BOOKLET

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Online J. Anim. Feed Res., 14 (5): 274-346; September 30, 2024

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Volume 14 (5); September 30, 2024

Research Paper

Effect of supplementation of cactus (*Opuntia ficus-indica*) cladodes, Acacia saligna, wheat bran and cotton seed cake on feed intake, digestibility, growth and carcass characteristics of goats

Berhe G, Aregawi T and Sisay A.

Online J. Anim. Feed Res., 14(5): 274-286, 2024; pii: S222877012400032-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.32

Abstract
The objective of this study was to evaluate **the** effect of supplementation of cactus (*Opuntia ficus-indica*) cladodes, *Acacia saligna*, wheat bran and cotton seed cake on growth, digestibility, intake and carcass characteristics of goats. A randomized complete block design was used in the experiment with 24 yearling central highland goats with an initial body weight of 15.6 - 16.1kg. The same amount of grass hay (GH) + 150 gDM/head/day wheat bran (WB) was given to all animals. The experimental diets consisted of 80 cotton seed cake (CSC) as treatment 1 (T1), 45CSC +160 cactus cladodes (CC) as T2, 45CSC+ 80 *Acacia saligna* (AS) as T3, and 45CSC+80CC+40AS as T4 (gram dry matter: DM, per day per goat), Data were gathered on the goats' growth, digestibility, intake, and carcass of major organs, edible and nonedible organs. The consumption of dry matter and organic matter was higher in goats fed T2 and T4 than in the T1 group. The DM, organic matter (OM) and crude protein (CP) digestibility, average daily body weight gain and feed conversion efficiency were higher in T4 and T3 goats when compared to T2 goats. Goats fed on T4 had higher hot carcass weight and dressing percentage on slaughter body weight basis than T2, T3, and T1 supplemented goats. Generally, the experimental diets improved goats' performance in descending order (T4 > T3 >T1 >T2). Supplementation of T4 (replacement of 35 g DM of cotton seed cake per day by 40g of *Acacia saligna* and 80g of cactus cladodes on dry matter bases) could be recommended to improve goat performance.

Keywords: Digestion, Dry matter, Feed conversion efficiency, Goat nutrition, Protein.

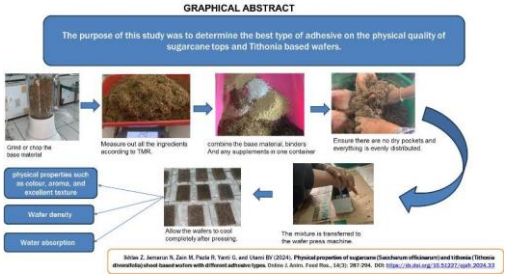
[Full text-PDF]

Research Paper

Physical properties of sugarcane (*Saccharum officinarum*) and tithonia (*Tithonia diversifolia*) shoot-based wafers with different adhesive types

Ikhlas Z, Jamarun N, Zain M, Pazla R, Yanti G, and Utami BV.

Online J. Anim. Feed Res., 14(5): 287-294, 2024; pii: S222877012400033-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.33



Abstract
Wafers (wafer-feed) are an effective processing technology and are expected to maintain the continuous availability of animal feed during the dry season. The purpose of this study was to determine the best type of adhesive on the physical quality of sugarcane tops and Tithonia based wafers. This study used the Split Split Plot Design (SSPD). The main plot as factor A was the type of adhesive, consisting of: Tapioca flour (A1), Pathi flour (A2), Gaplek flour (A3), Kariginan flour (A4), palm sugar (A5). The subplots as Factor B are temperature which consists of: 100oC (B1), 110oC (B2), and 120oC (B3), while the subplots as factor C are oven time consisting of: 10 minutes (C1), 15 minutes (C2), and 20 minutes (C3). The forage used was tabu pulp (*Saccharum officinarum*) and Tithonia (*Tithonia diversifolia*) in the ratio of 60:40. The best adhesive in making sugarcane tops and Tithonia based wafers is tapioca flour with a temperature of 120oC for 20 minutes, with physical properties such as colour, aroma, and excellent texture with a range (3.73, 3.70, and 3.63), density with a value of 5.68 g/cm3, and water binding capacity with a value of 104.22%. From the research it can be concluded that there are interactions on the physical properties of wafers (colour, aroma, and smell), density and water binding capacity.

Keywords: Binding, Physical qualities, Sugarcane shoots, Tithonia diversifolia, Wafer

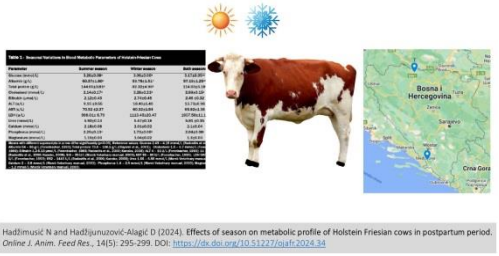
[Full text-PDF]

Short Communication

Effects of season on metabolic profile of Holstein Friesian cows in postpartum period

Hadžimusić N and Hadžijunuzović-Alagić D.

Online J. Anim. Feed Res., 14(5): 295-299, 2024; pii: S222877012400034-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.34



Hadžimusić N and Hadžijunuzović-Alagić D (2024). Effects of season on metabolic profile of Holstein Friesian cows in postpartum period. *Online J. Anim. Feed Res.*, 14(5): 295-299. DOI: https://dx.doi.org/10.51227/ojafr.2024.34

Abstract
The aim of the present study was to determine the metabolic profile of Holstein-Friesian cows in the postpartum period, as well as the effect of season on metabolic profile. The postpartum period is essential in the reproductive life of high yielding dairy cows because of its impact on future gravidity. This study included 60 cows up to 15 days after parturition, aged 2-8 years (the largest number of cows was between 3 and 5 years old) with no apparent clinical problems. Cows were sampled in summer season (n=30) and winter season (n=30). Parameters of metabolic profile were determined as follows: glucose, albumin, total protein, cholesterol, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), urea, calcium, phosphorus and magnesium. Statistical differences were considered significant at the $p<0.05$. Present research showed that all investigated parameters were within a reference range for cattle. Impact of season sampling was determined for glucose, albumin, total protein, cholesterol and phosphorus, while bilirubin, calcium, magnesium, urea as well as activities of ALT, AST and LDH were unaffected by the season of sampling. In conclusion, metabolic status is affected by the season and examination during the postpartum period can provide valuable information of cows' health status, in order to diagnose and moreover prevent postpartum diseases.

Keywords: Cows, Climate, Health status, Metabolic profile, Postpartum period.

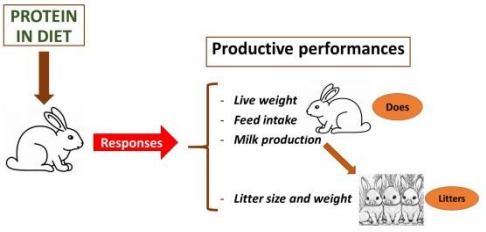
[Full text-PDF]

Research Paper

Effects of dietary protein content on the productive and reproductive performance of unselected rabbit does and their litters during first two lactations

Saidj D, Iles I, Moula N, Boukert R, Ain Baziz H, Dorbane Z, Mefti-Korteby H, Hornick JL, and Kadi SA.

Online J. Anim. Feed Res., 14(5): 300-308, 2024; pii: S222877012400035-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.35



Saidj D, Iles I, Moula N, Boukert R, Ain Baziz H, Dorbane Z, Mefti-Korteby H, Hornick JL, and Kadi SA (2024). Effects of dietary protein content on the productive and reproductive performance of unselected rabbit does and their litters during first two lactations. *Online J. Anim. Feed Res.*, 14(5): 300-308. DOI: https://dx.doi.org/10.51227/ojafr.2024.35

Abstract
The aim of the present study was to evaluate the influence of different dietary protein levels on the productive performance of unselected rabbit does and their litters during their first two lactations. For this purpose, fifty-two nulliparous rabbit does, 4.5 months of age and live weight of 3115 ± 71 g, were divided into three groups (17 or 18 females per group), kept in individual cages and each group received only one of the three experimental diets. These diets were iso-energetic (10.8 MJ DE/kg), but with increasing levels of crude protein (CP): 15%, 17% and 19 % for the low (L), medium (M) or high (H) diets, respectively. Breeding was carried out by natural copulation using 6 males of 5-6 months of age and 2865±21 g initial weight, controlled semi-intensive lactation and weaning at 35 days after birth. Female body weight, feed intake, milk production, litter size and weight were monitored at birth and weekly after parturition during the first two lactations. The protein intake of the rabbits increased with the amount of protein in the diet (L vs. M: +12.2%; L vs. H: +18.8%; $p < 0.001$), without any effect on milk production and feed intake. Milk production was unaffected by parity. Throughout the pre-weaning period, litter size and weight and maternal mortality were unaffected by dietary protein level. Dietary protein level had no effect on live weight, birth to weaning weight gain, milk production or feed intake during the first two consecutive lactations of rabbit does.

Keywords: Feed Intake, Litter parameters, Milk production, Protein content, Unselected rabbit does, Weight gain.

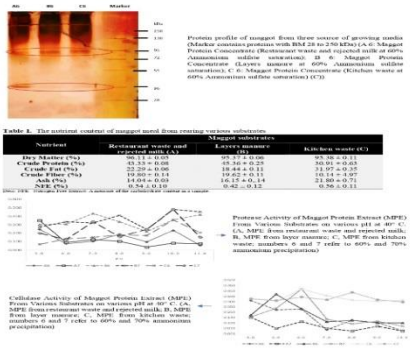
[Full text-PDF]

Research Paper

Nutrient profile, protease and cellulase activities of protein extracted from black soldier fly (*Hermetia illucens*) larvae reared on various substrates

Widiyastuti T, Rahayu S, Suryapratama W and Suhartati FM.

Online J. Anim. Feed Res., 14(5): 309-320, 2024; pii: S222877012400036-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.36



Abstract
The Black Soldier Fly (BSF; *Hermetia illucens*) larvae are recognized for their ability to convert diverse organic materials into protein-rich biomass, depending on the substrate they consume. The composition of these substrates can significantly impact the nutrient profile and enzyme activities of the resulting maggot protein extract (MPE). Therefore, this exploratory research aimed to assess the nutrient content, protease, and cellulase activity of MPE obtained from BSF maggots reared on different substrates, with a specific focus on substrates A (comprising restaurant waste and rejected milk), B (layer manure), and C (kitchen waste). The results showed that maggot meal from layer manure had the highest protein content (45.36%) and the lowest fat content (18.44%). Amino acids in maggot meal contained high levels of glutamic acid, aspartic acid, alanine, valine, leucine, and isoleucine. Lauric acids were found in maggot meal from kitchen waste (33.79%), layer manure (32.18%), and restaurant waste and rejected milk (22.94%). Maggot meal from layer manure had the highest oleic acid content (15.13%). The protein concentration of MPE from various substrates ranged from 0.56 to 0.601 mg/ml (at 60% w/v ammonium sulfate saturation) and 0.555 to 0.609 mg/ml (at 70% ammonium sulfate saturation). The protease activity of MPE from layer manure substrates exhibited optimum activity and stability in neutral to alkaline pH, with activity levels of 0.748 U/mg at pH 7.0 and pH 11.0 (at 60% w/v ammonium sulfate saturation) and 0.774 units/mg at 70% w/v ammonium sulfate saturation. The highest cellulase activity was found in MPE from kitchen waste, which remained stable at pH 5.0-11.0. In general, maggots from different substrate sources exhibited distinct nutrient profiles and enzyme activities. Protein extract from maggots grown in layer manure showed the most suitable nutrient profile for use as an alternative source of protein feed and protease enzymes.

Keywords: Amino acid, Chemical profile, Enzymes, Fatty acid, Maggot.

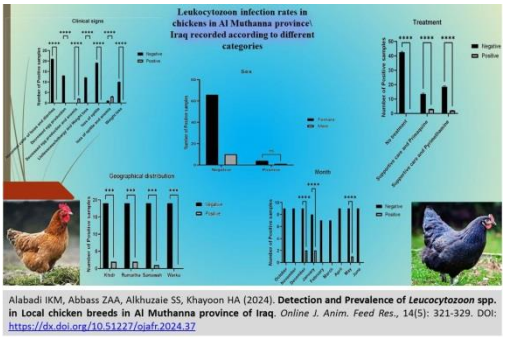
[Full text-PDF]

Research Paper

Detection and prevalence of *Leucocytozoon* spp. in Local chicken breeds in Al Muthanna province of Iraq

Alabadi IKM, Abbass ZAA, Alkhuzaie SS, Khayoon HA and Alsaadawi M.

Online J. Anim. Feed Res., 14(5): 321-329, 2024; pii: S222877012400037-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.37



Alabadi IKM, Abbass ZAA, Alkhuzaie SS, Khayoon HA (2024). **Detection and Prevalence of *Leucocytozoon* spp. in Local chicken breeds in Al Muthanna province of Iraq**. *Online J. Anim. Feed Res.*, 14(5): 321-329. DOI: https://dx.doi.org/10.51227/ojafr.2024.37

Abstract
Leucocytozoon species are avian haemoparasites with economic impacts on poultry production. The present study investigates the presence of *Leucocytozoon* in chickens of Al Muthanna province, Iraq. Eighty one blood samples were collected from chickens in Samawah, Rumaitha, Warkaa, and Kidhre regions to examine the prevalence of *Leucocytozoon*. An infection rate of 6.1% was found among chicken breeds. The study highlighted that the main symptoms of infection were decreased egg production, anemia, and loss of appetite. Notably, infection was more prevalent in the Rumaitha, Khidr and Samawah regions, while no cases were reported in Warka. Treatment methods included primaquine and pyrimethamine alongside care to manage the condition effectively. It is important to mention that the observed prevalence rate in chickens was lower compared to studies on birds in Iraq, where an overall blood parasite prevalence of 15% was documented. This difference could be attributed to factors like habitat variations, vector presence, or differing susceptibility among bird species. Our suggestion for future work can be the application of new programs for diagnosing and controlling parasites in chickens.

Keywords: Al-Muthana region, Avian health, Flocks, Hemiparasite, *Leucocytozoon* spp.

[Full text-PDF]

Research Paper

The health and economic dimensions of honey production in Imo state, Nigeria

Nwaiwu IUO, Kadiri FA, Osuji MN, Ukoha II, Anyiam KH, Anyanwu UG, Nwosu FO, Oshaji IO, Enoch OC, Bala MB, Isaiah GI, Obasi AC, Madu JA, Nwachukwu EU, and Nnorom EI.

Online J. Anim. Feed Res., 14(5): 330-338, 2024; pii: S222877012400038-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.38

Abstract
A study was conducted on honey production in Imo state of Nigeria, with a focus on the health and economic dimensions of the industry. The research was carried out using a multi-stage sampling procedure, and a sample size of 80 honey-producer respondents was selected. Data was collected through a well-structured questionnaire and analyzed using descriptive and inferential statistics. The study found that honey producers in the area had a mean age of 51 years, 11 years of education, 21 years of farming/bee-keeping experience, and a household size of 6 persons. The average annual household income was €709.10, with a farm size/number of hives kept of 72 hives per farmer and a quantity of honey produced per annum of 145 litres. The cost and returns analysis showed that the cost of production of honey per litre and profit per litre were €0.40 and €2.40, respectively. The study also determined the nutritional uses and health benefits of honey (e.g. healing wounds, treating ulcers, controlling sore throats and colds, boosting immunity, and as an antibacterial agent). Several factors, including uncontrolled bush burning, bee forage shortage, deforestation, theft of beehives, colony absconding, and poor agricultural practices which strongly constrain honey production has been observed. It is concluded that honey production is a very profitable venture with numerous uses and health benefits and venturing youths into honey production as a source of livelihood should be encouraged, and extension education should be tailored to technologies in beekeeping and the identification of genuine honey to minimize the success of adulteration, among others.
Keywords: Economic, Forage shortage, beekeeping, Honey, Health Benefits, Natural products

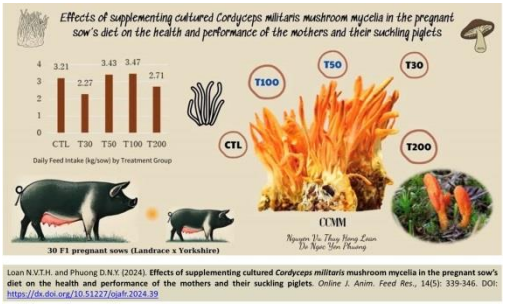
[Full text-PDF]

Research Paper

Effects of supplementing cultured *Cordyceps militaris* mushroom mycelia in the pregnant sow's diet on the health and performance of the mothers and their suckling piglets

Loan NVTH and Phuong DNY.

Online J. Anim. Feed Res., 14(5): 339-346, 2024; pii: S222877012400039-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.39



Abstract
Present study aimed to evaluate the effects of supplementing cultured Cordyceps mushroom mycelia (CMM) in the diets of pregnant sows on the productivity of the mothers and their suckling piglets during their first week of age. A total of 30 pregnant F1 (Landrace x Yorkshire) sows were randomly allocated to 5 dietary treatments with 6 replicates each: Control (sows fed the basal diet), and T30, T50, T100, and T200, where sows were fed the basal diet supplemented with 30, 50, 100, and 200 g of dried CMM, respectively. The animals were individually housed and fed twice daily. The performance and health status of the sows and their piglets were recorded accordingly. The results showed that the inclusion of CMM in the diets of pregnant and lactating sows affected the performance and health status of both the mothers and their piglets. For the piglets, the total number of piglets born and alive was higher in the T50, T100, and T200 groups compared to the control and T30 groups, but there was no effect on the survival rate at 7 days old. Daily gains per piglet were higher in the T30, T50, and T100 groups compared to T200 ($P<0.05$). For the sows, daily feed intake was lower in the T30 group compared to the other treatments ($P<0.05$). The values of gross energy in the milk produced by the sows were higher in the control, T30, T50, and T100 groups compared to T200 ($P<0.05$). Both the piglets and the sows on diets supplemented with CMM experienced fewer health problems than those on the Control diet ($P<0.05$). In conclusion, the supplementation of 50 and 100 g of CMM per day in the diets of pregnant and lactating sows improved litter size and health status but did not affect the performance of either the mothers or their piglets.
Keywords: Cordyceps mushroom mycelia, Health status, Pregnant sows, Suckling piglets, Weight gain.

