-lactation cows with a daily milk yield of 35-40 kg decreasing by 36% ($P < 0.05$), as well as in third-lactation cows with a daily milk yield of 20-25 kg decreasing by 44% ($P < 0.05$) and those with a daily milk yield of 35-40 kg - decreasing by 38% ($P < 0.05$) compared to similar parameters of first-lactation cows with a daily milk yield of 20-25 kg.

The analysis of the daily dynamics of ambient air temperature in the coldest period of the year for the climatic zone, where the Kyiv region is situated, has shown its maximum decrease during the night-time, but it increases during the daytime due to the sun, reaching its maximum at 6:00 p.m. Such daily changes in ambient air temperature affect the air temperature in different parts of a frame-type cowshed, which is designed for housing 1,000 cows (Figure 1). Moreover, the daily fluctuations in air temperature in the cowshed are influenced by its orientation relative to the cardinal directions. Specifically, the air temperature at the southern end of the cowshed remained above zero values throughout the 24-hour period and was, on average, 3°C higher at 12:00 p.m. and during the night compared to the center and northern end, where it dropped below zero.

The results of the present study agree with similar findings by Angrecka et al., (2020) who noted that the largest temperature difference between the ambient air and the cowshed air during the winter period was observed in the southern part of the premises, which is illuminated by the sun during the day. Additionally, the authors noted that the thermal balance of the premises depends on the wind and the location of the cowsheds relative to the cardinal directions. The studies of Cao et al. (2017) also indicated a possible decrease in air temperature inside easy-to-assemble cowsheds from -0.97 to 8.10°C during the winter period, when the ambient air temperature reached -20°C and below. Furthermore, differences in the heating of the cowshed's internal structural elements relative to the cardinal directions were reported, with the southern walls being warmer than the northern ones.

The fluctuations in air temperature in the cowshed, recorded during the night, suggest that the temperature was below the thermal comfort zone for cows, which, according to NRC (1981), should range from 13–18°C. However, studies by Butt et al. (2022) have explained the occurrence of cold stress in local cattle at temperatures ranging from 5.67 \pm 0.51 to 16.01 \pm 0.72°C. Additionally, based on their studies, Lees et al. (2019) suggest a slightly broader thermoneutral zone for cows, ranging from -0.5 to 20.0°C. In this regard, it is recommended to take into account breed, age, feeding levels, and productivity of the cattle.

The relative humidity of the ambient air depends on the weather conditions and season of the year for each climatic zone, which to a certain extent has an impact on the amount of water vapor in the cowshed air. Alongside that, the daily dynamics of the relative air humidity of the cowshed in its different parts also depended on the technological process. relative decrease in relative air humidity was observed during the daytime, when a significant amount of unorganized supply air enters the cowshed due to the technological processes, which involve the opening of gates, movement of motor vehicles, feeding and manure removal processes, as well as the movement of cattle into the milking hall and their subsequent return to the cowshed section.

The daily fluctuations of the relative air humidity in the center of the large frame-type cowshed were within the specified values and did not exceed 80% in the coldest period of the year. Yilmaz et al. (2020) consider that the relative air humidity in the cowshed should be within 40-80% in the coldest period of the year, which corresponds to most of the data obtained in the present study. Studies by Tang et al. (2019) have also shown that if the air humidity in the cowshed exceeded 80% during the winter, such a premise was unsuitable for keeping dairy cows. In contrast to these studies Jing and Jing (2021) expect that the upper limit of air humidity in cowsheds may be 85%, and when the air is almost saturated with humidity (100%), the wet surface of the skin increases heat loss from the cow's body, which fosters the development of pathogenic microflora and causes disease. However, in accordance with the data obtained in another study by Ma et al., (2017), the optimal relative air humidity in the cowshed during the winter should be maintained within a narrower range of 50-70%, which contradicts the above points of view and this discrepancy is explained by differences in cow-

keeping conditions, cowshed construction, and various microclimate support systems, particularly ventilation and sewage systems. The temperature-humidity index is recommended to be used for a more objective assessment of the microclimate of livestock premises, particular, cowsheds. *It is the most widely used criterion for assessing the influence of physical environmental factors on animal thermoregulation and more objectively reflects the combination of temperature and relative humidity in the environment* (Yan et al., 2019). Although this index is most often used to assess heat stress, it is also recommended to be used to assess cold stress in cattle. The threshold of cold stress is classified according to the temperature-humidity index (THI) as follows: $\text{THI} > 38$ (absence of stress); $25 < \text{THI} \leq 38$ (mild stress); $8 < \text{THI} \leq 25$ (moderate stress); $-12 < \text{THI} \leq 8$ (high stress); $-25 < \text{THI} \leq -12$ (extreme stress); $\text{THI} \leq -25$ (dangerous stress).

The data, obtained by the authors of the present study, showed that the temperature-humidity index in the frame-type cowshed during the day and evening, namely from 9:00 A.M. to 9:00 P.M., did not fall below the lower comfort limit in the coldest period of the year. At night, it reached its minimum and in the northern end and the center of the cowshed, it remained above the lower comfort limit (Figure 3). In accordance with the above-mentioned classification, the level of cold stress for the cows, kept in the large frame-type cowsheds in the present study, is estimated as mild.

One of the important parameters, that characterizes the ability of high-producing lactating cows, depending on milk productivity and age, to adapt to cold stress is the study of their metabolic status. The changes in external environmental factors, which are often observed during the winter period, as the ambient air temperature drops to -20°C and below, not only affects the microclimate of the premises where lactating cows are kept, but also changes affects fodder consumption and thermoregulation processes in animals, in particular, by intensifying heat generation processes. This, in turn, affects the intensity of metabolic processes in tissues, the functional state of the internal organs of the animals, as reflected in changes in blood chemistry values. The short-term cold stress did not have a significant influence on the parameters of protein metabolism in lactating cows' tissues, and changes in carbohydrate metabolism, particularly blood plasma glucose levels, were inconsistent. To a greater extent, cold stress primarily affected lipid metabolism in cows. Furthermore, total lipid levels in blood plasma increased with cow age, which is associated with the adaptive capacity of the metabolic system during cold stress and the increased use of lipids as an energy source for heat generation.

The results of the present study are difficult to compare with similar data from other researchers due to the climatic features of each geographical zone. The data obtained from Simmental cattle indicate a more pronounced effect of long-term cold stress on the metabolic status of cattle than what was observed in the present study on Holstein cows (Wang et al., 2023). In particular, this study showed that the concentration of glucose, enzymes of glucose metabolism, glucocorticoids, triiodothyronine and tetraiodothyronine in the blood plasma of Simmental cattle increased due to long-term cold stress ($P < 0.05$), but the levels of triglycerides, β -hydroxybutyrate, propionate, insulin and growth hormone were reduced ($P < 0.01$). The authors noted that long-term cold stress may suppress digestive function in Simmental cattle, increase energy metabolism, and disrupt stress hormone balance. The studies conducted on meat breed bulls and calves under extreme cold stress, indicate an increased ($P < 0.05$) level of cortisol and non-esterified fatty acids in the blood (Kim et al., 2023). However, a decreased blood glucose levels was observed in calves under cold stress. The authors noted that this metabolic response of cattle under cold stress characterizes their physiological adaptation to maintain homeostasis regardless of growth stage. One of the important criteria for assessing liver function is the activity of transaminases, in particular, ALT and AST in cow blood plasma. The activity of aspartate aminotransferase in cow's blood decreased as cows aged compared to first-lactation cows; however, no patterns were found regarding the changes in the activity of this enzyme in the blood plasma of cows concerning milk productivity (Table 3). The short-term cold stress did not significantly affect the activity of alanine aminotransferase and amylase lactating cows' blood plasma.

During the short-term cold stress, a decrease in the activity in blood plasma alkaline phosphatase was observed in cows as age and milk productivity increased (Table 3). The results of the study agree with similar data (Butt et al., 2022), which indicate a significant ($P < 0.05$) but slight effect of the cold stress on biochemical parameters in crossbred cattle, in particular, on the activity of AST, which decreased in cow's blood plasma of the cows from November to March by 7.8%, while the activity of ALT - conversely - increased by 1.3-fold during this period. The antioxidant enzyme activity in cattle was more strongly affected: despite stable superoxide dismutase activity, the activity of glutathione peroxidase decreased during the winter months 1.9-fold compared to the autumn and spring months.

CONCLUSION

The ambient air temperature in the climatic zone, where the Kyiv region is located, reaches -22.6°C at night during the coldest period of the year. The air temperature in the large frame-type cowshed depends on the ambient air temperature during the winter. In contrast to the southern end, the air temperature in the northern end and center of the cowshed drops below zero at night, and relative humidity rises to 87% which is outside the comfort zone for cows. The temperature-humidity index of the cowshed air is below 38 at night, which is classified as slight cold stress for cows. The cold stress does not affect the levels of total protein, urea, cholesterol, glucose, or calcium, but increases total lipid levels in the blood plasma. In second-lactation cows total lipids increase by 32.3% for those producing 20-25 kg of milk daily and by 1.6-fold for those producing 35-40 kg daily. In third-lactation cows, total lipids increase by 1.5-fold for those producing 20-25 kg of

milk daily compared to first-lactation cows producing 20-25 kg daily. The cold stress does not significantly affect ALT or amylase, but reduces AST activity in the blood plasma by 14.3% in second-lactation cows and by 17.8% in third-lactation cows producing 20-25 kg of milk daily, compared to first-lactation cows producing 35-40 kg daily. The activity of blood plasma alkaline phosphatase under cold stress decreases by 36% in second-lactation cows producing 35-40 kg of milk daily, by 44% in third-lactation cows producing 20-25 kg daily, and by 38% in those producing 35-40 kg daily, compared to first-lactation cows producing 20-25 kg daily. The study results may be used to assess the impact of cold stress on cattle metabolism during acclimatization, as well as to develop preventive measures to reduce potential economic losses.

DECLARATIONS

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Data availability

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

Ethical approval

All operations and manipulations of lactating cows during the study were humane and did not cause suffering to the animals. Any treatment of cattle was in accordance with the provisions of European legislation in the field of humane treatment of animals (Council Directive 86/609/EEC, 1986). The methodology of the experiment was agreed and verified by the Bioethical Commission of the National University of Bioresources and Nature Management of Ukraine, Kyiv (Protocol of ethical approval No. 54-17 dated 11/12/2018). The research was conducted to assess the intensity of cold stress in the climatic zone of Ukraine, which will be aimed at developing new and improving existing methods of insulating cowsheds.

Authors' contribution

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

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Competing interests

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

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METHANE EMISSION OF GOATS FED FIFTEEN DIETS: ON-FARM OBSERVATIONS

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 Supporting Information

ABSTRACT: It is suggested that the measurement of methane production from enteric fermentation must be done under situations similar to that of typical farming methods. It is against this background that this study measured methane emission from goats on a farm to ascertain the real situation on most farms. The objective of this study was to measure performance and methane emission from goats fed Ghanaian ruminant diets comprising of basal diets supplemented with browse leaves and to determine the effects of temperature and humidity on methane emission. Ten West African dwarf goats (5 males and 5 females; average weight 14 kg \pm 1.01) were fed fifteen Ghanaian ruminant diets for four months. Each diet was randomly fed twice in 24 hours for 2 days in a month. Methane emission, temperature and humidity were measured using handheld gas methane detector. Completely randomized design was used. Dry matter intake (DMI) was lowest ($P < 0.05$) when cassava (*Manihot esculenta*) peels were fed and highest ($P < 0.05$) when plantain peels were supplemented with *Moringa oleifera*. Weight gain, DMI and methane emission from manure increased with time. The highest enteric methane emission was recorded ($P < 0.05$) when untreated rice straw (749 ppm) was fed and the lowest was recorded ($P < 0.05$) when *Moringa oleifera* leaves (313 ppm) were fed. High environmental temperature favored low methane emission and high humidity was associated with high methane emission. In conclusion, feeding browse leaves alone and browse supplementation with basal diets resulted in lower methane emission than feeding basal diets alone. Moderate weight gains were recorded. High environmental temperature was inversely related to methane emission and high environmental humidity was directly related to methane emission. It is recommended that, browse leaves be incorporated in the feed of ruminants, especially when environmental temperatures are low and humidity is high.

Keywords: Basal diets, Browse leaves, Dry matter intake, Humidity, Temperature, Weight gain.

INTRODUCTION

Greenhouse gas emission is one of the drivers of Climate change (EPA, 2017). Agricultural activities are major sources of atmospheric greenhouse gas emissions, forming about thirty percent of the global anthropogenic emissions (Vermeulen et al., 2012; Rosenstock et al., 2016). Animal agriculture is a significant producer of greenhouse gases, forming about 14.5% of global emissions (Gerber et al., 2013a; Kristiansen et al., 2020) and 29.7% of the total Agricultural greenhouse gas emissions in Sub-Saharan Africa (FAOSTAT, 2024). The worldwide annual methane emission from ruminants is estimated to range between 80 and 95 million tons (Patra, 2014). The process of enteric fermentation contributes more than 90% of methane emissions from livestock (FAO, 2019) and forms 40% of the agricultural greenhouse gas emissions (Tubiello et al., 2013). This forms a major source of greenhouse gas emissions from the agricultural system (Steinfeld et al., 2006; Palangi et al., 2022). Methane represents 20% of the global anthropogenic greenhouse gas emission that causes global warming (Nisbet et al., 2016).

Methane is a potent greenhouse gas, next to carbon dioxide regarding its contribution to global warming (Martin et al., 2008; Olivier et al., 2018; IPCC, 2021). The United States Environmental Protection Agency (EPA) states that "methane is a powerful greenhouse gas, with a global warming potential more than 25 times greater than that of carbon dioxide over a 100-year time horizon" (EPA, 2017). Methane has a Global Warming Potential (GWP) of 85 times more than that of carbon dioxide over a 20-year time horizon, although carbon dioxide has thousands of years atmospheric lifetime but methane disappears in about 10-15 years (IPCC, 2021).

The rapid disappearance of methane and its high contribution to atmospheric temperature makes it a primary focus to curtail in an effective and timely manner in terms of climate change (Verde et al., 2023). According to the report of the International Energy Agency, reduction in methane emissions is one of the most effective interventions that should be included in economic terms, to rapidly decrease the rate of global warming and contribute immensely to activities to minimize the rise in global temperature (IEA, 2021).

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Methane emission is a loss of 2 to 12 % of dietary energy to the ruminant, depending on the composition of diet and intake level (McGinn et al., 2011; Goel and Makkar, 2012). Broucek (2014) suggested that the measurement of methane production from enteric fermentation must be done under situations similar to that of typical farming methods. It is against this background that this study measured methane emission from goats on a farm to ascertain the real situation on most farms. Also, methane emission in goats fed common ruminant diets have not been extensively studied in Ghana. The objective of this study was to measure performance and methane emission from goats fed Ghanaian ruminant diets and to determine the effects of temperature and humidity on methane emission.

MATERIALS AND METHODS

Study location

The study was carried out on Sakyi & Abban Farms at East Legon Hills, Accra (5° 43' 27.4" N 0° 05' 52.2 W) in the Coastal Savannah zone of Ghana. Total rainfall ranges from 508 mm to 743 mm per annum. Rainfall pattern is bimodal, with the major rains between May to August and minor rains in September- November. Temperature varies between 30 °C and 34 °C and relative humidity is from 53 % to 73 % (Sarkwa et al., 2020a).

Chemical analysis

Dry matter (DM), crude protein (CP) and ash were carried out using the methods of AOAC (2016). Fiber components were determined using the procedure of Goering and Van Soest (1970) and condensed tannins (CT) by butanol-HCl method as outlined by Iqbal et al. (2011) and validated by Sarkwa et al. (2023a).

Animal management and feeding

Ten West African dwarf goats (5 males and 5 females; average weight 14 kg \pm 1.01; two years old) were fed fifteen Ghanaian ruminant diets for four months. The goats were kept in a communal housing pen (60 m x 5 m). The goats were treated against ectoparasites and endoparasites before the start of the experiment as carried out by Sarkwa et al. (2023b). The fifteen diets were untreated rice straw (URS), urea treated rice straw (UT), plantain peels (PP), cassava peels (CP), *Moringa oleifera* (MO), *Albizia lebbek* (AL), *Leucaena leucocephala* (LL), *Millettia thonningii* (MT), yam peels (YP), *Mangifera indica* (MG), plantain leaves (PL), cassava leaves (CL), MO+PP, AL+PP and MO+CP.

Each diet was fed twice in 24 hours (day) and each of the fifteen diets was fed for two consecutive days in a month in no specific order or randomly. The feeding was carried out for four months. The fifteen diets were given separately to each of ten goats for 2 days and methane gas was measured for each feed. All the basal diets were cut into pieces of about 4cm in length. Untreated rice straw and urea treated rice straw were prepared as described by Sarkwa et al. (2021). Before the commencement of the experiment, each of the experimental diets was offered to the goats for 24 hours. All ten goats were offered 20 kg of experimental diets daily (10 kg in the morning and 10 kg in the afternoon). The quantity of browse leaves (AL, LL, MG, MO and MT) fed as supplement (MO+CP, MO+PP, AL+PP and AL+LL+PP) was 1000 g. However, goats fed sole basal diets or browse leaves were offered 2000 g of feed. In supplementing with browse leaves, each goat was offered 100 g of the browse leaves and 1900 g basal diet. Water was offered on *ad libitum* basis. Feed intakes were recorded daily by subtracting feed offered from feed residual or leftover. Weight gains were determined by weighing every month after starving the goats for 12 hours.

Methane emission, temperature and humidity measurements

Methane emission, temperature and humidity were measured daily using hand-held gas methane detector (GASTiger 2000, Stark Instrument Company, China). Enteric methane emission from each diet was measured after 12 hours of feeding a particular diet to the goats. Enteric methane emission was measured from goats by restraining them individually and about 30 meters away from the other goats. Then, the methane detector was placed very close to the mouth of the goats. This is because it has been reported that about 95 to 99 % of enteric methane is released through the mouth (Olijhoek and Lund, 2017). Manure from the goats excreta were heaped under a mango tree on the farm and methane emission was measured monthly.

Statistical analysis

Completely randomized design was used. Data obtained were subjected to analysis of variance using GenStat-2009 version 12.1 (GenStat, 2009) in accordance with the model below:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Which Y_{ij} is the response variable such as feed intake, feed leftover, total feed offered and enteric methane emission; μ was the overall mean; T_i is the experimental diets (15 diets); E_{ij} is the residual error.

Student Newmann keuls (SNK) test was used to separate significant ($P < 0.05$) means. Differences in monthly enteric methane emission, effect of sex on methane emission, monthly methane emission from manure and monthly feed intake were determined using error bars.

RESULTS

Chemical composition

The dry matter, crude protein, ash, neutral detergent fiber, acid detergent fiber and lignin of the experimental diets ranged between 840-946 g/kg, 31-330 g/kg DM, 50-200 g/kg DM, 202-620 g/kg DM, 175-548 g/kg DM and 105-201 g/kg DM respectively (Table 1). The condensed tannins content of the browse leaves were from 1.9 g/kg DM to 6.9 g/kg DM (Table 1).

Table 1 - Chemical Composition of Diets (g/kg DM)

Diets	DM(g/kg)	CP	Ash	NDF	ADF	Lignin	CT
AL	880	287	74.7	453	350	192	3.2
LL	840	271	102	300	176	139	6.9
MO	873	330	157	202	205	105	3.0
MT	894	234	109	534	391	139	3.1
Cassava Peels	946	31	73	363	274	201	-
UT	916	101	200	552	520	180	-
UNRS	936	66.8	173	620	548	191	-
Plantain Peels	900	80.7	143	371	270	199	-
Cassava leaves	879	120	83	354	250	125	1.9
Mango leaves	890	187	113	364	241	128	2.7
Yam Peels	889	90	50	370	380	160	-

AL:- *Albizia lebbek*, LL: *Leucaena leucocephala*, MO: *Moringa oleifera*, MT: *Milletia thonningii*, UT: Urea treated rice straw, UNRS: Untreated rice straw, CP: Crude Protein, NDF: Neutral Detergent fiber, ADF: Acid detergent fiber, CT: Condensed tannins.

Dry matter intake, feed leftover, total feed offered and methane emission of goats fed different diets

Dry matter intake was lowest ($P<0.05$) in goats fed cassava peels (CP) but was not different ($P>0.05$) from goats fed untreated rice (UNRS), plantain peels (PP) and yam peels (YP) (Table 2). Goats fed PP supplemented with *Moringa oleifera* (MO) recorded the highest ($P<0.05$) DMI but did not differ ($P>0.05$) from all the browse supplemented diets and solely fed browse leaves apart from goats fed urea treated rice straw (UT) (Table 2). Feed leftover and total feed offered on dry matter basis were in the range of 783 to 1418 g/d ($P<0.05$) and 1566 to 1884 g/d ($P<0.05$) respectively (Table 2).

Enteric methane emission was highest ($P<0.05$) in goats fed UNRS but was not different ($P>0.05$) from goats fed CP. The lowest ($P<0.05$) enteric methane emission was observed in goats fed MO but did not differ ($P>0.05$) from those fed *Albizia lebbek* (AL) and *Milletia thonningii* (MT) (Table 2). Goats fed MO+CP, MO+PP and AL+PP did not differ ($P>0.05$) from each other in enteric methane emission. Goats fed AL+ *Leucaena leucocephala* (LL)+PP and cassava leaves were not different ($P>0.05$) in terms of enteric methane emission but were higher ($P<0.05$) than those fed LL and *Mangifera indica* which differed ($P<0.05$) from each other (Table 2).

Table 2 - Dry matter intake, feed leftover, total feed offered and methane emission of goats fed different diets

Feeds	Dry Matter Intake (g/d)	Feed Leftover on DM basis (g/d)	Total Feed offered on DM basis (g/d)	Methane Emission (ppm)
Untreated Rice straw	454 ^c	1418 ^a	1872 ^b	749 ^a
Urea treated rice straw	608 ^b	1224 ^c	1832 ^c	517 ^{de}
Cassava peels (CP)	448 ^c	1310 ^b	1758 ^f	719 ^{ab}
Plantain peels (PP)	450 ^c	1350 ^b	1800 ^d	721 ^a
Yam peels	481 ^c	1297 ^b	1778 ^e	660 ^{abc}
<i>Moringa oleifera</i> (MO) + CP	830 ^a	1054 ^d	1884 ^a	589 ^{def}
<i>Moringa oleifera</i> (MO) + PP	839 ^a	958 ^e	1797 ^d	607 ^{def}
<i>Albizia lebbek</i> (AL) + PP	831 ^a	967 ^e	1798 ^d	596 ^{def}
AL + <i>Leucaena leucocephala</i> (LL) + PP	835 ^a	961 ^e	1796 ^d	560 ^{bd}
<i>Albizia lebbek</i> (AL)	817 ^a	943 ^e	1760 ^f	370 ^g
<i>Mangifera indica</i>	783 ^a	783 ^f	1566 ^h	426 ^{efg}
Cassava leaves (CL)	799 ^a	959 ^e	1758 ^f	555 ^{bd}
<i>Leucaena leucocephala</i> (LL)	811 ^a	949 ^e	1760 ^f	408 ^{fg}
<i>Milletia thonningii</i> (MT)	811 ^a	977 ^e	1788 ^{de}	387 ^g
<i>Moringa oleifera</i> (MO)	820 ^a	926 ^e	1746 ^g	313 ^g
SEM	±19.52	±24.86	±4.68	±45.11
P-values	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$

Means in the same column with different superscripts are different ($P<0.05$); SEM: Standard error of means.

Monthly dry matter intake of the diets can be seen in Figure 1. In general, there was improvement in dry matter intake with time (Figure 1). Dry matter intake in the first month had the lowest and the fourth month had the highest in all the 15 diets (Figure 1). Monthly intake of all diets were not different from each other except UNTRS (Figure 1). Intake of goats fed UNTRS for the first and second months were not different but the first month was different from the third and fourth months. Intake for the second and third months was not different from each other according to the error bars (Figure 1).

On the contrary, enteric methane emission decreased with time (Figure 2). The first month recorded the highest enteric methane emission while the fourth month recorded the lowest enteric methane emission (Figure 2). Enteric methane emission in goats fed UT in the fourth month was lower than the rest which was not different. Goats fed MO+CP, AL+PP and MO recorded enteric methane emission higher in the first month than the fourth month which did not differ from the other two months (Figure 2). Enteric methane emission from goats fed AL was highest in the first month but was not different from the second month. Goats fed AL recorded the lowest enteric methane emission in the fourth month but was not different from the third month. The highest methane emission in goats fed AL was recorded in the first month but the other three months were not different. In goats fed LL, enteric methane emission was lowest in the fourth month and was different from the other three months (Figure 2). In the first month, enteric methane emission from goats fed AL+LL+PP recorded the highest but was not different from the second month. Enteric methane emission from goats fed AL+LL+PP was lowest in the fourth month but not different from the third month according to the error bars (Figure 2).

Figure 3 shows enteric methane emission of males and females fed the experimental diets. Males recorded higher enteric methane emission but was not different from that of females (Figure 3). Methane emission from manure increased with time (Figure 4). The first month recorded the lowest methane emission from manure but it did not differ from the second month. The fourth month recorded the highest but it was not different from the third month (Figure 4).

Figure 5 shows an inverse relationship between methane and temperature: methane emission decreases with increase in temperature. Methane emission had direct relationship with humidity (Figure 6). Thus, methane emission increases with increase in humidity (Figure 6). There was improvement in weight gain with time (Figure 7). The fourth month recorded the highest weight gain of goats fed the experimental diets but it was not different from the second and third months (Figure 7). The first month recorded the lowest weight gain (Figure 7).