[Full text-PDF]

Archive

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EFFECT OF SUPPLEMENTATION OF CACTUS (*Opuntia ficus-indica*) CLADODES, *Acacia saligna*, WHEAT BRAN AND COTTON SEED CAKE ON FEED INTAKE, DIGESTIBILITY, GROWTH AND CARCASS CHARACTERISTICS OF GOATS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The objective of this study was to evaluate the effect of supplementation of cactus (*Opuntia ficus-indica*) cladodes, *Acacia saligna*, wheat bran and cotton seed cake on growth, digestibility, intake and carcass characteristics of goats. A randomized complete block design was used in the experiment with 24 yearling central highland goats with an initial body weight of 15.6 - 16.1 kg. The same amount of grass hay (GH) + 150 gDM/head/day wheat bran (WB) was given to all animals. The experimental diets consisted of 80 gDM of cotton seed cake (CSC) as treatment 1 (T1); 45 gDM of CSC + 160 gDM of cactus cladodes (CC) as T2; 45g DM of CSC + 80 gDM of *Acacia saligna* (AS) as T3; and 45 gDM of CSC + 80 gDM of CC + 40 gDM of AS as T4 (per day per goat). Data were gathered on the goats' growth, digestibility, intake, and carcass of major organs, edible and nonedible organs. The consumption of dry matter and organic matter was higher in goats fed T2 and T4 than in the T1 group. The dry matter (DM), organic matter (OM) and crude protein (CP) digestibility, average daily body weight gain and feed conversion efficiency were higher in T4 and T3 goats when compared to T2 goats. Goats fed on T4 had higher hot carcass weight and dressing percentage on slaughter body weight basis than T2, T3, and T1 supplemented goats. Generally, the experimental diets improved goats' performance in descending order (T4 > T3 >T1 >T2). Supplementation of T4 (replacement of 35 gDM of cotton seed cake per day by 40 gDM of *Acacia saligna* and 80 gDM of cactus cladodes on dry matter bases) could be recommended to improve goat performance.

Keywords: Digestion, Dry matter, Feed conversion efficiency, Goat nutrition, Protein.

INTRODUCTION

Goats and sheep are farmers' preferred livestock in arid areas. For farmers in arid and semi-arid areas, small-scale ruminant production is their primary source of income (Nampanzira et al., 2015; Timpanaro and Foti, 2024). However, during periods of insufficient feed supply, sheep and goats raised in these locations frequently suffer from substantial nutritional deficits, resulting in low productive and reproductive performance (Salem, 2010). Feed shortage, land scarcity and cost of feeds were the major constraints that hinder sheep and goat productivity (Diriba and Kebede, 2020). According to Endalew et al. (2016), in Ethiopia, there are significant limitations on livestock to have get year-round feed supplies, and the majority of feed resources are of low quality. Therefore, it could be necessary to use plants like cactus that are adapted to dry and semi-arid environments.

Cactus (*Opuntia ficus-indica*) has a high water retention capacity, high values in nutrients, and well-adapted to semiarid environmental conditions (Costa et al., 2013). The chemical composition of spineless cactus cladode consists of 9.2-10% dry matter, 5.62-7.9% crude protein, 25.97-38.7% neutral detergent fiber, and 15.08-27.2% acid detergent fiber (Shruthilaya et al., 2022) as well as water content (89.9%) and energy (8.4-9.2 MJ metabolizable energy per kg dry matter), minerals, and vitamin A for inclusion in ruminant diets (Abidi et al., 2009; Costa et al., 2013; Bezerra et al., 2021; Nyambali et al., 2022). Spineless cactus are often grown by smallholder farmers in arid and semiarid regions to use for animal feed, fences for dwellings and farm plots, and fruits for human consumption (Alary et al., 2007). Spineless cactus cladodes are low in phosphorous, fiber, and crude protein (Batista et al., 2003; Kumar et al., 2018). Due to these limitations, supplementation of protein source feeds like legume browse species is required to use spineless cactus cladodes for animals as forage.

As such in arid and semi-arid regions, browse species provide as a source of nourishment for ruminants, especially during the dry season when there is an abundance of low-quality fodder and crop residues (Amole et al., 2022). Farmers commonly use *Acacia saligna* to feed sheep and goats during dry season. Acacia leaves and twigs from both young and

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mature trees are regularly collected and given to grazing animals. This is a common practice used by farmers (Meneses et al., 2012). Nevertheless, its continued use as animal feed may be restricted due to the presence of secondary plant compounds. The primary anti-nutritional substances found in some Acacia species are condensed tannins, which reduce the digestibility of organic matter, dry matter, and crude protein content (Ben Salem et al., 1999). However, drying of *Acacia saligna* reported to reduces its tannin content than offering fresh leaves (Gebreslassie et al., 2021). In lambs, dried *Acacia saligna* leaves outperformed fresh leaves in terms of apparent digestibility of dry matter, organic matter, and crude protein content (Asefa and Tamir, 2006).

On smallholder mixed crop-livestock farms, such as those predominate in Ethiopia, supplementation with concentrate is not a feasible option due to the cost and limited availability. In such case; use of forages that are locally available, well adaptive and cheap in price like cactus cladodes and *Acacia saligna* could be feasible. Previous studies conducted on effect of cactus cladodes and legume browse species on the performance of goats and sheep in varied proportions shown that: supplementation with 300 gDM/day/head cactus and browse species mix (1:1 ratio) enabled body weight gain and prevented body weight loss of Somali goats (Taddesse et al., 2014). The total substitution of corn by cactus pear (280 gDM/kg), even if it resulted in reduced weight gain, the DM intake increases and improved the ability of sheep to digest the nutrients (Costa et al., 2013). To promote better animal performance, it is recommended to replace 63% of wheat bran by spineless cactus in sugar cane-based diets because of the optimal ruminal fermentation and higher volatile fatty acids synthesis for the animal (Lins et al., 2016). According to Aranda-Osorio et al. (2008), when cactus pear inclusion was raised from 15% to 30%, DM intake increases whereas feed conversion efficiency and overall live weight gain decreased. Other studies showed.

Spineless cactus species can replace ground corn as a source of energy in diets for finishing lambs without changing the ADG, DMI, and ingestive behavior and yield of commercial cuts (de Alencar Alves et al., 2023). Additionally, in sheep fed *Acacia saligna* and wheat bran, the average daily body weight gain was similar to that of sheep fed cotton seed cake and wheat bran (Yirdaw et al., 2017). Despite their abundant availability little research has been done undertaken in northern Ethiopia, especially Tigray region on the effect of supplementing of *Acacia saligna*, cactus and concentrate, on productive performance of goats. This study was thus designed to evaluate the effect of dietary supplementation of cactus cladodes, *Acacia saligna*, wheat bran and cotton seed cake on feed intake, growth, digestibility and carcass characteristics of goats.

MATERIALS AND METHODS

The study was conducted in the eastern zone of Wukro town in North Ethiopia's Tigray region, 820 kilometers north of Addis Ababa, 70 km south of Adigrat, the zonal city, and 40 km north of Mekelle, the regional capital. Its latitude is 13°47' 59.99" N and its longitude is 39°35' 59.99" E. This region is 1977 meters above sea level, with a distinct rainy season from July to September and followed by a lengthy dry season. The mean annual rainfall is 300-350 mm. The mean annual temperature in the study area varies from maximum of 31°c in May and minimum of 8.3°C in July with an overall mean range from 11.1 -28.3°C. The study area's primary farming method is a mixed crop livestock subsistence economy. In order to minimize production risk and optimize the return on their limited land capital resource, smallholder farmers integrate the production of crops and livestock (Abegaz, 2005).

Animal management and experimental design

To carry out the experiment, 24 yearling male Central highland goats were purchased from the local area market. Prior to the commencement of the feeding trial, the residence was maintained and cleaned. Following that, the goats spent 21 days getting used to the feed and the experimental housing. Within the experimental house individual pen was built with 0.75 meters of width and 1.2 meters of length. Goats were treated for worms and sprayed to defend against internal and external parasites during this time. They were also given antibiotics and ivermectin vaccinations to protect them from frequent diseases in the area. All of the goats were put in separate pens after the end of adaptation period.

The diets in the experiment consisted of grass hay as roughage, wheat, bran cotton seed cake, *Acacia saligna*, and cactus cladodes. Roughage feed was made using grass hay that was bought locally. During the feeding trial, the goats were given *ad libitum* access to the measured grass hay. The concentrate (cotton seed cake and wheat bran) purchased from a neighboring market. The closest local farmers to the study area provided fresh spineless cactus cladodes. The spineless cactus cladode was manually cut into 1-2 cm length strips by lengthwise cutting.

The chopped cactus cladodes were then dried in air in shade by being stretched out on a plastic sheet for eight days. *Acacia saligna* leaves were harvested from locally available trees and dried in air in shade for 4 days, by being spread out on plastic sheets until easily crushed by twisting. They were then stored in a well-ventilated, cool and dry area to prevent degradation or mold formation until the completion of the experiment. Then the feed ingredients were mixed properly in the required proportion for each treatment.

A randomized complete block design was used to conduct the feed trial. Six blocks (six animals per treatment) of experimental goats were assigned based on their initial body weight of 15.9±0.237 kg. After the adaption period was ended, their weight was determined by averaging two consecutive weighings that came after an overnight fast. Random assignments were made to each goat in a block to receive one of the four dietary treatments. The experimental diets were formulated according to Kearl (1982); (Table 1) to provide 48 gCP/day.

Table 1 - Amount of feed ingredients (supplement) in each experimental diet

Experimental diets/Treatments	Basal diet+ wheat bran (WB)	Supplement feed ingredients in gDM/head/day			CPI gDM/d
		CSC	AS	CC	
1	GH+150 WB	80	0	0	48.77
2	GH+150 WB	45	0	160	48.56
3	GH+150 WB	45	80	0	49.864
4	GH+150 WB	45	40	80	49.89

GH=grass hay, CSC=cotton seed cake, WB=wheat bran, AS=*Acacia saligna*, CC=cactus cladodes, CPI=crude protein intake, DM=dry matter, g=gram, d=day

Data collection procedures

Feeding trial

Before the feeding trial began, the goats in the experiment were gradually acclimated to the experimental diet and environment over a period of 21 days. Then the feeding trial continued for ninety days. The experimental goats had unrestricted access to water and basal feed (natural grass hay) during the feeding trial. Concentrate (wheat bran and cotton seed cake), *Acacia saligna* and spineless cactus cladode (*Opuntia ficus-indica*) were combined for each treatment in appropriate ratio as described in Table 1 and supplemented to goats. Additionally, salt was added to the experiment at 1% of the total experimental diet. The goats were given the experimental diet twice a day, at 8:00 a.m. and 5:00 p.m. Every day of the trial, the quantity of feed that each goat eaten and refused was recorded. Before fresh feed was supplied each morning, feed refusals were gathered, weighed, and individually recorded for every animal. To determine the chemical composition, representative samples of feed that was offered and refused by each animal were gathered.

Feed and nutrient intake, changes in body weight, and feed conversion efficiency

Using a hanging scale, the initial and final body weights of the experimental goats were determined in the morning before they were fed. The amount of feed declined was subtracted from the amount offered to determine each goat's daily feed consumption. The calculation of nutrient intake involved multiplying the feed intake by the appropriate percentage of each parameter's approximate chemical composition (dry matter, organic matter, crude protein, neutral detergent fiber, and acid detergent fiber). Throughout the trial, follow-up body weight measurements were made every ten days to know weight change. The average daily body weight growth and feed conversion efficiency were calculated using the formula below:

$$\text{Average daily body weight gain} = \frac{\text{Final body weight} - \text{Initila body weight}}{\text{Number of feeding days}}$$

$$\text{Feed conversion efficiency} = \frac{\text{Average daily body weight gain in gram}}{\text{Daily dry matter intake}}$$

Digestibility trial

After the end of the feeding trial, a digestibility trial was conducted. To gather feces for digestibility testing, a fecal collecting bag was fastened to each goat. For three days in order to allow for adaption, the goats were left in the presence of the fecal collection bags. Following that, feces collection took place for 7-day, every morning before feeding the goats. Every day, the feces were gathered and weighed, and at the end of the trial, 20% of each goat's daily feces were collected for analysis. Each goat's feces were collected and packed in polyethylene plastic bags, which were then stored in a deep freezer at -20°C until the digestibility study was completed. Following the completion of the collecting time, the feces were well combined, and 10% of the sample was dried for 72 hours at 60 degrees Celsius, crushed through a 1 mm sieve, and then stored at -20 degrees Celsius for chemical analysis. The following Equations were used to calculate apparent digestibility (Zeng et al., 2018).

$$\text{Digestibility of Nutrients} = \frac{\text{Nutrient Intake} - \text{Nutrient excreted in feces}}{\text{Nutrient Intake}} * 100$$

The equation for DM digestibility estimation (Somanjaya et al., 2022) was;

$$\text{DM digestibility} = \frac{\text{DM Intake} - \text{Feces DM}}{\text{DM Intake}} * 100$$

Carcass characteristics

Following an overnight fast, 20 experimental goats (five from each treatment) were slaughtered at the completion of the digestibility trial. Measurements included rib-eye muscle area (REMA), dressing percentage (DP), hot carcass weight (HCW), empty body weight (EBW), and slaughterer body weight (SBW) were done. Weighing and recording of the edible offals—heart, liver with gall bladder, kidney, empty stomach, testes, and head with tongue—were also done. Similarly, the weight and records of the inedible offals (skin with feet, lungs, trachea, esophagus, spleen, and penis) were also conducted. Each goat's empty body weight was calculated by deducting its slaughter body weight from the weight of its digesta (gut filling) (Soares et al., 2012). Whereas hot carcass weight was measured after the weight of the head, tail, thoracic, abdominal, and pelvic chambers, as well as the legs below the knee joints, were removed. Then the hot carcasses were divided along the dorsal midline. Following that, the left half of the goat carcass was dissected into normal commercial cuts, including the neck, proximal thoracic limb, proximal pelvic limb, steaks, and brisket. The hot goat carcass was placed in a freezer for 24 hours at 4°C to cold it down. Then, according to Koyuncu et al. (2007) measurements on commercial carcass cut and the REMA around the ribs, at the 11th and 12th rib positions were taken. After making the cut on the 11th and 12th ribs perpendicular to the dorsal bone, the cross-sectional area of the REMA (Longissimus dorsi) was drawn using plastic paper. Graph paper of 5 mm by 5 mm was used to trace the rib-eye muscle area once more, as measured using plastic paper. To determine the REMA in cm^2, the area of the squares that felt into the tracing paper on both sides was measured, and the average of two was used. Dressing percentage was computed using the empty body weight and slaughter body weight.

$$\text{Dressing percentage} = \frac{\text{HCW}*100}{\text{BWS}} \quad \text{and} \quad \text{Dressing percentage} = \frac{\text{HCW}}{\text{EBW}} * 100$$

Where HCW, SBW and EBW are hot carcass weight, slaughter body weight and empty body weight, respectively.

Chemical analysis

Feed and fecal samples were analyzed for ash, crude protein (CP; Nx6.25), and dry matter (DM) contents using the approved AOAC (1990) procedures. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined according to Van Soest et al., (1994). The ash content of the feed was ascertained by burning the samples in a muffle furnace for five hours at 550°C, while the dry matter content was determined by drying the samples in an oven at 105°C for overnight. Nitrogen (N) was determined by Kjeldahl method (CP = N×6.25).

Statistical analysis

The general linear model (GLM) procedure of SAS version 9.0 (SAS, 2002) was used to analyse the data on feed intake, digestibility, body weight increase, and carcass characteristics to the analysis of variance model for randomized complete block design. Tukey's test was used for mean separation and values were considered significant at P< 5%. The following statistical model was employed to analyze the data:

$Yij = \mu + T_i + B_j + e_{ij}$,

Where: Y_{ij} is the response variable (body weight gain, feed intake, digestibility, carcass characteristics; μ = the overall mean; Ti= the ith treatment effect; B_j = the jth block effect; e_{ij} = the random error.

RESULTS

Chemical composition of feed ingredients

The chemical composition of the feeds in the experiment are shown in Table 2. Cotton seed cake (CSC) contained more CP than wheat bran or *Acacia saligna*. Cactus cladode and hay had the lowest CP levels, with 6.13% and 5.38%, respectively. Cotton seed cake had 66.35% NDF and 37.62% ADF, whereas hay had 71.86% NDF and 45.87% ADF. *Acacia saligna* has a high ADL concentration.

Effect of concentrate, *Acacia saligna* and cactus cladodes on feed/ dry matter and nutrient intake of goats

Table 3 displays the results of the goats' nutritional and dry matter intake. The grass hay dry matter consumption was higher in sole concentrate (T1) and concentrate and *Acacia saligna* (T3) supplemented goats than concentrate + cactus (T2) and concentrate+ *Acacia saligna* + cactus (T4) supplemented goats. Similarly supplement dry matter intake was higher in cactus containing experimental diets (T2 and T4) supplemented goats than that of non-cactus containing diets (T3 and T1) supplemented goats. The total dry matter and organic matter intake was higher in concentrate + cactus (T2) and concentrate+ cactus + *Acacia saligna* (T4) supplemented goats than in sole concentrate (T1) supplemented goats. The reason for this could be the presence of cactus cladode, which had a lower NDF content compared to cotton seed cake and *Acacia saligna* which contains higher NDF content (Table 2). The amount of crude protein that the goats consumed across all experimental diets did not differ significantly. The NDF intake was highest in concentrate and cactus

supplemented goats (307.09 g/day) then followed by concentrate + *Acacia saligna* (289.45 g/day)>sole concentrate (284.36 g/day)>concentrate + *Acacia saligna*+ cactus (266.44 g/day) supplemented goats. Acid detergent lignin intake of goats was highest in concentrate + cactus supplemented goats then followed by concentrate + *Acacia saligna* >concentrate + *Acacia saligna* + cactus > sole concentrate supplemented goats.

Table 2 - Chemical composition of feed components on dry matter basis

Chemical composition / Parameters	Feed ingredients (supplements)				Basal diet
	Acacia saligna	Spineless cactus	Cotton seed cake	Wheat bran	Grass hay
DM	90.26	95.33	93.81	92.24	89.74
Ash	11.1	11.67	4.32	2.92	9.64
OM	88.89	88.33	95.68	97.07	90.36
CP	13.88	6.13	28.60	17.26	5.38
NDF	45.05	39.44	66.35	41.04	71.86
ADF	21.63	27.72	37.62	8.21	45.87
ADL	9.52	3.01	6.74	1.86	6.50

OM=organic matter, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, ADL=acid detergent lignin, CP=crude protein, DM=dry matter

Table 3 - Goats fed on grass hay and supplemented with concentrate, cactus cladodes and *Acacia saligna* and their dry matter and nutrient intake

Experimental diets/Treatments / Intake (gDM/d)	T1	T2	T3	T4	SEM	P-value
Grass hay DMI	264.28^{a}	229.53^{b}	244.45^{ba}	230.45^{b}	5.2	0.0023
Supplement DMI	208.15^{c}	296.63^{a}	258.26^{b}	289.45^{ba}	7.74	0.0001
Total DMI	472.44^{b}	526.16^{a}	502.71^{ab}	519.48^{a}	8.94	0.001
Nutrient intake						
OMI grass hay	239.28^{a}	208.68^{b}	221.81^{ba}	208.14^{b}	4.63	0.0022
OMI supplement	191.27^{b}	265.20^{a}	237.07^{a}	260.59^{a}	7.01	0.0001
OMI Total	430.57^{b}	473.89^{a}	458.89^{ab}	468.74^{a}	8.04	0.019
CPI grass hay	14.64^{a}	13.18^{b}	13.36^{b}	12.45^{b}	0.25	0.0008
CPI from supplement	39.09^{b}	40.69^{ab}	43.01^{ab}	44.19^{a}	1.06	0.018
CPI total	53.74	53.88	56.37	56.64	1.05	0.114
ADFI grass hay	119.76^{ab}	125.38^{a}	111.12^{bc}	104.15^{c}	2.48	0.0004
ADFI from supplement	33.87^{d}	86.18^{a}	58.29^{b}	47.28^{c}	2.26	0.0001
ADFI total	153.64^{c}	189.50^{a}	169.42^{b}	151.43^{c}	3.23	0.0001
NDFI grass hay	190.63^{a}	169.78^{b}	178.28^{ab}	166.40^{c}	3.75	0.003
NDFI from supplement	93.72^{c}	137.31^{a}	111.17^{b}	100.04^{bc}	4.31	0.0001
NDFI total	284.36^{bc}	307.09^{a}	289.45^{b}	266.44^{c}	5.76	0.0028
ADLI grass hay	16.87^{ab}	18.24^{a}	15.50^{bc}	14.60^{c}	0.34	0.0001
ADLI from supplement	5.36^{b}	12.53^{a}	12.70^{a}	10.83	0.54	0.0001
ADLI total	22.23^{d}	30.78^{a}	28.21^{b}	25.43^{c}	0.55	0.0001

$^{a, b, c, d}$ = means within a row not bearing a common superscript letter differ significantly($p<0.05$); SEM= standard error of mean, I=Intake, DM=dry matter, OM=Organic matter, CP=Crude Protein, NDF=Neutral Detergent Fiber, ADF=Acid Detergent Fiber , ADL=Acid detergent Lignin, T1= 150g DM of WB +80g DM of CSC, T2= 150g DM of WB + 45 g DM of CSC + 160g DM of cactus, T3= 150g DM of WB + 45g DM of CSC+ 80g DM of AS, T4= 150g DM of WB +45g DM of CSC + 80g DM of Cactus+40g DM of AS

Effect of concentrate, *Acacia saligna* and cactus cladodes on apparent dry matter and nutrient digestibility of goats

Table 4 displays the apparent dry matter and nutrient digestibility data for goats. Goats supplemented with concentrate plus *Acacia saligna* + cactus cladodes (T4) had greater digestibility of DM, OM, and CP than goats supplement with concentrate plus cactus (T2). The CP digestibility of goats was higher in *Acacia saligna* containing experimental diets (concentrate + *Acacia saligna* (T3) and concentrate + *Acacia saligna* +cactus (T4)). On the other hand, the NDF digestibility was higher in goats supplement sole concentrate (T1) than in concentrate + cactus (T2) and concentrate + *Acacia saligna* (T3). This might be due to the presence of higher amount of cotton seed cake containing high level of NDF in sole concentrate feeds. The ADF digestibility of goats was higher in T3 supplement than in concentrate + *Acacia saligna* +cactus (T4) supplement. However, the ADF digestibility of goats supplemented (T2) was the same to the ADF digestibility of goats' supplemented T1. The DM, OM, and CP digestibility of goats was higher in T4 compared to T2 supplement.

Effect of concentrate, *Acacia saligna* and cactus cladodes on body weight change and feed conversion efficiency of goats

Table 5 displays the body weight and feed conversion efficiency data for goats given grass hay supplemented with concentrate, *Acacia saligna*, and cactus cladode. In all experimental diets, there was no significant variation between the goats' initial and final body weights. However, numerically, the initial body weight ranged from 15.6-16 kg, while the final body weight ranged from 18.6-19.2 kg. In comparison to concentrate + cactus (T2) and sole concentrate (T1), the average daily body weight gain of goats in concentrate+ cactus+ Acacia saligna (T4) and concentrate + *Acacia saligna* (T3) revealed a significant difference ($p<0.05$). Goats' feed conversion efficiency varied between 0.049 to 0.79. There was significant ($p<0.05$) variation in the feed conversion efficiency of goats between the experimental diets; goats in T4 had a greater feed conversion efficiency than goats in T2.

Effect of concentrate, *Acacia saligna* and cactus cladodes on carcass characteristics

Table 6 displays the results of the carcass parameters and commercial cuts of goats. For all experimental diets, there was a significant difference ($p<0.01$) in the goats' hot carcass weight and dressing percentage. Goats supplemented with concentrate+ *Acacia saligna* + cactus (T4) had higher hot carcass weight and dressing percentage (on slaughter body weight) than goats supplemented with concentrate+ cactus (T2), concentrate + *Acacia saligna* (T3), and sole concentrate (T1). Goats supplemented with T4 exhibited significantly higher hot carcass weight and dressing percentage than goats supplemented with T2, T3, and T1 supplements.

Effect of concentrate, *Acacia saligna* and cactus cladodes on edible and non-edible carcass organs of goats

The results of weights of heart, kidney, small intestine, large intestine, testes, blood, and gut contents are presented in Table 7 and showed significant differences among experimental diets. The size of the heart was larger in concentrate supplemented (T1) goats than that of concentrate + *Acacia saligna*+ cactus (T4) supplemented goats. The size of kidney was ranged from 62 - 73.6 grams and it was higher in T4 supplemented goats than that T1 supplemented goats. Goats supplemented with T1 had larger small and large intestine weight with its contents, compared to goats supplemented with concentration + *Acacia saligna* (T3) and concentrate + *Acacia saligna* + cactus (T4). The size of the testes of goats in this study was ranged from 121.6-150.2 grams and it was higher in T3 than in T2 and T4 supplement. The gut content of goats was ranged from 2531-3530 grams and it was higher in goats supplemented T1 than T4.

Table 4 - Apparent dry matter and nutrient digestibility of goats fed on grass hay and supplemented Concentrate, *Acacia saligna* and cactus cladodes

Experimental diets/Treatments / Apparent Digestibility (g/kg)	T1	T2	T3	T4	SEM	P-value
DM	642.2^{b}	611.7^{c}	641.5^{b}	669.6^{a}	0.38	0.0003
OM	608.8^{a}	572.2^{b}	609.0^{a}	622.7^{a}	0.31	<0.0001
CP	717.6^{a}	672.1^{b}	717.8^{a}	736.9^{a}	0.42	<0.0001
ADF	486.6^{ab}	497.0^{ab}	512.6^{a}	471.5^{b}	0.67	0.031
NDF	612.86^{a}	560.3^{c}	595.3^{ab}	582.0^{bc}	0.46	0.0002

$^{a, b, c, d}$ = means within a row not bearing a common superscript letter differ significantly ($p<0.05$). DM=dry matter, OM=Organic matter, CP=Crude Protein, NDF=Neutral Detergent Fiber, ADF=Acid Detergent Fiber, T1= 150g DM of WB +80gDM of CSC, T2= 150g DM of WB + 45 g DM of CSC + 160gDM cactus, T3= 150g DM of WB + 45gDM of CSC+ 80gDM of AS, T4= 150g DM of WB +45gDM of CSC + 80gDM of Cactus+40gDM of AS, SEM=standard error of mean

Table 5 - Goats fed grass hay and supplemented with concentrate, *Acacia saligna*, and cactus cladodes, and their body weight change and feed conversion efficiency.

Experimental diets/Treatments / Parameter	T1	T2	T3	T4	SEM	P-value
IW (kg)	15.96	15.94	16.10	15.60	0.237	NS
FW (kg)	18.60	18.70	18.80	19.20	0.366	NS
BG (g/d)	25.55^{b}	26.22^{b}	28.88^{ab}	41.11^{a}	3.393	0.014
FCE	0.0541^{ab}	0.049^{b}	0.057^{ab}	0.079^{a}	0.006	0.019

$^{a, b, c, d}$ =Means with different superscripts in the same row differ significantly ($P<0.05$); BG=daily body weight gain; FW=final body weight; FCE = feed conversion efficiency; IW=initial body weight; SEM= standard error of mean, T1= 150g DM WB +80g DM of CSC, T2= 150g DM of WB + 45 g DM of CSC + 160g DM of cactus, T3= 150g DM of WB + 45g DM of CSC+ 80g DM of AS, T4= 150g DM of WB +45g DM of CSC + 80 g DM of Cactus +40g DM of AS

Table 6 - Carcass characteristics and commercial cuts goats fed on grass hay and supplemented with concentrate, *Acacia saligna* and cactus cladodes

Experimental diets/Treatments / Carcass characteristics (kg)	T1	T2	T3	T4	SEM	P-value
Final body weight	18.6	18.7	18.8	19	0.337	NS
Slaughter body weight (SBW)	17.80	17.80	17.60	18.20	0.26	NS
Hot carcass weight	5.9^{b}	6^{b}	5.84^{b}	6.7^{a}	0.13	0.002
Cold carcass weight	5.42^{b}	5.87^{ab}	5.68^{ab}	6.33^{a}	0.18	0.011
Empty Body Weight (EBW)	14.26	14.75	14.83	15.26	0.30	Ns
Rib-eye muscle area ($cm^{2)}$	4.95	4.82	5.27	5.87	0.41	NS
Dressing percentage on SBW	33.09^{b}	33.63^{b}	33.11^{b}	36.77^{a}	0.66	0.001
Dressing percentage EBW	41.27^{b}	40.5^{ab}	39.34^{b}	43.91^{a}	1.056	0.009
Neck	0.72	0.61	0.69	0.71	0.056	NS
Proximal thoracic limb	1.35^{b}	1.26^{c}	1.42^{b}	1.53^{a}	0.021	<0.0001
Steaks + Brisket	1.53	1.43	1.53	1.61	0.053	NS
Lumbar + abdominal region	0.81	0.78	0.85	0.88	0.038	NS
Proximal pelvic limb	1.64^{bc}	1.55^{c}	1.7^{ab}	1.81^{a}	0.025	0.002

$^{a, b, c, d}$ =Means with different superscripts in the same row differ significantly (P<0.05); SEM= standard error of mean, NS=non significance, T1= 150g DM of WB +80g DM of CSC, T2= 150g DM of WB + 45 g DM of CSC + 160g DM of cactus, T3= 150g DM of WB + 45g DM of CSC+ 80g DM of AS, T4= 150g DM of WB +45g DM of CSC + 80g DM of Cactus+40g DM of AS

Table 7 - Weight of edible and non-edible carcass organs of goats fed on grass and hay supplemented Concentrate *Acacia saligna* and cactus cladodes

Treatments / Carcass organs	T1	T2	T3	T4	SEM	P-value
Edible carcass organs (g)						
Tongue, ear, head	1252.2	1252.2	1295.4	1281.2	37.31	Ns
Heart	78.8^{a}	70.2^{ab}	71.8^{ab}	65.40^{b}	2.73	0.032
Liver with gallbladder	252.6	242.36	224.4	242.6	11.5	Ns
Kidney	62^{b}	62.6^{b}	63^{b}	73.6^{a}	1.9	0.003
Small and large intestine and its contents	1395.4^{a}	1316.2^{ab}	1185.6^{b}	1212.6^{b}	21.22	0.000
Testes	132.8^{ab}	123.8^{b}	150.2^{a}	121.6^{b}	4.42	0.002
Empty stomach	436.8	458.8	469.0	469.8	21.55	Ns
Blood	580^{ab}	680^{ab}	720^{ab}	780^{a}	41.63	0.032
Total edible carcass organs (kg)	4.18	4.20	4.14	4.24	0.085	Ns
Nonedible carcass organs (g)						
Skin with feet, tail	2070	2062	2124	2084	58.89	Ns
Spleen	25.4	25	23.6	26	2.25	Ns
Penis	33.6	33.2	31.0	29	2.85	Ns
Lungs, trachea, esophagus	263.6	263.8	273.2	263.8	21.58	Ns
Gut content	3530^{a}	3041^{ab}	2763^{ab}	2531^{b}	205.97	0.027
Total nonedible carcass organs (kg)	5.72	5.16	4.94	5.07	0.27	Ns

$^{a, b, c, d}$ =Means with different superscripts in the same row differ significantly (P<0.05); T1= 150g DM of WB+80gDM of CSC, T2= 150g DM of WB + 45g DM of CSC + 160gDM cactus, T3= 150gDM of WB + 45gDM of CSC+ 80gDM of AS, T4= 150gDM of WB +45gDM of CSC + 80gDM of Cactus+40gDM of AS,Ns=non significance, g=gram, Kg=kilgram

DISCUSSION

Chemical composition of feed ingredients

Cotton seed cake contained higher crude protein content than wheat bran and *Acacia saligna*. Cactus cladode and hay had the lowest crude protein levels, with 6.13% and 5.38%, respectively. Cotton seed cake had 66.35% NDF and 37.62% ADF, whereas hay had 71.86% NDF and 45.87% ADF. *Acacia saligna* has a high acid detergent lignin (ADL) concentration of 9.52%. The crude protein (CP) content of cotton seed cake analyzed in this particular study (28%) exhibited similarity to the value of 28% as reported by Negussie et al. (2015). But it was lower than the value of 38% reported by Al-asa et al. (2023). Such discrepancies can potentially be attributed to variances in soil fertility and the technique employed for cotton seed extraction.

Similar values of 16.2% and 17.7%, as reported by Yirdaw et al. (2017) and Negussie et al. (2015), were obtained for the CP content of wheat bran in this study. However, it was greater than the 15.1% CP value that Al-asa et al.(2023) reported. Wheat bran's NDF content was comparable to the 39.5% recorded by Negussie et al. (2015). On the other hand, wheat bran's NDF content was less than the 35.5% that Yirdaw et al. (2017) reported. Wheat bran's ADF concentration turned out to be lower than the 12.6% and 11.6% reported by Yirdaw et al. (2017) and Negussie et al. (2015), respectively. *Acacia saligna*'s CP content was comparatively close to the 14.20% recorded by Yirdaw et al. (2017). However, it was less than the 16.4%–28.3% range number that Gebremeskel et al. (2019) reported. *Acacia saligna*'s NDF content was less than the value 44.29% reported by Yirdaw et al. (2017). However, it was greater than the value range of 28%–36% reported by Gebremeskel et al. (2019). The present investigation found that the CP content of spineless cactus cladodes was higher than the values 3.6% and 3.26% reported by Negussie et al. (2015) and de Oliveira et al. (2022), respectively. But it was similar to the values of 5.99%, 5.86% and 6.1% reported by Bezerra et al. (2021) and Alkhtib et al. (2023), respectively. Additionally, this study's NDF content for spineless cactus cladode was greater than the values reported by Negussie et al. (2015); Bezerra et al. (2021), and Alkhtib et al. (2023) of 23.3%, 26.5%, 25.97%, and 26%, respectively. In this experiment, the ADF content of cactus cladodes was more than the reported values of 15% and 23.39% by Negussie et al. (2015); Bezerra et al. (2021). The age of the cladodes, the fertility of the soil, and the harvest season are a few possible explanations for this discrepancy. With the exception of cotton seed cake, the inclusion of feed ingredients in this study had no discernible impact on animal feed intake. Dry matter intake (DMI) was negatively affected when the neutral detergent fiber (NDF) level exceeded 60%.

Effect of concentrate, *Acacia saligna* and cactus cladodes on dry matter and nutrient intake of goats

Grass hay dry matter intake was higher in sole concentrate (T1) and concentrate + *Acacia saligna* (T3) supplemented goats than concentrate + cactus (T2) and concentrate + *Acacia saligna* + cactus (T4) supplemented goats. Similarly, supplement dry matter intake of goats was higher in cactus containing diets (concentrate + cactus and concentrate + cactus + *Acacia saligna*) than the non-cactus containing diets (concentrate+*Acacia saligna* and sole concentrate). Goats supplemented cactus containing experimental diets (T2 and T4) consume more in order to fulfill their dietary needs due to the presence of cactus which contains low protein level compared to other feed ingredients. There is a direct correlation between an increase in dry matter intake and an increase in organic matter intake. In a similar manner, as organic matter intake increases intake of dry matter increases (Widyobroto et al., 2016). Goats that were supplied concentrate plus cactus or concentrate plus cactus and *Acacia saligna* consumed more total dry matter and total organic matter than those that were fed solely concentrate. Given that cotton seed cake has a higher NDF concentration than cactus cladode, which has a lower NDF level (Table 2). The total dry matter intake found to be similar to the values 407.3 – 647.1 g/day, 481.5- 586.3 g/d and 349-515 g/d reported by Worku and Urge (2014); Gebremariam et al. (2006) and Megersa et al. (2012) respectively. But it was lower than the value, 754-822 g/d reported by Hidosa (2017). The total organic matter intake was similar to the values 339.3- 538.7g/d, 384.8- 515.1 g/d and 316-475g/d reported by Worku and Urge (2014); Gebremariam et al. (2006) and Megersa et al. (2012), respectively. However, organic matter intake was comparatively lower than the value 741.1- 827 g/day reported by Hidosa (2017). The observed disparity in intake could potentially be explained by the increased consumption of dry matter and the enhanced nutritional value of grass hay in earlier research. Their increased intake as the number of cactus cladodes grows may have been caused by factors such as the cactus pear's high palatability, low fiber content, and high passage rate (Costa et al., 2012).

The amount of crude protein consumed by goats across all experimental diets did not differ significantly. But numerically it was higher in *Acacia saligna* containing diets (T3 and T4) supplemented goats than goats supplemented non *Acacia saligna* containing diets (T1 and T2). The study found that the goats' crude protein intake was comparable to the values reported by Gebremariam et al. (2006) and Megersa et al. (2012), which were 48.6-55.5 g/d and 16-69 g/d, respectively. It was lower than the value 43.9-110 g/h/d reported by Worku and Urge (2014). The Neutral detergent fiber (NDF) intake of goats was higher in T2 (307.09 g/day) followed by T3 (289.45 g/day)>T1 (284.36 g/day)>T4 (266.44 g/day). The study's NDF consumption for goats was comparable to Gebremariam et al. (2006)'s reported value of 235.1–388.0 g/d. In line with this discovery, Sileshi et al. (2021) observed that the experimental animals' daily consumption of NDF and acid detergent lignin (ADL) decreased when dietary energy and protein levels rose. Goats' body weight, the kind and quality of the feed, the amount provided, the breed, and the feed's palatability are some of the variables that might affect how much feed they consume. Han et al. (2019) further emphasized that palatability plays a significant role in determining feed consumption in livestock. Additionally, the dietary NDF content, which is known for its slow degradation and low rate of passage through the rumen, is considered to be limiting. Small ruminants' intake and digestion of roughages are greatly influenced by the fiber content, particularly the NDF (Mertens, 2002; Harper and Mcneill, 2015). The NDF consumption of the goats in this study increased linearly with increasing cactus cladodes from 80 gDM/day to 160 gDM/day in the experimental diet, which is similar to the finding of Costa et al. (2012).