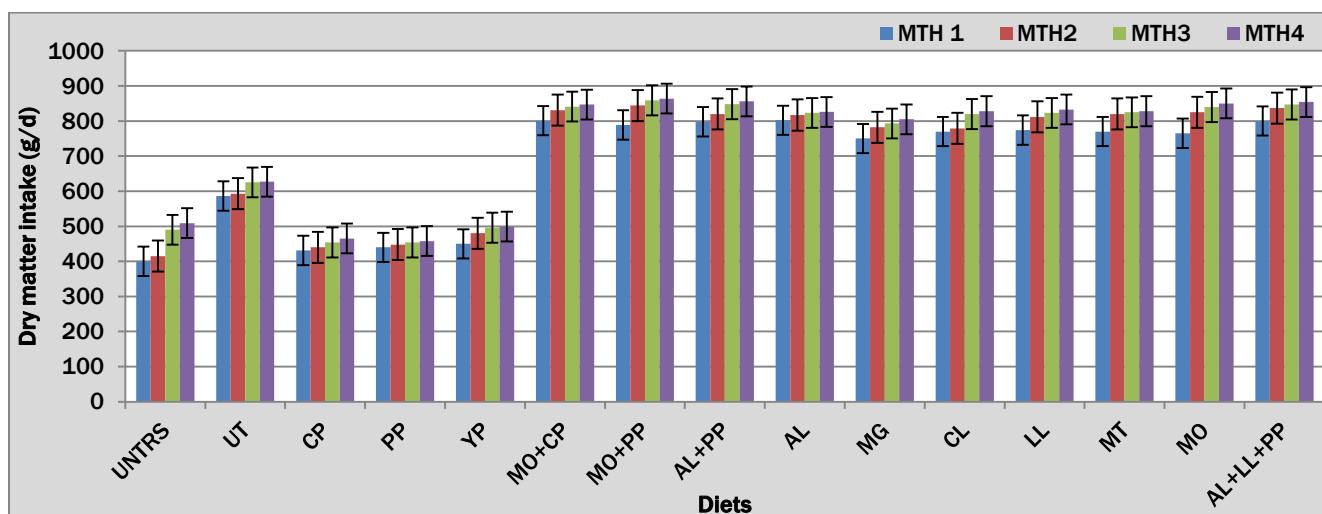


Figure 1 - Monthly dry matter intake of goats fed 15 diets.

UNTRS: Untreated rice straw; UT: urea treated rice; CP: cassava peels, PP: plantain peels, YP: yam peels, MO: *Moringa oleifera*, AL: *Albizzia lebbek*, MG: *Mangifera indica*, CL: cassava leaves, LL: *Leucaena leucocephala* and MT: *Milletia thonningii*.

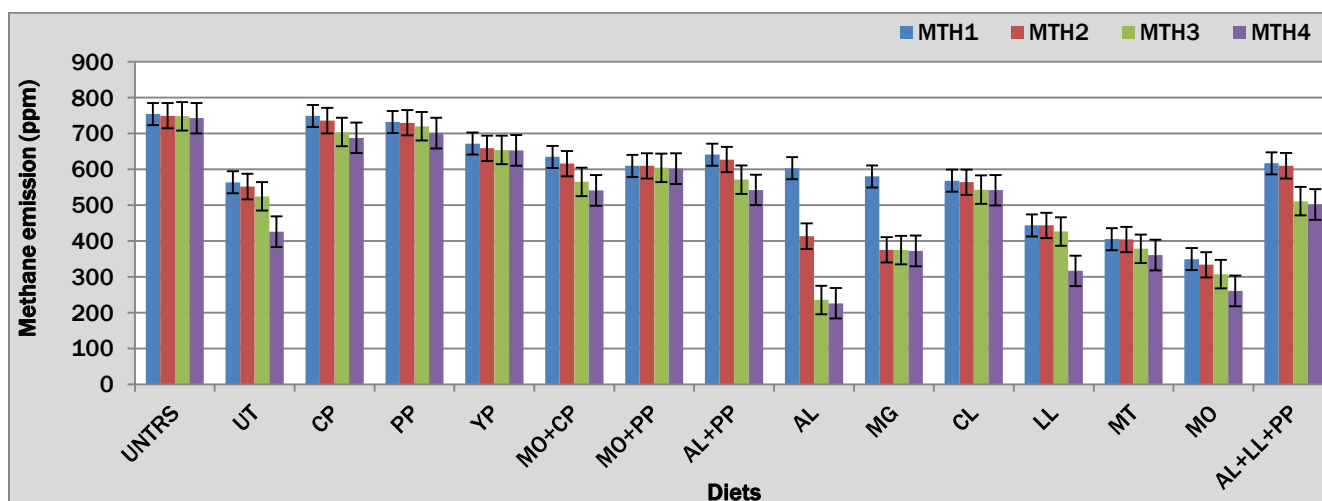


Figure 2 - Monthly enteric methane emission of goats fed 15 diets.

UNTRS: Untreated rice straw; UT: urea treated rice; CP: cassava peels, PP: plantain peels, YP: yam peels, MO: *Moringa oleifera*, AL: *Albizzia lebbek*, MG: *Mangifera indica*, CL: cassava leaves, LL: *Leucaena leucocephala* and MT: *Milletia thonningii*.

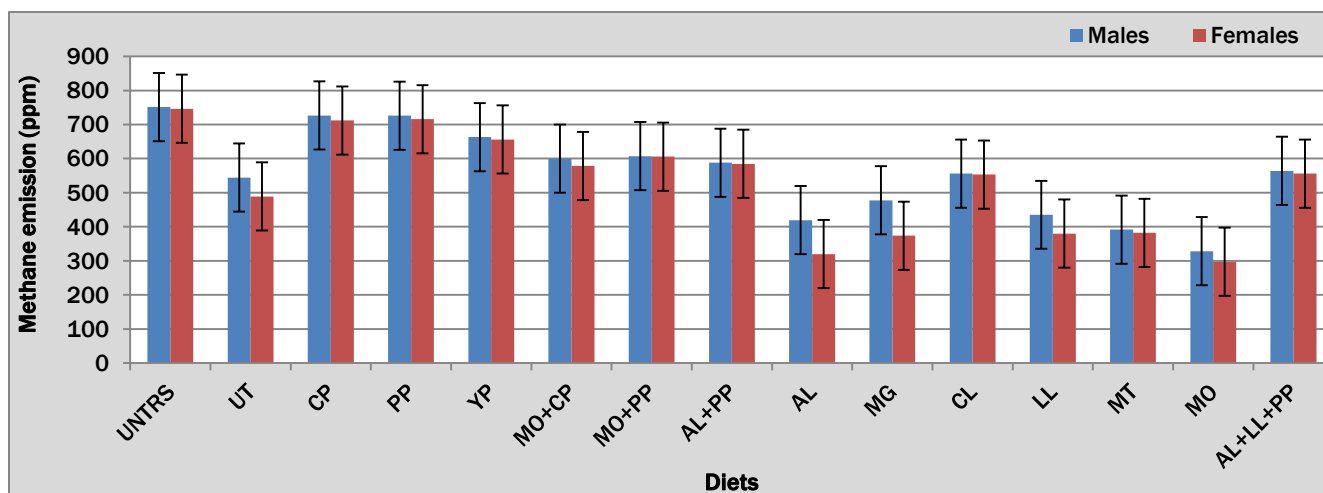


Figure 3 - Effect of sex on enteric methane emission of goats fed 15 diets.

UNTRS: Untreated rice straw; UT: urea treated rice; CP: cassava peels, PP: plantain peels, YP: yam peels, MO: *Moringa oleifera*, AL: *Albizia lebbek*, MG: *Mangifera indica*, CL: cassava leaves, LL: *Leucaena leucocephala* and MT: *Milletia thonningii*.

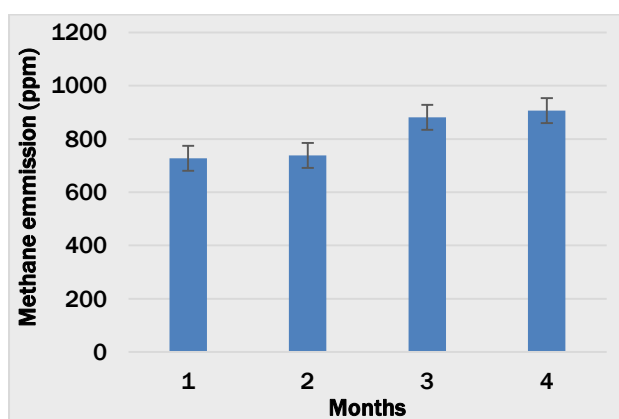


Figure 4 - Monthly methane emission from goats manure.

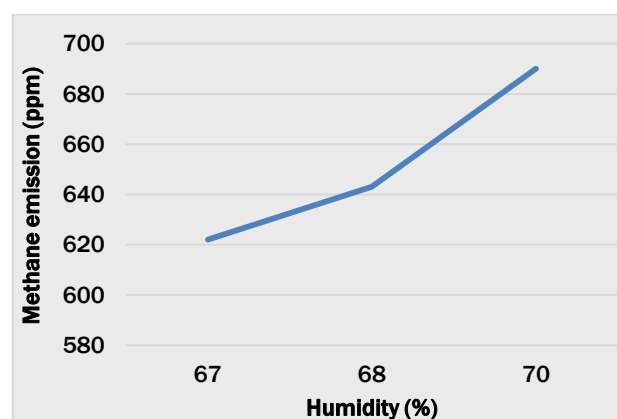


Figure 6 - Relationship between enteric methane emission and humidity.

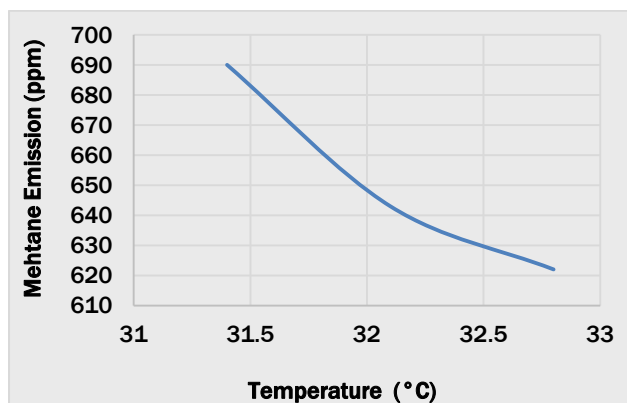


Figure 5 - Relationship between enteric methane emission and temperature.

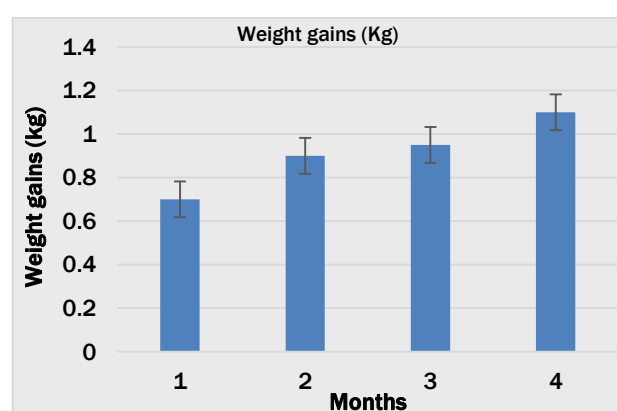


Figure 7 - Monthly weight gains of goats fed 15 diets.

DISCUSSION

Chemical composition

The high crude protein and ash, low condensed tannins, low to moderate fiber components of the browse leaves may have contributed to the gain in weight and lower methane emission recorded in this study and this is similar to earlier reports (Yisehak et al., 2014; Sarkwa et al., 2020a, 2020b; Adogla-Bessa et al., 2022). Also, Patra et al. (2017) and Jayanegara et al. (2012) reported that plant secondary substances such as tannins have inhibitory effects on

methanogenesis and this may be the reason why the browse diets recorded lower methane because they contained condensed tannins. The basal diets were high in fiber components and methane emission. This is in line with reports by Jayanegara et al. (2009) and Jin et al. (2012) that high ADF and NDF levels resulted in low digestibility and high formation of methane by altering short chain fatty acid proportion to acetate formation which yields more hydrogen.

Intake

Annan and Tuah (1999) reported intake of cassava peels between 255 to 347 g/d and for cassava peels supplemented with *Ficus exasperata* (browse leave) intake of 383-475 g/d. Similar trend of intakes were recorded in the present study but with higher values. Dry matter intakes of feeds containing different levels of condensed tannins were similar (Animut et al., 2008a). This is corroborated by the current study where the different browse leaves with different condensed tannins levels fed solely and as supplements had similar dry matter intake. This may be due to the fact that the condensed tannins levels of the browse leaves were also similar and low. The present study has confirmed an earlier study by Bhatta et al. (2002), that dietary condensed tannins levels below 60 g/kg did not reduce feed intake. In the present study, condensed tannins levels were low and there was high intake of the browse leaves as supplement compared to the basal diets.

Enteric and manure methane emission

Condensed tannins containing diets reduced enteric methane emission in goats irrespective of its content (Puchala et al., 2005 and 2012; Animut et al., 2008a). In this present study, sole browse leaves and their supplementation recorded lower enteric methane than the basal diets that did not contain condensed tannins and this is similar to earlier reports (Carula et al., 2005; Tavendale et al., 2005; Puchala et al., 2005 and 2012; Animut et al., 2008a). Condensed tannins from different sources had similar effects on enteric methane emission in goats, most likely by altering the activities of methanogens, although alteration in activities of bacteria and protozoa may also contribute to it (Animut et al., 2008b; Sarkwa et al., 2023a). This has been confirmed by the current study in which goats fed the different browse leaves emitted similar amount of enteric methane. Feeding two browse leaves as supplement recorded lower enteric methane than supplementing with one browse leaf. This has confirmed earlier reports that feeding combinations of diets resulted in lower enteric methane emission than feeding one diet (Naumann et al., 2015; Sarkwa et al., 2023b).

Forage size has immense effect on enteric methane emissions. Animals spend significant amount of their energy to the process of chewing (Gerber et al 2013b). Reduction of particle size of fodder mechanically helps to increase digestibility by enhancing accessibility of substrate to microbes, thereby reducing enteric methane emission and energy expenses and improving the passage rate of digesta and animal productivity (Hristov et al 2013). In the present study, the basal diets were all cut into pieces and this may have contributed to improve digestibility, feed intake and weight gain and lower enteric methane emission especially in the case of the urea treated rice straw. Browse feeding and reduction in the size of feed are good feeding practices that may have contributed to improve performance and lower enteric methane emission in this current study. This supports a report by Mayuni et al (2019) that greenhouses gases are reduced with better feeding practices.

It has been suggested that nutritional strategies and management practices are traditional options by which enteric methane emission can be reduced in goats (Pragna et al., 2018). This is supported by the current study where browse leaves containing condensed tannins and urea treated rice straw recorded lower methane emission than the non tanniferous diets and untreated rice straw. Methane emission was higher initially than the later stages (Animut et al., 2008a; Sarkwa et al., 2023b). Similar trend was observed in this current study where enteric methane emission decreased with time. The reduction in enteric methane emission with time maybe due to decrease population and activities of protozoa and methanogens in the rumen due to regular feeding of tanniferous diets. A report showed that male ruminants generally have higher enteric methane (CH₄) emissions compared to females (Hegarty et al., 2007). This is in line with the current study and this may likely be due to differences in body size, metabolic rate and hormone levels between the sexes.

Methane from manure increased with time and this may be due to increase in the quantity. This study supports earlier report by Jabab (2023) who reported high methane emission from manure in the wet season due high quantity of manure as a result of high intake of feed. Methane emission from manure in intensively managed farms with manure storage system was higher than extensive system because it is mostly exposed to air (Knapp et al., 2014). Anaerobic digestion is a natural process in which the microorganisms consume organic matter under an oxygen-free environment and this result in greenhouse gas generation such as carbon dioxide and methane (Knapp et al., 2014). In the current study, manure was stored in a heap under a tree in the open or not stored under anaerobic conditions and therefore, additional generation of methane may have been minimal.

Methane emission, temperature and humidity

Enteric methane production is also influenced by environmental temperature (Nonaka et al., 2008). At high temperatures feed intake and rate of passage in the rumen becomes slow and this increases digestibility and decreases

methane production in the rumen (Kurihara et al., 1995; Kurihara, 1996; Bhatta et al., 2006; Nonaka et al., 2008). These findings are in line with present study because increased in temperature caused decreased in enteric methane emission. The current study recorded an increase in enteric methane emission as relative humidity increased which is in line with an earlier report by Hansen et al (2012). The findings of the current study reveal that, when temperatures are low and humidity is high, enteric methane emission will be high and therefore, more concerted effort will be needed to mitigate methane emission.

Weight gains

The results on weight gains in this current study are similar to earlier reports where browse leaves were fed solely (Sarkwa et al., 2020b) and as supplements (Adogla-Bessa et al., 2022; Idan et al., 2023a and 2023b). This may be due to the improvement in feed intake and reduction in methane emission with time. The improvement in feed intake and reduction in methane emission may imply that less feed energy is lost as a result of low methane production which may have made more energy available to the goats to use for weight gains. The results on weight gains in this current study corroborate earlier studies that enteric methane production is a loss of energy that is due to inefficient digestion in the rumen (Johnson and Johnson, 1995), which decrease metabolisable energy intake (MEI) by the animal (McGin et al., 2011; Goel and Makkar, 2012) and may potentially be used for meat production (Eckard et al., 2010).

CONCLUSION

Feeding browse leaves alone and browse supplementation with basal diets resulted in lower enteric methane emission than feeding basal diets alone. Moderate weight gains were recorded in goats fed the experimental diets. Feed intake, methane emission from manure and weight gains increased with time while enteric methane emission decreased with time. High environmental temperature resulted in low enteric methane emission and high environmental humidity favored high enteric methane emission. It is recommended that, regular incorporation of browse leaves in the feeding of ruminants should be encouraged especially when environmental temperatures are low and humidity is high. This will enhance climate smart and sustainable goat production and contribute to reduce the impact of climate change.

DECLARATIONS

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Data availability

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

Authors' contribution

FO Sarkwa, EC Timpong-Jones, T Adogla-Bessa and V Antwi contributed to the research, data analysis, and manuscript writing.

Conceptualization: FO Sarkwa and EC Timpong-Jones

Data curation: FO Sarkwa and V Antwi

Funding acquisition: FO Sarkwa, EC Timpong-Jones and T Adogla-Bessa

Methodology: FO Sarkwa and EC Timpong-Jones

Investigation, project administration, resources, software, analysis, visualization & writing original draft: FO Sarkwa

Supervision: FO Sarkwa and EC Timpong-Jones

Validation: FO Sarkwa and T Adogla-Bessa

Writing, review & editing: FO Sarkwa, EC Timpong-Jones and T Adogla-Bessa

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Ethical approval

Ethical clearance was sought from Ethics Committee of College of Basic and Applied Sciences, University of Ghana, Legon (ECBAS 032/21-22). The authors also complied with the ARRIVE guidelines.

Consent to publish

Not applicable.

Competing interests

The authors declare no competing interests.

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ESTIMATION OF CRUDE FIBER CONTENT OF A FEED FROM ITS ADF VALUE WHERE THERE IS NO LABORATORY SERVICE

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✉ Supporting Information

ABSTRACT: Because of the cost and inaccessibility of laboratory facilities, animal feed formulation at the farm level, in many parts of Ethiopia, is based on feed database information. However, nowadays many laboratories are phasing out the Weende crude fiber (CF) method of analysis. The fiber content of feeds available in most feed databases (including the sub-Saharan Africa feeds composition database) are a result of detergent method analysis (NDF, ADF and lignin). However, CF is still used in poultry feed formulation and forage analysis for horses, in addition to the neutral detergent fiber (NDF) fraction for determining fiber in different countries. Since there is a statistically ($P < 0.01$) difference between the CF and acid detergent fiber (ADF) value of a feed, ADF can't be used directly in place of CF. Therefore, this work aims to formulate a regression equation that could roughly estimate the CF level of a feed from its NDF and ADF values. Considering the strong multicollinearity between NDF and ADF, this study developed separate models for ADF and NDF and compared them based on R^2 and Akaike Information Criterion (AIC), and the ADF-based model provided a better fit. The equations $0.79 \times \text{ADF} - 0.46$, $0.01 + 0.79 \times \text{ADF}$, and $1.37 + 0.62 \times \text{ADF}$ have effectively predicted CF for cereal grains and beans, pulses and byproducts, and also oilseed meals and cakes, respectively. For grass forages, the equation $3.38 + 0.76 \times \text{ADF}$, tested on 10 forages, showed potential but remains unreliable due to its R^2 value below 0.8. Finally, it is concluded that this approach provides a practical alternative for estimating CF where laboratory services or database information are unavailable.

Keywords: Crude Fiber, Estimation, Feed database information, Prediction, Regression.

INTRODUCTION

Dietary carbohydrates can be divided into two basic fractions: fiber and non-fiber carbohydrates (Mirzaei-Aghsaghal and Maheri-Sis, 2011). Fiber is any component in feed that is not digested by mammalian enzymes (Jha and Mishra, 2021). Based on its solubility in water fibers can be grouped into soluble fiber (which dissolves in water) and insoluble fiber. The proximate analysis system developed by the Weende Experiment Station in Germany classified carbohydrates in feed into a more digestible component called nitrogen-free extract (NFE) and a less digestible fibrous component called crude fiber (Singh and Kim, 2021). Crude fiber is a plant cell structural component, including cellulose, hemicelluloses, lignin, and pectin (An et al., 2022; Musa, 2021). The proximate analysis system underestimates the true fiber in the feed. A major problem with this procedure is that the acid and base used in the analysis solubilize some of the true fiber (particularly hemicelluloses, pectin, and lignin), and some cellulose is partially lost too (Musa, 2021). The proximate analysis system only represents a small fraction of the fiber content (average 80% of hemicellulose or pentosans, 50-90% lignin and 50-80% cellulose recovery) (Van Soest and McQueen, 1973). The CF method has a complete recovery of pectins (Möller, 2014).

The other analysis process using neutral and acid detergents by Vax Soest (1963) categorized fiber into neutral detergent fiber (NDF) comprising of cellulose, hemicellulose and lignin, and acid detergent fiber (ADF), largely consisting of cellulose and lignin (Singh and Kim, 2021). The Van Soest detergent fiber system is also affected by unreliability and falls short of accounting for all non-starch polysaccharides (NSP) in the poultry feed ingredients (Singh and Kim, 2021). Non-starch polysaccharides are complex carbohydrates found predominantly in plant cell walls and include components like cellulose, hemicellulose, and pectin. Unlike starch, NSPs cannot be digested by non-ruminants due to their structural complexity and cross-linking, which limits their availability as an energy source.

The Neutral Detergent Fiber (NDF) method has been criticized for not adequately recovering pectin, which is an important part of the cell wall matrix in plants. This omission can lead to an incomplete understanding of the fiber content and its digestibility in poultry diets (Van-Soest et al., 1991). A relatively new feed composition analysis method is Near Infrared Reflectance spectroscopy (NIR). Though NIR method allows rapid and least cost determination of multiple nutrients and characteristics of feeds or forage, it is not available in many places of Ethiopia including the pioneer agricultural university-Haramaya.

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Because of the cost and inaccessibility of laboratory facilities, animal feed formulation at the farm level, in many parts of Ethiopia, is based on feed database information. However, nowadays many laboratories are phasing out the CF method of analysis. The fiber content of feeds available in most feed databases (including the sub-Saharan Africa feeds composition database) is more of a result of detergent method analysis (NDF, ADF and lignin). However, CF is still used in poultry feed formulation (Singh and Kim, 2021) and forage analysis for horses, in addition to the NDF fraction for determining fiber (Hoffgård, 2022) in different countries. Therefore, the objective of this work is to formulate a regression equation that could roughly estimate the CF level of a feed from its NDF and ADF values.

MATERIALS AND METHODS

Regression models were developed to estimate the CF level of feeds (that could be determined using the Weende proximate analysis system) from their ADF and ADF level using R 4.4.1. The data were taken from Makkar et al. (2024) and INRAE (2024a). First, two candidate models (i.e. using NDF or ADF as predictor variables) were developed for each feed category (i.e. grass forages, cereals grain and bran, pulse seed and byproducts, and oilseed byproducts) and compared using the analysis of variance (ANOVA) in R. Finally, the best-performed model for each feed category was selected for its applicability using a paired sample t-test between the actual and predicted CF values for its non-significance tells us its goodness of fit.

The sample size is determined using Green (1991) and Memon et al. (2020) formula: $n=104+k$; where, n and k are the number of sample size and predictors, respectively. The predator in this case was one (i.e. NDF or ADF) and the minimum sample size would be 105.

The model used in this study was: $Y = \beta_0 + \beta_1\chi_1 + e$; where Y is the response variable (i.e. CF), β_0 is the intercept of the regression line, corresponding to the predicted values when χ_1 (i.e. NDF or ADF) are zero. $\beta_1\chi_1$ is the regression coefficient (β_1) on the independent variables (χ_1 i.e. NDF or ADF). e is the model error (residuals), which defines how much variation is introduced in the model when estimating Y.

RESULTS AND DISCUSSION

Table 1 presents the correlation between CF, NDF, and ADF. The strong correlation between NDF and ADF suggests the presence of high multicollinearity between them indicating they can't be used together in model formulation to predict CF from detergent fiber results. Therefore, separate Models were formulated for ADF and NDF. Choct (2016) noted that, though, the proportion of cellulose and to a lesser extent lignin extracted can be highly variable depending on the ingredient, CF, more or less, represents cellulose and lignin (i.e. ADF) content. However, since there is a significant ($P<0.01$) difference between ADF and CF (Table 1) we can't directly use ADF in place of CF.

Figures 1 and 2 as a partial residual plot showing the linearity assumption of a predictor's (i.e. ADF and NDF) relationship with the dependent variable CF. Both figures show a linear relationship of predator with CF. Where a partial residual plot shows the linear relationship between predictors and dependent variables a linear model can be used (Fox, 2015).

The partial residual plot indicates both the magnitude of the linearity variance and the linearity magnitude and position (Roy et al., 2020).

Table 2 below presents the model parameters and parameters used to compare the models in estimating CF of different categories of feeds from their ADF and NDF contents. In all feed categories, the R^2 value of model one was higher, and the Akaike Information Criterion (AIC) value was lower than model 2. The Delta AIC (ΔAIC) values were much greater than 10. The R^2 value of model one ranges from 0.75 to 0.94. Except for grass forages, the R^2 value of model one of the feed categories was greater than 0.80. The R^2 value equal to 0.8 clearly indicates a very good regression model performance, regardless of the ranges of the ground truth values and their distributions (Chicco et al., 2021). Lower AIC indicates a better fit. If $\Delta AIC > 10$, there is strong evidence that the model with the lower AIC is better (Burnham and Anderson, 2002).