Effect of concentrate, *Acacia saligna* and cactus cladodes on apparent dry matter and nutrient digestibility of goats

The dry matter, organic matter and crude protein digestibility of goats was higher in concentrate + *Acacia saligna* + cactus than concentrate + cactus. This might be due to the positive associative effect of combined feed ingredients (*Acacia saligna* and cactus). Dry matter digestibility and organic matter digestibility of goats in this study was similar to the range value of 42.11% - 69.96% reported by Hidosa (2017). The increased crude protein digestibility of goats in T3 and T4 compared to goats in T2 can be attributed to the presence of *Acacia saligna*. This notion is supported by the fact that the digestibility of nutrients in ewes is enhanced by an increased level of *Acacia saligna* supplementation, as stated by Maamouri et al. (2011). The NDF digestibility was higher in goats supplemented T1 than in goats supplemented T2 and T4. This may be due to the presence of higher amount of cotton seed cake containing high level of NDF in T1 (Table 2). Similarly NDF digestibility of goats was higher in T3 supplemented than in T2 supplemented. The ADF digestibility of goats was higher in T3 supplemented than in T4 supplement. Gebremariam et al. (2006) observed that the digestibility coefficients for ADF, CP, and NDF decreased as the quantity of cactus increased in the diet. In a similar way, the digestibility coefficients of DM, OM, CP, and NDF decrease as the level of cactus cladodes increases from 80 g/d to 160 g/d. Dietary OM digestibility was reduced when barely replaced by cactus. Contrary to this, studies by Salem (2010) and Costa et al. (2013) showed that the digestibility coefficients of DM, OM, and CP in sheep increased linearly as the amount of cactus cladodes in the diet increased. This discrepancy can result from the cladodes' age, the fertility of the soil, or the harvesting season or the species of the animals.

Effect of concentrate, *Acacia saligna* and cactus cladodes on body weight change and feed conversion efficiency of goats

There was a significant difference ($p<0.05$) in growth rate of goats between the concentrate + *Acacia saligna* + cactus (T4) and concentrate + *Acacia saligna* (T3) supplements compared to the sole concentrate (T1) and concentrate + cactus (T2) supplements. The combination of cactus and *Acacia saligna* in the experimental diet increase weight gain. This coincides to the finding of de Oliveira et al. (2022), who reported goats fed on a combination of spineless cactus and Leucaena hay gained weight on a daily basis at a rate that was 68.5% more than that of goats grazing only on pasture. Enhancing the ruminal microorganism population's growth performance with values of 42.84 g/day is primarily attributable to the synchronization of the crude protein of leucena hay and the energy of the spineless cactus. The range reported by Rahman et al. (2014) and de Oliveira et al. (2022) which were 30.8-43.5 g/d and 13.5-42.84 g/d, respectively, was within the range of daily growth rate found in our investigation. On the other hand, the gains were lower than the 41.67–60.65 g/d range found by Hidosa (2017).

There are reasons for the reduced growth rate of goats observed in this study. These include the small quantity of roughage feeds (natural grass hay) that are ingested, the portion of crude protein in the hay that is indigestible, and the feeds' palatability (Mulligan et al., 2001). Furthermore, the animals' actual consumption of the intended supplement feed and their possible reaction to weight growth were factors. For example, in treatment 2 (T2), leftover feed resulted in an actual consumption of just 40.69 DM g/d of crude protein, compared to the targeted intake of 48.56 DM g/d. This disparity suggests that the goats may not have eaten all of the supplement feed that was provided, which may have affected their overall nutritional intake and consequent weight increase.

When comparing cactus-containing diets, it was found that goats supplemented with T4 had a higher average daily weight gain than those supplemented with T2. This aligns with findings from Costa et al. (2013), who observed that the daily weight gain of sheep decreases as the amount of cactus supplementation increases. However, this is inconsistent with the study by Nyambali et al. (2022), which reported that heifers fed diets with 10% and 20% cactus had lower average daily gains (ADG) compared to those fed commercial diets. In the current study, the weight gain of goats was highest in those supplemented with T4 compared to goats supplemented with T1 and T2. This observation is supported by the findings of Degen et al. (2000) who suggested that the provision of *Acacia saligna* had a positive effect on body mass change in animals.

Goats supplemented with T4 had a greater feed conversion efficiency ($p<0.05$) than goats supplemented with T2. Goat feed conversion efficiency was found to be comparable to the range of 0.05-0.09 reported by Rahman et al. (2014); Hidosa (2017). Diets using 80 gDM cactus (concentrate + *Acacia saligna*+cactus) had a higher feed conversion efficiency than diets containing 160 gDM cactus (concentrate + cactus). This finding is comparable to that of Costa et al. (2013), who found that sheep's feed conversion efficiency drops when cactus levels rise. Diets containing cactus pear forage are beneficial when there is sufficient nitrogen available, as proven by Misra et al. (2006). This could be because cactus and Acacia saligna are available at the same time as a source of energy and protein, respectively, which could support ruminal microbial communities and improve animal performance. The nutritional composition of the experimental diets used in this study comparatively proved to be fairly adequate in increasing the goats' body weight. In contrast to sheep in other studies findings, goats in this study grew at a relatively slower rate than the recommended growth rate. In line with this finding Awassi lambs grow faster than Baladi kids and can get to the desirable market weight (Haddad and Obeidat, 2007). This could be because goats have different nutritional needs than sheep and consume less concentrated feed, which ultimately helps sheep perform better in feedlots (Sheridan et al., 2003).

Effect of concentrate, *Acacia saligna* and cactus cladodes on carcass parameters and commercial cuts of goats

The hot carcass weight and dressing percentage of goats supplemented with concentrate, cactus, and *Acacia saligna* were significantly higher than those of goats supplemented with the other treatments. Goats with concentration + cactus + *Acacia saligna* (containing 80 gDM cactus) supplement had a higher hot carcass weight than goats with concentrate + cactus (containing 160 gDM cactus). This was in line with the recommendation made by Bezerra et al. (2021), who found that hot carcass weight decreased from 15.6 to 14.4(kg) with cactus supplementation to lambs increases from 51.9 gDM to 75.3 gDM. The higher hot carcass weight of goats in concentrate+ cactus + *Acacia saligna* might be due to the lower NDF content of cactus cladodes and *Acacia saligna*. Concurred to this study, Mirzaei-Alamouti et al. (2021) reported a linear increases in hot carcass weight was found by decreasing dietary NDF concentration on a quantitative level. In this study, the dressing percentage and hot carcass weight of goats were found to be lower than those reported by Worku and Urge (2014), which were 35.4%–41.1% and 47.1%–58.9% and 6–9.7 (kg), respectively. There is no significance difference in rib eye muscle area of goats among experimental diets. The rib eye muscle area in this study was similar to the value 4.3- 7.7cm^2 reported by Worku and Urge (2014). The larger body weight of goats, the kind of feed, the breed of animals, and the setting in which the trials were conducted in the prior study could all be contributing factors to the discrepancy. The proximal pelvic limb was varied in weight from 1.558-1.815 (kg). The proximal pelvic limb of goats in T4 was larger than in goats supplemented with T2 and T1 supplement. The proximal thoracic limb was varied in weight from 1.268 -1.533 (kg). The proximal thoracic limb of goats in T4 was larger than in T2, T3 and T4 supplement. Goats' neck and proximal pelvic leg weight had greater than those of values of 0.47 kg and 1 kg respectively reported by Atay et al. (2011). The kind of feed, age, breed, and quantity of concentrate supplementation may all have a role in this discrepancy. When goats were supplemented with T4, their carcass qualities improved. Quantitatively speaking, goats fed this experimental diet (T4) had a greater rib eye muscle area compared to goats fed the other treatments.

Effect of concentrate, *Acacia saligna* and cactus cladodes on edible and non-edible offal components of goats

The kidney and spleen weight of goats in this study was similar to the values, 42-63g and 59-78g reported by Worku and Urge (2014) but lower than the values of 82g and 97g respectively reported by Atay et al. (2011). Kidney weight of the experimental goats increases significantly as body weight increases. Similarly, spleen weight increases as body weight increases numerically. The testes weight of goats in this study ranged from 121.6-150.2 grams and it was higher in goats supplemented with T3 than in goats supplemented with T2 and T4. The testis weight of goats was higher than the value 80g reported by Atay et al. (2011). But it was lower than the value 155g-206.3g reported by Alemu et al. (2010) and Worku and Urge (2014). There was no significance difference in empty stomach weight of goats in all experimental diets. But numerically, it was smaller in sole concentrate supplemented goats than in goats supplemented the rest treatments. The kind of feed, particularly the amount of fiber, affected the weight and growth of reticulo-rumen (Cardoso et al., 2016; Klein et al., 1987). The goats' empty stomach weight was lower than the value, 573-795g reported by Worku and Urge (2014). The gut content of goats was ranged from 2531-3530 grams and it was higher in goats supplemented with T1 than in goats supplemented with T4. The gut content of goats was lower than the range of 4.2-5.8 kg reported by Worku and Urge (2014). The reason for the lighter weight of goat offal in this study might be due to lower quality of hay and amount of concentrate feed left, as well as the initial body weight of the animals. As recommended by Bezerra et al. (2021) up to 50 gDM of spineless cactus could be included in the diet of confined lambs. Similarly, in this study it is possible to include up 80g DM/d spineless cactus in the experimental diet to improve goat performance. Therefore, T4 (150 wheat bran, 45 cotton seed cake, 40 *Acacia saligna* and 80 cactus cladodes (gDM/d) could be recommended to small holder farmers to feed their animals in order to improve their performance.

CONCLUSION

The study demonstrates that dietary supplementing goats with diets containing concentrate and browse plants improves some performance parameters. Goats supplemented cactus containing diets consume more DM and OM than those of goats which utilized non-cactus containing diets. Goats on T4 diet (concentrate + cactus + *Acacia saligna*) exhibited higher DM, OM, and protein digestibility than those on the T2 diet (concentrate + cactus). The study observed larger testis size in goats fed with concentrate + *Acacia saligna* (T3) compared to those on concentrate + cactus (T2) and concentrate + *Acacia saligna* + cactus (T4). Goats on the T4 diet achieves suitable performance in most cases: higher efficiency (better utilization of the feed), relatively high growth rate, high dressing percentage, and hot carcass weight, larger weights of kidney, proximal thoracic limb, and proximal pelvic limb. Since both cactus and *Acacia saligna* are locally available year-round, providing a reliable forage source that meets maintenance requirements of animals and boosts economic returns for producers. Further research could be done evaluation of performance of goats by supplementing minerals and vitamins on top of the diets used in this experiment or other proportions of these feed ingredients.

DECLARATIONS

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Data availability

The data that support the study findings are available from the corresponding author upon request.

Author contribution

Genet Berhe: Conceptualization; Methodology; Data curation; Formal analysis and writing original draft of the manuscript, Amsalu Sisay and Teferi Aregawi: reviewing; editing; supervision; validation.

Consent to publish

All participants have consented to the submission of the research article to the journal.

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Ethics committee approval

The experiment was approved by the Ethics Committee of the Tigray Agricultural Research Institute following guidelines of the European Union directive number 2010/63/EU (2010) regarding the care and use of animals for experimental and scientific purposes.

Competing interests

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

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PHYSICAL PROPERTIES OF SUGARCANE (*Saccharum officinarum*) AND TITHONIA (*Tithonia diversifolia*) SHOOT-BASED WAFERS WITH DIFFERENT ADHESIVE TYPES

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ABSTRACT: Wafers (wafer-feed) are an effective processing technology and are expected to maintain the continuous availability of animal feed during the dry season. The purpose of this study was to determine the best type of adhesive on the physical quality of sugarcane tops and Tithonia based wafers. This study used the Split Split Plot Design (SSPD). The main plot as factor A was the type of adhesive, consisting of: Tapioca flour (A1), Pathi flour (A2), Gaplek flour (A3), Karagenan flour (A4), palm sugar (A5). The subplots as Factor B are temperature which consists of: 100°C (B1), 110°C (B2), and 120°C (B3), while the sub-plots as factor C are oven time consisting of: 10 minutes (C1), 15 minutes (C2), and 20 minutes (C3). The forage used was Sugarcane tops *(Saccharum officinarum*) and Tithonia (*Tithonia diversifolia*) in the ratio of 60:40. The best adhesive in making sugarcane tops and Tithonia based wafers is tapioca flour with a temperature of 120°C for 20 minutes, with physical properties such as colour, aroma, and excellent texture with a range (3.73, 3.70, and 3.63), density with a value of 5.68 g/cm^3, and water binding capacity with a value of 104.22%. From the research it can be concluded that there are interactions on the physical properties of wafers (colour, aroma, and smell), density and water binding capacity. For further research, the best wafers obtained were continued to the in vitro digestibility stage to see the digestibility of wafers as ruminant feed.

Keywords: Binding, Physical qualities, Sugarcane shoots, *Tithonia diversifolia*, Wafer

INTRODUCTION

Feed is one of the factors that greatly affect the success of livestock business both in terms of quality and quantity. Feeding that has good quality and in accordance with the needs of livestock will produce livestock that have high productivity. The availability of forage is very abundant during the rainy season, while it is very limited during the dry season, so it is necessary to find alternative feed to meet the needs of forage for livestock.

Alternatives that can be used to meet the needs of forage for ruminants are by utilising sugarcane tops and tithonia. One hectare of sugarcane plantation will produce 110 tonnes/year consisting of 72 tonnes of bagasse and 38 tonnes of sugarcane tops (Conrad and Prasetyaning, 2014). The amount of sugarcane production must be utilised as a forage source of energy for ruminants (Pazla et al., 2021; Pazla et al., 2023; Arief et al., 2023).

Explained that sugarcane tops can be used as a source of fibre feed because it has high crude fibre and its availability is large and rarely used by humans. The content of sugarcane tops is dry matter 60.62%, organic matter 87.81%, crude protein 8.53%, crude fat 1.64%, crude fiber 38.72%, cellulose 29.08%, and Total digestible Nutrient (TDN) 57.30%. Therefore, sugarcane tops have the potential to be used as an energy source animal feed for ruminants (Pazla et al., 2022).

Tithonia plants harvested six times a year can produce dry biomass production of 4.10 -10.20 tonnes / ha or fresh production of 24.00 - 46.80 tonnes / ha / year (Jama et al., 2000). Tithonia has not been widely utilized by the community because it is often regarded as a weed that cannot be used as animal feed, even though tithonia can be a ruminant feed because it contains quite good nutrients (Pazla et al., 2023b; Jamarun et al., 2023). The nutritional content of tithonia is 25.57% dry matter, 84.01% organic matter, 22.98% crude protein, 18.17% crude fibre, 61.12 neutral detergent fiber (NDF), 40.15% acid detergent fiber (ADF), 34.59% cellulose, 20.97% hemicellulose, and 4.57 lignin (Pazla et al., 2021b). Hence, tithonia has the potential so that it can be used and developed as a protein source feed for ruminants.

To increase shelf life, it is necessary to have a touch of technology to reduce water content, one of which is wafer feed processing. Wafer is one of the effective feed processing technologies and is expected to maintain the continuity of animal feed, especially in the dry season and has a dense and compact square physical form that greatly facilitates storage and handling. The advantages of processing feed into wafers include increasing density, reducing storage space,

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reducing transportation costs, making it easier to control, monitor and regulate livestock feed intake, consistent and guaranteed nutrient content, reducing dust and respiratory problems in livestock (Kaliyan and Morey, 2009). The physical quality of wafers is strongly supported by adhesives. Adhesives are additional ingredients that are deliberately added to the formulation of feed ingredients to unite all the raw materials used (Adelina et al., 2023). Some natural ingredients that have been used for wafers include wheat flour, corn flour, rice flour, onggok, and palm sugar (Retnani et al., 2023). This study aims to determine the physical quality of colour, aroma, texture, density, water binding capacity and various types of adhesives used to make sugarcane tops and tithonia-based wafers.

MATERIALS AND METHODS

Materials

The main materials are sugarcane tops (*Saccaharum officinarum*) and tithonia (*tithonia diversifolia*) in a ratio of 60:40. Sugarcane tops are taken in the Puncak Lawang, Agam area and Tithonia is taken in Padang Panjang, both materials were dried and mashed. Types of adhesives are: tapioca flour, pathi flour, karagenan, flour, cassava flour, palm sugar were obtained at belimbing traditional market.

Methods

This research method uses a complete randomized design factorial pattern with 3 factors. Factor A is the application of various types of adhesives consisting of: Tapioca flour (A1), Patin flour (A2), cassava flour (A3), Karagenan flour (A4), palm sugar (A5). Factor B drying temperature in the oven consisting of: 100^{o}C (B1), 110^{o}C (B2), 120^{o}C (B3), while as factor C is the length of drying time in the oven consisting of: 10 minutes (C1), 15 minutes (C2), and 20 minutes (C3). Each treatment was repeated three times.

Research procedure

Tools used for the manufacture of wafers are mier, grinder machine, wafer felting machine, wafer mould, analytical scales, oven and vernier. The combination of sugarcane tops and tithonia is added to various types of adhesives according to the treatment of factors A, B, and C. The wafers are ready to be analyzed.

Parameters measured

Determination of texture, colour and aroma

Observations of physical properties were made by scoring each wafer criterion, which can be seen in Table 2.

Water absorption (Trisyulianti et al., 2003)

Water absorption was obtained from measuring the weight of wafers before and after soaking with water for 5 minutes. The percentage of water absorption was obtained by the formula:

DSA (%) = (BA-BB)/BA x 100%

When. DSA = water absorption (%); BA = Initial Weight (g); BB = Final Weight (g)

Density measurements (Trisyulianti et al., 2003)

Density is an important factor in the physical properties of wafers as a guide to obtain an overview of the desired wafer strength. Wafer density value can be calculated by the formula

K = W/(P x T x L)

When. K = Density (g cm^{-3}); W = Weight of Test Sample (g); P = Length of Test Sample (cm); L = Width of Test Sample (cm); T = Thickness of Test Sample (cm).

Table 1 - Chemical composition of the feed ingredients making up the treatment ration (%DM)

Feed Ingredients / Nutritional Content (%)	*Saccaharum officinarum*	*Tithonia diversifolia*
Dry matter	60.02	81.79
Organic matter	86.60	83.49
Crude protein	7.88	21.85
Crude fat	1.50	3.21
Crude fiber	40.83	21.21
NFE	36.39	37.23
ADF	55.80	39.80
NDF	86.83	60.31
Cellulose	33.84	25.83
Hemicellulose	31.03	20.51

NFE= Nitrogen Free Extract, ADF = acid detergent fiber, NDF = neutral detergent fiber

Table 2 – Wafer characteristics assessment criteria

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

Source; (Solihin et al., 2015)

Statistical analysis

This study used Split split plot design (SPPD). Data were analysed using Analysis of Variance (ANOVA). Significant differences among treatments were further tested using Tukey's test. All procedures were performed using SPSS 20 statistical package software. The results were analysed using generalised linear models method with IBM SPSS Statistics 26.0 version (IBM Corp., NY, USA).

RESULT

Physical properties of wafers

Wafer colour

The average colour score of sugarcane tops and tithonia-based wafers using several types of adhesives according to the treatment can be seen in Table 3. Data of table 3 shows that the manufacture of sugarcane tops and tithonia-based wafers with different types of adhesives has a very significant effect (P<0.01) on the colour of the wafers produced, on the colour of water there is an interaction (P<0.01) between the type of adhesive, temperature and length of oven time.

Wafer aroma

Wafer aroma is an indicator that can be used to determine the presence or absence of damage through changes in aroma that occur in wafers, so as to determine the quality of wafers. The average aroma score of sugarcane tops and tithonia-based wafers using several types of adhesives according to the treatment can be seen in Table 4. Data of table 4 shows that the manufacture of sugarcane tops and tithonia-based wafers with different types of adhesives has a very significant effect (P<0.01) on the aroma of wafers produced, on the aroma of water there is an interaction (P<0.01) between the type of adhesive, temperature and length of oven time.