Table 1 - Correlation and difference between variables in different feed categories

Parameters	grass forages (Roughages)	Cereal grain and bran	pulse seed and byproducts	Oilseed byproducts
Correlation (NDF and ADF)	0.85	0.86	0.91	0.92
Mean difference between ADF and CF (i.e ADF-CF)	6.14	2.63	4.14	8.61
t-value	-21.97	-11.069	-8.7222	-11.739
SEM	0.74	1.07	1.51	1.81
P-value	< 0.01	< 0.01	< 0.01	< 0.01

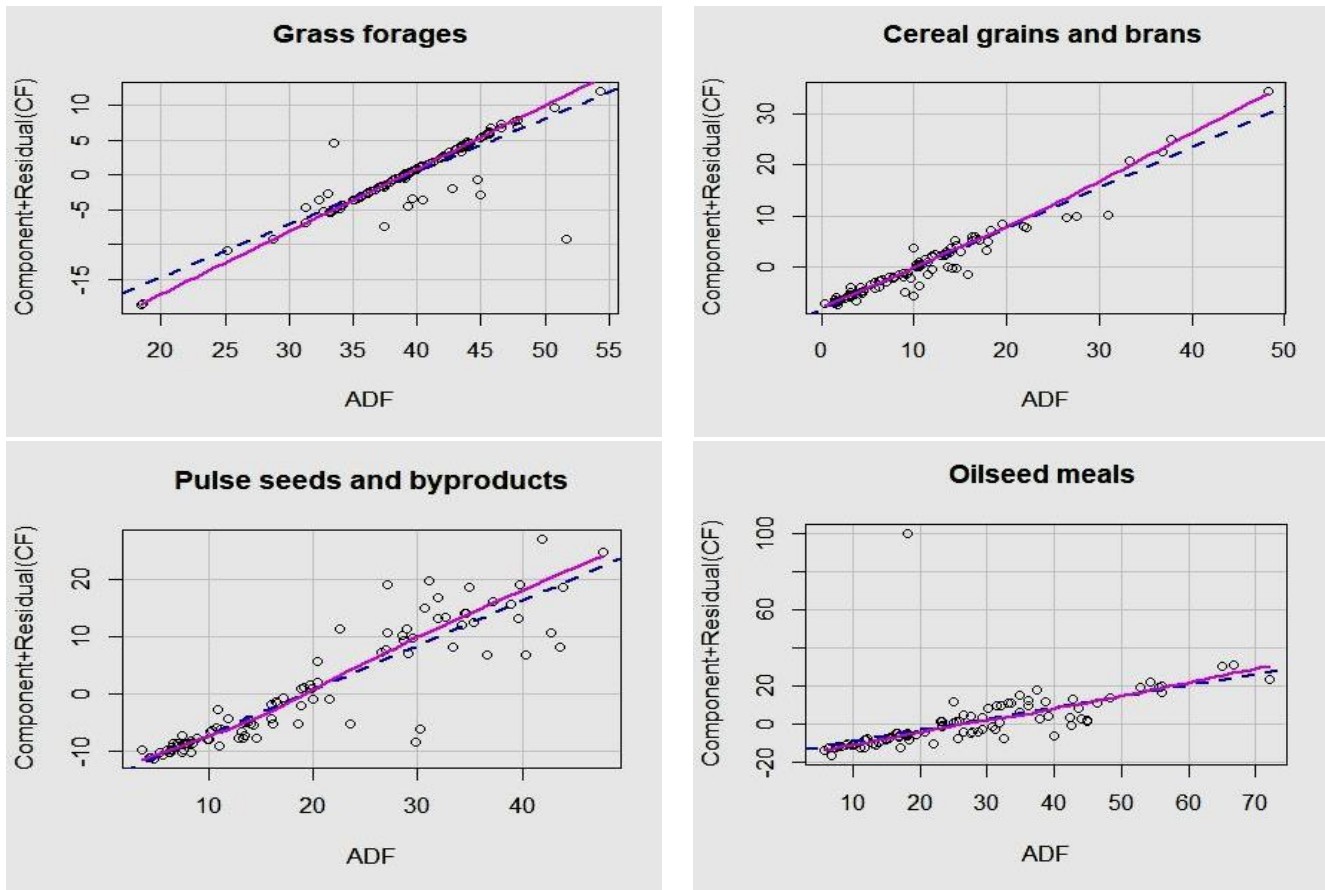


Figure 1 - Trends of the relation between crude fiber (CF) and acid detergent fiber (ADF)

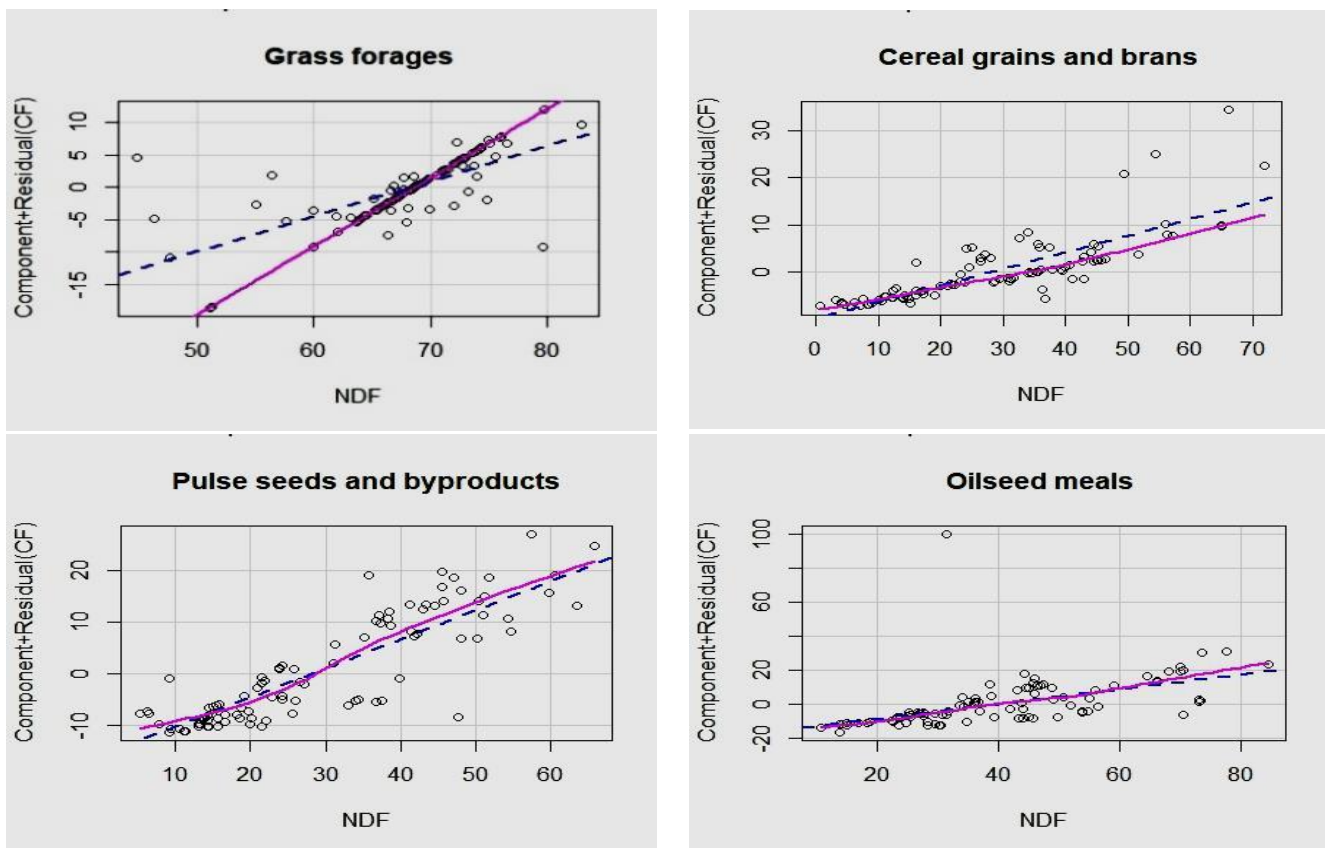


Figure 2 - Trends of the relation between crude fiber (CF) and the neutral detergent fiber (NDF).

Table 2 - Estimation of CF of a feed based on its NDF and ADF values.

Models	Model parameters				Model comparison parameters		
	β_0 (Intercept)	β_1 (Coefficient)	R ²	P-value	RSS*	AIC	Δ AIC
grass forages (roughages)							
Model-1: CF~ADF	3.38 ± 1.73	0.76 ± 0.04	0.7454 ^a	<0.001	684.54 ^b	508.24	73.65
Model-2:CF~NDF	-3.90 ± 3.71	0.55 ± 0.05	0.4932 ^b	<0.001	1362.49 ^a	581.89	
SE			0.07		0.60		
P-value			**		**		
Cereals grains and brans							
Model-1: CF~ADF	-0.46 ± 0.27	0.79 ± 0.02	0.9403 ^a	<0.001	297.33 ^b	413.27	184.11
Model-2:CF~NDF	-1.72 ± 0.80	0.35 ± 0.02	0.6555 ^b	<0.001	1716.87 ^a	597.38	
SE			0.10		0.61		
P-value			**		**		
Pulse and their byproducts							
Model-1: CF~ADF	0.01 ± 0.78	0.79 ± 0.03	0.8308 ^a	<0.001	1808.20 ^b	607.50	46.66
Model-2:CF~NDF	-0.65 ± 1.05	0.56 ± 0.03	0.7373 ^b	<0.001	2808.19 ^a	654.16	
SE			0.08		0.91		
P-value			**		**		
Oilseed meals and cake							
Model-1: CF~ADF	1.37 ± 0.89	0.62 ± 0.03	0.8025	<0.001	2282.64 ^b	632.20	72.93
Model-2:CF~NDF	-0.19 ± 1.51	0.45 ± 0.04	0.6070	<0.001	4541.94 ^a	705.13	
SE			0.07		1.11		
P-value			**		**		
RSS: Residual Sum of Squares							

Crude fiber estimation from ADF of grass forages

Table 3 presents the difference between the actual and estimated CF values of 11 grass forages. The paired t-test result indicated no significant difference ($P>0.05$) between the actual and the predicted values of CF. The predicted CF value of orchard grass was 34.54 and 37.58% of DM. In agreement with this finding, the CF value of Orchard grass was reported to range from 30.2% (Joanna et al., 2007) to 46.45% of DM (Farshadfar, 2012). Glatter et al. (2021) also reported the CF value of meadow hay (35.6%) which is similar to the predicted value of Orchard grass Orchard grass. The 32.94% predicted value of the bamboo leaves reported in this research agrees with the 33.19 % reported by Antwi-Boasiako et al. (2011). Shahowna et al. (2013) reported 54.3 and 47.4 % CF for fresh and fermented sugar cane bagasse, similar to the 48.07% predicted CF value for the same feed using the regression equation developed for grass forages or roughages. The predicted CF value of German grass (33.93%) was within the range of CF value of the German grass at the 3rd (28.30%) and 1st (38.93%) cut reported by Islam et al. (2018). Rahman et al. (2024) also reported the CF value of German grass to be 34.7%. This sight variation in CF content is due to the difference in stage maturity when the sample was analyzed. The predicted CF value (29.14%) for roadside grass was within the range of 32.25 to 36.09 % reported by Haryono et al. (2020) for different cultivars of the roadside grass.

Table 3 - Differences between original CF and model-1 predicted CF for some grass forages*

Feed type/name	Original CF (%)	Literature Source	Predicted CF (%)
Orchardgrass (<i>Dactylis glomerata</i> L): mid-bloom	33.00	Schroeder (2004)	34.54
Orchard grass (<i>Dactylis glomerata</i> L): late-bloom	37.00	"	37.58
Sorghum-Sudan-grass Timothy:			
Late vegetative	27.00	Schroeder (2004)	25.42
Mid-bloom	31.00	"	30.74
Late bloom	31.00	"	45.18
Roadside grass (<i>Stenotaphrum secundatum</i>)	46.27	Selim et al. (2022)	29.14
Banana leaves	29.35	"	31.35
Bamboo leaves	35.56	"	32.94
Sugar cane bagasse	37.89	"	48.07
German grass (<i>Echinochloa polystachya</i>)	39.84	"	33.93
King grass (<i>Pennisetum purpureum</i>)	36.10	Tuturoong et al. (2019)	35.22
Mean	34.91 ^a		34.92 ^a
	SE of the difference	2.59	
Original vs Predicted CF	t-value	-0.0041	
	P-value	0.9968	

* The original crude fiber (CF) used in this table are not used in the model formulation

Crude fiber estimation from ADF of cereals grain and bran

Table 4 presents the differences between the original CF and the CF predicted from ADF using the model for some cereals grain and brans. The insignificant ($P>0.05$) difference observed between the actual and the predicted CF value suggests the good fit of the model in predicting CF from ADF. The CF value of 3.32% predicted from ADF of maize grain was found within the range (2.8 to 4.5%) reported by [Fufa et al. \(2019\)](#) and [Rose and Gupta \(2018\)](#), respectively. The predicted value also falls within the range 2.62 to 3.93 reported by [Radosavljević et al. \(2020\)](#).

[Hossain et al. \(2008\)](#) reported 11.38% CF for wheat bran, similar to the 11.64% CF predicted from the ADF of wheat bran. In agreement with this [Liu et al. \(2024\)](#) also reported 10.94% of CF for wheat bran. The CF values of 3.2% and 5.23% predicted from ADF of wheat and barley grain, respectively, were similar to 3.0% and 5.23% reported for wheat and barley grain by [Hossain et al. \(2008\)](#) and [Venslovas et al. \(2024\)](#), respectively. The CF values of 5.81, 5.44, and 7.05% predicted from ADF of Sorghum grain were similar to 5.90, 5.40, and 6.50 % reported by [Kumar et al. \(2019\)](#) for different sorghum varieties. In agreement with this finding [Treviño-Salinas et al. \(2021\)](#) also reported 6.07 to 9.09 % of CF for different varieties of sorghum grains. The CF value of 4.17% predicted from the ADF of Sorghum HB2 was similar to the 4.17% reported by [Banna and Arifuddin \(2024\)](#).

Table 4 - Differences between original CF and model-1 predicted CF for some cereals grain and bran *

Feed type/name	ADF (%)	Original CF (%)	Literature source	Predicted CF (%)
Maize grain	4.79	1.10	Jaishankar et al. (2021)	3.32
Wheat bran	15.32	14.07	Ning et al. (2022)	11.64
Wheat	4.63	2.71	"	3.20
Barley grains	7.20	9.0	Asma et al. (2021)	5.23
Corn	3.96	1.82	Sheikhhasan et al. (2020)	2.67
Sorghum HB1	7.94	1.96	Salinas et al. (2006)	5.81
Sorghum HB2	5.86	2.67	"	4.17
Sorghum HB3	9.51	4.17	"	7.05
Sorghum HB4	24.24	9.02	"	18.69
Sorghum HB5	25.47	6.80	"	19.66
Sorghum HB7	7.47	1.71	"	5.44
Mean		5.00 ^a		7.90 ^a
SE of the difference			2.23	
Original vs Predicted CF		t-value	-1.9943	
		P-value	0.07409	

* The ADF and original CF used in this table are not used in model formulation. CF: crude fiber;; ADF: acid detergent fiber

Crude fiber estimation from ADF of pulse seeds and byproducts

Table 5 presents the differences between the original CF and the CF predicted from ADF using the model formulated for pulse seeds and byproducts. The insignificant ($P>0.05$) difference observed between the original and the predicted CF value suggests the ability of the model to predict CF from ADF. The CF values of 27.98%, 32.48%, and 9.16% predicted from ADF of the cowpea haulms, cowpea Pod husks, and faba bean seeds, respectively, were similar to 27.5%, 31.8 %, and 8.9 % reported by [Antwi et al. \(2014\)](#), [Abebe and Alemayehu \(2022\)](#) and [Micek et al. \(2015\)](#), respectively for similar ingredients. The CF value of 5.15% predicted from ADF of cowpea seed was also comparable to the 5.66% reported by [Gutema and Tolesa \(2024\)](#). The CF value of 11.31% predicted from ADF of Lupin seed was found within the range (10.0 to 16.0%) reported by [Abraham et al. \(2019\)](#). [Uzun and Okur \(2023\)](#) also reported 11.75 % CF for Blue lupin. The CF values of 5.15% and 7.36% predicted values from ADF of the Adzuki bean and pigeon pea were similar to the 4.71 ± 0.54 % and 6.6% reported by [Sai-Ut et al. \(2010\)](#) and [Saxena et al. \(2010\)](#), respectively. The CF values of 4.32%, 7.81% and 5.90% predicted from ADF of Chickpea, Faba bean, and Common vetch were comparable to the 3.9 %, 7.72 %, and 3.80–7.17 % reported by [INRAE \(2024b\)](#), [Smit et al. \(2021\)](#), and [Huang et al. \(2017\)](#), respectively.

Crude fiber estimation from ADF of Oilseed byproducts

Table 6 presents the differences between the original CF and the CF predicted from ADF using the model formulated for oilseed byproducts. The insignificant ($P>0.05$) difference between the original and the predicted CF suggests the ability of the model to predict CF from ADF. The CF values of 8.31%, and 4.64% predicted from ADF of rapeseed cake and Soybean meal were similar to the 7.9 % and 4.40% reported by the [National Dairy Development Board \(2012\)](#) of India and [Makkar et al. \(2024\)](#), respectively. In agreement with this finding [Tang et al. \(2024\)](#) also reported 8.26 % CF for rapeseed cake.

The CF value of 10.93% predicted from ADF of canola meal was in the range 8.97 to 11.4 % reported by [Birmani et al. \(2019\)](#). [Kaiser et al. \(2022\)](#) also reported 10.1% CF of the canola meal. The CF value of 17.92% predicted from ADF of Noug seed cake was similar to the 17.65 % reported by [Amare et al. \(2021\)](#). The CF value of 17.12% predicted from ADF of Sesame meal was less than the 9.86 % reported by [Elfaki and Unal \(2023\)](#). The Difference is due to their ADF content. The former had 25.41% ADF, while the latter had 13.83%. The CF value of 15.11% predicted from ADF of Sunflower cake was within the range (11.6-23.89%) reported by [Swain et al. \(2023\)](#). The CF values of 8.44% and 4.68% predicted from the ADF of flaxseed cake and soybean meal were similar to the 8.8 % and 5.44 % reported by [Nehmeh et al. \(2022\)](#) and [Etiosa et al. \(2018\)](#), respectively. [Dunmire et al. \(2021\)](#) also reported CF values ranging from 4.27 to 5.17% for soybean meal produced from different varieties of soybean, which is similar to the above-mentioned predicted CF value for soybean meal.

Table 5 - Original CF and model-1 predicted CF for some pulse seeds and byproducts *

Feed type/name	NDF (%)	ADF (%)	Original CF (%)	Literature source	Predicted CF (%)
Cowpea haulms	49	35.4	29.9	Li et al. (2021)	27.98
Cowpea Pod husks	54.2	41.1	31.8	"	32.48
Faba bean seeds	22.06	11.58	9.72	Meng et al. (2021)	9.16
cowpea seed	16.6	6.5	5.6	Makkar et al 2024	5.15
Lupins (al bus)	17.2	14.3	10.27	Sipas et al. (1997)	11.31
Adzuki bean	12.70	6.50	4.76	"	5.15
Pigeon pea	13.70	9.30	8.07	"	7.36
Chickpea Kabuli	11.90	5.46	2.93	"	4.32
Faba bean	12.79	9.87	8.41	"	7.81
Common vetch	21.90	7.46	5.10	"	5.90
		Mean	11.656 ^a		11.662 ^a
Original vs Predicted CF	SE of the difference			4.5733	
	t-value			-0.012801	
	P-value			0.9901	

* The NDF, ADF, and original CF used in this table are not used in the model formulation. CF: crude fiber; NDF: neutral detergent fiber; ADF: acid detergent fiber

Table 6 - Original CF and model-1 predicted CF for some Oilseed byproducts *

Feed type/name	NDF (%)	ADF (%)	Original CF (%)	Literature sources	Predicted CF (%)
Rapeseed cake	17.80	26.03	11.19	Renata et al. (2018)	8.31
Soybean meal	8.21	5.28	3.89	Tanawong (2013)	4.64
Canola meal	22.64	15.42	10.50	"	10.93
Noug seed cake	34.5	26.7	22.0	Moges et al. (2016)	17.92
Sesame seed meal	37.50	24.25	9.00	Mahmoud and Wafaa (2014)	16.41
Cottonseed Cake	61.53	17.70	12.10	Idrissou et al. (2020)	12.34
Sesame meal	39.35	25.41	3.28	Omer et al. (2019)	17.12
Flaxseed cake	14.2	11.4	8.1	Niyonshuti and Kirkpinar (2024)	8.44
Sunflower cake	32.59	36.56	22.16	Renata et al. (2018)	15.11
Soybean	9.36	13.99	5.34	"	4.68
		Mean	10.76		11.59
Original CF vs Predicted CF	SE of the difference			2.6353	
	t-value			-1.6653	
	P-value			0.1198	

CF: crude fiber; NDF: neutral detergent fiber; ADF: acid detergent fiber

CONCLUSION

Where there is no laboratory service and the database information for crude fiber values for a feed in question, it is possible to estimate the CF value from the ADF value of a feed. Since there is a statistically significant ($P < 0.01$) difference between the CF and ADF value of a feed, ADF can't be used directly in place of CF. The regression equation $0.79 \times \text{ADF} - 0.46$, $0.01 + 0.79 \times \text{ADF}$, and $1.37 + 0.62 \times \text{ADF}$ can be used for cereals grains and brans, pulse and their byproducts, and oilseed meals and cake, respectively. However, for the R^2 value less than 0.8, the regression equation $3.38 + 0.76 \times \text{ADF}$ formulated for grass forages, even though tested on 10 forages and found effective, the model is not reliable.

DECLARATIONS

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Supplementary Information

The supplementary materials are available at <https://ojafr.com/> (Volume 15, Issue 2; Pages 79-88).

Author's Contribution

I contribute to data analysis, interpretation, discussion, and the write-up of the manuscript.

Competing interests

The author has not declared any competing interests.

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

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EVALUATION of ECONOMIC EFFICIENCY of SHEEP FARMING ENTERPRISES in KARS PROVINCE by DATA ENVELOPMENT ANALYSIS

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Supporting Information

ABSTRACT: In this study, it was aimed to determine the economic activities of sheep farming enterprises in Kars province, Türkiye by Data Envelopment Analysis (DEA). For this purpose, data obtained from face-to-face surveys conducted on 99 sheep farming enterprises in Kars Province were used. In the DEA applied to determine the economic efficiency of sheep enterprises, the Charnes Cooper Rhodes (CCR) was used according to the input-oriented scale. According to the study findings, the average age of the owners of the enterprises, all of whom were male, was 46 years, and their experience was average 9 years. It was determined that the majority of the farm owners (76.8%) were primary and secondary school graduates. It was determined that 67.7% of the enterprises were farming only Akkaraman, 3% were farming only Morkaraman, and 29.3% were farming both of the breeds. According to the DEA results used to determine the economic efficiency of enterprises, 41 enterprises (41.4%) were determined to be effective and 58 (58.6%) were determined to be inefficient. Consequently, it was concluded that inefficient enterprises need to reduce their input costs to become economically effective. In addition, it has been considered that it is very important for enterprises to make progress in the stages of obtaining, processing, branding and marketing high value-added products (milk/dairy products and meat/meat products, wool) from sheep farming to increase their income and profitability.

Keywords: Data envelopment, Economic efficiency, Kars, Sheep farming.

INTRODUCTION

Sheep farming is one of the important animal production activities that meet many human needs, both directly (meat, milk, wool and offal) and indirectly (sausage casing, surgical thread, medicine and cosmetics) (Günlü and Mat, 2021). In addition, sheep farming is a livestock subsector that can utilize agricultural lands that are not suitable for plant production and low-yield pastures and creates employment in rural areas.

There are 1.3 billion sheep worldwide (FAO, 2024). Sheep farming is mainly concentrated between 35-55° north latitudes in Europe and Asia and 30-45° south latitudes in South America, Australia, and New Zealand. Türkiye is located between 36-42° north latitude, with wide pasture areas suitable for sheep breeding. Türkiye ranks 7th in the world with approximately 42 million sheep (FAO, 2024; Tüik, 2024). Türkiye is a deep-rooted agricultural and animal husbandry country in which sheep have been bred for thousands of years. Sheep farming in Türkiye is not only an economic activity but also an important part of Turkish culture and lifestyle. Sheep farming, an indispensable element of nomadic life in the past, continues to be an important source of income for many people living in rural areas.

However, in recent years, difficulties in employing labor (shepherds, etc.), increasing input costs, inefficient/decreased pastures, animal diseases, problems in marketing animal products obtained from sheep (low demand or sales below cost) and migration from rural areas to urban areas have negatively affected the profitability and sustainability of sheep farming (Aksoy and Yavuz, 2012; Demir et al., 2015). Specifically in sheep farming, for successful policies aimed at solving existing problems and for economically effective enterprises, primarily, the structural characteristics of the sheep farming sector must be well known and the deficiencies must be identified. Data Envelopment Analysis, which was also used in the current study, is used to reveal the efficiency and profitability of sheep farming enterprises, especially in economic terms.

Data Envelopment Analysis (DEA) is a nonparametric analysis method used to measure the relative efficiency of decision-making units (DMUs) that produce one or more outputs using multiple inputs. In economic analyses, it is often preferred to evaluate the efficiency of various DMUs, such as firms in different sectors, public institutions or geographical

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regions. There are different DEA models such as CCR (Charnes, Cooper, Rhodes), BCC (Banker, Charnes, Cooper) and SBM (Slacks-Based Measure). There are many software programs available for DEA analyses (Deat, DEA Solver, Frontier Analyst, etc.). One of the most important advantages of DEA is that it reduces the efficiency values of DMUs with multiple inputs and outputs to a single result. As an efficiency measurement technique, DEA is based on linear programming, based on the relationship of multiple inputs and outputs. Linear programming is a mathematical technique aimed at the most efficient use of limited resources within a specific purpose (Gonzalez et al., 2022). Mathematically, the efficiency measurement of DEA can be expressed as the division of the sum of weighted outputs of the DMU by the sum of weighted inputs (Stichhauerova and Pelloneova, 2019). Data envelopment analysis, which is used in many sectors, is used in the field of livestock to measure the technical and economic efficiency of enterprises and clinics (Demir et al., 2012; Sariözkan et al., 2023; Aydın et al., 2024).

In this study, it was aimed to reveal the economic activities of sheep farming enterprises in Kars Province using DEA and to present suggestions for inefficient enterprises to become effective.

MATERIALS AND METHODS

In the present study, data from 99 sheep farming enterprises in Kars province were used. Data for one year (2024) for sheep farming enterprises were obtained from face-to-face surveys (general information about the farm and owner of the farm, information about the cost items of the farm, and information about the income items of the farm). The results of this study were examined in three parts.

1) Descriptive Information about the sheep enterprises

The owners were asked survey questions that included general information about themselves and their enterprises. The answers to the questions as a result of the survey were presented as percentages.

2) Cost, Income and profitability status of the enterprises

Survey questions were asked to obtain seven input and five output data of the sheep enterprises.

Economic analysis method for determining the total cost, income and profit situations (Demir et al. 2012; Sariözkan et al. 2023);

Total cost (TL) = Feed (roughage, concentrated feed) cost+ labor cost (family labor, foreign labor) + veterinary-health cost + maintenance-repair (vehicle, etc.) cost + depreciation cost (tractor, tool-equipment) + general administrative expense cost (transportation + communication, etc.) + other cost (shipping, invoices, shepherd dog, etc.)

Total Income (TL) = Milk sales income (yoghurt, cheese, clotted cream) + animal sales income (sheep/yearling, lamb) + inventory value increase (IVI) + fertilizer income + government support

Profitability = Total Income-Total cost

Cost and income items of the sheep enterprises are given in Table 1.

Table 1- Cost and income items

Costs Items		Income Items	
1.	Feed costs	1.	Milk sales
2.	Labor costs	2.	Animal sales
3.	Veterinary and health costs	3.	Inventory value increases (IVI)
4.	Maintenance and repair costs	4.	Fertilizer sales
5.	Depreciation costs	5.	Government support
6.	General administrative costs		
7.	Other costs		

3) Data Envelopment Analysis (DEA)

In the created DEA economic efficiency model, 7 input variables (feed expense, labor expense, veterinary-health expense, maintenance-repair expense, depreciation expense, general administrative expense and other expenses) belonging to the enterprises were taken into account.

The obtained data were analyzed using the input-oriented CCR technique. In DEA, it is assumed that each unit has "m" inputs, "s" outputs and "n" decision-making units on the problem to be analyzed. The *i*th input amount of the *j*th decision-making unit is $X_{ij} \geq 0$ and the *Y_j* parameter shows the *i*th output amount used by the *j*th decision-making unit. The mathematical expression of the CCR technique of the input-oriented fractional DEA model, where $Y_{ij} \geq 0$, is as follows.

$$h_k = \frac{\sum_{r=1}^s (u_r y_{rk})}{\sum_{i=1}^m (v_i x_{ik})} \quad (1)$$

$$\frac{\sum_{r=1}^s (u_r y_{rj})}{\sum_{i=1}^m (v_i x_{ij})} \leq 1; \quad j=1,2,\dots,n$$

$$u_r \geq 0; \quad r=1,2,\dots,s$$

$$v_i \geq 0; \quad i=1,2,\dots,m$$

The CCR data envelopment analysis model can be transformed into the following model.

$$h_k = \sum_{k=1}^n \lambda_k \frac{\sum_{r=1}^s (u_r y_{rk})}{\sum_{i=1}^m (v_i x_{ik})} \quad k=1,2,\dots,n$$

$$\sum_{i=1}^m (v_i x_{ik}) u_r \geq 0; \quad r=1,2,\dots,s \quad v_i \geq 0; \quad i=1,2,\dots,m$$

$$\sum_{r=1}^s (u_r y_{rj}) - \sum_{i=1}^m (v_i x_{ij}) \leq 0; \quad j=1,2,\dots,n$$

Enb: Maximization; urk": Weight assigned to the rth output by decision unit k; vik": Weight assigned to the ith input by decision unit k; Yrk": Output produced by decision unit k for the rth output; Xik": ith input used by decision unit k; Yrj": rth output produced by jth DMU; Xij": ith input used by jth DMU (Aydın et al., 2014).

The problem here has been processed n times to determine the efficiency of all DMU scores, and weighted inputs and outputs have been selected to optimize the efficiency score of each decision-making unit. The efficiency value of each DMU is in the range of [0, 1]. If the efficiency score of the DMU is 1, the relevant decision-making unit is considered effective, while if the efficiency score is less than 1, it is not considered effective (Aydın et al., 2014; Khezrimotlagh et al., 2021).

The analysis was applied to a total of 99 sheep enterprises. For inputs, the 1st enterprise was coded as "G1 {I}", 2nd enterprise was coded as "G2 {I}",... and 99th enterprise was "G99 {I}", and for outputs, the 1st enterprise was coded as "C1 {O}", 2nd enterprise was "C2 {O}",... and 99th enterprise was "C99 {O}". In this study, MS Excel and EMS 1.3.0 were used for DEA, and IBM SPSS 25.0 package program was used for the independent sample t-test.

RESULTS AND DISCUSSION

According to the findings of this study, the average age of the owners of the enterprises, all of whom were male, was 46. It was determined that 3% of the owners of the enterprises were illiterate; 76.8% were primary and secondary school graduates; 19.2% were high school graduates; and 1% were university graduates. It was observed that the average experience of owners was 9 years (39.4% had 1-5 years, 25.3% had 6-10 years, 16.2% had 11-15 years, and 19.2% had 16 years and above experience). All owners who participated in the survey had their own enterprises. The survey revealed that 67.7% of the enterprises were farming only Akkaraman, 3% were farming only Morkaraman, and 29.3% were farming both the breeds. The actual capacity of the enterprises was 365 sheep on average. The 25% of the owners of the enterprises stated that they preferred sheep farming because they "like sheep farming", 37.8% because "there is pasture availability", 12.2% because "the current sector is profitable", 10.8% because "they want to earn additional income" and 14.2% because "there is government support".

The proportional distribution of annual average cost and income items of Sheep farming enterprises in Kars is given in Table 2-3. The proportional distributions of the annual average cost and income items of sheep farming enterprises in Kars are presented in Tables 2-3. The proportional distribution of cost items of sheep farming enterprises, from largest to smallest, was as follows: 50.7% feed costs, 35.3% labor costs, 5.6% veterinary and health costs, 2.9% depreciation, 2.6% general administrative costs, 2.1% maintenance and repair costs, and 0.8% other costs (Table 2).

When the distribution of annual average income items of enterprises was examined, it was determined that inventory value increases had the highest share (58.6%), followed by animal sales income (36.4%), government support (3.4%), fertilizer income (1.1%), and milk sales income (0.5%) (Table 3). The efficiency scores of the enterprises according to the DEA results applied to the data of sheep farming enterprises are given in Table 4.

Table 2 - Distribution rates of cost items (%).

Cost Items	Share of total costs (%)
Feed costs	50.7
Labor costs	35.3
Veterinary and health costs	5.6
Maintenance and repair costs	2.9
Depreciation costs	2.6
General administrative costs	2.1
Other costs	0.8

Table 3 - Proportional distribution of income items (%).

Income Items	Share total income (%)
Inventory value increases (IVI)	58.6
Animal sales	36.4
Government support	3.4
Fertilizer sales	1.1
Milk sales	0.5

Table 4 - Economic efficiency scores of sheep farming enterprises

Decision unit (Sheep farming No)	Efficiency score	Benchmarks (Reference set)	Number of references shown by another decision unit	Efficiency status
Sheep farm 5	0.52	Sheep farm 3 (0.15) Sheep farm 4 (0.18) Sheep farm 6 (0.27) Sheep farm 34 (0.15) Sheep farm 36 (0.04) Sheep farm 62 (0.01) Sheep farm 83 (0.25)	0	Inefficient
Sheep farm 7	0.70	Sheep farm 4 (0.09) Sheep farm 6 (0.72) Sheep farm 34 (0.05) Sheep farm 36 (0.18) Sheep farm 59 (0.24) Sheep farm 83 (0.10)	0	Inefficient
Sheep farm 8	0.87	Sheep farm g 6 (1.19) Sheep farm 9 (0.04) Sheep farm 36 (0.38)	0	Inefficient
Sheep farm 12	0.80	Sheep farm 3 (0.06) Sheep farm 6 (0.94) Sheep farm 9 (0.30) Sheep farm 11 (0.09) Sheep farm 83 (0.02)	0	Inefficient
Sheep farm 14	0.72	Sheep farm 4 (0.21) Sheep farm 49 (0.39) Sheep farm 62 (0.35) Sheep farm 83 (0.01) Sheep farm 96 (0.28) 98 (0.04)	0	Inefficient
Sheep farm 15	0.94	Sheep farm 2 (0.20) Sheep farm 6 (1.40) Sheep farm 47 (0.36) Sheep farm 83 (0.38)	0	Inefficient
Sheep farm 16	0.71	Sheep farm (0.12) Sheep farm 6 (1.11) Sheep farm 19 (0.02) Sheep farm 38 (0.04) Sheep farm 39 (0.10)	0	Inefficient
Sheep farm 17	0.61	Sheep farm 3 (0.29) Sheep farm 62 (0.03) Sheep farm 74 (0.00) Sheep farm 83 (0.37)	0	Inefficient
Sheep farm 18	0.97	Sheep farm 3 (0.48) Sheep farm 13 (0.09) Sheep farm 49 (1.98) Sheep farm 90 (0.25)	0	Inefficient
Sheep farm 20	0.72	Sheep farm 39 (0.10) Sheep farm 74 (0.03) Sheep farm 83 (0.64) Sheep farm 90 (0.02) Sheep farm 96 (0.30)	0	Inefficient
Sheep farm 21	0.66	Sheep farm 6 (0.03) Sheep farm 11 (0.08) Sheep farm 36 (0.05) Sheep farm 47 (0.00) Sheep farm 49 (0.51) Sheep farm 83 (0.24)	0	Inefficient
Sheep farm 22	0.93	Sheep farm 6 (0.47) Sheep farm 13 (0.00) Sheep farm 31 (0.51) Sheep farm 39 (0.14)	0	Inefficient
Sheep farm 25	0.76	Sheep farm 39 (0.02) Sheep farm 49 (0.70) Sheep farm 74 (0.02) Sheep farm 83 (0.19) Sheep farm 96 (0.21)	0	Inefficient
Sheep farm 27	0.90	Sheep farm 39 (0.26) Sheep farm 83 (0.22) Sheep farm 96 (0.42) Sheep farm 98 (0.04)	0	Inefficient
Sheep farm 28	0.88	Sheep farm 34 (0.52) Sheep farm 36 (0.10) Sheep farm 39 (0.37) Sheep farm 49 (0.05) Sheep farm 59 (0.17)	0	Inefficient
Sheep farm 29	0.97	Sheep farm 4 (0.21) Sheep farm 39 (0.07) Sheep farm 47 (0.10) Sheep farm 65 (0.39) Sheep farm 83 (0.02) Sheep farm 96 (0.10)	0	Inefficient
Sheep farm 32	0.99	Sheep farm 36 (0.13) Sheep farm 39 (0.09) Sheep farm 45 (0.17) Sheep farm 59 (0.04) Sheep farm 68 (0.56)	0	Inefficient
Sheep farm 33	0.88	Sheep farm 4 (0.38) Sheep farm 34 (0.15) Sheep farm 38 (0.03) Sheep farm 39 (0.04) Sheep farm 47 (0.12) Sheep farm 59 (0.19)	0	Inefficient
Sheep farm 35	0.76	Sheep farm 39 (0.07) Sheep farm 74 (0.03) Sheep farm 83 (0.30) Sheep farm 96 (0.51) Sheep farm 98 (0.09)	0	Inefficient
Sheep farm 41	0.67	Sheep farm 1 (0.25) Sheep farm 3 (0.10) Sheep farm 6 (0.05) Sheep farm 36 (0.07) Sheep farm 62 (0.03) Sheep farm 83 (0.25)	0	Inefficient
Sheep farm 42	0.88	Sheep farm 6 (0.50) Sheep farm 36 (0.17) Sheep farm 39 (0.03) Sheep farm 59 (0.17) Sheep farm 81 (0.01)	0	Inefficient
Sheep farm 43	0.66	Sheep farm 4 (0.05) Sheep farm 6 (0.52) Sheep farm 47 (0.08) Sheep farm 60 (0.01) Sheep farm 83 (0.42)	0	Inefficient
Sheep farm 44	0.84	Sheep farm 36 (0.60) Sheep farm 48 (0.15) Sheep farm 83 (0.19)	0	Inefficient
Sheep farm 46	0.70	Sheep farm 3 (0.08) Sheep farm 9 (0.22) Sheep farm 13 (0.27) Sheep farm 36 (0.51)	0	Inefficient
Sheep farm 50	0.89	Sheep farm 60 (0.01) Sheep farm 62 (0.54) Sheep farm 73 (0.50)	0	Inefficient
Sheep farm 51	0.94	Sheep farm 1 (0.11) Sheep farm 2 (0.04) Sheep farm 3 (0.28) Sheep farm 4 (0.23) Sheep farm 11 (0.12) Sheep farm 83 (0.72)	0	Inefficient
Sheep farm 52	0.57	Sheep farm 3 (0.00) Sheep farm 4 (0.06) Sheep farm 6 (0.74) Sheep farm 38 (0.00) Sheep farm 39 (0.20) Sheep farm 45 (0.11) Sheep farm 47 (0.09)	0	Inefficient
Sheep farm 53	0.89	Sheep farm 3 (0.35) Sheep farm 6 (0.18) Sheep farm 62 (0.03) Sheep farm 83 (0.24)	0	Inefficient
Sheep farm 56	0.81	Sheep farm 49 (0.42) Sheep farm 74 (0.10) Sheep farm 83 (0.33) Sheep farm 96 (0.25)	0	Inefficient
Sheep farm 57	0.86	Sheep farm 47 (0.11) Sheep farm 49 (0.70) Sheep farm 83 (0.13) Sheep farm 96 (0.03)	0	Inefficient
Sheep farm 58	0.96	Sheep farm 6 (0.34) Sheep farm 34 (0.02) Sheep farm 38 (0.01) Sheep farm 39 (0.11) Sheep farm 47 (0.21) Sheep farm 49 (0.16)	0	Inefficient
Sheep farm 61	0.77	Sheep farm 39 (0.03) Sheep farm 40 (0.00) Sheep farm 49 (0.70) Sheep farm 74 (0.03) Sheep farm 76 (0.00) Sheep farm 83 (0.18) Sheep farm 96 (0.21)	0	Inefficient
Sheep farm 63	0.97	Sheep farm 39 (0.15) Sheep farm 74 (0.03) Sheep farm 83 (0.24) Sheep	0	Inefficient

		farm 96 (0.45) Sheep farm 98 (0.02)		
Sheep farm 64	0.92	Sheep farm 30 (0.24) Sheep farm 39 (0.21) Sheep farm 49 (0.46) Sheep farm 59 (0.08) Sheep farm 62 (0.04) Sheep farm 83 (0.01)	0	Inefficient
Sheep farm 66	0.95	Sheep farm 3 (0.05) Sheep farm 4 (0.27) Sheep farm 19 (0.00) Sheep farm 34 (0.20) Sheep farm 39 (0.20) Sheep farm 49 (0.44) Sheep farm 96 (0.13)	0	Inefficient
Sheep farm 67	0.90	Sheep farm 3 (0.09) Sheep farm 6 (0.28) Sheep farm 47 (0.01) Sheep farm 49 (0.22) Sheep farm 83 (0.28)	0	Inefficient
Sheep farm 69	0.83	Sheep farm 4 (0.06) Sheep farm 9 (0.06) Sheep farm 47 (0.23) Sheep farm 74 (0.08) Sheep farm 83 (0.33)	0	Inefficient
Sheep farm 70	0.96	Sheep farm 6 (0.31) Sheep farm 34 (0.44) Sheep farm 47 (0.08) Sheep farm 65 (0.14) Sheep farm 74 (0.05) Sheep farm 96 (0.10)	0	Inefficient
Sheep farm 71	0.81	Sheep farm 62 (0.03) Sheep farm 74 (0.09) Sheep farm 83 (0.30) Sheep farm 96 (0.51) Sheep farm 98 (0.05)	0	Inefficient
Sheep farm 72	0.89	Sheep farm 6 (0.04) Sheep farm 9 (0.01) Sheep farm 11 (0.68) Sheep farm 83 (0.05)	0	Inefficient
Sheep farm 75	0.99	Sheep farm 3 (0.53) Sheep farm 39 (0.44) Sheep farm 49 (0.25)	0	Inefficient
Sheep farm 77	0.48	Sheep farm 1 (0.11) Sheep farm 2 (0.02) Sheep farm 3 (0.10) Sheep farm 4 (0.18) Sheep farm 6 (0.12) Sheep farm 11 (0.01) Sheep farm 83 (0.29)	0	Inefficient
Sheep farm 78	0.97	Sheep farm 3 (0.01) Sheep farm 6 (0.43) Sheep farm 59 (0.35) Sheep farm 83 (0.06)	0	Inefficient
Sheep farm 79	0.57	Sheep farm 4 (0.14) Sheep farm 34 (0.04) Sheep farm 36 (0.16) Sheep farm 49 (0.00) Sheep farm 59 (0.08) Sheep farm 83 (0.41)	0	Inefficient
Sheep farm 80	0.73	Sheep farm 6 (0.28) Sheep farm 9 (0.04) Sheep farm 36 (0.28) Sheep farm 83 (0.40)	0	Inefficient
Sheep farm 82	0.72	Sheep farm 34 (0.15) Sheep farm 39 (0.27) Sheep farm 45 (0.22) Sheep farm 59 (0.63)	0	Inefficient
Sheep farm 84	0.76	Sheep farm 3 (0.07) Sheep farm 4 (0.04) Sheep farm 6 (0.19) Sheep farm 9 (0.06) Sheep farm 36 (0.01) Sheep farm 45 (0.27) Sheep farm 48 (0.07) Sheep farm 83 (0.26)	0	Inefficient
Sheep farm 85	0.98	Sheep farm 3 (0.02) Sheep farm 39 (0.04) Sheep farm 49 (0.84) Sheep farm 62 (0.01) Sheep farm 83 (0.03) Sheep farm 96 (0.02)	0	Inefficient
Sheep farm 86	0.79	Sheep farm 62 (0.29) Sheep farm 83 (0.29) Sheep farm 96 (0.34) Sheep farm 98 (0.18)	0	Inefficient
Sheep farm 87	0.95	Sheep farm 2 (0.11) Sheep farm 3 (0.18) Sheep farm 4 (0.26) Sheep farm 6 (0.35) Sheep farm 11 (0.32) Sheep farm 83 (0.50)	0	Inefficient
Sheep farm 88	0.64	Sheep farm 4 (0.14) Sheep farm 6 (0.97) Sheep farm 34 (0.00) Sheep farm 38 (0.04) Sheep farm 39 (0.12) Sheep farm 47 (0.03) Sheep farm 65 (0.00)	0	Inefficient
Sheep farm 89	0.64	Sheep farm 3 (0.19) Sheep farm 62 (0.08) Sheep farm 74 (0.06) Sheep farm 83 (0.35)	0	Inefficient
Sheep farm 91	0.84	Sheep farm 3 (0.39) Sheep farm 13 (0.33) Sheep farm 24 (0.14) Sheep farm 49 (0.21) Sheep farm 62 (0.03)	0	Inefficient
Sheep farm 92	0.95	Sheep farm 49 (0.36) Sheep farm 74 (0.11) Sheep farm 83 (0.47) Sheep farm 96 (0.20)	0	Inefficient
Sheep farm 93	0.71	Sheep farm 6 (0.09) Sheep farm 49 (0.40) Sheep farm 83 (0.49)	0	Inefficient
Sheep farm 94	0.74	Sheep farm 3 (0.04) Sheep farm 6 (0.26) Sheep farm 13 (0.02) Sheep farm 31 (0.67) Sheep farm 39 (0.08)	0	Inefficient
Sheep farm 97	0.91	Sheep farm 49 (0.34) Sheep farm 74 (0.04) Sheep farm 83 (0.53) Sheep farm 96 (0.24)	0	Inefficient
Sheep farm 99	0.91	Sheep farm 3 (0.05) Sheep farm 60 (0.18) Sheep farm 62 (0.21) Sheep farm 73 (0.27) Sheep farm 83 (0.01)	0	Inefficient

The efficiency scores of the enterprises, according to the DEA results applied to the data of sheep farming enterprises, are presented in Table 4. According to the DEA results for economic efficiency, 41 enterprises were found to be efficient (efficiency score = 1) and 58 enterprises were found to be inefficient (efficiency score <1). Sheep enterprise 1, was referenced a total of 3 times (by enterprises 41, 51, and 77); enterprises 3, a total of 21 times (by enterprises 5, 12, 17, 18, 41, 46, 51, 52, 53, 66, 67, 75, 77, 78, 84, 85, 87, 89, 91, 94, and 99); enterprise 4, a total of 16 times (by enterprises 5, 7, 14, 16, 29, 33, 43, 51, 52, 66, 69, 77, 79, 84, 87, and 88); enterprise 6, a total of 25 times (by enterprises 5, 7, 8, 12, 15, 16, 21, 22, 41, 42, 43, 52, 53, 58, 67, 70, 72, 77, 78, 80, 84, 87, 88, 93, and 94); enterprise 39, a total of 22 times (by enterprises 16, 20, 22, 25, 27, 28, 29, 32, 33, 35, 42, 52, 58, 61, 63, 64, 66, 75, 82, 85, 88, and 94); enterprise 49, a total of 19 times (by enterprises 14, 18, 21, 25, 28, 56, 57, 58, 61, 64, 66, 67, 75, 79, 85, 91, 92, 93 and 97); enterprise 62, a total of 13 times (by enterprises 5, 14, 17, 41, 50, 53, 64, 71, 85, 86, 89, 91 and 99); enterprise 83, a total of 39 times (by enterprises 5, 7, 12, 14, 15, 17, 20, 21, 25, 27, 29, 35, 41, 43, 44, 51, 53, 56, 57, 61, 63, 64, 67, 69, 71, 72, 77, 78, 79, 80, 84, 85, 86, 87, 89, 92, 93, 97 and 99); enterprise 96, a total of 17 times (by enterprises 14, 20, 25, 27, 29, 35, 56, 57, 61, 63, 66, 70, 71, 85, 86, 92 and 97). The DEA input-oriented efficiency scores of sheep farming enterprises and residual values of variables (input elements that need to be reduced) are presented in Table 5.

Table 5 - Residual values of variables with Charnes Cooper Rhodes (CCR) input-side efficiency score of sheep farming enterprises (TL).

Sheep farming No	Efficiency score	Feed	Veterinary and health costs	Labor	Other	Maintenance and repair	Depreciation	General and administrative
Sheep farm 5	0.52	54,664.86	2,295.92	0	0	0	0	0
Sheep farm 7	0.70	1,581.05	37,643.95	0	0	0	0	0
Sheep farm 8	0.87	0	6,102.08	0	816.21	0	816.21	0
Sheep farm 12	0.80	1,524.67	0	8,683.37	0	0	0	0
Sheep farm 14	0.72	2,395.98	57,047.15	0	0	0.03	0.03	0
Sheep farm 15	0.94	2,577.01	1,988.13	71,353.78	0	5,881.55	5,881.55	0
Sheep farm 16	0.71	0	0	23,968.5	1,630.77	0	1,630.77	0
Sheep farm 17	0.61	279,649.7	0	82,452.11	0	17,306.71	17,306.71	0
Sheep farm 18	0.97	69,792.27	89,336.86	0	0	0	0	5,845.92
Sheep farm 20	0.72	0.02	6,675.77	0	0	0	0	0
Sheep farm 21	0.66	0	3,988.99	0	0	0	0	0
Sheep farm 22	0.93	74,907.35	0	199,210.4	2,369.11	0	2,369.11	0
Sheep farm 25	0.76	4,970.83	3,458.58	0	0	18,864.82	18,864.82	1,426.27
Sheep farm 27	0.90	185,964.0	14,823.47	0	0	16,890.67	16,890.67	0
Sheep farm 28	0.88	614.07	881.77	0	0	9,617.28	9,617.28	0
Sheep farm 29	0.97	0	0	116,046.0	0	0	0	0
Sheep farm 32	0.99	41,799.67	0	0	21.22	0.76	21.98	1,255.95
Sheep farm 33	0.88	776.81	18,495.58	0	0	0	0	0
Sheep farm 35	0.76	27,139.62	9,722.76	0	0	11,717.66	11,717.66	1,925.6
Sheep farm 41	0.67	12,811.87	72,350.3	0	0	0	10,811.87	0
Sheep farm 42	0.88	6,417.32	0	0	0	0	0	192.52
Sheep farm 43	0.66	1,683.2	33,757.45	0	0	4,423.12	4,423.12	0
Sheep farm 44	0.84	19,960.12	2,679.09	0	0	0.0	0	711.33
Sheep farm 46	0.70	1,762.75	25,624.66	0	3,020.99	6,910.47	9,931.45	0
Sheep farm 50	0.89	285,668.7	54,678.61	147,082.5	0	0	0	0
Sheep farm 51	0.94	445.96	10,618.17	0	0	0	0	0
Sheep farm 52	0.57	17,865.6	0	0	0	0	0	0
Sheep farm 53	0.89	407,122.6	24,345.54	0	0	30,570.06	30,570.06	0
Sheep farm 56	0.81	1,351.85	24,156.89	3,751.86	0	3,745.07	3,745.07	0
Sheep farm 57	0.86	1,887.04	5,741.02	54,863.97	0	0	0	0
Sheep farm 58	0.96	56.47	1,344.45	0	0	0	0	0
Sheep farm 61	0.77	1,126.66	8,680.62	0	0	12,701.15	12,701.15	0
Sheep farm 63	0.97	287,770.0	31,201.36	0	0	24,185.29	24,185.29	0
Sheep farm 64	0.92	236.36	21,399.65	0	0	23,509.92	23,509.92	2,316.47
Sheep farm 66	0.95	652.58	0	0	0	10,876.34	10,876.34	0
Sheep farm 67	0.90	0	4,646.2	75,661.98	0	0	0	2465
Sheep farm 69	0.83	1,723.96	16,154.48	0	0	17,424.54	17,424.54	0
Sheep farm 70	0.96	14,869.92	15,038.91	132,530.6	0	11,869.92	0	5,319.75
Sheep farm 71	0.81	3,110.58	20,414.81	0	0	16,005.73	16,005.73	1,911.08
Sheep farm 72	0.89	75,168.67	0	26,897.12	0	0	0	3,061.97
Sheep farm 75	0.99	41,664.99	0	207,492.7	633.08	0	0	0
Sheep farm 77	0.48	1,966.47	46,820.7	0	0	0	0	0
Sheep farm 78	0.97	91,704.97	2,684.57	0	0	230.94	230.94	2,877.76
Sheep farm 79	0.57	1,223.17	29,123.08	0	0	0	0	0
Sheep farm 80	0.73	2,261.32	0	0	0	0	0	67.84
Sheep farm 82	0.72	1,096.97	7,579.32	0	8,651.54	0	8,651.54	0
Sheep farm 84	0.76	11,458.4	0	0	0	0	0	0
Sheep farm 85	0.98	77,828.88	0	0	0	0	0	2,334.87
Sheep farm 86	0.79	426,808.5	66,926.16	0	0	11,668.68	11,668.68	0
Sheep farm 87	0.95	621.73	14,803.18	0.01	0	0	0	0
Sheep farm 88	0.64	12,653.3	0	0	0	0	0	0
Sheep farm 89	0.64	344,101.5	0	53,012.13	0	16,910.61	16,910.61	0
Sheep farm 91	0.84	280,702.6	0	0	0	9,740.96	9,740.96	0
Sheep farm 92	0.95	0	39,388.17	225,498.4	0	8,614.59	8,614.59	0
Sheep farm 93	0.71	2,254.31	1,096.5	72,024.61	0	791.9	791.9	0
Sheep farm 94	0.74	12,936.66	0	73,280.32	415.83	0	415.83	2,623.93
Sheep farm 97	0.91	82,928.75	13,725.94	0	0	26,353.74	26,353.74	4,645.58
Sheep farm 99	0.91	185,451.2	0	14,095.73	0	6,771.26	6,771.26	0

Table 6 - Average economic data for effective and ineffective sheep farming enterprises

Variable (average)	Effective	Ineffective
Actual capacity (head)	409	334
Total cost, TL (\$)	1,271,524 (36,855.7)	1,370,421 (39,722.3)
Total income, TL (\$)	2,826,651 (81,931.9)	2,171,374 (62,938.3)
Profit TL (\$)	1,555,127 (45,076.1)	800,953 (23,216.0)
\$1= 34.5 TL (28.11.2024)		

The average actual capacity and economic data (income, costs, and profit) of the effective and ineffective sheep farming enterprises are presented in Table 6.