Wafer texture

The average texture score of sugarcane tops and tithonia-based wafers using several types of adhesives according to the treatment can be seen in Table 5. Data of table 5 shows that the manufacture of wafers based on sugarcane tops and tithonia with different types of adhesive has a very significant effect (P<0.01) on the texture of the wafers produced, on the texture of water there is an interaction (P<0.01) between the type of adhesive, temperature and length of oven time.

Wafer density

The density value indicates the density of the wafer feed and also determines the physical shape of the wafer feed produced. The average density of sugarcane tops and tithonia-based wafers using several types of adhesives according to the treatment can be seen in Table 6. The data of table 6 shows that the manufacture of sugarcane tops and tithonia-based wafers with different types of adhesives has a very significant effect (P<0.01) on the density of wafers produced, on the density of wafers there is an interaction (P<0.01) between the type of adhesive, temperature and length of oven time. The highest density was found in the type of tapioca starch adhesive with a temperature of 120^{o}C which was oven for 20 minutes with a value of 5.68 g/cm-3. And the lowest was found in the type of keriginan flour adhesive with a temperature of 100^{o}C which was oven for 20 minutes.

Water absorption

The average water absorption of sugarcane tops and tithonia-based wafers using several types of adhesives according to the treatment can be seen in Table 7. Data of table 7 shows that the manufacture of sugarcane tops and tithonia-based wafers with different types of adhesives has a very significant effect (P<0.01) on the water absorption of wafers produced, on the water absorption of wafers there is an interaction (P<0.01) between the type of adhesive, temperature and length of oven time. The lowest water absorption was found in the type of tapioca flour adhesive with a temperature of 120^{o}C which was oven for 20 minutes with a value of 104.22%. The highest was found in the type of pathi flour adhesive with a temperature of 100^{o}C which was oven for 10 minutes with a value of 119.58%.

Table 3 - Average colour values of sugarcane tops and fermented tithonia-based wafers

Factor A	Factor B	Factor C		
		C1	C2	C3
A1	B1	$3.23^{bcdefg} \pm 0.35$	$2.70^{abc} \pm 0.26$	$2.97^{abcde} \pm 0.26$
	B2	$3.00^{abcde} \pm 0.20$	$3.03^{abcdef} \pm 0.25$	$3.17^{abcdefg} \pm 0.21$
	B3	$3.07^{abcdefg} \pm 0.15$	$2.70^{abc} \pm 0.10$	$3.73^{g} \pm 0.15$
A2	B1	$2.57^{a} \pm 0.25$	$2.60^{ab} \pm 0.20$	$2.70^{abc} \pm 0.36$
	B2	$2.77^{abc} \pm 0.15$	$3.13^{abcdefg} \pm 0.15$	$2.83^{abc} \pm 0.15$
	B3	$3.13^{abcdefg} \pm 0.21$	$3.17^{abcdefg} \pm 0.25$	$3.03^{abcdef} \pm 0.21$
A3	B1	$3.00^{abcde} \pm 0.36$	$3.17^{abcdefg} \pm 0.15$	$2.57^{a} \pm 0.31$
	B2	$2.90^{abcd} \pm 0.10$	$2.80^{abc} \pm 0.10$	$3.00^{abcde} \pm 0.26$
	B3	$3.20^{abcdefg} \pm 0.26$	$3.03^{abcdef} \pm 0.15$	$2.60^{ab} \pm 0.10$
A4	B1	$3.03^{abcdef} \pm 0.15$	$3.20^{abcdefg} \pm 0.10$	$3.07^{abcdefg} \pm 0.15$
	B2	$3.30^{cdefg} \pm 0.26$	$3.30^{cdefg} \pm 0.20$	$3.07^{abcdefg} \pm 0.15$
	B3	$3.57^{efg} \pm 0.15$	$3.50^{defg} \pm 0.10$	$3.67^{fg} \pm 0.06$
A5	B1	$2.93^{abcde} \pm 0.06$	$2.70^{abc} \pm 0.20$	$2.83^{abc} \pm 0.21$
	B2	$3.00^{abcde} \pm 0.17$	$2.77^{abc} \pm 0.06$	$2.30^{abcde} \pm 0.35$
	B3	$3.03^{abcdef} \pm 0.12$	$2.97^{abcde} \pm 0.15$	$2.27^{abcdef} \pm 0.15$

Note: Statistical further test using generalized linear models method, Different superscripts in the columns above indicate significant differences (P<0.01), ± standard deviation. Factor A : A1: tapioca flour; A2: patin flour; A3: cassava flour; A4: karagenan flour; A5: palm sugar; and Factor B : B1: 100°C; B2: 110°C; C3: 120°C.

Table 4 - Mean value of aroma of sugarcane tops and tithonia based wafers

Factor A	Factor B	Factor C		
		C1	C2	C3
A1	B1	$3.40^{e} \pm 0.35$	$2.50^{a} \pm 0.26$	$3.03^{bcde} \pm 0.26$
	B2	$3.03^{bcde} \pm 0.20$	$3.17^{cdf} \pm 0.25$	$3.27^{ef} \pm 0.21$
	B3	$3.23^{cdf} \pm 0.15$	$2.67^{abcd} \pm 0.10$	$3.70^{f} \pm 0.15$
A2	B1	$2.33^{a} \pm 0.91$	$2.50^{ab} \pm 0.25$	$2.33^{a} \pm 0.20$
	B2	$2.50^{ab} \pm 0.15$	$2.33^{a} \pm 0.15$	$2.50^{ab} \pm 0.25$
	B3	$2.50^{ab} \pm 0.21$	$2.33^{a} \pm 0.25$	$2.50^{ab} \pm 0.21$
A3	B1	$2.57^{ab} \pm 0.35$	$2.40^{a} \pm 0.15$	$2.50^{ab} \pm 0.31$
	B2	$2.43^{ab} \pm 0.10$	$2.50^{ab} \pm 0.10$	$2.43^{ab} \pm 0.26$
	B3	$2.60^{ab} \pm 0.26$	$2.37^{a} \pm 0.15$	$2.43^{ab} \pm 0.10$
A4	B1	$2.70^{abcd} \pm 0.15$	$3.37^{ef} \pm 0.10$	$3.43^{ef} \pm 0.15$
	B2	$3.63^{f} \pm 0.26$	$3.57^{ef} \pm 0.20$	$3.53^{ef} \pm 0.15$
	B3	$3.53^{ef} \pm 0.15$	$3.57^{ef} \pm 0.10$	$3.70^{f} \pm 0.06$
A5	B1	$2.50^{ab} \pm 0.06$	$2.53^{ab} \pm 0.20$	$2.33^{a} \pm 0.21$
	B2	$2.67^{abcd} \pm 0.17$	$2.37^{a} \pm 0.06$	$2.30^{a} \pm 0.35$
	B3	$2.50^{ab} \pm 0.12$	$2.50^{ab} \pm 0.15$	$2.27^{a} \pm 0.15$

Note: statistical further test using generalized linear models method, Different superscripts in the columns above indicate significant differences (P<0.01), ± standard deviation. Factor A : A1: tapioca flour; A2: patin flour; A3: cassava flour; A4: karagenan flour; A5: palm sugar; and Factor B : B1: 100°C; B2: 110°C; C3: 120°C.

Table 5 - Mean Tekstur value of sugarcane tops and tithonia based wafers

Factor A	Factor B	Factor C		
		C1	C2	C3
A1	B1	$3.53^{ef} \pm 0.15$	$3.30^{cdef} \pm 0.20$	$3.00^{abc} \pm 0.20$
	B2	$3.50^{def} \pm 0.10$	$3.00^{abc} \pm 0.10$	$3.10^{abcde} \pm 0.10$
	B3	$3.00^{abc} \pm 0.17$	$3.00^{abc} \pm 0.10$	$3.63^{f} \pm 0.06$
A2	B1	$2.63^{a} \pm 0.06$	$2.90^{abc} \pm 0.20$	$2.80^{ab} \pm 0.10$
	B2	$2.80^{ab} \pm 0.17$	$3.03^{abcd} \pm 0.21$	$3.00^{abc} \pm 0.10$
	B3	$3.00^{abc} \pm 0.20$	$3.07^{abcde} \pm 0.15$	$2.87^{abc} \pm 0.06$
A3	B1	$2.97^{abc} \pm 0.06$	$3.23^{bcdef} \pm 0.06$	$3.17^{bcdef} \pm 0.12$
	B2	$3.17^{bcdef} \pm 0.15$	$3.00^{abc} \pm 0.10$	$3.00^{abc} \pm 0.20$
	B3	$3.30^{cdef} \pm 0.10$	$3.27^{bcdef} \pm 0.12$	$3.10^{abcde} \pm 0.10$
A4	B1	$3.03^{abcd} \pm 0.06$	$3.03^{abcd} \pm 0.12$	$3.07^{abcde} \pm 0.21$
	B2	$3.20^{bcdef} \pm 0.10$	$3.30^{cdef} \pm 0.10$	$3.23^{bcdef} \pm 0.15$
	B3	$3.23^{bcdef} \pm 0.25$	$3.30^{cdef} \pm 0.20$	$3.60^{f} \pm 0.10$
A5	B1	$3.03^{abcd} \pm 0.12$	$3.17^{bcdef} \pm 0.06$	$3.27^{bcdef} \pm 0.06$
	B2	$3.03^{abcd} \pm 0.32$	$3.17^{bcdef} \pm 0.06$	$3.03^{abcd} \pm 0.06$
	B3	$3.07^{abcde} \pm 0.15$	$3.17^{bcdef} \pm 0.15$	$3.23^{bcdef} \pm 0.06$

Note: statistical further test using generalized linear models method, Different superscripts in the columns above indicate significant differences (P<0.01), ± standard deviation. Factor A : A1: tapioca flour; A2: patin flour; A3: cassava flour; A4: karagenan flour; A5: palm sugar; and Factor B : B1: 100°C; B2: 110°C; C3: 120°C.

Table 6 - Average values of sugarcane tops and tithonia based wafer density (g/cm³)

Factor A	Factor B	Factor C		
		C1	C2	C3
A1	B1	$4.29^{abcd} \pm 0.09$	$5.36^{mno} \pm 0.06$	$5.20^{ijklmno} \pm 0.20$
	B2	$5.43^{no} \pm 0.04$	$4.97^{ghijklmno} \pm 0.08$	$5.19^{ijklmno} \pm 0.9$
	B3	$5.38^{mno} \pm 0.12$	$5.50^{o} \pm 0.07$	$5.68^{q} \pm 0.04$
A2	B1	$5.28^{klmno} \pm 0.04$	$4.99^{ghijklmno} \pm 0.04$	$4.74^{cdfghij} \pm 0.10$
	B2	$4.81^{dfghijkl} \pm 0.02$	$4.90^{fghijklmn} \pm 0.02$	$4.62^{bcdfgh} \pm 0.05$
	B3	$5.10^{hijklmno} \pm 0.05$	$5.19^{ijklmno} \pm 0.03$	$5.21^{jklmno} \pm 0.03$
A3	B1	$4.35^{bcd} \pm 0.04$	$4.41^{bcdf} \pm 0.03$	$3.79^{a} \pm 0.05$
	B2	$4.22^{abc} \pm 0.04$	$4.35^{bcd} \pm 0.05$	$4.73^{bcdfghij} \pm 0.03$
	B3	$4.90^{fghijklmn} \pm 0.02$	$4.36^{bcd} \pm 0.04$	$5.05^{ghijklmno} \pm 0.05$
A4	B1	$4.42^{bcdf} \pm 0.03$	$4.67^{bcdfghi} \pm 0.03$	$5.25^{jklmno} \pm 0.04$
	B2	$5.22^{jklmno} \pm 0.04$	$5.00^{ghijklmno} \pm 0.02$	$5.26^{jklmno} \pm 0.04$
	B3	$5.30^{lmno} \pm 0.03$	$5.32^{lmno} \pm 0.02$	$5.62^{p} \pm 0.02$
A5	B1	$4.55^{bcdfg} \pm 0.04$	$4.76^{dfghijk} \pm 0.04$	$4.22^{abc} \pm 0.03$
	B2	$4.21^{abc} \pm 0.06$	$4.20^{ab} \pm 0.02$	$4.60^{bcdfgh} \pm 0.02$
	B3	$4.48^{bcdf} \pm 0.05$	$4.91^{fghijklmn} \pm 0.04$	$4.89^{fghijklm} \pm 0.03$

Note: statistical further test using generalized linear models method, Different superscripts in the columns above indicate significant differences (P<0.01), ± standard deviation.
Factor A : A1: tapioca flour; A2: patin flour; A3: cassava flour; A4: karagenan flour; A5: palm sugar; and Factor B : B1: 100°C; B2: 110°C; C3: 120°C.

Table 7 - Mean value of water absorption of sugarcane tops and tithonia based wafers (%)

Factor A	Factor B	Factor C		
		C1	C2	C3
A1	B1	$114.43^{cdefg} \pm 2.67$	$111.25^{abcde} \pm 1.55$	$115.85^{cdefg} \pm 4.38$
	B2	$114.09^{cdefg} \pm 1.65$	$119.17^{fg} \pm 4.38$	$116.30^{defg} \pm 1.35$
	B3	$115.56^{cdefg} \pm 1.28$	$113.01^{abcdefg} \pm 2.20$	$104.22^{a} \pm 0.97$
A2	B1	$119.58^{g} \pm 2.02$	$117.95^{efg} \pm 0.75$	$114.35^{cdefg} \pm 4.67$
	B2	$118.89^{fg} \pm 1.90$	$115.98^{cdefg} \pm 1.12$	$116.55^{defg} \pm 3.92$
	B3	$117.39^{defg} \pm 3.04$	$115.62^{cdefg} \pm 2.88$	$115.33^{cdefg} \pm 4.51$
A3	B1	$117.74^{efg} \pm 1.57$	$117.33^{defg} \pm 1.25$	$114.82^{cdefg} \pm 3.70$
	B2	$115.97^{cdefg} \pm 0.22$	$113.86^{cdefg} \pm 3.04$	$118.18^{efg} \pm 1.33$
	B3	$116.63^{defg} \pm 0.86$	$115.79^{cdefg} \pm 2.81$	$116.509^{defg} \pm 1.08$
A4	B1	$118.32^{efg} \pm 0.81$	$114.97^{cdefg} \pm 0.98$	$110.61^{abcd} \pm 1.19$
	B2	$116.63^{defg} \pm 2.30$	$112.78^{abcdefg} \pm 1.49$	$109.16^{abc} \pm 0.90$
	B3	$116.66^{defg} \pm 0.65$	$112.87^{abcdefg} \pm 1.54$	$105.94^{ab} \pm 1.90$
A5	B1	$118.14^{efg} \pm 2.48$	$116.76^{defg} \pm 1.61$	$115.20^{bcdefg} \pm 3.07$
	B2	$113.19^{bcdefg} \pm 5.47$	$112.73^{abcdefg} \pm 0.99$	$117.88^{efg} \pm 2.73$
	B3	$114.40^{cdefg} \pm 2.15$	$116.78^{defg} \pm 1.01$	$112.39^{abcdef} \pm 0.75$

Note: statistical further test using generalized linear models method, Different superscripts in the columns above indicate significant differences (P<0.01), ± standard deviation.
Factor A : A1: tapioca flour; A2: patin flour; A3: cassava flour; A4: karagenan flour; A5: palm sugar; and Factor B : B1: 100°C; B2: 110°C; C3: 120°C.

DISCUSSION

Wafer colour

Based on the interaction of factor A (application of different types of adhesive), factor B (drying temperature in the oven), and Factor C (length of time in the oven), the best wafer colour is found in the $A_1B_3C_3$ treatment, namely sugarcane tops and tithonia-based wafers (40: 60) using tapioca starch adhesive type which is baked at 120°C with a time of 20 minutes with an average of 3.73. The combination of sugarcane tops and tithonia gives a dark brown colour given the type of tapioca starch adhesive. Dark brown colour changes also occur due to a fairly high heating process of 120°C and are supported by the material of sugarcane tops and tithonia relatively the same. Lee et al. (2017) stated that the colour change that occurs during the heating process makes the material a darker brown colour. Type of adhesive Tapioca flour gives the best wafer colour, this is thought to occur because tapioca flour has the ability to bind the source of feed ingredients (sugarcane tops and Tithonia) in dry form is also getting better. Harahap et al. (2021) reported that tapioca flour is best used as a feed adhesive.

Wafer aroma

The aroma of wafers is an indicator that can be used to determine the presence or absence of damage through changes in aroma that occur in wafers, so as to determine the quality of wafers. The interaction that occurs between factor A, factor B, and factor C obtained the highest value in the $A_1B_3C_3$ treatment, namely sugarcane tops and tithonia-based wafers using tapioca starch adhesive material in the oven for 120°C for 20 minutes with a value of 3.70. The aroma of wafers is considered very well in terms of wafer quality which ranges from 3 - 3.9. Sugarcane shoots and tithonia have a distinctive aroma so that the aroma produced is quite good in wafer quality. In this study, the aroma produced is good enough so that it is not rancid. Reed (2005). That changes in the rancid aroma of wafers are caused by the fermentation of wafers which makes the aroma rancid.

Wafer texture

Wafer texture is an indicator to see how strong, dense, and rough or slimy so that the quality of a wafer is obtained. The interaction that occurs between factor A, factor B, and factor C obtained the highest value in the $A_1B_3C_3$ treatment, namely sugarcane tops and tithonia-based wafers using tapioca starch adhesive material in the oven for 120°C for 20 minutes with a value of 3.63. The texture in the $A_1B_3C_3$ treatment was considered very good with the description (Has a firm, dense texture (not easily broken). Animal feed wafers that have a firm and dense texture are good and not easily broken when given to livestock. The best wafer texture is found in the $A_1B_3C_3$ treatment, this occurs because the use of tapioca which is baked at 120°C for 20 minutes provides a better texture than other treatments. Tapioca is one of the best types of adhesives in making wafers. Tapioca flour produces the best physical properties of wafers (Fayzullahogglu, 2017; Sandi et al., 2015; Sudekum et al., 2008). A good texture is also supported by the oven temperature of 120°C and an oven time of 20 minutes, where the higher the temperature and the longer the oven time, the better the texture of the wafers will be.

Wafer density

The interaction that occurred between factor A, factor B, and factor C obtained the highest value of wafer density in the $A_1B_3C_3$ treatment, namely sugarcane tops and tithonia-based wafers using tapioca starch adhesive material in the oven for 120°C for 20 minutes with a value of 5.68. This happens because in the $A_1B_3C_3$ treatment using tapioca flour in the manufacture of wafers, tapioca contains starch which is very good as a designer material in the manufacture of wafers, so as to get a very good density value, this also happens because the temperature and time used is 120°C for 20 minutes longer than other treatments so that starch is more optimal in unification with sugarcane tops and tithonia. Tapioca starch contains 17% amylose and 83% amylopectin which is hygroscopic (Da Silva et al., 2022). The ratio of amylose and amylopectin affects the starch gelatinisation process and digestibility (Retnani et al., 2023). Amylopectin is sticky while amylose is hard (Deng et al., 2010 and Hadipernata et al., 2023). The gelatinisation process during heating will cause the formation of hydrogen bonds that will bind the feed components resulting in a compact texture and not easily destroyed, low water absorption thus increasing feed efficiency (Retnani et al., 2023) and causing changes in the physical characteristics of feed (Zhu et al, 2016 and Milawarni et al., 2020).