According to the study findings, the average actual capacity, average total income, and average net profit of effective enterprises were higher than those of ineffective enterprises, and their average total costs were lower. Concerning the profitability, it was determined that economically effective enterprises made an average of 754,174 TL (\$21,860.1) more profit than enterprises that were ineffective (Table 6).

In Kars Province, located in the Eastern Anatolia Region of Türkiye, animal husbandry is the most important source of income for the local people, as the climatic conditions are not suitable for agriculture and there are large pasture areas. For sustainable sheep and cattle farming, which is extremely important for the people of the region, breeders must have economically effective enterprises. In the current study, the economic effectiveness of sheep enterprises in Kars Province were revealed through the DEA method, which is used to determine the effectiveness of enterprises in many areas. Although there are studies supporting the findings of the current study in terms of the gender, age, and education status of enterprise owners (Dossa et al., 2008; Demir et al., 2015; Tamer and Sarıözkan, 2017), this study have shown that the education level of those engaged in sheep farming, which is a sub-sector of livestock, is lower and the average age is higher compared to other livestock sub-sectors (broiler/egg chicken farming, cattle farming; Sarıözkan and Sakarya, 2006; Yalçın, et al., 2010). The average experience of the owners of the enterprises participating in the survey was determined as 9 years, which is consistent with the study conducted (Demir et al., 2015) in Ardahan Province. The fact that 39.4% of the participants had 1-5 years of experience shows that the support and incentives given by the government in recent years, as well as the presence of pastures in the region, have caused farm owners to turn to sheep farming, which they like. It has been thought that owners prefer Akkaraman or Morkaraman breeds due to factors such as the good adaptation of these breeds to the region, their resistance to diseases and the lower losses of sheep/yearlings and maternal animals, especially lambs than other breeds. When the proportional distribution of input items of the enterprises was examined, it was determined that the largest proportion belonged to feed cost (50.7%). This was followed by labor (35.3%), veterinary-health (5.6%), maintenance-repair (2.9%), depreciation (2.6%), general administrative (2.1%), and other costs (0.8%). Data from previous studies (Demir et al., 2015; Tamer and Sarıözkan, 2017) conducted in different regions of Türkiye are consistent with the findings of the current study. In the study conducted by Tamer and Sarıözkan (2017), the proportional distribution of costs is as follows, respectively: feed (59.5%), labor (23.2%), veterinary-health (6%), depreciation (3.3%), maintenance-repair (2.7%), general administrative (2.7%) and other costs (2.1%). In the study conducted by Demir et al. (2015) feed (48.9%) and labor (16.9%) costs had the largest share in costs, as in the current study. In the proportional distribution of the income items of enterprises, the highest share belongs to inventory value increase while the lowest share belongs to milk sales income. The largest share in income belongs to inventory value increase was similar to the study of Tamer and Sarıözkan (2017). In the current study, the reasons for the low milk sales income were listed as the low amount of milk obtained from sheep, milk being given to lambs, insufficient labor force, and insufficient demand for milk and dairy products obtained from these animals (Morris, 2017).

CONCLUSION

In this study, the efficiency scores of sheep farming enterprises in Kars Province were determined for the first time using Data Envelopment Analysis, and it was determined that 41 out of 99 enterprises were effective and 58 were ineffective. It has been determined that ineffective enterprises must reduce the costs of some input items specific to their own enterprises. It was thought that ineffective enterprises need to make progress in obtaining, processing, branding, and marketing high value-added products (milk/dairy products and meat/meat products) from sheep farming, in addition to reducing their costs in order to become effective. In addition, the productivity and quality of the pasture assets in Kars province should be increased by improving them and exchanging grazing them. Effective organizational structures (cooperatives and producer unions) regarding sheep farming need to be established in the region. Thus, it is possible for enterprises to increase their incomes and make farming more profitable. This DEA study can also provide guidance for enterprises in this province to achieve economically effective and profitable production.

DECLARATIONS

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Data availability

The data and materials of this study are available from the corresponding author.

Authors' contribution

M.Küçükoflaz, and E. Aydın: designed the research, supervision, writing, and editing.
C.İ. Zaman, and A.K. Aydın: investigation, collecting the data.
S. Sarıözkan: writing, and review.
M. Ayyıldız Akın: analysis.

Consent to publish

All authors have read and approved the final version of the manuscript and give their consent for publication.

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Ethical considerations

No invasive intervention was performed on the animals. This study does not present any ethical concerns.

Competing Interests

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

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FILLETING ATTRIBUTES, LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF SOME LOCAL FISH SPECIES COLLECTED FROM YANBU FISH MARKET (RED SEA COAST, SAUDI ARABIA)

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Supporting Information

ABSTRACT: Analyzing the filleting attributes of fish is essential for evaluating the commercial viability of fish products. This study assesses the filleting attributes, length-weight relationships, and condition factors of three commercially important fish species (*Lethrinus nebulosus*, *Epinephelus tauvina*, and *Plectorhinchus gaterinus*) from the Yanbu fish market in Saudi Arabian Red Sea coast. Fillet production results indicated a decreasing trend in edible portions among these species, with *Lethrinus nebulosus* yielding the most, followed by *Plectorhinchus gaterinus* and *Epinephelus tauvina*. Fish with smaller heads and medium-sized skeletons produced higher edible fillet yields. Linear regression analysis revealed no significant differences, establishing a linear correlation between net edible weight and fillet yield. The length-weight relationship analyses for *Lethrinus nebulosus*, *Plectorhinchus gaterinus*, and *Epinephelus tauvina* indicated positive allometric growth. Condition factor analysis showed that *Lethrinus nebulosus* had the lowest mean condition factor (1.05 ± 0.05), while *Epinephelus tauvina* had the highest mean condition (1.67 ± 0.15). A robust association between weight and fillet yield components was also observed. These findings enhance our understanding of the biological and economic characteristics of these species along the Yanbu coastline, supporting fisheries management and postharvest research in line with conservation and restoration efforts.

Keywords: Condition factor, Edible weight, Filleting yield, Fish products, Postharvest characteristics.

INTRODUCTION

The study of fish filleting attributes including the length-weight relationship (LWR) and condition factor (K), is critical to assessing the viability of commercial fish products (Rasyadi, et al., 2023). These parameters help determine the health and growth rates of fish, offering insights for fisheries management and postharvest processing (Akintola, et al., 2022). For instance, evaluating the weight-to-length ratio provides key indicators for the economic viability of fish species as food sources and assesses their role in sustaining industries related to postharvest technology, including canning and processing sectors (Adam Sulieman et al., 2011; Ikape and Solomon, 2018; Tahany et al., 2022).

The length-weight relationship research is a common way to study fish in order to see how body weight composition and they are. It is useful for managing fisheries in the field of postharvest technology and fish handling. Many authors have studied how the length and weight of tropical fish are related, and how healthy they are (Zafar et al., 2002; Hossain et al., 2006; Muchlisin et al., 2010; Khillare and Khandare, 2020) while the condition factor shows how healthy and heavy a fish is based on its environment and biology.

Accordingly, the weight to length ratio of a fish may vary depending on the species and location (Blackwell et al., 2000; Fernandes, et al., 2020). The condition factor (K) is a tool used in fisheries science to assess the health and nutrition of fish. It assists them in monitoring the amount of food the fish are consuming, their age, and their rate of growth (Getso et al., 2017).

Fisheries in the Red Sea region, specifically along the Yanbu coast of Saudi Arabia, provide substantial protein resources and support industries that contribute to the national economy. In Saudi Arabia, traditional fishing methods account for a significant portion of the national fish catch, including the Red Sea region, where fish species such as grouper, snapper, and emperors are abundant (total catch from marine fisheries in Saudi Arabia, 2022). However, limited research has focused on the processing attributes, length-weight relationships, and condition factors of local fish species in this area, particularly those sold in the Yanbu fish market.

This research aims to fill this gap by analyzing the processing attributes, length-weight relationships and condition factors of selected fish species, contributing to a better understanding of the health and viability of these commercially valuable fish.

RESEARCH ARTICLE
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MATERIALS AND METHODS

Sampling site

The studied commercial fish samples were purchased from Yanbu Fish Market at Yanbu City near to Red Sea coast, Saudi Arabia/al-Madinah/Yanbu/Yanbu Al-Bahr (Al-Balad) located at 24° 04'11.7"N 38° 03'13.4"E.

Experimental fish species

A total of 60 individuals, belonging to three families, were procured for this research. These families include, Lethrinidae, Serranidae and Haemulidae, representing three species, namely, *Lethrinus nebulosus* (Emperors or scavengers), *Epinephelus tauvina*, Local name (Arabian grouper or greasy rockcod) and *Plectorhinchus gaterinus*.

Experimental trial

A total of 60 fish samples were utilized, 20 individuals regarding each species. Fish samples were purchased from Yanbu Fish Market and put in to sterile polythene bags and taken in icebox and transported to the Taibah University, College of Science, Department of Biology laboratory where data were processed: Their total and standard length were recorded (in cm) using measuring tape meter and total body weights were recorded in grams using an electronic weighing balance. The samples were then filleted, eviscerated, beheaded using a sharpen knives. The weight of viscera, fillets, heads, and skeletons (skeleton) were weighed separately using weighing balance. A pooled mean of these weights was calculated and used to estimate the percentage of each part of the dress out-fillets, head, gut and skeleton relative to the weight of whole fish and recorded the findings in Table 1.

Length-weight relationship

The equation $W=aL^b$ was used to calculate the relationship between the length and weight of the fish samples. In this study, used the least-square method to find the values of constant a and b. Values transformed the data using logarithms and used the formula: $\log W= \log a + b \log L$.

Which, W is the fish's body weight in grams, L is the total length in centimeters, a is the intercept of the regression curve and b is the regression coefficient.

Condition factor

Fulton's condition factor (K) measures the health condition of the fish by using the formula:

$$K=100W/L^3,$$

where W is the weight and L is the length of the fish. W represents weight in grams and L represents total length in centimeters. The condition factor was figured out using a formula created by [Fagbua et al. \(2019\)](#).

Statistical analysis

The gathered numbers and data in this study were used to analyze with the statistical method ANOVA and software SPSS version 17. Followed by Duncan multiple range tests, and the difference between species was investigated by independent sample T-test correlations between body size (weight and length and condition factor) and edible and inedible parts comparing with condition factor were analyzed by Pearson's coefficient for linear regression (r). The differences were considered significant at $P<0.05$ and $P<0.01$. All data were recorded as mean±Standard error.

RESULTS

Length-weight relationship

The length-weight relationships (LWR) of the studied fish species from the Yanbu Fish Market are presented in Tables 2 and Figures 7-9. The 95% confidence interval values of the exponent 'b' in the relationship varied among investigated fish (*Lethrinus nebulosus*: 0.93, *Plectorhinchus gaterinus*: 1.09 and *Epinephelus tauvina*: 1.34). Analysis of studied fish species statistically showed that all the species exhibited positive allometric growth pattern. Their 'b' values were less than 3. There was correlation between the length and the weight of all the studied fish species.

Filleting yield composition

The study found that the fish's filleting yield composition, body parts, and amount of meat varied a lot. The *Lethrinus nebulosus* had the highest fillet percentage (37.4%), followed by *Plectorhinchus gaterinus* (36.5%) and *Epinephelus tauvina* has the lowest fillet (34.2%). While in the case of inedible parts all studied fish species were recorded a high percentage showed (59.2%, 61.1% and 61.7% respectively) as presented in Table 1, Figures 1-6 and 16, 17. There was strong correlation between weight and their filleting yield components Tables 4-7.

Condition factor (K)

The mean condition factors (K) of all studied species are shown in Table 3, and Figures 10-15. The results exhibited variability in the condition factor for studied samples. As shown in the Figure 17, the condition factor for the 3 species recorded range between 1.05 and 1.67. *Lethrinus nebulosus*, had (1.05±0.05), *Plectorhinchus gaterinus* (1.24±0.06) and *Epinephelus tauvina* (1.67±0.15). The study revealed a negative correlation between condition factor and total length, while a positive correlation was observed between condition factor and total body weight across all examined fish (Tables 4–7). Additionally, it highlighted variations in the condition factor in relation to total length among the three studied fish from Yanbu Fish Market, as shown in Figures 10–18.

Table 1 - Average (%) Body weight composition and filleting yield of three fish type procured from Yanbu Fish Market

Fish species	Total body (g)	Total length (cm)	Standard length (cm)	Head %	Viscera %	Fin %	Skeleton %	Fillet %	Skin %	Edible parts %	Inedible parts %
<i>Lethrinus nebulosus</i>	299.0±24.06 ^a	29.47±1.44 ^a	24.43±0.52 ^a	28.08±0.052 ^a	4.97±0.067	2.17±0.23	13.43±0.40 ^c	37.44±0.40 ^a	10.80±0.77 ^a	37.44±0.40 ^a	59.27±0.97
<i>Epinephelus tauvina</i>	245.57±23.1 ^b	24.75±0.87 ^b	20.63±0.77 ^b	23.28±1.04 ^b	4.76±0.90	1.97±0.08	21.82±0.59 ^a	34.30±1.17 ^b	8.63±0.92 ^b	34.29±1.17 ^b	61.77±0.54
<i>Plectorhinchus gaterinus</i>	340.73±14.09 ^a	30.40±0.46 ^a	25.70±0.47 ^a	25.01±0.17 ^b	6.03±0.54	1.61±0.21	16.00±0.62 ^b	36.59±0.92 ^a	11.87±0.72 ^a	36.37±0.92 ^a	61.19±1.20
P-value	0.008 ^{**}	0.001 ^{**}	0.001 ^{**}	0.006 ^{**}	0.401	0.130	0.0001 ^{**}	0.041 [*]	0.002 ^{**}	0.405	0.173

Average ± Standard error. * denoted is significant at the 0.05 level. ** denoted is significant at the 0.01 level. Sample size = 60. The values in the table represent the mean ± SE. P-values indicate the significance of differences calculated from the test comparing the means of the studied fish species. Different superscript letters indicate statistically significant differences between fish species (Tukey's test, $p < 0.05$). Values sharing the same letter within a column are **not** significantly different.

Table 3 - Condition factor regression analysis of three fish species collected from Yanbu Fish Market

Fish species	TW+SE	SL+SE	95% CI for b	R ²	K+SE	Condition factor Equation	KP
<i>Lethrinus nebulosus</i>	299.00+24.06	29.47+1.44	0.935-1.167	0.413	1.05+0.05	$K = 1.85 - 0.3^* (TL)$ $K = 0.74 + 1.03^* (TW)$	(-) (+)
<i>Epinephelus tauvina</i>	245.57+23.17	24.75+0.87	1.346-1.991	0.323	1.67+0.15	$K = 4.14 - 0.10^* (TL)$ $K = 0.83 + 3.6^* (TW)$	(-) (+)
<i>Plectorhinchus gaterinus</i>	340.73+14.09	30.40+0.46	1.098-1.379	0.518	1.24+0.06	$K = 4.39 - 0.10^* (TL)$ $K = 0.38 + 2.5^* (TW)$	(-) (+)

K: Condition factor; TL: Total length; TW: Total weight; SE: Standard error; SL: Standard length; KP: condition factor pattern; R² : correlation coefficient, Sample size : 60

Table 4 - Correlation of the Body weight composition of *Lethrinus nebulosus* collected from Fish Yanbu Market

Parameters	Edible part	Inedible part	Total Weight	Head	Viscera	Fin	Skeleton	Skin
Edible part								
Inedible part	0.591**							
TL	-0.057	0.324						
Head	0.542*	0.763**	0.397					
Viscera	0.448*	0.644**	0.044	0.410				
Fin	0.502*	0.243	-0.369	0.169	0.703**			
Skeleton	0.053	0.145	-0.019	0.051	-0.297	-0.423		
Skin	-0.345	-0.248	-0.086	-0.657**	-0.063	-0.165	-0.331	
Total Weight	-0.234	-0.102	0.638**	0.029	-0.238	-0.405	-0.125	0.082

*: Correlation is significant at the 0.05 level (2-tailed). **: Correlation is significant at the 0.01 level (2-tailed).

Table 5 - Correlation of the Body weight composition of *Epinephelus tauvina* collected from Fish Yanbu Market

Parameters	Head	Vesera	Fin	Skeleton	Skin	Total weight	Total length	Edible part
Head								
Vesera	-0.812**							
Fin	0.203	-0.187						
Skeleton	0.385	-0.581**	0.276					
Skin	-0.595**	0.846**	-0.292	-0.723**				
Total weight	0.657**	-0.812**	0.142	0.370	-0.712**			
Total length	0.320	-0.085	-0.039	-0.178	0.113	0.409		
Edible part	0.388	-0.489*	0.250	0.414	-0.374	0.533*	0.290	
Inedible part	-0.457*	0.316	-0.376	-0.390	0.376	-0.246	-0.321	-0.458*

*: Correlation is significant at the 0.05 level (2-tailed). **: Correlation is significant at the 0.01 level (2-tailed).

Table 6 - Correlation of the Body weight composition of *Plectorhinchus gaterinus* fish collected from Fish Yanbu Market

Parameters	Total Weight	Edible part	Inedible part	Total Length	Head	Vesera	Fin	Skeleton
Total weight (TW)								
Edible part	-0.560**							
Inedible part	0.329	-0.557**						
Total Length	0.191	0.253	-0.485*					
Head	0.376	-0.458*	0.878**	-0.605**				
Vesera	-0.593**	0.605**	-0.714**	0.310	-0.812**			
Fin	-0.085	0.494*	-0.325	0.210	-0.349	0.466*		
Skeleton	0.528*	-0.399	0.576**	0.102	0.482*	-0.743**	-0.240	
Skin	0.109	-0.505*	0.479*	-0.171	0.213	-0.287	-0.319	0.117

*: Correlation is significant at the 0.05 level (2-tailed). **: Correlation is significant at the 0.01 level (2-tailed).

Table 7 - Correlation of the Body length and condition factor of three fish species (*Lethrinus nebulosus*, *Epinephelus tauvina* and *Plectorhinchus gaterinus* collected from Fish Yanbu Market

Parameters	Total weight	Condition factor
Total length		
Total weight	0.638**	
Condition Factor	-0.376	0.445*

*: Correlation is significant at the 0.05 level. **: Correlation is significant at the 0.01 level.

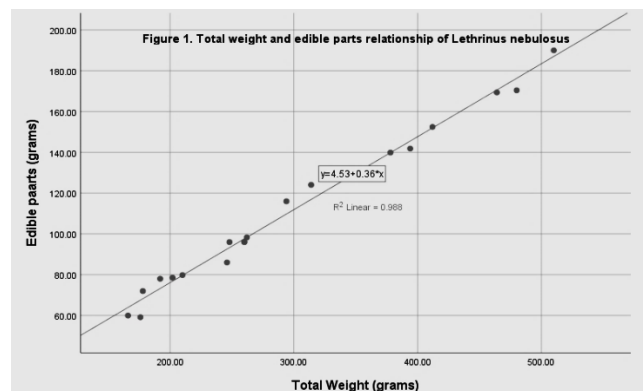


Figure 1 - Total weight and edible parts relationship of *Lethrinus nebulosus*.

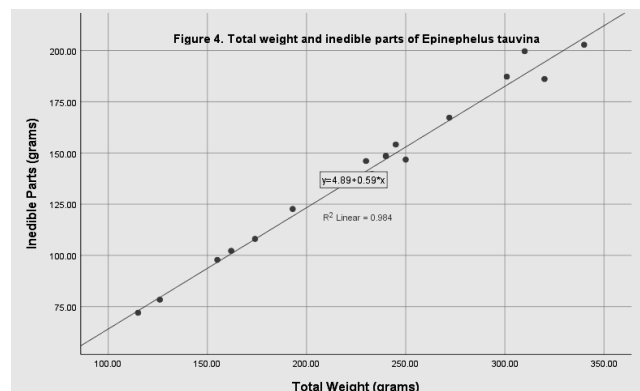


Figure 4 - Total weight and inedible parts relationship of *Epinephelus tauvina*.

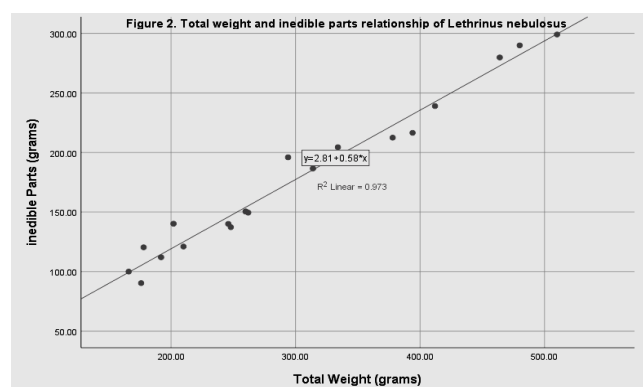


Figure 2 - Total weight and inedible parts relationship of *Lethrinus nebulosus*.

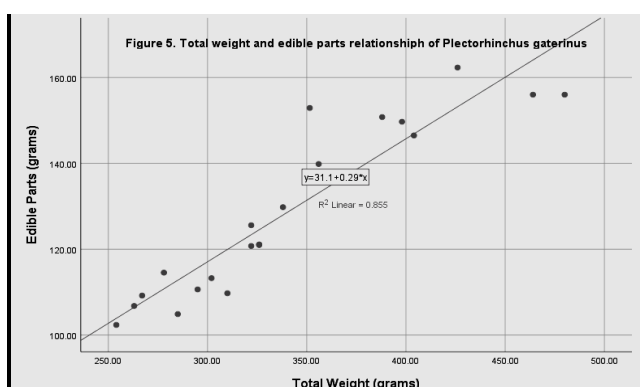


Figure 5 - Total weight and edible parts relationship of *Plectorhinchus gaterinus*.

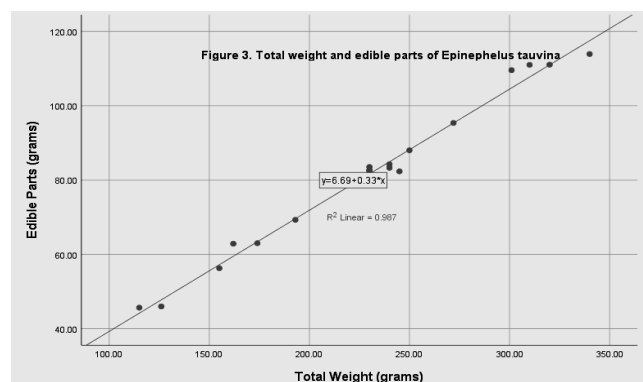


Figure 3 - Total weight and edible parts relationship of *Epinephelus tauvina*.

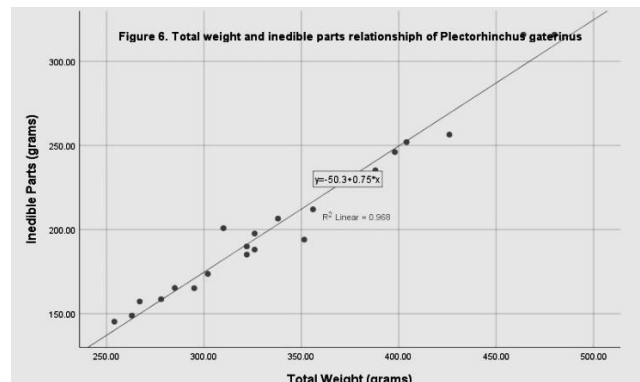


Figure 6 - Total weight and inedible parts relationship of *Plectorhinchus gaterinus*.

1. Variations of weight parameters with total weight of three studied fish from Yanbu fish Market

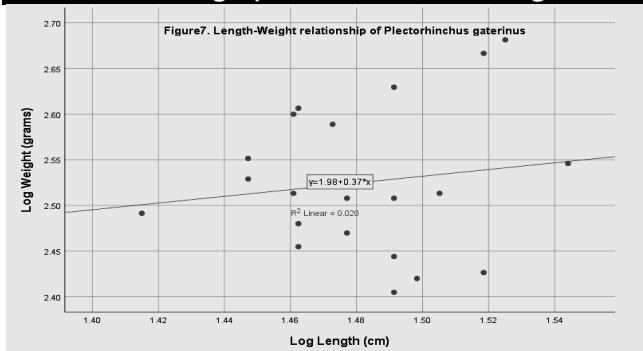


Figure 7 - Length-weight relationship of *Plectorhinchus gaterinus*.

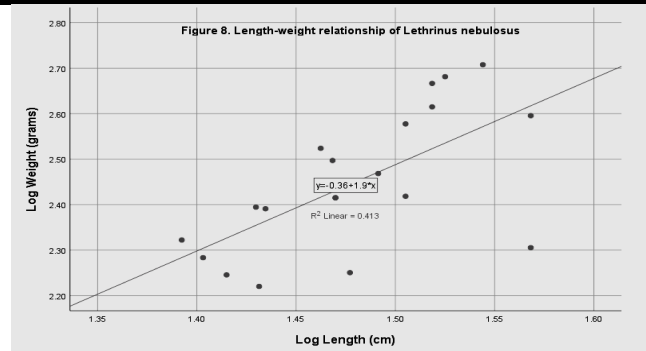


Figure 8 - Length-weight relationship of *Lethrinus nebulosus*.

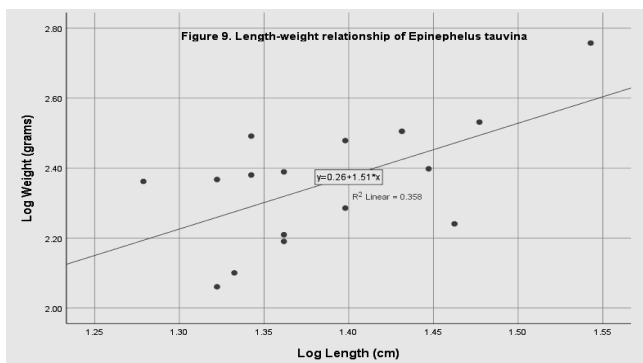


Figure 9 - Length-weight relationship of *Epinephelus tauvina*.

2. Variations of length- weight relationship (LWR) of three fish species collected from Yanbu fish Market

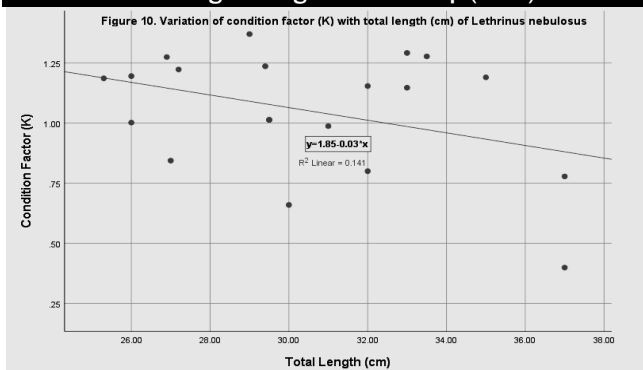


Figure 10 - Variation of condition factor (K) with total length (cm) of *Lethrinus nebulosus*.

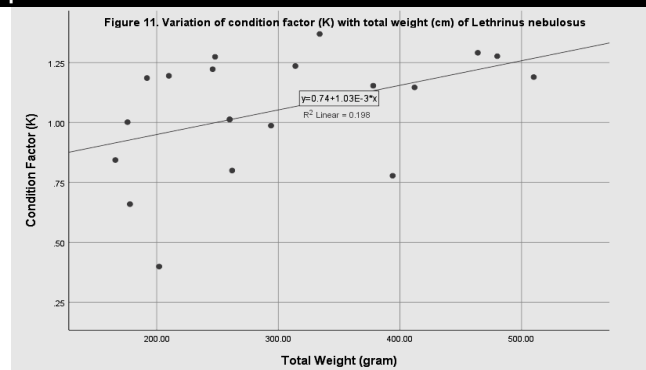


Figure 11 - Variation of condition factor (K) of total weight (g) *Lethrinus nebulosus*.

3. Variations of condition factor (K) with total length (cm) of three studied fish from Yanbu fish Market

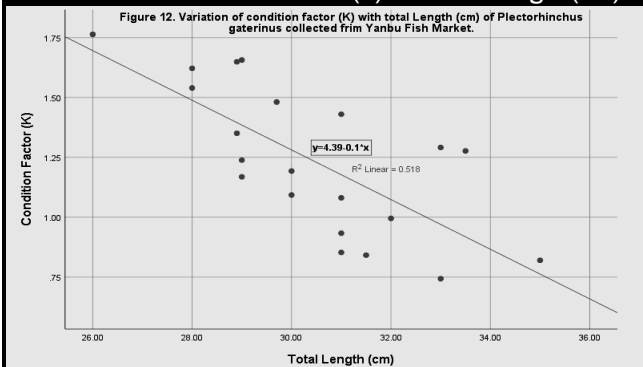


Figure 12 - Variation of condition factor (K) with total length (cm) of *Plectorhinchus gaterinus* collected from Yanbu Fish Market.

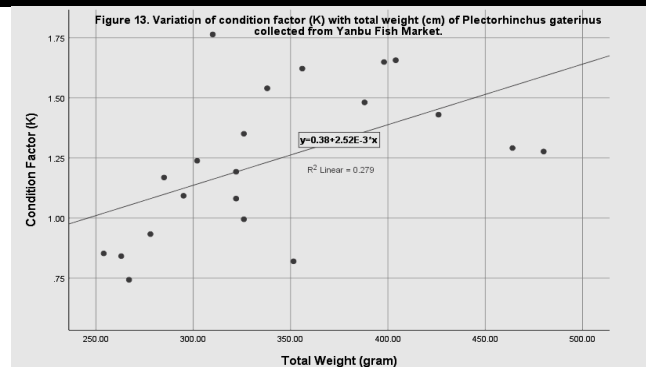


Figure 13 - Variation of condition factor (K) with total weight (grams) of *Plectorhinchus gaterinus* collected from Yanbu Fish Market.

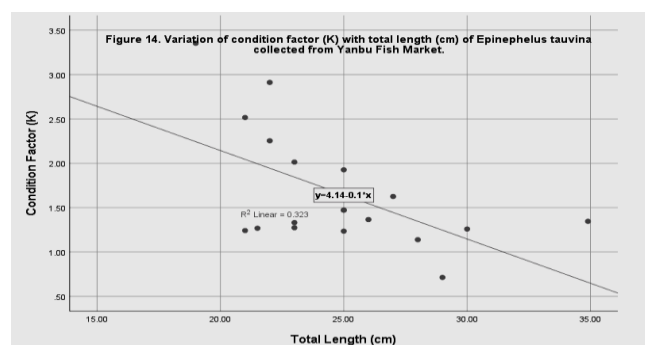


Figure 14 - Variation of condition factor (K) with total length (cm) *Epinephelus tauvina* collected from Yanbu Fish Market.

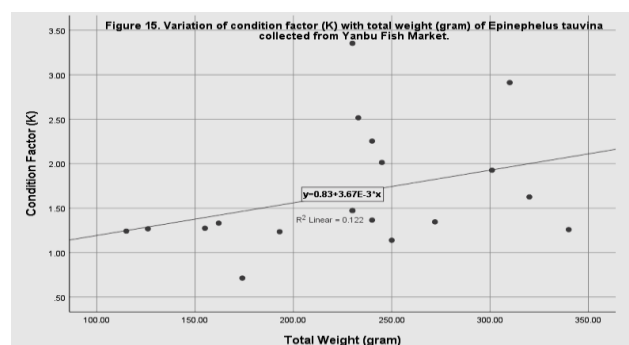
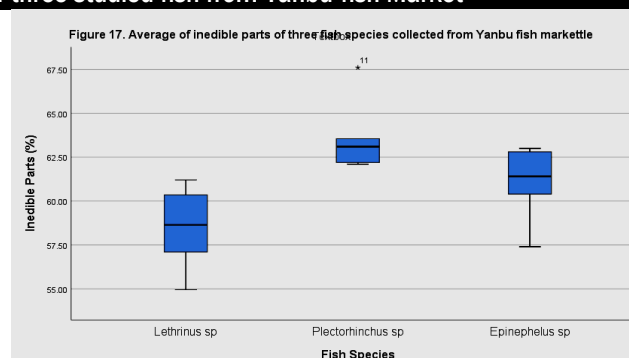
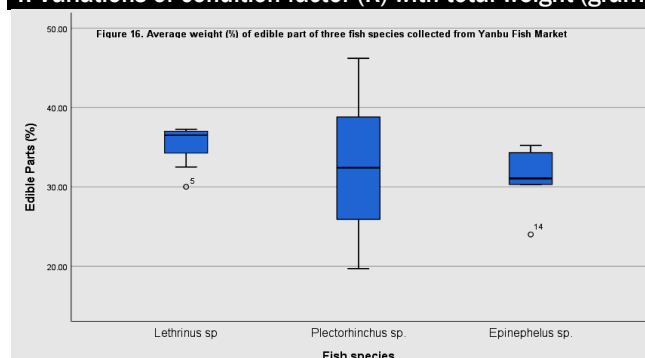


Figure 15 - Variation of condition factor (K) with total weight (g) *Epinephelus tauvina* collected from Yanbu Fish Market.

4. Variations of condition factor (K) with total weight (grams) of three studied fish from Yanbu fish Market



Figures 16 and 17 - Average of edible and inedible of three fish species collected from Yanbu fish market

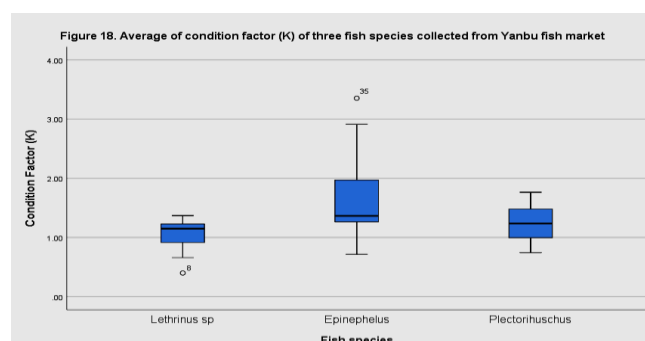


Figure 18 - Average of condition factor (K) of three fish species collected from Yanbu fish market

DISCUSSION

Length-weight relationship

The length-weight relationship (LWR) and condition factor (K) are essential for evaluating fish health and postharvest quality (Moutopoulos and Stergiou, 2002; Jayasankar et al., 2024). While Fetouh and El-Far (2023), stressed the importance of LWR in modeling aquatic ecosystems This study's findings indicate that allometric growth patterns for fish in the Yanbu region differ from documented LWR ranges in tropical species, which typically exhibit 'b' values between 2.5 and 3.5 (Pauly, 1997; Rosemonde, et al., 2019). The positive allometric growth observed implies that Yanbu's environmental conditions may limit fish weight gain relative to length increases, possibly due to unique habitat variables like salinity or food availability. The length-weight relationship (LWR) of the fish species analyzed in this study, specifically *Lethrinus nebulosus*, *Plectorhinchus gaterinus*, and *Epinephelus tauvina*, revealed a significant positive correlation. All species exhibited positive allometric growth patterns with 'b' values less than three, suggesting that the weight of these fish increases more slowly compared to their length.

This result aligns with the works of Moutopoulos and Stergiou (2002) and Jayasankar et al. (2024), who noted that LWR is valuable for fish population studies, as it reflects environmental conditions affecting growth. The values observed for each species (*Lethrinus nebulosus*: 0.93, *Plectorhinchus gaterinus*: 1.09, and *Epinephelus tauvina*: 1.34), suggest that

the growth patterns of fish species vary based on specific environmental and genetic factors (Hossain et al., 2006; Muchlisin et al., 2010; Famoofo and Abdul, 2020).

This relationship had avital role in fisheries biology because it allows estimation of average weight of the fish of a given length group (Kirankaya, 2014). The findings of this study revealed that the growth pattern of the studied fish species in the Yanbu Fish Market registered as positive allometric, because the b-values ranged from 1.51 to 1.98. This means that the studied fishes do not grow symmetrically as reported by Prasad and Verma (2023) or the fish becomes thinner with increase in length as mentioned by Gusau et al. (2021). Notable among them includes the results of Kaushik (2004) and in commercial fish landings in central market, and also in an investigation of some morphometric parameters of fish species of Lower Nun River in Niger Delta (Abowei, 2010; David et al., 2025). Also, Usman (2012), observed allometric growth pattern in Kontagora Reservoir, while Rosemonde et al. (2019) and Suleiman et al. (2021) made similar findings in an evaluation of length-weight relationship of fish species of Ebonyi River. Also, the same findings were observed by Usman (2012) and Sulaiman et al. (2022), which these results agreed with the findings of the present study. However, the b-values recorded for all the species in the present study is below the documented values of 2.5 to 3.5 for tropical fish species (Pauly, 1997; Rosemonde et al., 2019).

Filleting yield composition

The filleting yield of each species showed notable differences. *Lethrinus nebulosus* recorded the highest fillet percentage (37.4%), followed by *Plectorhinchus gaterinus* (36.5%), and *Epinephelus tauvina* with the lowest yield (34.2%). However, non-edible parts constituted a high percentage, exceeding 59% for all species. These findings correspond with other studies indicating that fish species with higher proportions of head and skeleton yield less edible fillet, a factor that affects their commercial value (Di Blase and Marchisio, 1991; Shehawy, et al., 2016). The correlation between body weight and filleting yield suggests that species with a higher condition factor may yield better fillets relative to their body structure. The filleting yield results further emphasize the impact of fish body composition on commercial yield. The higher percentage of inedible parts in the analyzed fish species presents a potential loss for fisheries, as consumers typically discard these components. The study suggests the utilization of these inedible portions for fish silage or meal production, a sustainable alternative practiced in other regions with similar high-nutrient waste products (FAO, 2010; Report of the FAO/CECAF 2020; Fisheries statistics: Saudi Arabia 2016–2021, 2023).

Condition factor (K)

The condition factor (K) results for the three fish species ranged between 1.05 and 1.67, reflecting variations in health and nutritional status. *Lethrinus nebulosus* had a mean K value of 1.05 ± 0.05 , *Plectorhinchus gaterinus* recorded 1.23 ± 0.06 , and *Epinephelus tauvina* had the highest at 1.67 ± 0.15 . The K values align with findings by Ahmed et al. (2011), Kumolu-Joh and Ndimele (2011) and it is contrary with Usman (2012). Shalloof et al. (2024) noted that higher condition factors are indicators of better nutritional status and growth rates in fish populations. Additionally, these findings reveal a negative correlation between condition factor and total length and a positive correlation with total body weight, implying that growth rates may impact the physiological state of fish over time. This result of Figures 9, 12, and 14 are not the same as the one found by Oniye et al. (2006), Ayoade and Ikulala (2007) and Kirankaya (2014). Finally, the condition factor observed among the fish species reflects their adaptation to local environmental factors, with variations in K values suggesting differential access to nutrients and feeding habits. High K values generally indicate robust health and favorable growth conditions, as noted by Ayoade and Ikulala (2007) and Rosemonde et al. (2019) who associated condition factors with environmental quality. For the Yanbu fish market, improving fish yield through selective handling or habitat management could increase the commercial value of species with lower K values, thus enhancing their marketability and nutritional value for consumers.

CONCLUSION

In conclusion, the fish species in this study showed a clear relationship among their lengths and weights. Also, the study revealed that the edible and inedible parts of studied fish, was a result of its structural body and processing skills. Fish with small heads and skeletons produces high fillet (edible) and it is suggested to use the parts of fish that people don't eat to make fish silage or fish meal in different fishing industries and poultry meal.

DECLARATIONS

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Data availability

The original data supporting this study are included in the article, is available upon reasonable request to the corresponding author.

Author contribution

Prof. H.A. Sulieman contributed to the study design and experiment scheduling, while T. Hassan conducted the data analysis. Both authors reviewed the analyzed data and approved the final manuscript draft.

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Ethical considerations

The present research work does not contain any studies performed on animal/human subjects by any of the authors.

Consent to publish

All authors agree to the publication of this manuscript.

Competing Interests

The authors have not declared any competing interest.

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PROSPECTS FOR USING *Hermetia illucens* LARVAE IN THE DIET OF FARM ANIMALS: A REVIEW

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Supporting Information

ABSTRACT: *Hermetia illucens* larvae is a promising raw material as an alternative ecological raw material for obtaining feed ingredients. The aim of this review is to gain a comprehensive understanding of the current state of research in this topic by critically analyzing existing studies. Based on the review, recommended doses of defatted *Hermetia illucens* larval meal in the diet were identified. Replacing fish meal with *Hermetia illucens* larval meal in the amount of 25 and 50% ensures stable weight gain and high-quality fish products. When feeding largemouth bass and red hybrid tilapia, the recommended proportion of replacing fish meal with insect meal is no more than 30%. Substitution of vegetable protein with *Hermetia illucens* protein in the diet of sea bass in the amount of 40% improves the histological condition of intestinal tissue. Replacing linseed fat in the amount of 30 and 60 g/kg of feed with fat from *Hermetia illucens* larvae in feeding rabbits revealed a negative effect on meat quality: a high content of saturated fatty acids is observed. As a positive effect of *Hermetia illucens* fat, a decrease in meat oxidation can be noted. The use of full-fat *Hermetia illucens* meal in the diet of piglets should be limited to 2%. However, the protein of the *Hermetia illucens* larvae has great potential and can be partially replaced in combination with the protein of other insects. A number of studies presented in this review have proven the economic efficiency of using *Hermetia illucens* larval meal in feed production: the cost of *Hermetia illucens* larval meal is lower than the cost of fish meal by 0.35 USD/kg, which increases the profitability of using this type of raw material by 25%. The problems of the widespread use of *Hermetia illucens* larval meal in animal feeding have been identified, which consist in the low attractiveness of meat and fish products grown on feed using insects. In order to reduce the negative attitude of consumers to such food products, it is necessary to increase public awareness of the environmental friendliness and safety of using such components in animal feeding.

Keywords: Fat sources, Feed components, *Hermetia illucens*, Insect flour, Protein sources.

INTRODUCTION

Fishmeal and fish fat are the main sources of animal protein and fat in compound feeds for various animals, birds and fish. Its deficit, constantly fluctuating quality and the lack of implemented alternatives make the compound feed industry highly dependent on this product (Muin and Taufek, 2024). A topical issue in the field of feed production is the introduction of alternative ecological feed ingredients. Insects have long been considered by the scientific and industrial community as a worthy alternative to fishmeal and fish fat (Vastolo et al., 2024). Silkworms, mealworms, housefly larvae, *Hermetia illucens* larvae, grasshoppers, termites, common mosquitoes, etc. are considered as a source of feed protein and fat (Henry et al., 2015). The authors (Téguia et al., 2002; Henry et al., 2015; Yu, et al., 2020; Wendin and Nyberg, 2021; Giotis and Drichoutis, 2021; Pahmeyer et al., 2022; Nampijja. et al., 2023; Roccatello R et al., 2024; Muin and Taufek, 2024) consider their advantages (highly reproducible, rich amino acid composition, high digestibility, etc.) and disadvantages (for example, the unattractiveness of food products grown on feed containing insects). Therefore, meal from insects (houseflies) was used in the study (Téguia et al., 2002). According to the results of Téguia et al. (2002), it was found that feed conversion and weight gain in the control and experimental groups were almost the same. However, the authors noted that in the experimental group, whose diet included meal from insects, an increase in the liver and goiter was observed. The authors conclude that it is necessary to analyze the toxicity of meal from insects, in particular, flour from housefly larvae, since most likely it was the cause of negative changes in the organs of birds.

Over the past few years, the number of studies on the use of the *Hermetia illucens* fly larvae as an alternative ecological raw material for obtaining feed ingredients has increased significantly (Barragan-Fonseca et al., 2017; Lee et al., 2020; Sadykova et al., 2021; Dong et al., 2024; Daniso et al., 2024; Zhan et al., 2024; Maltseva et al., 2024). *Hermetia illucens* larvae flour (Muin and Taufek, 2024) has a lower feed conversion ratio (1.69) and a higher protein efficiency ratio (1.97) compared to fishmeal, which has a feed conversion ratio of (2.43), protein efficiency ratio (1.37) when fed to tilapia. Despite the significant results obtained in this area, a comprehensive understanding of the effects of alternative ecological feed ingredients on the animal organism and the identification of further development paths

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are needed. The aim of this study is to gain a comprehensive understanding of the current state of research in this topic by critically analyzing existing studies.

Useful nutrients of the *Hermetia illucens* larvae

In the studies (Sealey et al., 2011; Park, 2016; Liland et al., 2017; Ewald et al., 2020; Lee et al., 2020; Sadykova et al., 2021; Tuichiev, 2023; Nampijja et al., 2023; Rudoy et al., 2023; Zhan et al., 2024), the authors noted the beneficial properties of the *Hermetia illucens* larvae, examining each of the components in more detail: protein and amino acids, fat and fatty acids, vitamins, chitin, peptides. To compare different studies on similar parameters, let's look at each of them in more detail.

Protein and amino acids

The *Hermetia illucens* larvae has an amino acid composition similar to that of fish meal (Tuichiev, 2023) and chicken egg white (Sadykova et al., 2021). The protein content and amino acid composition may differ depending on the substrate on which the larvae are raised. When grown on animal waste, larvae will have a lower protein content than larvae grown on food production waste, especially with a high protein content (Sealey et al., 2011). Therefore, the choice of substrate will have a significant impact on the quality of the product, as well as the cost price. The predominant amino acids in the protein of the *Hermetia illucens* larva are glutamic and aspartic acid and leucine (Sadykova et al., 2021). The protein is highly digestible (Muin et al., 2024) and can be used in feeds both in starter feeds and in grower feeds (Nampijja et al., 2023).

Fat and fatty acids

Sealey et al. (2011) investigated the change in fatty acid composition depending on the substrate. Thus, when growing larvae on cow manure, the predominant fatty acids were: lauric (12:0) 23.6%; palmitic (16:0) 19.8%; oleic acid (18:1n-9) 22.7%. When adding fish by-products - waste from fish processing plants - to this substrate, the amount of lauric acid (12:0) sharply increased 37.1%; palmitic (16:0) slightly decreased 17.3%; and oleic acid (18:1n-9) 18.8%. In another study by Caligiani et al. (2018) the predominant fatty acid was also lauric acid (12:0) more than 40%. Lauric acid has a bactericidal effect, suppressing the development of pathogenic microflora (Setianto et al., 2017). This is confirmed by the study (Kumar et al., 2021; Biasato et al., 2022), where researchers came to the conclusion that the fat of the *Hermetia illucens* larva has an immunostimulating effect and helps prevent intestinal enteritis in rainbow trout (*Oncorhynchus mykiss*) caused by soybean fat, which is currently used in feed. Lauric acid also has a positive effect on the microbial population of the intestines of animals, their vital organs and blood composition (Khan et al., 2021; Zhan et al., 2024). When analyzing the fat of the *Hermetia illucens* larvae (Cruz et al., 2023) a volatile compound, limonene, was discovered, which is used in the pharmaceutical, cosmetic and food industries due to its antioxidant and insecticidal properties. Ewald et al. (2020) conducted research on the effect of substrate on fatty acid composition. It was found that regardless of the substrate, the main fatty acid is lauric acid (saturated fatty acid). In this regard, researchers concluded that it is impossible to completely replace fish fat with fat from the larvae of *Hermetia illucens*. This is supported by another study (Kumar et al., 2021), where 16% of fish fat was replaced by *Hermetia illucens* larval fat. Histological examination of the liver revealed hyperplasia of the bile ducts, which may indicate excessive release of bile to facilitate lipid digestion (especially containing semi-saturated fatty acids, which is lauric acid). Therefore, *Hermetia illucens* larval fat can only partially replace fish fat due to the high concentration of lauric acid.

Vitamins

The larvae contain various fat-soluble vitamins and carotenoids (Liland et al., 2017; Sadykova et al., 2021; Papin et al., 2025), that have a positive effect on the digestibility of compound feed and participate in vital processes. According to Sadykova et al. (2021), the amount of carotenoids is 0.23 mg/100 g, Vitamin E — 3.1 current. equiv./100 g, Vitamin B1 (Thiamine) — 53 µg / 100 g. In Liland et al. (2017), the content of Vitamin E in the control (when hatched on a substrate — crushed wheat) was 53 mg/kg⁻¹, when seaweed was added to the substrate in an amount of 50%, the content of Vitamin E increased 4 times and amounted to 187 mg/kg⁻¹, with 100% replacement of the substrate with seaweed, the content of Vitamin E was 249 mg/kg⁻¹. Papin et al. (2025) argue that in order to obtain larvae with a given set of essential vitamins and carotenoids, it is necessary to adjust the composition of the substrate. In addition, the researchers of this study found that *Hermetia illucens* larvae are able to bioaccumulate vitamin E and carotenoids. To achieve higher concentrations of beneficial vitamins and carotenoids, additional research on insect cultivation is necessary.

Chitin

Chitin is a valuable feed raw material, has a positive effect on weight gain, has a negative effect on pathogenic microorganisms. Shaikhiev et al. (2022) determined the dynamics of changes in the chitin content in the insect during growth: larval stage — 3.6%, prepupal stage — 3.1%, puparia stage — 14.1%, imago stage — 2.9%. In other studies, the chitin content in the larvae was 3.85% (Smets et al., 2020), 7.8% (Soetemans et al., 2020), and 4.65% (Yu et al., 2020).

This difference may be due to the substrate on which the larvae were raised. Kim et al. (2025) presented the results of the antitumor effect on the animal's body (laboratory mice were used in the study), chitin helps to reduce the mass of adipose tissue in obesity and increases the diversity of intestinal microbiota with beneficial microorganisms, increases the number of lactobacilli. In this research, chitin obtained from *Hermetia illucens* was introduced into the stomach every other day using a probe as a chitin solution at a dosage of 10 mg/kg. When examining the larvae for protein content, the indicators are overestimated due to the presence of non-protein nitrogen in insects. Non-protein nitrogen also includes chitin. In the standard method, protein content is calculated by determining the total nitrogen content and using the nitrogen-to-protein conversion coefficient. This coefficient is 6.25. Janssen et al. (2017) determined the coefficient to be 5.60. These studies obtain indicators for identification of protein content in insects, without overestimating them.