Water absorbency of wafers

Water absorption is a variable that shows the ability of wafer feed to attract surrounding water (air humidity) which binds to material particles or is retained in the pores between material particles. The interaction that occurred between factor A, factor B, and factor C obtained the lowest value of wafer water absorption in the $A_1B_3C_3$ treatment, namely sugarcane tops and tithonia-based wafers using tapioca starch adhesive material in the oven for 120°C for 20 minutes with a value of 104.22. This happens because in the $A_1B_3C_3$ treatment using tapioca flour in the manufacture of wafers, tapioca contains quite high starch, starch will undergo a galatinisation process during the heating process. So that it can reduce the water absorption of the wafer. Harahap et al. (2021) reported that tapioca flour is best used as a feed adhesive. This is related to the starch content in the adhesive material which will undergo a gelatinisation process during heating. The higher the starch content, the higher the gelatinisation process will be because the granule structure is tighter which will glue the feed, so the lower the water absorption. Water absorption is related to particle density. The higher the water absorption, the lower the particle density, and vice versa. The water absorption capacity of the cloud is inversely proportional to the density and texture will cause the water absorption capacity to decrease (Silaban et al., 2020). High particle density indicates better wafer quality because wafers are denser, harder, more durable and easier to handle, store or transport (Adelina et al., 2021) but has disadvantages because livestock have difficulty consuming them (Muralidharan et al., 2016) and reduce palatability (Retnani et al., 2023 and Sukaryana et al., 2020).

CONCLUSION

From the research it can be concluded that there are interactions on the physical properties of wafers (colour, aroma, and smell), density and water binding capacity. The best adhesive in making sugarcane tops (*Saccharum officinarum*) and Tithonia (*Tithonia diversifolia*) based wafers is tapioca flour with a temperature of 120°C for 20 minutes, with physical properties such as colour, aroma, and excellent texture with a range (3.73, 3.70, and 3.63), density with a value of 5.68 g/cm3, and water binding capacity with a value of 104.22%.

DECLARATION

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Author participation

N. Jamarun and M. Zain contributed to research concepts, technical and logistic support, and supervised the research. R. Pazla and G. Yanti contributed to experimental design, data collection and execution. Z. Ikhlas contributed to data collection, analyses and the write up of the manuscript. B.V Utami contributed to writing the final drafted manuscript. All authors have read and approved the final manuscript

Data availability

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

Competing interests

We declare that there are no conflicts of interest with any financial organisation regarding the material discussed in this paper.

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EFFECTS OF SEASON ON METABOLIC PROFILE OF HOLSTEIN FRIESIAN COWS IN POSTPARTUM PERIOD

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ABSTRACT: The aim of the present study was to determine the metabolic profile of Holstein-Friesian cows in the postpartum period, as well as the effect of season on metabolic profile. The postpartum period is essential in the reproductive life of high yielding dairy cows because of its impact on future gravidity. This study included 60 cows up to 15 days after parturition, aged 2-8 years (the largest number of cows was between 3 and 5 years old) with no apparent clinical problems. Cows were sampled in summer season (n=30) and winter season (n=30). Parameters of metabolic profile were determined as follows: glucose, albumin, total protein, cholesterol, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), urea, calcium, phosphorus and magnesium. Statistical differences were considered significant at the $p<0.05$. Present research showed that all investigated parameters were within a reference range for cattle. Impact of season sampling was determined for glucose, albumin, total protein, cholesterol and phosphorus, while bilirubin, calcium, magnesium, urea as well as activities of ALT, AST and LDH were unaffected by the season of sampling. In conclusion, metabolic status is affected by the season and examination during the postpartum period can provide valuable information of cows' health status, in order to diagnose and moreover prevent postpartum diseases.

Keywords: Cows, Climate, Health status, Metabolic profile, Postpartum period.

INTRODUCTION

Metabolic profile of cows is of a great importance in order to monitor health status, but also to diagnose and prevent metabolic and nutritional diseases in dairy cattle. The concept "metabolic profile" refers to the examination of blood biochemical parameters and is immensely important for the health condition of the herd (Puppel and Kuczyńska, 2016). However, multiple variables should be considered in order to accurately interpret the obtained data, such as physiological state of an animal. The periparturient period, also known as the transition period, lasts from 3 weeks before to 3 weeks after calving (i.e., the pregnant, nonlactating state to the nonpregnant, lactating state) (Erdoğan and Aliç, 2020) and is often a disastrous experience for the cow due to metabolic overload (Wang et al., 2014). Dairy cows experience severe metabolic stress after calving, because they cannot meet the enormous energy and protein demands for milk production. The postpartum period is essential in the reproductive life of high yielding dairy cows because of its impact on future gravidity. Immediately after calving a negative energy balance (NEB) occurs; NEB may reduce the conception rate to insemination due to detrimental effect it has on the oocyte that is released after ovulation. Cows under negative energy balance show extended periods of an ovulation. Postpartum anestrus, along with infertility is enhanced by losses of body condition during the early postpartum period associated with negative energy balance (Nigussie, 2018; Mekuriaw, 2023). NEB status can be assessed by changes in blood metabolites. Examination of metabolic status during postpartum period could provide valuable information of cows' health status, in order to diagnose and moreover prevent postpartum diseases.

The aim of the present study was therefore to determine metabolic profile of Holstein-Friesian cows in the postpartum period, as well as the effect of season on metabolic profile.

MATERIALS AND METHODS

Present study included 60 cows up to 15 days after parturition, aged 2-8 years (the largest number of cows was between 3 and 5 years old) with no apparent clinical problems. The research was conducted in two different geographical areas, specifically in the northern area – farm T and in the southern part of Bosnia and Herzegovina – farm V (Figure 1). Cows were sampled in summer season (June to August; n=30) and winter season (November to February, 2023; n=30). Blood samples were taken in the morning between 09:00 and 11:00 hrs, approximately two hours after feeding, via puncture of a coccygeal vein into two vacutainers. Blood from each animal was taken into two vacutainers. Blood samples for letter assessment of serum parameters was collected using 5-mL vacutainers containing no additives. Blood samples for plasma analyses was collected into 5-mL vacutainers containing sodium heparin. After collection vacutainers were stored

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on ice and transported to the lab, within two hours. Upon arrival, vacutainers with heparin were LC 320, 3000 rpm / 10 min, and plasma was frozen at −20 °C. Blood samples to harvest serum were stored on ice after collection, allowed to clot at 5 °C, and centrifuged at 1,000 × g to harvest serum. Serum was immediately frozen at −20 °C for later analyses.

Parameters of metabolic profile were determined using the "Beckmann DU-64 UV/VIS" spectrophotometer, as follows: glucose, albumin, total protein, cholesterol, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), urea, calcium, phosphorus and magnesium. Statistical analysis was done using SPSS 10.00 software program. Differences were considered statistically significant at the level $p < 0.05$.

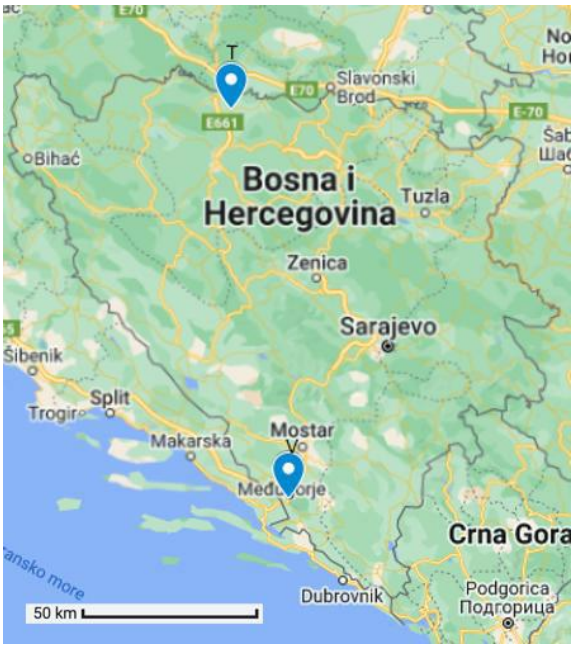

Figure 1. The image shows two geographical locations in Bosnia and Herzegovina. The northern location is marked with (T) for Topola, while the southern location is marked with (V) for Višići.

RESULTS AND DISCUSSION

Results are shown in Table 1. In postpartum cows, the metabolic profile is critical for health monitoring, disease diagnosis, treatment, and prognosis. Since glucose is a master regulator of hormones and metabolites that regulate reproductive processes, blood glucose concentrations in postpartum cows are of great significance, especially due to the fact that low blood glucose levels in postpartum dairy cows are linked to infertility. Glucose is a critical nutrient in the postpartum dairy cow since glucose is required for milk synthesis by the mammary gland. A variety of other tissue types, including those involved in reproduction, demand glucose. Moreover, glucose is a valuable parameter for determining dietary sufficiency. Glucose levels of Holstein-Friesian postpartum cows during summer and winter season is given in Table 1. Present study has identified statistically significant higher values of glucose concentrations during summer, compared to winter. The higher glucose levels during summer could be explains by higher supply of forage in summer, compared to the winter. Seasonal effects on blood glucose levels were confirmed in previous studies (Giri et al., 2017; Cerutti et al., 2018). However, obtained values were within the reference range (Table 1).

Table 1 - Seasonal Variations in Blood Metabolic Parameters of Holstein-Friesian Cows

Parameter	Summer season	Winter season	Both seasons
Glucose (mmol/L)	3.28±0.08^{a}	3.06±0.06^{b}	3.17±0.05^{ab}
Albumin (g/L)	60.37±1.80^{a}	53.78±1.51^{b}	57.19±1.25^{ab}
Total protein (g/L)	144.65±3.81^{a}	82.32±4.96^{b}	114.03±5.18^{c}
Cholesterol (mmol/L)	2.14±0.17^{a}	3.28±0.21^{b}	2.69±0.15^{c}
Bilirubin (µmol/L)	2.12±0.45	2.74±0.45	2.46 ±0.32
ALT (u/L)	9.15 ±0.55	18.46±1.46	13.73±0.98
AST (u/L)	70.52 ±2.37	60.32±1.54	65.92±1.36
LDH (u/L)	998.01± 8.79	1113.45±20.47	1007.50±11.11
Urea (mmol/L)	5.92±0.13	5.47±0.18	5.81 ±0.35
Calcium (mmol/L)	2.18±0.08	2.01±0.02	2.1±0.04
Phosphorus (mmol/L)	2.29±0.13^{a}	1.73±0.06^{b}	2.04±0.08^{c}
Magnesium (mmol/L)	1.15±0.03	1.04±0.02	1.3±0.03

Means with different superscripts in a row differ significantly ($p<0.05$). Reference values: Glucose 2.49 – 4.16 mmol/L (Radostits et al., 2000); Albumin 54 – 86 g/L (Forenbacher, 1993); Total protein 73.8 – 106.2 g/L (Olayemi et al., 2001); Cholesterol 1.5 – 6.7 mmol/L (Forenbacher, 1993); Bilirubin 1.2-5.13 µmol/L (Forenbacher, 1993; Radostits et al., 2000; Kaneko, 2008); ALT 4 – 11 U/L (Forenbacher, 1993); 11 – 40 U/L (Radostits et al., 2000; Kaneko, 2008), 9.6 – 35 U/L (Merck Veterinary manual, 2003); AST 35 – 80 U/L (Forenbacher, 1993); LDH 500 – 1500 U/L (Forenbacher, 1993); 692 – 1445 U/L (Radostits et al., 2000; Kaneko, 2008); Urea 1.66 – 6.66 mmol/L (Merck Veterinary manual, 2003); Calcium 2 – 2.8 mmol/L (Merck Veterinary manual, 2003); Phosphorus 1.4 – 2.5 mmol/L (Merck Veterinary manual, 2003); Magnesium 0.7 – 1.2 mmol/L (Merck Veterinary manual, 2003).

Blood proteins are important markers of animal health. Albumin concentration, but also total proteins concentration was found significantly lower during winter season versus summer season (Table 1). Statistically significant higher values obtained in summer season could be explained by the quality of animal nutrition; pasturage used at the examined localities originates from lawns of diverse botanical composition and hence its quality (Hadžimusić and Hrković-Porobija, 2018). Moreover, some types of grasses in the early stages of growth contain large amounts of water and excess protein

and total nitrogen. Higher protein values could also be explained also by possible dehydration of animals. However, albumin concentration changes may indicate deteriorated liver function as a result of inflammatory conditions (Bobbo et al., 2017). Cholesterol concentration determined by present study was found significantly higher during winter season. Similar findings were reported by Dar et al. (2019). Moreover, Yokus and Cakir (2006), observed significantly higher medium cholesterol values (4.71 ± 1.75 mmol / L) during winter, compared to summer season (4.22 ± 1.16 mmol / L).

Bilirubin concentration determined by this study was within the reference range. Moreover, this research showed that season has no effect on billirubin levels. Significant changes in bilirubin concentrations are associated with bile flow disorders (Puppel and Kuczyńska, 2016). The liver enzymes determined by present study (ALT, AST and LDH) were within the normal range and showed no seasonal effect. The impact of pregnancy on the level of AST and ALT activity is quite controversial. Several studies have shown an increase in the activity of liver enzymes during the postpartum period, while some authors on the contrary reported a decrease in activity during the same period (Hadžimusić and Krnić, 2012). Plasma ALT activity is affected by age and muscle activity. Physiological changes of ALT activity are related to pregnancy and the beginning of lactation, when the level of ALT activity is reduced. However, result of our study shown that ALT activity was within the reference range. It is well known that LDH is not an organ-specific enzyme. LDH activity is therefore reported in muscle, heart, kidneys, and liver (Krsmanović et al., 2016). Present research showed LDH activity within reference range, as well no seasonal effect was determined.

Blood urea nitrogen concentration is affected by many factors, such as dietary protein intake and rumen degradability, dietary amino acid composition, liver and kidney function, muscle tissue breakdown (Puppel and Kuczyńska, 2016). Urea concentration determined by study was within the normal range and did not differ significantly during summer and winter season of sampling. In contrast to present study some authors (Giri et al., 2017; Cerutti et al., 2018) reported a seasonal effect on blood urea nitrogen of cattle. However, in contrary with their research, present study included only postpartum cows.

Minerals play a role in almost all living systems, either as structural elements or as regulators of majority of metabolic processes. Calcium is the most abundant mineral in an organism. Since phosphorus and calcium also play a role in bone formation, these two minerals are sometimes considered together. Moreover, hypophosphatemia is often connected with moderate hypocalcaemia (Hadžimusić and Krnić, 2012). In relation to calcium and phosphorus, magnesium metabolism is in many respects specific in. It is well known that magnesium acts as an antagonist to calcium, while its deficiency emphasizes the effect of calcium. Along with hypocalcaemia hypomagnesaemia can cause pasture tetany in cows and goats. Hypocalcaemia may be a major cause for the emergence of hypomagnesaemia. Values of investigated minerals were within the reference intervals for cows, and showed no seasonal effect, except for the phosphorus. While the calcium and magnesium were unaffected by the season, significantly higher concentrations of phosphorus were determined during summer season compared to the winter season. Present result was in agreement with previous studies which reported that phosphorus levels in cows mainly depends on climatic variation and nature of feed (Shrikhande et al., 2008; Kubkomawa et al., 2015; Coates et al., 2019).

CONCLUSION

In this study, the metabolic profile of postpartum Holstein-Friesian cows was analyzed to understand the seasonal variations in blood glucose, blood proteins, cholesterol, bilirubin, liver enzymes, blood urea nitrogen, and essential minerals. The results highlighted several significant findings that are crucial for optimizing the health and productivity of postpartum dairy cows. Present research showed blood glucose levels higher in the summer compared to winter, although the difference was not statistically significant. Both albumin and total protein concentrations were significantly lower in winter compared to summer. This difference is likely due to the quality of nutrition, as summer pasturage is richer in diverse botanical composition, which enhances protein intake. These findings suggest that improving winter feeding strategies could enhance protein levels and overall cow health. Cholesterol concentrations were significantly higher in winter, aligning with findings from previous studies. Bilirubin and urea levels showed no significant seasonal variation. Liver enzyme activities (ALT, AST, and LDH) also remained within normal ranges and exhibited no significant seasonal effects. Among the minerals studied, only phosphorus showed significant seasonal variation, with higher levels in summer. Calcium and magnesium levels remained stable across seasons.

DECLARATIONS

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Authors' contribution

N.Hadžimusić, Dž.Hadžijunuzović-Alagić: Conceptualized and designed the study, conducted the experiments, and drafted the manuscript. Dž. Hadžijunuzović-Alagić., N. Hadžimusić: Analyzed the data, interpreted the results, and revised the manuscript. N. Hadžimusić , Dž. Hadžijunuzović-Alagić: Assisted with sample collection and laboratory analyses, and contributed to the manuscript writing. Dž. Hadžijunuzović-Alagić., N. Hadžimusić: Supervised the project, provided critical feedback, and helped shape the research and manuscript.

Data availability

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

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Ethical regulations

This study did not involve any excessive experimental procedures on animals. The blood sampling conducted was part of the routine health monitoring and systematic veterinary examination of Holstein-Friesian cows. Given that the blood samples were collected as a part of standard veterinary care and did not require any invasive procedures beyond those typically performed during routine check-ups, approval from an ethics committee was not required. All procedures adhered to the established guidelines for animal care and welfare, ensuring minimal stress and discomfort to the animals involved.

Consent to publish

All authors have reviewed and approved the final manuscript for publication.

Competing interests

The authors declare that they have no competing interests.

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EFFECTS OF DIETARY PROTEIN CONTENT ON THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF UNSELECTED RABBIT DOES AND THEIR LITTERS DURING FIRST TWO LACTATIONS

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ABSTRACT: The aim of the present study was to evaluate the influence of different dietary protein levels on the productive performance of unselected rabbit does and their litters during their first two lactations. For this purpose, fifty-two nulliparous rabbit does, 4.5 months of age and live weight of 3115 ± 71 g, were divided into three groups (17 or 18 females per group), kept in individual cages and each group received only one of the three experimental diets. These diets were iso-energetic (10.8 MJ DE/kg), but with increasing levels of crude protein (CP): 15%, 17% and 19 % for the low (L), medium (M) or high (H) diets, respectively. Breeding was carried out by natural copulation using 6 males of 5-6 months of age and 2865±21 g initial weight, controlled semi-intensive lactation and weaning at 35 days after birth. Female body weight, feed intake, milk production, litter size and weight were monitored at birth and weekly after parturition during the first two lactations. The protein intake of the rabbits increased with the amount of protein in the diet (L vs. M: +12.2%; L vs. H: +18.8%; $p < 0.001$), without any effect on milk production and feed intake. Milk production was unaffected by parity. Throughout the pre-weaning period, litter size and weight and maternal mortality were unaffected by dietary protein level. Dietary protein level had no effect on live weight, birth to weaning weight gain, milk production or feed intake during the first two consecutive lactations of rabbit does.