Antimicrobial peptides

Lee et al. (2020) and Peng et al. (2024) discovered antimicrobial peptides in the hemolymph of the *Hermetia illucens* fly larvae. They have antibacterial properties and a wide range of effects on various pathogenic microorganisms. Cecropins, natural broad-spectrum peptides, are given special attention due to their high efficiency. This peptide was first obtained from the pupa of the giant silkworm. Currently, insects are the source of this peptide. It is noteworthy that the *Hermetia illucens* larvae contains more than 36 genes (Lee et al., 2020) that encode cecropins. For comparison, the larvae of the *Musca domestica* fly contain only 12 genes that encode cecropins. Cecropins have shown their effectiveness in the fight against gram-positive bacteria, including *Escherichia Coli*, *Acinetobacter baumannii* and *Klebsiella pneumonia*, which causes pneumonia.

The research conducted by Kumar et al. (2021) demonstrated that the effectiveness of using flour from the larvae of the fly *Hermetia illucens* as a component that reduces the degree of inflammation when using soy flour, which may contain anti-nutrients and cause inflammatory processes in the animal's body. Peng et al. (2024) note that peptides obtained from the larvae of *Hermetia illucens* can be used in the development of industrial antimicrobial drugs.

REVIEW OF THE RESULTS OF STUDIES ON FEEDING ANIMALS, BIRDS AND FISH WITH PROCESSED PRODUCTS OF THE LARVAE OF *Hermetia illucens*

A review of studies on the use of defatted *Hermetia illucens* larval meal, fat and full-fat *Hermetia illucens* larval meal was conducted. The main results are summarized in Table 1.

The results of a review of scientific studies on the use of *Hermetia illucens* in feeding various animals, birds and fish allowed us to determine the recommended doses of introducing low-fat flour from the larvae of *Hermetia illucens* into the diet. Skimmed flour from the larvae of *Hermetia illucens* can replace fish meal without negative consequences in quantities up to 30% (Sealey et al., 2011; Li et al., 2021; Nampijja et al., 2023; Dong et al., 2024; Daniso et al., 2024; Muin and Taufek, 2024; Marcheva G et al., 2024). Such a replacement ensures stable weight gain and high-quality products. The increase in the proportion of defatted meal from the larvae of *Hermetia illucens* should be done individually depending on the species and age of the animal.

For fish, the recommended proportion of fish meal replacement with flour from *Hermetia illucens* insects is from 30 to 40% (Sealey et al., 2011; Dong et al., 2024; Daniso et al., 2024; Muin and Taufek, 2024). For broiler chickens, the replacement of fish meal with flour from *Hermetia illucens* was 540 g/kg (Nampijja et al., 2023). In the feed recipe for Chinese soft-shelled turtles, fish meal can be replaced with defatted *Hermetia illucens* larval meal in an amount of no more than 10%. The researchers had note that the negative impact of higher doses of *Hermetia illucens* larval meal may be associated with the high chitin content and the increased protein content in the feed due to it, when using the standard nitrogen-to-protein conversion factor of 6.25. This is confirmed by another study (Yu et al., 2020), where the authors note the inability of monogastric animals (pigs, horses, poultry, etc.) to digest chitin, and therefore the apparent digestibility of nutrients is overestimated. An increase in the amount of low-fat flour from *Hermetia illucens* larvae in the diet of animals causes negative consequences: there is a decrease in growth (Li et al., 2021; Dong et al., 2024), deterioration of blood parameters (Marcheva et al., 2024), development of intestinal dysbiosis (Dong et al., 2024). The use of fat from the larvae *Hermetia illucens* has a negative impact on the quality of meat – the content of saturated fatty acids increases, in addition, blood parameters deteriorate: there is a violation of cholesterol metabolism and the formation of blood clots (Zotte et al., 2018). As a positive effect of *Hermetia illucens* fat, a decrease in the oxidizability of meat can be noted.

The use of full-fat flour from *Hermetia illucens* in the diet of piglets should be limited to 2%. The high fat content of *Hermetia illucens* meal has a negative impact on the vital organs of the animal: the size of the liver and small intestine increases. This is consistent with the results of numerous studies (Sealey et al., 2011; Zotte et al., 2018; Ewald et al., 2020; Kumar et al., 2021), where the authors note the high content of saturated fatty acids, which increases the load on the digestive organs for its digestion. Thus, according to numerous studies, the prospect of using *Hermetia illucens* larval meal as a partial replacement for fish meal has been confirmed. *Hermetia illucens* larval meal has a beneficial effect on the growth and development of animals, their survival. But, in addition to these indicators, an important economic indicator is one that determines the feasibility of using this raw material in feed production (Vastolo et al., 2024).

Table 1 — Average Value of lignin parameters from sugarcane bagasse

Object of feed testing	The substrate on which the larvae were grown	Proportion of larvae added (whole, larvae meal or fat)	Evaluated Indicators	Results	References
<i>Oncorhynchus mykiss</i>	Dairy cow manure (experimental group 1) and dairy cow manure with fish by-products added in an amount of 25 to 50% (experimental group 2)	25 and 50% replacement of fish meal on defatted flour from HI larvae	Weight gain, hepatosomatic index, intraperitoneal fat and muscle mass to total body mass ratio, fish fillet appearance, amino acid composition and fatty acid composition	Weight gain in fish and hepatosomatic index in the control and experimental group 2 did not differ significantly, in contrast to the experimental group 1; the highest amount of intraperitoneal fat was observed in the control; the ratio of muscle mass to body weight in all the studied samples had an insignificant difference; assessment of the appearance of the fillet did not reveal a significant difference in all the tested samples; replacement of fish meal with experimental group 2 or experimental group 1 in the amount of 25 and 50% does not have a negative effect on trout growing	Sealey et al. (2011)
<i>Micropterus salmoides</i>	No data	10, 20, 30 and 40% fish meal replacement on defatted flour from HI larvae	Rate of weight gain, morphology and intestinal microbiota, amino acid composition and fatty acid composition of fish meat	The efficiency of the experimental feed was observed when replacing fish meal with <i>Hermetia illucens</i> larval meal (HI) in an amount of no more than 30%. When replacing 40%, a decrease in the height of the villi, an increase in their width and an increase in the number of goblet cells were observed in the intestinal morphology, which contributes to intestinal dysbacteriosis. The rate of weight gain for the control sample and samples where fish meal was replaced by 10-30% was equally high (723-749%). In the experiment where 40% HI was present, the growth rate dropped sharply (624%). The content of polyunsaturated fatty acids decreased with the introduction of HI. With the introduction of HI, the amount of tyrosine and histidine increased. The introduction of HI flour did not have a significant effect on other amino acids.	Dong et al. (2024)
<i>Sparus aurata</i> L.	No data	20 and 40% replacement of vegetable protein on defatted flour from HI larvae	Histological condition of intestinal tissues	The absence of animal protein (fish meal or HI meal) in the control negatively affects the intestinal condition: moderate to severe gastritis is observed. Also, in the experiment with HI content of 20%, a mild degree of gastritis was observed. Replacing vegetable protein with HI protein in the amount of 40% improves the histological condition of intestinal tissues.	Daniso et al. (2024)
Red Hybrid Tilapia	Soybean curd residue	30% HI meal is introduced into the feed, partially replacing fish meal, soybean meal, rice bran meal and corn meal	Dry matter digestibility coefficient, weight gain, feed conversion coefficient	The body weight gain in the experimental feed was 1.4 times higher than in the control. The feed conversion ratio in the experiment was 1.7, in the control — 2.4. The dry matter digestibility ratio in the experiment was also higher (1.97) than in the control (1.37). 100% survival was observed. The addition of 30% HI flour is effective in feeding tilapia.	Muin and Taufek, (2024)
Rabbits	No data	Replacement of linseed fat with HI larval fat in the amount of 30 and 60 g/kg of feed	Degree of oxidation of meat, composition of fatty acids of meat, indicators of atherogenicity and thrombogenicity	The meat of the rabbit, whose diet included HI fat, showed significantly higher atherogenicity and thrombogenicity than the control sample. Increasing the amount of HI fat in the sample from 30 to 60 g / kg increased the level of atherogenicity, while increasing the level of flaxseed fat decreased this indicator. HI fat has a high content of lauric fatty acid (12: 0) and myristic fatty acid (14: 0). When feeding rabbits with food with HI fat, the amount of these acids also increases, which	Zotte et al. (2018)

				contributes to an increase in atherogenicity and thrombogenicity. The meat of the rabbit, which was fed with food with flax fat, is more susceptible to oxidation than when using HI fat.	
Broiler chickens	Beer waste	Replacement of fish meal with HI meal in quantities of 250, 500, 750 and 1000 g/kg dry matter i.e. replacing fish meal with HI flour 25%, 50%, 75% and 100% respectively	Weight gain, feed intake, dry matter digestibility, nutritional composition of meat	Weight gain, feed intake and dry matter digestibility decreased with increasing proportion of HI meal. The authors attribute the decrease in digestibility to the chitin content. Replacing fish meal with HI meal increased the fat content and decreased the protein content. Increasing the proportion of HI meal increased the proportion of saturated fatty acids and Omega-6 fatty acids, but decreased the proportion of Omega-3. Replacing fish meal with HI meal is possible up to 540 g/kg (i.e. the possible replacement of fishmeal with HI flour is 54%). Such a replacement will be cost-effective and will not have a significant effect on the studied parameters.	Nampijja et al. (2023)
Pigs	No data	Replacement of soybean meal (in control 228 g/kg) with defatted meal from HI in the amount of 30, 60, 90 and 120 g/kg i.e. replacing soybean meal with flour from HI 13%, 26%, 40% and 52% respectively	Growth indicators and blood biochemical parameters	Replacement of soybean meal with HI flour is permissible in quantities up to 120 g/kg i.e. to 52%. At such values, growth rates and blood parameters did not change.	Marcheva et al. (2024)
Chinese soft-shelled turtles	No data	Replacement of fish meal with defatted HI meal in quantities of 5, 10, 15 and 20%	Growth indicators, biochemical index of blood serum, antioxidant properties, amino acid composition of turtle meat	The growth rates when replacing fish meal with HI meal in the amount of 5 and 10% showed no difference compared to the control. When the proportion of HI meal increased to 15 and 20%, the growth rate decreased. The authors also associate this result with the chitin content and overestimated protein levels in the feed with the standard conversion of nitrogen to protein (with a coefficient of 6.25). The fat content increased linearly with an increase in the proportion of HI meal. An analysis of the amino acid composition showed a decrease in phenylalanine, tyrosine and arginine in the experimental samples compared to the control. Blood analysis showed that with a 10% replacement of fish meal, the maximum activity of alkaline phosphatase was observed, which affects the immune response of the animal's body. The optimal proportion of replacing fish meal with HI meal is 10%.	Li et al. (2021)
Piglets	No data	Replacement of fish meal with full fat HI flour in the amount of 1.2 and 4%	Organ condition, feed digestibility	With an increase in the proportion of full-fat HI meal, an increase in the weight of the liver and small intestine was observed. The size of the remaining organs did not show significant differences. The digestibility of protein and fat decreased with an increase in the proportion of HI meal in the feed. The minimum was observed at 4%. Thus, the optimal proportion of replacing fish meal with full-fat HI meal is 2%.	Yu et al. (2020)

ECONOMIC EFFICIENCY OF USING *Hermetia illucens* LARVAE

According to Chia et al. (2019), up to 70% of livestock production costs are spent on feed, especially protein components. Recently, there has been a rapid increase in prices for fishmeal and soybean meal. This is especially true for small farms, whose budget forces them to look for alternative sources of high-quality protein at an affordable, stable price. According to Chia et al. (2019), the cost of fishmeal is 1.2 US dollars/kg, while the cost of *Hermetia illucens* meal is 0.85 US dollars/kg. This increases the profitability of using this type of raw material by 25%. Pahmeyer et al. (2022) calculated the costs of obtaining *Hermetia illucens* larvae and the payback period of an automated module for their production depending on the cost of the finished product. Thus, with the cost of larvae of 3.55 US dollars/kg, the payback period will be 5 years (the module capacity was 1.1 tons/month). The average cost of fishmeal in the world is 1.23 US dollars/kg. At this cost, the payback period will be 10 years, which is not profitable. According to Zagorovskaya (2020), for Black soldier fly meal to be in demand on the market, its cost should be 1.3-1.62 US dollars/kg. For this, the production volume should be at least 30-50 tons/month. Therefore, when scaling the automated module (Pahmeyer et al., 2022) in order to increase its capacity, the cost of production per 1 kg will be significantly reduced and will be consistent with the results presented in the work (Chia et al., 2019). An additional economic effect is the reduction in feed conversion ratio values when using BSF larval protein, which increases weight gain with the same feed consumption (Yu et al., 2020; Nampijja et al., 2023; Muin and Taufek, 2024). Thus, using *Hermetia illucens* fly larvae is beneficial both in terms of animal production and economic efficiency.

TECHNOLOGIES AND METHODS FOR OBTAINING PROTEIN FROM *Hermetia illucens* LARVAE

From a review of studies by various researchers (Table 1), it was determined that the most effective way to feed animals is to use defatted flour from the larvae of the fly *Hermetia illucens*. There are several technologies and methods for obtaining this product. To identify the advantages and disadvantages of each of them, let us consider these technologies in more detail.

The process of converting larvae into protein concentrate begins with cleaning the substrate on which they were reared by sieving and rinsing with cold water (Bußler et al., 2016; Biasato et al., 2022; Cruz et al., 2023; Maltseva et al., 2024). Next, the larvae are inactivated by freezing at minus 20 degrees Celsius (Bußler et al., 2016; Cruz et al., 2023) or by grinding and pasteurization (Biasato et al., 2022). Various methods have been used to separate the fractions.

1) The authors of the study (Bußler et al., 2016) presented several stages of fractionation: in the first stage, the protein portion was separated from the fat by pureeing the frozen mass with distilled water in a 1:1 ratio, re-freezing, freeze-drying, grinding, chemical fat extraction using a solvent (hexane) and re-grinding. A high-protein fraction was obtained from defatted flour using aqueous extraction of soluble proteins with distilled water in an alkaline medium. Next, centrifugation and protein precipitation were carried out due to acid hydrolysis. Insoluble proteins were freeze-dried and ground. Using this method, the following products are obtained: fat, defatted flour with high (water-soluble proteins) and low (water-insoluble proteins) protein content.

2) In another study (Biasato et al., 2022), after the pasteurization process, protein and fat were separated by centrifugation. Using this method, 2 fractions were obtained: fat and partially defatted protein meal. The authors noted that insect meal after such treatment can be stored for 6 months at a temperature of no more than 20 degrees Celsius, in a dry place.

3) In Cruz et al. (2023), after freezing, the larvae were again washed under running water, placed in a sodium hydrochloride solution for sterilization, then washed again under running water and placed in a boiling solution of sodium bisulfite, which acts as an antioxidant. Then the larvae were dried at a temperature of 60 degrees Celsius, crushed and sent for fat extraction. Extraction was carried out with supercritical CO₂, which is an environmentally friendly method compared to the hexane extraction method. In addition, this method speeds up the extraction process and allows preserving a large number of carotenoids in the larval fat. Using this method, 2 fractions were obtained: fat and partially defatted protein flour.

4) A mechanical method for separating the larvae into fractions is described in the work (Maltseva et al., 2024). It consists of drying the larvae, grinding them, heating the ground mass using a microwave and squeezing out the fat on a screw press. Microwave heating is used to quickly heat the raw material, reduce the viscosity of the fat and increase the intensity of its separation from the protein part, as well as for the purpose of disinfection. Using this method, 2 fractions were obtained: fat and partially defatted protein flour.

Thus, both chemical and mechanical methods can be used to obtain feed components from the *Hermetia illucens* larva. The choice of method will depend on the technical, financial capabilities and scale of production.

CONSUMER WILLINGNESS TO CONSUME FOOD GROWN ON INSECT-FED FEED

Despite the potential of using insects in feed as a high-quality, efficient and cost-effective raw material, there are limitations in the sale of finished products grown on such feed among consumers. Food products grown on feed using

insects are less attractive than food products obtained using traditional technologies (Wendin and Nyberg, 2021; Giotis and Drichoutis, 2021; Roccatello R et al., 2024). The main reasons preventing the choice of such products are disgust and doubts about the safety of food products (Roccatello R et al., 2024). The authors state (Wendin and Nyberg, 2021; Giotis and Drichoutis, 2021; Roccatello R et al., 2024) that raising public awareness of the environmental friendliness and safety of using such components in animal feeding reduces negative consumer attitudes towards such food products. In addition, studies (Giotis and Drichoutis, 2021) note that consumers are more likely to purchase food grown using insect feed than to use insects as food. Wendin and Nyberg (2021) and Giotis and Drichoutis (2021) argue that the palatability of food products obtained using innovative feeds (insect feeds) is influenced by their taste. This factor also relates to public awareness of the benefits of such products.

CONCLUSION

Numerous studies have shown that both fish meal and insect meal play a key role in normal development, weight gain and feed efficiency. A review of the studies showed that fish meal remains a key source of animal protein and essential amino acids and cannot be fully replaced by *Hermetia illucens* larval meal. In addition, larval fat is less effective when used in animal feed, so the whole larvae can be used in animal feed only in small quantities (about 2%), especially when feeding monogastric animals. Therefore, it should be defatted to minimize the final amount of fat in the feed. At the same time, the fat of the larvae contains lauric acid, which has a bactericidal effect and has an immunostimulating effect, and a volatile compound — limonene, which has antioxidant and insecticidal properties. Such properties make the fat of the *Hermetia illucens* larvae a useful and valuable raw material both in the feed industry and in the pharmaceutical, cosmetic and food industries. The results of the studies of the economic efficiency of using *Hermetia illucens* larvae in feed production showed that larval meal can be economically advantageous when replacing fish meal. There are some differences in the results of the studies, where according to some data, the cost of *Hermetia illucens* larval meal is currently lower than the cost of fish meal, while others only determined the prospects and ways to reduce the cost of *Hermetia illucens* larval meal. This may be due to the technologies used to process the larvae into feed components, as well as the costs of growing larvae, which differ in different countries (in particular, due to climatic conditions, the cost of electricity, etc.). Therefore, one of the areas of further research is to reduce the cost of producing insect meal, in particular from *Hermetia illucens* larvae. The chitin contained in the larvae can have both positive and negative effects. Therefore, its use is limited to the feeding object. However, the protein of the *Hermetia illucens* larvae has great potential and can be partially replaced in combination with the protein of other insects, which are used as feed additives. One of the problems of its widespread use is the low attractiveness of meat and fish products grown on feeds using insects. To reduce the negative attitude of consumers to such food products, it is necessary to increase public awareness of the environmental friendliness and safety of using such components in animal feeding. This direction is fundamental in the further development of the use of insects in the feed industry. It is also necessary to continue research to improve existing technologies for processing *Hermetia illucens* larvae into feed components, reducing the cost of the final product.

DECLARATIONS

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Data availability

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

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Authors participation

T. Maltseva contributed for the write up of the study design, review and analysis of research. D. Rudoy performed supervision and editing. A. Olshevskaya performed final revision of the manuscript. M. Odabashyan reviewed and analyzed studies. V. Shevchenko critically revised the manuscript for important academic contents.

Competing interests

The authors declare that they have no competing interests.

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EFFECTIVENESS OF *Lactobacillus fermentum* CMUL-54 AND *Lactobacillus fermentum* B978 AS PROBIOTIC CANDIDATES PRODUCING MANNANASE, CELLULASE AND PROTEASE ACTIVITIES FOR POULTRY

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 Supporting Information

ABSTRACT: The present research investigated the potential of *Lactobacillus fermentum* strains CMUL-54 and B978 as a probiotic candidates with mannanase, cellulase, and protease activities. The materials used in this research included *L. fermentum* CMUL-54, *L. fermentum* B978, MRS Broth containing oxgall, and various equipment and chemicals for analyzing probiotic candidates, mannanase, cellulase, and protease activities. This study utilized quantitative analysis conducted using a paired two-sample t-test with ten replications. The results revealed that *L. fermentum* CMUL-54 could be significantly ($P < 0.01$) used as a probiotic candidate, showing resistance to temperatures of 42°C ($9.9 \times 10^9 \pm 0.71$ CFU/ml), gastric pH ($72.35 \pm 0.80\%$), bile salt resistance ($87.69 \pm 3.66\%$), and hydrophobicity test to the intestine ($92.40 \pm 0.30\%$). *Lactobacillus fermentum* CMUL-54 also exhibited significant inhibitory zones against lactic acid bacteria (LAB) and pathogenic bacteria such as *Escherichia coli* (13.27 ± 0.13 mm), *Salmonella enteritidis* (13.91 ± 0.13 mm), *Staphylococcus aureus* (17.75 ± 0.24 mm), high activity mannanase (12.36 ± 0.61 U/ml), cellulase (12.42 ± 0.24 U/ml) and protease (11.30 ± 0.08 U/ml). It is concluded that *L. fermentum* CMUL-54 exhibited superior probiotic properties compared to *L. fermentum* B978, thus positioning it as a more promising candidate for improving broiler performance through enhanced digestion and overall health.

Keywords: Enzyme activity, *Lactobacillus fermentum* CMUL-54, *L. fermentum* B978, Probiotics

INTRODUCTION

Broiler chickens are a type of poultry that have a rapid growth period, and can be marketed from three to six weeks of age. Therefore, broilers require very high-quality feed intake. Notably, good quality feed comes at a fairly high price, which increases the cost of rations for poultry, especially broilers. Hence, nutritional optimization is required to maximize nutrient provision, optimize feed, and manage production costs. One way to optimize nutrition is by adding feed additives in the form of microbes (probiotics).

Probiotics are living microorganisms that enhance the health of their host by improving the balance of intestinal microflora when ingested adequately (Hill et al., 2014; Harumdewi et al., 2018; Srifani et al., 2024a). The addition of probiotics as feed additives in broiler diets improves the health of broiler and the digestibility of feed. This resulted in improved body weight gain and feed conversion ratio (Melia et al., 2022) and increased the intake of vitamins and other feed substances (Sugiharto et al., 2018; Sabo et al., 2020). Probiotics can also increase the number of beneficial microbes in the digestive tract and stimulate the growth of broiler digestive organs (Mirsalami and Mirsalami, 2024). Furthermore, the use of probiotics in poultry rations can replace antibiotics which have negative impacts including the occurrence of antibiotic resistance residues that can be passed on to humans and endanger health. In addition to producing residues, antibiotics can also cause normal imbalances in the intestinal flora of poultry (Zhou et al., 2020; Xing et al., 2021).

Bacteria can be considered probiotic if they meets several criteria: they must be non-pathogenic, part of the normal intestinal microbiota of a particular host, and remain functional in environments with high gastritis acid and bile salts within the small intestine. They can also grow and metabolize quickly, be available in large quantities in the digestive tract, and be able to colonize the intestinal tract at a certain period. Additionally, they can efficiently produce organic acids and antimicrobial properties against pathogenic bacteria in the digestive tract. According to He et al. (2023), the selection of probiotic strains must meet several criteria, including being non-pathogenic, capable to producing antimicrobial substances, resistant to acidic conditions in the gastritis and bile salts in the small intestine. They also be able to

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modulate immune responses and influence metabolic processes in the intestine. Notably, one type of probiotic bacteria is lactic acid bacteria (LAB).

The potential of LAB, such as *Lactobacillus*, can vary depending on the source of microbial isolation. Research by Rahmiati and Mugi (2017) discovered that bacterial isolates from various sources have different characteristics and abilities, both microscopic and macroscopic. Kim et al. (2019) also isolated four types of microbes from various sources and tested them as probiotics, yielding diverse results in terms of gastric pH survivability and bile salts. However, the four microbes were equally effective reducing the odor of pig manure waste. One of the LAB that can be used as probiotics is *Lactobacillus fermentum*.

Lactobacillus fermentum is a LAB, gram-positive, facultative anaerobic, and non-pathogenic and also helps maintain microbes in the digestive tract (Karlyshev et al., 2015). Malik and Javed (2024) added that LAB can be cellulolytic as it has the ability to produce cellulase to degrade cellulose. In addition to having LAB properties, *L. fermentum* can be employed as a probiotic (Barone et al., 2016) in rations to improve broiler performance.

Seftiadi et al. (2020) isolated LAB from decomposed palm kernel cake (PKC), where the identified bacteria were *Lactobacillus* sp. It exhibited cellulase activity of 18.4U/ml, mannanase 24.86U/ml, and protease 10.45 U/ml. Furthermore, Mirnawati et al. (2022) conducted sequencing tests using 16S rRNA where the identified bacteria are *L. fermentum* CMUL-54 and assessed the nutritional content with PKC fermentation (fermentation time is four days). The results revealed crude protein at 26.31%, crude fiber at 15.71%, crude fat at 1.45%, nitrogen retention at 63.92%, and metabolic energy at 2752.69 kcal/kg (Mirnawati et al., 2023). The same study also reported enzyme activities such as cellulase activity (18.01% U/ml), mannanase (24.95 U/ml), and protease (10.55 U/ml) (Mirnawati et al., 2023).

This study was conducted using *Lactobacillus fermentum* strains (CMUL-54 and B978), which are cellulolytic and mannanolytic as a probiotic candidate for broiler.

MATERIALS AND METHODS

Study periods and location

This research was executed from 08 January to 30 April 2024 in Animal Biotechnology Laboratory, the Non-Ruminant Nutrition Laboratory, the Feed Industry Technology Laboratory, Andalas University and the Bacteriology Laboratory of Bukittinggi Veterinary Center, West Sumatera, Indonesia.

Research design

This research was undertaken in the laboratory in several stages. The first step was to isolate *L. fermentum* CMUL-54 and *L. fermentum* B978 on De Man Rogosa and Sharpe (MRS) broth (oxoid CM359B). After that, the bacteria were tested for their ability to produce cellulase enzymes on carboxymethyl cellulase (CMC), mannanase enzyme on mannan, and protease enzyme on casein.