Keywords: Feed Intake, Litter parameters, Milk production, Protein content, Unselected rabbit does, Weight gain.

INTRODUCTION

The main cost in raising rabbits is feed, which accounts for more than 70% of the total production cost. Rabbits does are highly fertility and can be simultaneously be pregnant and suckling. The digestible protein consumed by rabbit does hence could be used both for body growth, milk production and fetal growth. A negative protein balance can result if the does are pregnant and lactating simultaneously, especially if the feed intake does not match demand (Parigi-Bini et al., 1992; Martínez-Paredes et al., 2022).

Previous studies have reported the significant effect of energy balance on rabbit does production (Xiccato, 1996; Xiccato et al., 1999), as well as the requirement for milk, in late pregnancy, body fat, energy and protein requirements also become relevant because of the development of the pregnant uterus and the fœtal protein turnover (Arias-Alvarez et al., 2009). Saidj et al. (2016) showed that there was no effect of the energy content of the feed neither on their weight or milk production of local Algerian does, nor on their litter. However, a significant decrease in feed intake in females fed most energetic diet was recorded. The protein content of the diet has a significant impact on the cost of feeding, which is the highest cost item in rabbit production - up to 70%. Diets with lower protein content allow for a significant reduction in feed costs and in excretion of nitrogen (Maertens, 1999). According to Mathika (2023), rabbits are likely to have a variable response to the level of protein in their diet. Recently, Martínez-Paredes et al. (2022) suggested that an excess of CP may increase the rate of stillbirth at first parturition.

Therefore, the aim of the present work was to study the influence of different diet protein level on productive performances of local unselected rabbit does and their litters during their two first lactations.

MATERIALS AND METHODS

Ethical consideration

The experiment was carried out in accordance with the guidelines for Experimental Animals approved by the Algerian Association of Animal Experimentation Sciences (58 AASEA: N°45/DGLPAG/DVA/SDA/19).

Animals and experimental design

A total of fifty-two (52) nulliparous local unselected rabbit does, 4.5 months old and 3115±71 g weight with variable phenotype, were individually weighed and subsequently kept in individual galvanized cages, disposed on flat deck system

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and received one of the three experimental diets. Reproduction was performed by natural mating using six males (2 per group) of 5 to 6 months old and 2865±21 g initial weight with semi-intensive rhythm (mating between 10 to 12 days post-partum). The pregnancy diagnosis was made by abdominal palpation 10 days post coitum. Each cage was provided of nest box with a gate to isolate the females of their litters. Each group made up of 17 or 18 females and 2 males. All animals were subject to the same breeding conditions. Reproduction rhythm was semi-intensive, and the kits weaning done at 35 days postpartum. The live weight of does, feed intake, milk production and litters enumeration and weight were controlled at birth and weekly after partum during the two first lactations. The nest boxes were always closed except for the moment of lactation; the does were subjected to control nursing: they had access to their litter once a day (between 08.00 and 09.30). For milk yield measurement, differential weighing of does was performed immediately before and after suckling from parturition to 21d post-partum (Parigi-Bini et al., 1992).

Experimental diets

Does were divided into three groups and offered one of the three diets formulated to be isoenergetics and to meet the requirements (10.8 MJ DE/kg) according to De Blas and Mateos (2020) but with different and increasing crude protein (CP) content i.e.15, 17 or 19% CP for Low (L), Mean (M) or High (H) diet respectively. The ingredients and nutritional characteristics of the three diets are shown in Table 1. Diets were provided ad libitum between parturition and weaning. The chemical analyses were performed at alimentation laboratory of Higher National Veterinary School, Algiers, Algeria, according to ISO methods and considering the recommendations proposed by the EGRAN group (EGRAN, 2001): dry matter (ISO 6496:1999), crude ash (ISO 5984:2002), crude protein (N×6.25, Dumas method, ISO 16634-2:2009). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed at nutritional laboratory of animal production department, Liège University, Belgium, according to sequential method of Van Soest, ashless, without sodium sulphite, and using crucibles (Tecator apparatus) (AFNOR 1997, ISO 16472: 2006 and ISO 13906: 2008). The gross energy (ISO 9831:1998) was analyzed at Animal production laboratory Agro-Bio Tech de Gembloux, Belgium. The digestible energy was estimated with Allix software (2002) when the feeds were formulated.

Table 1 - Nutritional characteristics of the experimental diets

Ingredients (%) \ Diets	L	M	H
Corn Grain	35.0	28.0	23.0
Alfalfa meal	44.1	43.5	43.6
Barley Grain	4.4	6.3	5.6
Soybean Meal	11.7	17.1	22.5
Wheat Bran	2.0	2.3	2.5
Di-Calcium Phosphate	1.8	1.8	1.8
Premix[1]	1.0	1.0	1.0
Chemical composition (%)			
Dry matter	89.3	89.5	89.6
Ash	7.71	10.07	8.40
Crude protein	15.5	17.5	18.9
Crude fibre	11.88	13.88	13.03
ADF	15.89	17.14	16.01
NDF	35.33	45.08	41.79
Ether extract	1.67	2.0	2.33
Gross energy (MJ/kg)	18.26	18.17	18.34
Digestible Energy (MJ/kg)	**10.9**	**10.9**	**10.9**

L: Low-protein content; M: Medium-protein content; H: High-protein content; [1] Composition of mineral-vitamin complement: vitamin B: 6.100 mg; folic acid: 200mg; vitamin D1: 200mg; biotin: 4mg; Choline chloride: 18mg; Co :40 mg; Fe: 4000 mg; Cu: 1000 mg; Mn: 2000 mg; Iodine: 80 mg; Zn: 6000 mg; Se: 8 mg; Mg: 26000mg; Sulphur: 6800mg.

Statistical analysis

Statistical analysis of the data was performed using the Statistical Analysis Systems Software (SAS, 2005). The data were analyzed using the mixed model procedure of SAS software (SAS, 2005) to identify significant sources of variation. To test the effects on production performances, two fixed effects were considered: diet (L, M, and H) and parity (first and second). The litter size was considered as a covariate in the model for the live weight, milk yield, feed intake, and mortality rates. The least-squares means were compared using Tukey HSD tests (SAS, 2005). The number of kits was compared between groups using a non-parametric procedure (Kruskal-Wallis test) (SAS, 2005). The significance threshold was set at $P<0.05$.

RESULTS

Live weight, feed intake and milk production of does

During the experimental period, the live weight of the does was not different regardless the protein level of the ingested feed ($p>0.05$). However, a significant difference in weight gain was noted at 21 days postpartum ($p<0.05$) and a trend towards significance in weight gain at weaning ($p=0.09$). Females that were fed the lowest protein diet tended to gain more weight (Table 2).

Table 2 - Effect of an increase in the protein content of the diet on the body weight of rabbit does and their feed intake (g) during their first two reproductive cycles (LSM ± SEM).

Parameters	Diets			Parity		P-value		
	L	M	H	P1	P2	Diet	Parity	Cov.
Number of observations	31	32	33	50	46	-	-	-
Live Weight at Parturition (g)[A]	3776±76.7	3859 ±74.0	3742±76.4	3640±62.6	3945±66.3	0.53	0.002	0.06
Live Weight During Lactation (g)								
1st Week [B]	4085±92.1	4111±83.5	3914±87.3	4022±76.7	4052±76.0	0.23	0.79	0.55
2nd Week [C]	4156 ±89.1	4124±78.6	3975±83.8	4068±72.0	4102 ±71.1	0.28	0.75	0.46
3rd Week [D]	4219±94.0	4146±82.9	3998±88.3	4219±75.8	4023±74.8	0.22	0.08	0.47
4th Week [E]	4280±86.6	4180±76.5	4054±81.4	4247±69.9	4095±69.1	0.17	0.14	0.085
5th Week (Weaning) [F]	4281±92.4	4199 ±81.6	4060±86.8	4273±74.6	4086±73.7	0.22	0.09	0.26
Weight Gain Parturition-3rd week [D]	407±51.5[a]	230±45.5[b]	328±48.4[ab]	491±41.5	153±50.0	0.04	<0.001	0.16
Weight Gain Parturition-Weaning [F]	467±59.1[a]	284±52.1[b]	391±55.4[ab]	542±47.7	220±47.1	0.07	<0.001	0.04
Feed Intake (g)								
1st Week [B]	1287±79.2	1414±69.9	1444±74.6	1364±64.3	1400±63.4	0.32	0.71	0.003
2nd Week [C]	1831±82.5	1734±74.4	1810±77.5	1804±67.3	1780±65.6	0.64	0.81	0.023
3rd Week [D]	1979±80.7	1943±72.8	1871±77.9	2067±65.8	1795±65.5	0.62	0.007	0.002
Total Feed Intake 21 d [D]	5102±197.7	5088±178.4	5150±191.1	5234±161.1	4993±160.4	0.97	0.311	0.007
4th Week [E]	2324±91.2	2251.58±82.3	2376±85.6	2416±74.4	2218±72.5	0.57	0.07	<0.001
5th Week (Weaning) [F]	2913±130.5	2768±117.6	2935±122.3	2756±106.5	2987±103.8	0.57	0.14	<0.001
Total Feed Intake 35 d [F]	10351±374.0	10090±337.3	10428±360.9	10414±305.2	10165±303.8	0.77	0.58	<0.001
Daily Feed Intake [F]	295.7 ±10.7	288.3 ±9.6	297.9±10.3	298±8.7	290±8.7	0.77	0.58	<0.001
Daily protein intake [F]	44.5±1.9[a]	50.7±1.8[b]	54.8±1.7[c]	50.4±1.6	49.5±1.6	<0.001	0,70	<0.001

L: Low-protein content; M: Medium-protein content; H: High-protein content; P: parity; Cov.: covariate; SEM: standard error of the mean; a, b, c: Means with different letters on the same row between diets (Low, Medium and High) and parities (P1 and P2) differ significantly (P<0.05); A Covariate: Total litter size at partum; B Covariate: Total litter size at 07 days; C Covariate: Total litter size at 14 days; D Covariate: Total litter size at 21 days; E Covariate: Total litter size at 28 days; F Covariate: Total litter size at 35 days.

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Table 3 - Effect of an increase in the protein (%) content of the diet on the milk production of local rabbits does during their first two reproductive cycles (LSM ± SEM)

Parameters (g)	Diets			Parity		P-value		
	L	M	H	P1	P2	Diet	Parity	Cov.
Number of observations	31	32	33	50	46	-	-	-
1st Week [B]	703±33	739 ±31	741±32	699±28	756±27	0.664	0.174	<0.001
2nd Week [C]	1083±46	1085±42	1063.±44	1062±38	1092±37	0.924	0.592	<0.001
3rd Week [D]	1217±49	1300±45	1265±47	1241±41	1280±39	0.484	0.515	<0.001
Lactation [D]	3016±122	3132±111	3080±116	3007±100	3144±97	0.785	0.352	<0.001

L: Low-protein content; M: Medium-protein content; H: High-protein content; P: parity. Cov.: covariate; SEM: standard error of the mean; [B] Covariate: Total litter size at 07 days; [C] Covariate: Total litter size at 14 days; [D] Covariate: Total litter size at 21 days.

Table 4 - Effect of increasing dietary protein (%) on rabbit litter performance over the first two reproductive cycles (LSM ± SEM)

Parameters	Diets			Parity		P-value		
	L	M	H	P1	P2	Diet	Parity	Cov.
Number of observations	31	32	33	50	46	-	-	-
Total Litter Size at								
Birth	6.74	6.91	7.00	6.02	7.83	0.890	<0.001	-
Live at Birth	5.13	6.12	5.66	5.10	6.24	0.463	0.013	-
1st Week	3.77	4.85	4.44	3.50	5.30	0.322	0.001	-
2nd Week	3.71	4.76	4.34	3.42	5.22	0.361	0.001	-
3rd Week	3.71	4.76	4.31	3.42	5.20	0.359	0.001	-
4th Week	3.71	4.76	4.28	3.40	5.20	0.353	0.001	-
5th Week (Weaning)	3.65	4.76	4.25	3.36	5.17	0.320	0.001	-
Mortality (%) at								
Birth [A]	25.2±5.28	11.8±5.10	18.1±5.17	16±4.34	20.77±4.55	0.193	0.457	0.800
Birth to Weaning [E]	31.1±7.06	24.8±6.49	30.7±6.82	34.9±5.39	22.85±5.71	0.990	0.823	<0.001
Total litter weight (g) at								
Birth [A]	394±11.2	420 ±10.8	392±10.9	394±9.1	410±9.6	0.142	0.253	<0.001
Live at Birth [A]	313±21.2[a]	382±19.9[b]	359±20.9[ab]	346±17.2	356±18.1	0.062	0.705	<0.001
1st Week [B]	658±25.8	674±22.7	685±24.2	648±20.8	698±20.6	0.748	0.107	<0.001
2nd Week [C]	1216±49.5	1172±43.3	1157±47	1132±40	1231±38.9	0.675	0.091	<0.001
3rd Week [D]	1717±69.5	1724±61.2	1694±66.4	1665±56.4	1759±54.9	0.942	0.254	<0.001
4th Week [E]	2640±110.8	2573±97.6	2591±105.8	2528±90.1	2675±87.6	0.900	0.265	<0.001
5th Week (Weaning) [F]	3835±149.2	3579±131.3	3786±142.3	3584±121.3	3883±118.1	0.380	0.091	<0.001

L: Low-protein content; M: Medium-protein content; H: High-protein content; P: parity; Cov.: covariate. SEM: standard error of the mean; a,b,c, Means with different letters on the same row between diets (Low, Medium and High) and parities (P1 and P2) differ significantly (P<0.05); [A] Covariate: Total litter size at partum; [B] Covariate: Total litter size at 07 days; [C] Covariate: Total litter size at 14 days; [D] Covariate: Total litter size at 21 days.; [E] Covariate: Total litter size at 28 days; [F] Covariate: Total litter size at 35 days

Of the total number of 52 nulliparous rabbits, 100% of the females was receptive. Also, the fertility rate found in the trial was 100%. No effect of parity on the weight of does was observed ($p>0.05$). However, parity's effect on weight gain was significant ($p<0.001$). During the experiment, no significant interaction was detected between the dietary protein content and the parity on live weight and weight gain of the does ($p>0.05$). There was no effect of litter size at weaning, used as a covariate, on the weight of does between parturition and weaning ($p>0.05$). On the other hand, the effect of the covariate on the weight gain of does was significant ($p<0.05$) over the same period. The protein content of the diet did not influence the milk yield produced ($p>0.05$). The effect of parity on milk production was not significant, although the quantity produced in the second lactation was higher by +5.4% (Table 3).

Litter size and weight

Regardless of the feed distributed, litter size, litter weight and nest mortality showed no significant difference between birth and weaning ($p>0.05$), except for a trend towards significance in live litter weight at birth ($p=0.06$); live litters fed the M feed were significantly heavier than those fed the lower protein L feed (Table 4). The effect of parity on litter size was significant ($p<0.05$) between parturition and weaning. Litters from second pregnancy were larger than those from the first pregnancy. On the other hand, there was no significant difference due to parity on still birth, nest mortality and litter weight at pre-weaning ($p>0.05$). However, a statistical trend ($p=0.09$) was found on litter weight at weaning. The effect of litter size under the dam was highly significant ($p<0.001$) and taken into account as a covariate to eliminate its effect on litter weight.

DISCUSSION

The choice of this topic was motivated by the availability of a unique feed for rabbits in the local market, which is distributed to all rabbits of different sex and/or physiological stages. This work has already been initiated on local nulliparous pregnant rabbits from the same population (Saidj et al., 2019); the results showed that variations in the protein content of the feed had no effect neither on the weights and intakes of the females during their first management, nor on their metabolic profile. However, the bibliography showed a considerable variation in the nutritional needs of does according to their production and reproduction cycles (Parigi-Bini and Xiccato, 1993; Pascual et al., 2003, Maertens and Coudert, 2006; De Blas and Mateos, 2020). Of the total number of nulliparous rabbits, the receptivity was higher than that found by Ilés (2015) on the same population, estimated at 69.2%, but on a greater number of observations (253). Also, the fertility rate found was higher than that measured by Fellous et al. (2012) on the same local population which was 85.9% in first parity. These same authors emphasize that among the local population, the fertility rate decreased with parity. In the second parity during the present experiment, females were presented to male at the 10th day post-partum and all the females were receptive. These results showed that the experimental breeding conditions were adequate with natural reproduction and without the use of synthetic hormones, but a number of does reduce.

Live weight, feed intake and milk production of does

The use of high protein diets doesn't show effect on local unselected rabbit doe's weight at partum and between partum and weaning, and consecutively on does' weight gain during lactation. Does given H diet showed significantly higher protein intake a day at lactation (58.08 g for H group vs. 52.94 g for M group vs. 44.34g for L group) ($p<0.01$), but no difference was detected in the feed intake a day between partum and weaning (294.5g for L group vs 311.1g for M group vs. 305.8 g for H group), the digestible energy intake a day and their milk yield. Brun and Lebas (1997) showed that the use of feed with high protein does not affect prolificacy. In this work, litter size and weight at partum and at weaning were not affected by the diets but the effect of litter size on milk production during 21 days post-partum was observed in all the three diets ($p<0.001$). Saidj et al. (2021) reported, on the same rabbit population and with the same diets, that lactation peak occurs at different times (16th, 20th and 20th day PP at first lactation and 15th, 17th and 20th days post-partum at second lactation for L, M and H groups respectively) and lactation peak tended to occur later.

Sanchez et al. (1985) reported that between 21 and 28 days of lactation, live weight was lower in rabbits fed a diet containing 17.5% CP than those fed a diet with 19 or 20.5% CP. According to Odi (1990), a decrease in body weight of rabbits could be expected at 21 days post-partum. Partridge et al. (1983) point out that the mobilization of body reserves starts at about 11 days and continues at the time of peak milk production between 18 and 21 days PP, coinciding with a period of catabolism of the body. This variation in female body weight at this time of reproduction is not always evident (Odi, 1990). The gradual increase in female body weight throughout the trial is not found by Sakr (2012) who observed a decrease in female rabbit body weight between the first parturition and day 25 postpartum. The results obtained in the present study showed that the female rabbits completed their growth progressively while ensuring their gestation and lactation simultaneously.

In growing rabbits, Ouhayoun and Cheriet (1983) observed that for a given feed energy concentration, the variation of protein content (17.2 and 13.8%) had no significant effect on the growth rate and the slaughter yield, which is also found in growing piglets. Also, in growing piglets, Quiniou et al. (1994) did not notice any difference in the protein content of the feed (17.8, 15.5 and 13.6% BW) on weight gain and carcass characteristics. However, carcass fatness was reduced in rabbits fed the highest protein diets (Ouhayoun and Cheriet, 1983). On the other hand, Ouhayoun and Dalmas (1983)

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found a significant increase in weight at 11 weeks of age and overall growth rate with increasing feed protein level (10.4, 13.8, and 17.2%).