Method

The method used in this study was quantitative analysis through a two-sample paired t-test with ten replications. The research began with assessing the ability of *L. fermentum* CMUL-54 derived from degraded palm kernel cake (PKC) and *L. fermentum* B978 derived from LIPI (Indonesia Institute of Science) as a probiotic. Probiotic candidate tests that will be performed include the resistance of 42 °C, gastric pH survivability, bile salts resistance, hydrophobicity test on the intestine, antagonistic activity, and enzyme activity (mannanase, cellulase, and protease).

Probiotic testing

Resistance to 42 °C

Resistance test at 42 °C by growing bacteria on MRS Broth media (oxoid CM359B) and placing it at 42 °C, then bacterial growth is observed through colonization and colony formation based on the standard plate count method (Zawistowska-Rojek et al., 2022).

Gastric pH survivability

The experiment utilized MRS Broth media mixed with HCl 37% (Merck KGaA) to obtain pH 2.5 and for the control, MRS Broth is not given addition of HCL 37% with pH of 6.8. The media was sterilized with an autoclave at 121 °C for duration of 15 minutes. Bacteria were isolated from up to 0.5 ml of MRS Broth-HCl and incubated at 37 °C for 3 and 6 hours. Then, the absorbance was measured at a wavelength of 600 nm.

Bile salt resistance

The experiment involved with adding bile salt concentrations of 0%, 0.3%, and 0.5% to MRS Broth media. The media was sterilized with an autoclave at 121 °C for duration 15 minutes. Then, 5 ml of MRS Broth containing 0%, 3%, and 5%

oxgall (Sigma-Aldrich, St. Louis, MO, USA) was added with 0.5 ml of bacterial isolates. Next, the mixture was incubated for 5 hours at 37°C. The treatments were compared with the control, which consisted of MRS Broth with no additional bile salt (0% concentrations). Growth was measured by analysing the absorbance at a wavelength of 600 nm.

Hydrophobicity test on intestine

The hydrophobicity test uses stainless steel plates. The stainless steel can be thoroughly cleaned by immersing it in a hot detergent solution (temperature 40-45°C) for 24 hours. Then, the plate was rinsed with hot water until it was not longer foamy and slippery, dried, and marked. To prepare the growth media, 5.22 g MRS Broth was dissolved into 100 ml of distilled water. The growth media and stainless steel were sterilised in the autoclave (temperature 121°C) for 15 minutes. Then, the stainless steel plate was placed into 25 ml of MRS Broth inoculated with 1 ml of bacterial isolate in an erlenmeyer and incubated (temperature 37°C) for 24 hours. After incubation, the stainless steel was swabbed evenly. The swab was homogenized after being placed into a tube containing 10 ml of phosphate buffer solution (A) and then measured at a wavelength of 600 nm. 1 ml of the media's liquid was removed and diluted in 9 ml of phosphate buffer solution for the measurement of liquid phase growth (Ao). Then, the absorbance at a wavelength of 600 nm is measured.

Antagonistic activity

The antagonistic effects of *L. fermentum* strains (CMUL-54 and B978) against several pathogens were determined by the agar well diffusion method (Hossain, 2024). *Lactobacillus fermentum* isolates were cultured in MRS Broth at 37°C for 24 hours, and the targeted pathogens were also pre-cultured under the circumstances of brain heart infusion (BHI) (Liofilchem, Italy). Mueller Hinton Agar plates were subsequently covered with 200 µL of the test pathogen (10^7 CFU/ml). Cell-free supernatant previously centrifuged at 6,000 rpm for 10 minutes was streaked as much as 100 µL on Petri dish. Then, petri dish were incubated (37°C for 24 hours). The antagonistic activity of *L. fermentum* was assessed in terms of inhibition zone formation (mm) around the wells. Each *L. fermentum* isolate was subjected to this procedure four times, with the average outcome being recorded. The target pathogens assessed were *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enteritidis*.

Enzyme activity testing

Mannanase activity

Bacterial isolates were taken 1 ml reacted with 1 ml of manan substrate (0.5 manan plus 10 ml phosphate buffer); all solutions were reacted in a test tube and then placed in a water bath (60°C) for duration at 30 minutes. Take 1 ml of the previous solution, add 1 ml of Nelson AB. After that, the solution is heated over a stove (temperature 100°C) for 30 minutes. After 30 minutes, remove test tube and allow it to cool briefly. After cooling, add 1 ml of phosphomolybdate and 1 ml of distilled water. Absorbance was measured using a spectrophotometer UV-VIS 1800 (Shimadzu USA MFG inc.) with a wavelength of 575 nm.

Cellulase activity

Bacterial isolates were taken in 1 ml and reacted with 1% CMC (carboxymethyl cellulose) (Himedia). All solutions were reacted in a test tube and then placed in a water bath at 60°C for 30 minutes. Take 1 ml of the previous solution, add 1 ml of Nelson AB. After that, the solution is heated over a stove (temperature 100°C) for 30 minutes. After 30 minutes, remove test tube and allow it to cool briefly. After cooling, add 1 ml of phosphomolybdate and 1 ml of distilled water. Absorbance was measured using spectrophotometer UV-VIS 1800 (Shimadzu USA MFG inc.) with a wavelength of 575 nm.

Protease activity

Pipette 2.5 ml of 1% casein solution and add 1.5 ml of 0.1 M phosphate buffer at pH 7 in a test tube, homogenized with a vortex mixer (Raypa) with vibration of 3. The sample was incubated in a water bath at 37°C for 10 minutes, adding 1 ml of bacterial isolate. Then, the reaction was incubated in a water bath at 50°C for 10 minutes. For control, enzyme activity was stopped by adding 5 ml of 20% Trichloroacetic acid (TCA) (Himedia) solution, homogenized with a vortex, and then cooled in the refrigerator for 30 minutes to coagulate the protein. The reaction for enzyme activity was carried out, and the solution that has been incubated, was then centrifuged (Sigma) at 5,000 rpm at 4°C for duration at 15 minutes, thereafter filtered, and supernatant was observed. Then, the supernatant was pipetted 2 ml and then put into a test tube and add 5 ml of 0.5N NaOH (Himedia) and 0.5 ml of folin ciocalteu (Merck KGaA) reagent to test tube, and cool for 10 minutes. Absorbance was measured using spectrophotometer uv-vis 1800 (Shimadzu USA MFG inc.) a wavelength of 650 nm.

Statistical analysis

This study used a paired two-sample t-test with ten replications. Tukey test at a confidence level of 0.01 ($P < 0.01$) was used to see the difference in each sample.

RESULTS

Probiotic testing

Probiotic testing of *Lactobacillus fermentum* strains (CMUL-54 and B978) can be observed in Table 1.

Resistance to 42 °C

Figure 1 displays the incubation results of *L. fermentum* CMUL-54 and *L. fermentum* B978 after incubation at 42 °C. The growth of *L. fermentum* CMUL-54 was better than that of *L. fermentum* B978. Total colonies from *Lactobacillus fermentum* CMUL-54 had $9.9 \times 10^9 \pm 0.71$ CFU/ml. Meanwhile, *L. fermentum* B978 had $8.7 \times 10^9 \pm 1.75$ CFU/ml (Table 1).

Table 1 - Probiotic test of *Lactobacillus fermentum* CMUL-54 and *Lactobacillus fermentum* B978

Probiotic test	<i>Lactobacillus fermentum</i> CMUL-54	<i>Lactobacillus fermentum</i> B978
Resistance to 42 °C (CFU/ml)	$9.9 \times 10^9 \text{ }^a \pm 0.71$	$8.7 \times 10^9 \text{ }^b \pm 1.75$
Gastric pH survivability (%)	$72.35 \text{ }^a \pm 0.80$	$68.87 \text{ }^b \pm 0.57$
Bile salts resistance (%)	$87.69 \text{ }^a \pm 3.66$	$78.20 \text{ }^b \pm 3.57$
Hydrophobicity test to Intestine (%)	$92.40 \text{ }^a \pm 0.39$	$85.57 \text{ }^b \pm 1.10$
Antagonistic activity (mm)		
<i>Escherichia coli</i>	$13.27 \text{ }^a \pm 0.13$	$12.24 \text{ }^b \pm 0.5974$
<i>Salmonella enteritidis</i>	$13.91 \text{ }^a \pm 0.13$	$12.81 \text{ }^b \pm 0.23$
<i>Staphylococcus aureus</i>	$17.75 \text{ }^a \pm 0.24$	$16.94 \text{ }^b \pm 0.15$

a,b; Means within a row with different superscripts different significantly (P<0.01).

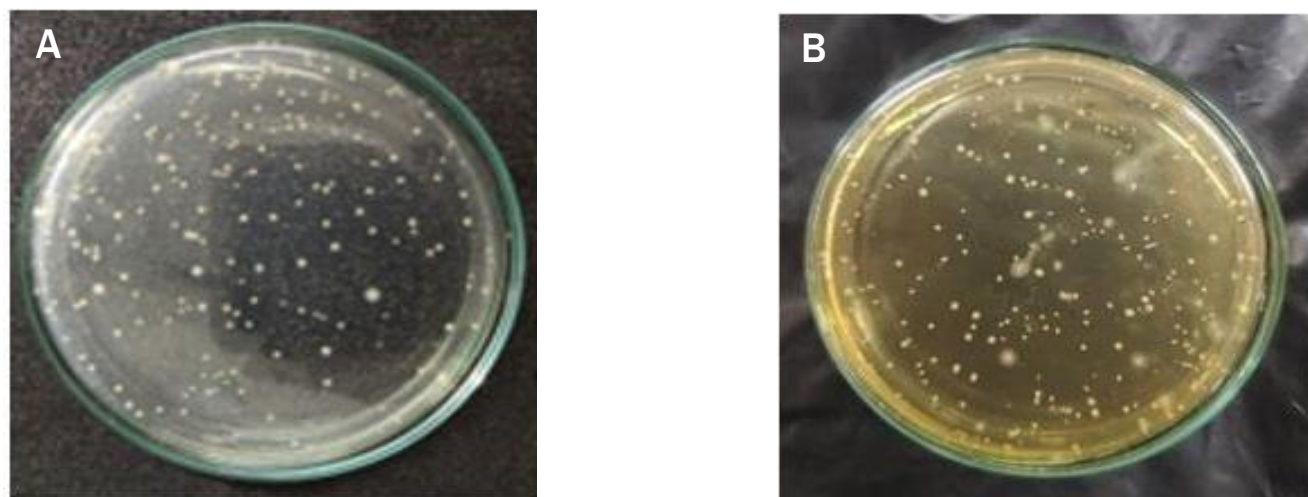


Figure 1 - Resistance of *L. fermentum* CMUL-54 (A) and *L. fermentum* B978 (B) at 42 °C.

Gastric pH survivability

The results of the bacterial resistance test are provided in Table 1, where the resistance of *L. fermentum* CMUL-54 ($72.35 \pm 0.80\%$) is higher than that of *L. fermentum* B978 ($68.87 \pm 0.57\%$). At the 3-hour time interval, *Lactobacillus fermentum* CMUL-54 showed higher resistance than *Lactobacillus fermentum* B978. This higher resistance value indicates that *L. fermentum* CMUL-54 is better able to survive at pH 2.5 conditions in a short time. At the 6-hour time interval, *L. fermentum* CMUL-54 also showed higher resistance compared to *L. fermentum* B978. Although both strains experienced a decrease in resistance, *L. fermentum* CMUL-54 remained superior in terms of resistance to acidic conditions. There is a significant negative relationship between *Lactobacillus fermentum* CMUL-54 and *L. fermentum* B978 (Table 1).

Bile salt resistance

The bile salt resistance test results can be observed in Table 1, where *L. fermentum* CMUL-54 ($87.69 \pm 3.66\%$) is higher than *L. fermentum* B978 ($78.20 \pm 3.56\%$). At 0.3% bile salt concentration, *L. fermentum* CMUL-54 showed higher resistance compared to *L. fermentum* B978. This higher resistance value indicates that *L. fermentum* CMUL-54 is better able to survive in lower bile salt conditions. At 0.5% bile salt concentration, *L. fermentum* CMUL-54 also showed higher

resistance compared to *L. fermentum* B978. Although both strains experienced a decrease in resistance as the bile salt concentration increased, *L. fermentum* CMUL-54 remained superior in terms of resistance at higher bile salt conditions.

Hydrophobicity Test on Intestine

Cell wall components such as phospholipids and lipopolysaccharides play a vital role in the hydrophobic interaction of bacterial cells. Table 1 indicated that the hydrophobicity value of *L. fermentum* CMUL-54 ($92.40 \pm 0.39\%$) is higher than *L. fermentum* B978 ($85.57 \pm 1.10\%$). *Lactobacillus fermentum* CMUL-54 showed a higher resistance value compared to *L. fermentum* B978. This higher resistance value indicates that *L. fermentum* CMUL-54 has a better ability to attach to hydrophobic surfaces in the gut. The difference in resistance values between the two strains was statistically significant indicating that *L. fermentum* CMUL-54 is superior in hydrophobicity compared to *L. fermentum* B978.

Antagonistic activity

The results in Table 1 showed that the inhibition of *Lactobacillus fermentum* CMUL-54 is higher than *Lactobacillus fermentum* B978. The average inhibition power produced by each bacterium ranged from 12.24 to 17.75 mm. The difference in the diameter of the inhibition zone between the two strains was statistically significant, indicating that *Lactobacillus fermentum* CMUL-54 was more effective in inhibiting the growth of the pathogenic bacteria.

Enzyme activity testing

The enzyme activity testing of *Lactobacillus fermentum* strains (CMUL-54 and B978) can be observed in Table 2.

Table 2. Enzyme activity of *Lactobacillus fermentum* CMUL-54 and *Lactobacillus fermentum* B978

Enzyme activity (U/ml)	<i>Lactobacillus fermentum</i> CMUL-54	<i>Lactobacillus fermentum</i> B978
Mannanase Activity	$12.36^a \pm 0.61$	$9.78^b \pm 0.22$
Cellulase Activity	$12.42^a \pm 0.24$	$8.94^b \pm 0.54$
Protease Activity	$11.30^a \pm 0.08$	$8.87^b \pm 0.13$

a,b: Means within a row with different superscripts different significantly ($P < 0.01$).

Mannanase activity

The research results on mannanase activity are summarized in Table 2, where the enzyme activity in *Lactobacillus fermentum* CMUL-54 is higher than in *L. fermentum* B978. Mannanase activity from *L. fermentum* CMUL-54 had 12.36 ± 0.61 U/ml; however, *L. fermentum* B978 had 9.78 ± 0.22 U/ml. There was a significant difference between the mannanase activities of the two bacterial strains. This indicates that *L. fermentum* CMUL-54 is more effective in producing mannanase enzyme than *L. fermentum* B978.

Cellulase activity

The results of cellulase activity research can be observed in Table 2, where the highest activity value is reported in *L. fermentum* CMUL-54. Cellulase activity from *L. fermentum* CMUL-54 was 12.42 ± 0.24 U/ml. Nevertheless, *L. fermentum* B978 had 8.94 ± 0.54 U/ml. There was a significant difference between the cellulase activities of the two bacterial strains. This indicates that *L. fermentum* CMUL-54 is more effective in producing cellulase enzyme than *L. fermentum* B978.

Protease activity

The results of protease activity research are provided in Table 2, where the activity value of *L. fermentum* CMUL-54 is higher than that of *L. fermentum* B978. Protease activity from *L. fermentum* CMUL-54 had 11.30 ± 0.08 U/ml, but *L. fermentum* B978 had 8.87 ± 0.13 U/ml. These results indicate that *L. fermentum* CMUL-54 is more effective in producing protease enzymes than *L. fermentum* B978 in probiotic applications.

DISCUSSION

Resistance to 42 °C

Microbes that are resistant at a temperature of 42 °C is a normal body temperature in poultry and their digestive system since, at this temperature, microbes can live and multiply (Yang et al., 2014; Mhone et al., 2022; Srifani et al., 2024b). The growth of *Lactobacillus fermentum* CMUL-54 is better than that of *L. fermentum* B978. Note that bacterial growth is influenced by several factors, one of which is temperature. According to Pellisery et al. (2020), based on the temperature of microbial growth can be divided into mesophiles (20-45 °C) and thermophiles (45-65 °C). *Lactobacillus fermentum* can grow well at 42 °C. Therefore, it can be categorized into mesophile bacteria. These bacteria can be used as probiotics since they can live in poultry's body and digestive tract.

Gastric pH survivability

Resistance to acidic environments is a crucial requirement for LAB as probiotics. In accordance with the statement of [Mulaw et al. \(2019\)](#), probiotic microbes must be able to pass through an acidic gastritis. Note that the gastritis has very high acidity; thus, the microbes that live in the gastritis must be able to survive at pH 3 ([Sanhueza et al., 2015](#)) or pH 4, which is the pH of the gastric mucus layer ([Garcia et al., 2017](#)). As such, microbes that cannot withstand gastric pH due to high acidity can damage cell membranes and intracellular components, ultimately causing death ([Guan and Liu, 2020](#)). pH below 2 can directly activate pepsinogen which in turn produces pepsin, a protease with an optimal acidic pH. Pepsin contributes importantly to first-line feed digestion during feed retention in poultry ([Svihus, 2014](#)). Proventriculus and gizzard are estimated to have the longest feed retention time, ranging from 30 minutes to 2 hours, before the partially digested chyme is discharged into the small intestine ([Han et al., 2019](#)). So during this interval, probiotic isolates must endure the low pH of proventriculus and gizzard.

Based on the result in Tabel 1, *Lactobacillus fermentum* CMUL-54 (72.35%) is higher than *Lactobacillus fermentum* B978 (68.87%). [Mulaw et al. \(2019\)](#) stated that the resistance LAB isolates at pH 2.5 for 3 hours exceeded 50%. These results indicated that these two bacteria can be used as probiotics in terms of resistance to acidic pH. This supports [Skenderidis et al. \(2020\)](#) results, who found that high quality probiotics are resistant to acidic pH and less impacted by it.

Bile salt resistance

Resistance to bile salts is a critical criterion for probiotic candidates, as bile salts serve as potent emulsifiers and exposure to bile in gastrointestinal tract offers significant toxicity for bacterial species, hindering their survival and functionally in gut ([Shimizu et al., 2023](#); [Foley et al., 2023](#)). Bile is one of the complex conditions in the digestive tract that probiotics must be able to tolerate. Bile contains antimicrobial properties and is an important component of the body's physiocochemical defense system ([Long et al., 2017](#)). Bile can damage to bacterial membranes. Probiotics must exhibit resistance to bile salts to endure in gastrointestinal tract and fulfill their functional role as probiotics ([Zhang et al., 2020](#)). Elevated resistance to bile salt in bacterial isolate enhances their ability to colonize the host gastrointestinal tract. So, evaluating the potential capacity of probiotics to thrive in presence of bile salt is essential.

Resistance to bile salts is related to the ability of isolates to produce the enzyme bile salt hydrolase (BSH). Some types of *Lactobacillus* have BSH enzymes that can hydrolyze bile salts, thus changing the physico-chemical properties of bile salts to be non-toxic to LAB ([Morinaga et al., 2022](#)). Additionally, BSH enzyme activity can improve bacterial survival in the gut and provide favorable characteristics for probiotic bacteria.

Hydrophobicity test on intestine

A high level of hydrophobicity indicates the presence of hydrophobic molecules on the surface of the bacterial cells being tested. [Yang et al. \(2022\)](#) stated that bacteria with a high level of hydrophobicity have the ability to settle on the intestinal surface, multiply, and enter the tissue. One thing that affects the ability to hydrophobize is the origin of the bacteria. Meanwhile, [Panjaitan et al. \(2018\)](#) stated that the value of microbial hydrophobicity is influenced by bacterial strains, growth media, bacterial age, and bacterial surface structure. *Lactobacillus fermentum* CMUL-54 comes from bacterial isolation from decomposed palm kernel meal ([Mirnawati et al., 2023](#)), while *L. fermentum* B978 is obtained from LIPI isolation. The diversity of these factors causes each species and strain to be used to demonstrate various levels of hydrophobicity.

Antagonistic activity

These results are lower than the results of research by [Srifani et al. \(2024b\)](#) on the ability of LAB isolates isolated from soymilk waste to inhibit *Escherichia coli* by 22.25 mm, but inhibit *Staphylococcus aureus* and *Salmonella enteritidis* from this study is higher than [Srifani et al. \(2024b\)](#) (*Staphylococcus aureus* by 15.15 mm, and *S. enteritidis* by 12.5mm). According to [Riyanto et al. \(2020\)](#), the strength of an antibacterial power can be measured based on the size of the inhibition formed like considered very strong if it is 20 mm or more, the servant area between 10-20 mm suggests strong, while between 5-10 mm indicates moderate. If it is 5 mm or below, then the antibacterial is considered weak. One that can inhibit pathogenic bacteria is the content of organic acids present in LAB. Organic acids such as acetic acid and lactic acid significantly inhibit gram-negative bacteria since these compounds act as the main antimicrobial for the inhibitory activity of probiotics against pathogens ([Chizhayeva et al., 2022](#)). Moreover, the main targets of organic acids are the bacterial cell wall, cytoplasm, and specific metabolism of bacteria, which can cause damage and the death of pathogens ([Nair et al., 2017](#)).

Mannanase activity

Mannanase activity produced by microbes varies depending on the source. This enzyme can be produced from various sources, including animals, plants, and microorganisms such as bacteria, molds, and yeasts ([Kuo et al., 2022](#)). The microbial source of this research is *L. fermentum* CMUL-54, obtained from decomposed PKC isolation ([Mirnawati et al.,](#)

2023), while *L. fermentum* B978 was obtained from LIPI. The ability of microbes to produce mannanase has a role in degrading mannose and manooligosaccharides. In accordance with the opinion of Chen et al. (2023) mannanase is an enzyme capable of hydrolyzing manan substrates into manooligosaccharides and small amounts of mannose, glucose, and galactose. So, adding mannanolytic microbes to the ration can produce improvements and increase the nutritional value to ensure that it can be optimally utilized by livestock, especially poultry.

Cellulase activity

Cellulolytic bacteria such as *Lactobacillus fermentum* are able to degrade cellulose. In accordance with the opinion of Gurovic et al. (2023), microbes can degrade cellulose since they produce degrading enzymes. Note that cellulase enzymes are generally produced by microbes and can also be produced by animals and plants. However, microbes are the most widely used since microbial growth is faster, they can grow on cheap substrates, and their enzyme production can be more easily increased, such as by using cellulolytic bacteria. Opinion of Murtiyaningsih and Hazmi (2017), cellulolytic bacteria can hydrolyze cellulose by synthesizing cellulase complex enzymes. The isolation of cellulolytic bacteria can improve and increase nutrition in the ration so that poultry can optimally utilize it.

Protease activity

Protease is an enzyme that can degrade proteins. According to Rio et al. (2021), protease plays a role in hydrolyzing proteins into amino acids. Microbes are the most widely used source of enzymes. Similarly, Adrio and Demain (2014) mentioned that the selection of microbes as enzyme producers is based on their ability since microbes can be used to meet the high demand for enzymes and support sustainable production. Furthermore, using proteolytic bacteria such as *L. fermentum* can improve the nutritional value of the ration to ensure that it can be optimized optimally by poultry.

CONCLUSION AND RECOMMENDATION

Based on this study, it can be deduced that both *Lactobacillus fermentum* strains (CMUL-54 and B978) have the potential to be employed as probiotics. However, *L. fermentum* CMUL-54 has the highest results, such as resistance to 42 °C ($9.9 \times 10^9 \pm 0.71$ CFU/ml), gastritis pH survivability ($72.35 \pm 0.80\%$), bile salt resistance ($87.69\% \pm 3.66\%$), and hydrophobicity to the intestine ($92.40 \pm 0.39\%$). In addition, it can also inhibit pathogenic bacteria (*Escherichia coli* 13.27 ± 0.13 mm, *Salmonella enteritidis* 13.91 ± 0.12 mm and *Staphylococcus aureus* 17.75 ± 0.15 mm) and have enzyme activities (mannanase 12.36 ± 0.61 U/ml, cellulase 12.42 ± 0.24 U/ml, and protease 11.30 ± 0.08 U/ml). the conclusions from this study suggest that *L. fermentum* CMUL-54 exhibits superior probiotic properties compared to *L. fermentum* B978, making it a more promising option for enhancing broiler performance through improved digestion and overall health.

DECLARATION

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Data availability

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

Author's contribution

Mirnawati and Harnentis contributed to research concepts, technical and logistic support, and supervised the research. G. Yanti contributed to experimental design, data collection and execution. A.R. Iryos contributed to data collection, analyses and write up of the manuscript. A. Srifani contributed to writing the final drafted manuscript.

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Ethical approval

This research does not necessitate ethical approval due to its utilization of neither human or animal as research.

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Competing interests

The authors have not declared any competing interests.

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
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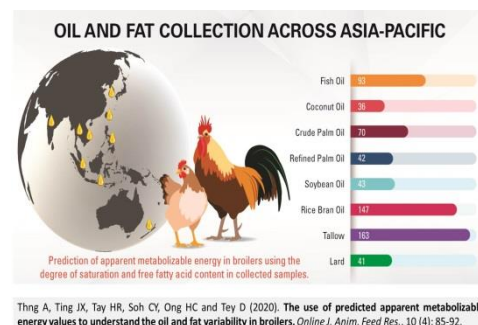
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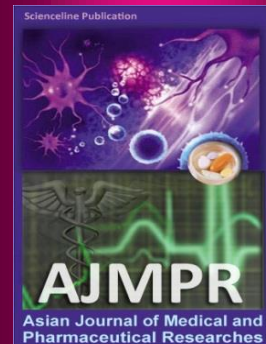
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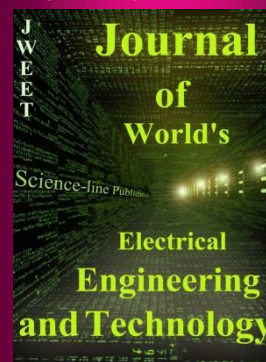
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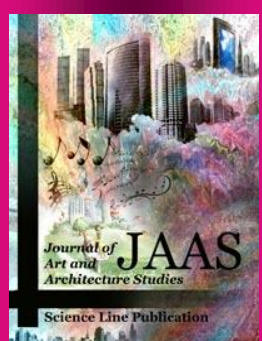
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