According to Renouf et al. (2009), in fattening rabbits, the decrease in diet crude protein level from 16% to 14.5% had no effect on live weight, feed intake or animal mortality. However, with the highest phosphorus level, the decrease in protein level tended to reduce feed intake, while the opposite effect was observed with the lower phosphorus level.

In rabbits fed diets containing different protein levels (13.5%, 17.5% and 21% CP/kg DM), Partridge and Allan (1982) recorded a decrease in feed intake in females fed the lowest protein diet (13.5%) and an increase in milk production with an increase in the protein content of the diet, without any change in the chemical composition of the milk; in contrast to The present study results which show no effect of the dietary protein content on milk performance or feed intake of does.

In the present trial, the protein intake of rabbits increased with the protein level of the diet (L vs. M: +12.2%; L vs. H: +18.8%; $p < 0.001$) without affecting milk production. The present results corroborate those found in pigs. Indeed, studies have shown the absence of a significant influence of a severe protein ration on milk production and litter growth (Revell et al., 1998; Mejia-Guadarrama et al., 2002). Jang et al. (2014) tested on pregnant sows, 4 feeds with different protein contents (11%, 13%, 15% and 17%). The results showed no significant difference in the amount of milk produced, although the daily feed intake did not vary significantly during lactation, leading to a higher protein intake in sows fed the higher protein diet (+38% protein intake between the 11 and 17% CP diets).

On the other hand, amino acids can vary milk production. In fact, in rabbits, Taboada et al. (1994) observed a positive and significant effect of the lysine content of the feed on milk production, with a better result in females fed the 0.82% lysine feed compared to the 0.68% one, knowing that the protein content of the different feeds is 18% CP. In sows and rats, an intake of amino acids in the diet, during a period of growth when the mammary cells are still multiplying, stimulates the proliferation of these cells and consequently, increases the amount of milk produced (Knight and Peaker, 1984; Jansen and Binard, 1991; Luise et al., 2023). This difference in milk production is thought to be related to dietary amino acid intake and is not due to hormonal secretion, as lysine and methionine do not stimulate galactopoietic hormone secretion to any great extent (Kuhara et al., 1991). According to Rulquin (1992), in dairy cows, supplementation with certain amino acids such as methionine and lysine (with a 14% crude protein feed), increased milk production at the beginning of lactation. However, in mid-lactation, no increase in milk production was observed. Partridge and Allan (1982) found no change in the chemical composition of milk in rabbits fed diets with different protein levels (13.5%, 17.5% and 21%). On the other hand, in dairy cows, Rulquin (1992) has shown that the composition of milk, and more specifically the composition of milk proteins, can be improved by a complementary supply of certain amino acids such as methionine and lysine (14% of crude protein); during the beginning and middle of lactation, an increase in protein content is noticed.

This physiological phenomenon could be explained, in part, by the availability of the different amino acids that make up milk at the mammary level. In pregnant sows, Jang et al. (2014) using 4 feeds of different protein levels (11%, 13%, 15% and 17%) observed no difference in the chemical composition of colostrum (at day 01 postpartum), nor milk at day 21 of lactation. On the other hand, Zhang et al. (2011) reported a positive and linear effect of the lysine level of iso-protein feeds (13% crude protein and lysine levels of 0.46, 0.56, 0.65 and 0.74%) on the protein level of the colostrum (+5%), knowing that the females concerned ingested the same amount for the different groups.

In the current study, there was no effect of parity on milk production. These results do not corroborate those found by Xiccato et al. (2004) who determined an increase in milk production by 8% and 10% respectively of the rabbits in the second and third litter compared to the first, while equalizing the litters involved in the experiment. In this work, litter equalization was not performed. However, litter size, which had a highly significant effect ($p< 0.001$), was taken into account as a covariate to eliminate its effect on milk production.

Litter size has a strong influence on milk production during the two lactations studied ($p<0.001$). Indeed, according to Zerrouki et al. (2005), the milk production of the local population increases with the number of young until reaching 7 kits / litter where a production plateau is reached. Chibah-Ait Bouziad et al. (2015) confirm that regardless of the litter size at birth and/or the genetic type of the females, the increase in milk production per day and per 21 days follows the number of kits breastfed, even if they are adopted, without any relation to the number of kits at birth. In addition, the increase in milk production following the number of lactations is known in mammals, which is due to various influencing factors such as physiology, weight and maturity of the udder. In other species as prolific as the rabbit (such as sows and rats), litter size and/or parity are directly correlated to milk production with the growth of the mammary gland during lactation, which would cause a direct effect on the intensity of the mother's milk production (Farmer and Palin, 2005; Hurley, 2001).

Litter size and weight

The results obtained herein are similar to those of Brun and Lebas (1997) who showed that in crossbred rabbits (2066 X 1077) an increase in dietary protein from 14.9% to 20.6% DM had no effect on litter size at birth or weaning. Likewise, Partridge and Allan (1982) did not find that weaned litter size varied with dietary protein levels (13.5, 17.5 and 21 %). However, Brun and Lebas (1997) reported that the highest protein diet increased the average weight of a young rabbit at weaning (29 days) by 8.2% and the average litter size by 6.5%, in contrast to the lack of effect in the present

trial. Also, in the long term, female rabbits receiving the lower protein diet weaned more litters than females receiving the high crude protein diet (Brun and Lebas, 1997).

In Rex rabbits, Ren et al. (2004) recommend the use of a diet with 10.5 MJ/Kg of digestible energy and 17.5% crude protein for pregnant rabbits, which is favorable for improving the size and weight of total and live litters at birth. On the other hand, these same authors indicate that in lactating rabbits, the crude protein content should reach 19.5% for an energy content of 10.7 MJ/Kg, in order to improve the size and weight of litters at weaning. As for the effect of parity on prolificacy and litter size traits, the obtained results corroborate those obtained by Zerrouki et al. (2005) on the same population concerning litter size at birth. However, these authors did not find an effect of litter size under the dam, contrary to the present work results which show a significant effect of parity on litter size during lactation ($p<0.05$).

Furthermore, the positive effect of parity on litter weight at birth increased with the order of parity, with higher individual weanling weights in multiparous animals (Zerrouki et al., 2005). In crossbred rabbits (New Zealand X Californian), Xiccato et al. (2004) found no significant difference in litter size regardless of parity. On the other hand, litter weight at birth, at weaning and weight gain during the birth-weaning period increased significantly with parity (+7.7%, +10.3% and 6.7% respectively for the parameters mentioned).

CONCLUSION

The local unselected rabbit does don't require high level of protein to meet the needs of reproduction and production. Similarly, the size and weight of live litters and the mortality rate under the dam are not affected by the protein content of the diet throughout the pre-weaning period. Although there are no overall effects of the protein content of the feed on the zootechnical parameters of the rabbits, the females gained more weight by receiving more proteins, which can have a positive effect on the longevity of their reproductive career, thus avoiding negative and harmful energy balance on their production career.

DECLARATIONS

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Authors' contribution

All authors contributed equally to the study.

Availability of data

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

Consent to publish

All authors have reviewed and approved the final manuscript for publication.

Competing interests

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

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NUTRIENT PROFILE, PROTEASE AND CELLULASE ACTIVITIES OF PROTEIN EXTRACTED FROM BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE REARED ON VARIOUS SUBSTRATES

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ABSTRACT: The Black Soldier Fly (BSF; *Hermetia illucens*) larvae are recognized for their ability to convert diverse organic materials into protein-rich biomass, depending on the substrate they consume. The composition of these substrates can significantly impact the nutrient profile and enzyme activities of the resulting maggot protein extract (MPE). Therefore, this exploratory research aimed to assess the nutrient content, protease, and cellulase activity of MPE obtained from BSF maggots reared on different substrates, with a specific focus on substrates A (comprising restaurant waste and rejected milk), B (layer manure), and C (kitchen waste). The results showed that maggot meal from layer manure had the highest protein content (45.36%) and the lowest fat content (18.44%). Amino acids in maggot meal contained high levels of glutamic acid, aspartic acid, alanine, valine, leucine, and isoleucine. Lauric acids were found in maggot meal from kitchen waste (33.79%), layer manure (32.18%), and restaurant waste and rejected milk (22.94%). Maggot meal from layer manure had the highest oleic acid content (15.13%). The protein concentration of MPE from various substrates ranged from 0.56 to 0.601 mg/ml (at 60% w/v ammonium sulfate saturation) and 0.555 to 0.609 mg/ml (at 70% ammonium sulfate saturation). The protease activity of MPE from layer manure substrates exhibited optimum activity and stability in neutral to alkaline pH, with activity levels of 0.748 U/mg at pH 7.0 and pH 11.0 (at 60% w/v ammonium sulfate saturation) and 0.774 units/mg at 70% w/v ammonium sulfate saturation. The highest cellulase activity was found in MPE from kitchen waste, which remained stable at pH 5.0-11.0. In general, maggots from different substrate sources exhibited distinct nutrient profiles and enzyme activities. Protein extract from maggots grown in layer manure showed the most suitable nutrient profile for use as an alternative source of protein feed and protease enzymes.

Keywords: Amino acid, Chemical profile, Enzymes, Fatty acid, Maggot.

INTRODUCTION

Maggots are insect larvae that rapidly decompose waste substances, such as kitchen waste (Green and Popa, 2012), straw (Zheng et al., 2012; Liu et al., 2021), as well as manure (Lalander et al., 2013; Banks et al., 2014). The nutrient composition of the body and the survival rate of larvae are strongly influenced by the quality and quantity of larval development media (Gobbi et al., 2013; Makkar et al., 2014). The nutrient composition of the larval body and the survival rate of the larvae are profoundly shaped by the characteristics of the larval development media, both in terms of its quality and quantity. This influence has been noted in previous studies (Gobbi et al., 2013; Makkar et al., 2014) which emphasize that the nutritional makeup and overall well-being of the larvae are significantly dependent on the specific attributes and abundance of the media in which their development takes place.

Katayane et al. (2014) reported that the protein content and dry weight of larvae reared on palm kernel cake or hereinafter reffered to as palm kernel cake (PKC) media were higher than those reared on faecal dung media. This is presumably because the quality of poultry waste protein is lower due to high levels of non-protein nitrogen (NPN: Nitrogen compounds that are not part of proteins, often found in faecal matter) in faeces compared to the PKC (Arief et al., 2012). Several studies have reported that substrates with low nutritional quality will produce fewer Black Soldier Fly (BSF or Black Soldier Fly: A type of fly whose larvae are known for decomposing organic waste) larvae because the growth media contains fewer or limited nutrients (Tschirner and Simon, 2015; Suciati and Faruq, 2017; Maulana et al., 2021). The high production of organic waste in Indonesia is a potential source of substrates for rearing maggots. Maggot meal, characterized by its high protein and fat content, serves as a viable replacement for fish meal in poultry diets, offering an alternative source of nutrition for poultry.

The high protein and fat content of maggots, as highlighted by Rambet et al. (2015), underscores the advantages of using them as a feed source. It is reported that maggot meal (*Hermetia illucens*) contains nutrients, including 35-57% crude protein and 15-49% crude fat (Dordević et al., 2008; Bosch et al., 2014; Abduh et al., 2022), as well as 2.63% calcium and 0.28% phosphorus (Dengah et al., 2015). The quantities of amino acids and the types of fatty acids present in Black Soldier Fly (BSF) larvae are significantly influenced by the specific makeup of the fly's food (Shumo et al., 2019;

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Spranghers et al., 2017). At the same time, the composition of the larvae's diet has an impact on the diverse quantities of amino acids and fatty acids (Tschirner and Simon, 2015; Spranghers et al., 2017) and but not by the different substrate media where the larvae are grown.

The larvae are esteemed for their remarkable nutritional attributes, with a particular emphasis on the substantial quantities and exceptional quality of both their protein and fat content. This unique combination of protein and fat characteristics positions them as highly valuable constituents when incorporated into animal feed formulations, offering a distinct advantage in enhancing the nutritional quality of such feeds and contributing to the overall health and well-being of the animals that consume them (Wang et al., 2017; Wang and Shelomi, 2017; Gold et al., 2018). Maggot flour derived from BSF larvae is considered a viable and appropriate component for animal feed due to its rich content of crucial amino acids, essential fats, and calcium, all of which are vital for promoting and sustaining the growth of livestock. However, the high chitin content in maggot flour has rendered it unpopular as poultry feed. Chitin, a polymer of N-acetyl-D-glucosamine and a small amount of D-glucosamine, is challenging for poultry to digest (Rinaudo, 2006; Hahn et al., 2018; Van Huis, 2020). Additionally, chitin constitutes 8-24% of maggot biomass (Dossey et al., 2016; Soetemans et al., 2020). Numerous researchers have observed that incorporating insect-derived elements into animal feed typically leads to a decrease in the performance of poultry. Elevating the proportion of maggot meal within the animal feed, particularly when reaching levels of 10% and beyond, has been observed to lead to a decrease in the average daily gain (ADG) in avian species, as documented by (Moula and Detilleux, 2019). This dietary adjustment has also been associated with a reduction in egg weight and feed consumption, while simultaneously resulting in an increase in feed conversion rates, as demonstrated in Widjastuti et al. (2014). Hence, the separation of chitin from maggot biomass via protein extraction is a required step.

The BSF larvae's capability to transform organic waste can be attributed to their innate aptitude for generating both protease and cellulase enzymes within their gastrointestinal system. These enzymes enable them to effectively break down proteins and cellulose present in the organic matter used as their rearing substrate. A multitude of hydrolytic enzymes are synthesized by diverse bacterial populations inhabiting the gastrointestinal tract of BSF larvae (Dong et al., 2009; Yu et al., 2011). In the study conducted by Kim et al. (2011), it was revealed that the digestive extracts obtained from BSF larvae's gastrointestinal system exhibited elevated levels of amylase, lipase, and protease enzymatic activities. Maggot protein extract contains proteases and cellulases, which, when added to feed, are expected to enhance feed digestion in the poultry digestive tract and improve feed efficiency. However, there has been limited information regarding protease and cellulase activity in protein extracts. Given this, this study aims to explore protein extracted from BSF larvae, along with its essential nutritional profiles, protease, and cellulase activity. The extracted protein is further investigated for its specific use as poultry feed. The application of maggot protein extract as a feed supplement serves a dual purpose, acting both as a protein supplement and as hydrolytic enzymes that optimize the digestion and absorption processes in the poultry digestive tract.

This study ventures into this uncharted domain, aiming to uncover the intricate interplay between the specific protein compositions inherent in black soldier fly (BSF) larvae and their suitability as a functional feed. Diverging from conventional research approaches that predominantly focused on broader facets such as nutrient content, enzymatic activities, and the overall nutritional profile of the larvae, our investigation hones in on the specific exploration of protein profiles. By shedding light on these profiles and their potential implications in the domain of functional feed, this research aspires to establish a foundational comprehension that may herald innovative strategies in animal nutrition and feed development. This shift in emphasis towards the nuanced intricacies of protein profiles represents a valuable opportunity to delve deeper into uncharted realms and expand our understanding of the black soldier fly larvae's potential role as a substantive protein source in animal diets.

MATERIALS AND METHODS

This exploratory research was conducted using BSF maggots reared in various substrates, categorized into three groups: A (restaurant waste and rejected milk), B (laying hens' manure), and C (kitchen waste). The subjects under observation were BSF maggots within the age range of 10 to 15 days, a phase corresponding to their pre-pupae development, with a physical length ranging from 10 to 15 millimeters. The research implementation has followed the procedures outlined in the research guidelines provided by the Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia in 2022, as stated in the Decision Letter (SK) number 2127/UN23/PT.01.02/2022. The study followed the guidelines for the ethical treatment of animals as set forth, ensuring that no undue harm or stress was caused to the organisms involved.

Nutrient content of maggot meal

Fresh maggots were subjected to a series of meticulous preparation steps to render them into a dry and analyzable form. This involved an initial cleansing process in running water, followed by a careful drying phase at a temperature of 60°C, which spanned a duration of 24 to 48 hours. Subsequently, the dried maggots were finely ground into a powder-like consistency, facilitating further nutritional assessment. The evaluation of nutrient levels was executed employing a

comprehensive analytical approach. Firstly, the proximate method as stipulated by the Association of Official Agricultural Chemists (AOAC, 2019) was employed for the proximate analysis, providing insights into fundamental nutritional components. Additionally, the quantification of fatty acids was conducted using the sophisticated Gas Chromatography (GC) technique, which specializes in the separation and measurement of volatile compounds within the sample. Furthermore, the examination of amino acids was carried out through the application of High-Performance Liquid Chromatography (HPLC), a specialized analytical method designed for the separation and quantification of compounds within liquid samples. These analytical methodologies collectively offered a comprehensive understanding of the nutritional composition of the processed maggot material.

Production of maggot protein extract (MPE)

The extraction and preparation of the maggot-derived protein involved a series of methodical steps. Approximately 50 grams of freshly collected maggots, roughly 15 days old, were initially immersed in a phosphate buffer solution with a concentration of 100 millimolar (mM) and a pH value of 7.2. To facilitate the subsequent separation of the exoskeletal components from the valuable body fluids, the maggots were thoroughly crushed using a mortar. The body fluids, now freed from the exoskeletal remnants, underwent further refinement. They were subjected to centrifugation at 2000 revolutions per minute (rpm) at a temperature of 4°C for a duration of 10 minutes. The resulting supernatant was subsequently treated with ammonium sulfate solutions at concentrations of 70% and 80% (v/w), causing protein precipitation. Following this step, another centrifugation process was carried out at a speed of 10,000 rpm, still maintaining a temperature of 4°C, and lasting for 30 minutes. The outcome of this procedure was the collection of a protein-rich precipitate, which was then reconstituted by suspension in a phosphate buffer solution (100 mM, pH 7.2). This resulting extract was identified as Maggot Protein Extract (MPE), stemming from the larvae of the maggots, and was thoughtfully preserved in a refrigerated environment, ensuring its integrity and stability for subsequent analytical investigations.

Protein and enzyme assay

The quantification of protein concentration within the MPE was carried out employing the Bradford method, utilizing bovine serum albumin (BSA) as the reference standard protein, following the protocol outlined by (Waterborg, 2003). To determine the protein content, precisely 0.1 milliliters of the MPE sample was meticulously combined with 2.9 milliliters of the Bradford reagent. The resulting mixture was thoroughly vortexed and left to incubate for a duration of 3 minutes to facilitate the protein-reagent interaction. Subsequently, the absorbance of the prepared sample was assessed at a wavelength of 595 nanometers, yielding valuable data regarding the protein concentration within the MPE.

The determination of protease activity was conducted in accordance with the procedure outlined by Walter (in Matthews, 1987), and it was performed as follows:

1. Initially, a volume of 0.1 milliliters of Maggot Protein Extract (MPE) was mixed with 0.25 milliliters of a 1% casein solution in distinct pH buffers, specifically 50 millimolar (mM) citrate-phosphate buffers at pH 5.0 and 6.0, 50 mM Tris-Cl buffers at pH 7.0, 8.0, and 9.0, and 50 mM Borate-NaOH buffers at pH 10 and 11.0.
2. After this preparation, the mixture was incubated for a duration of 10 minutes at a temperature of 40°C.
3. To halt the enzymatic reaction, 0.5 milliliters of 0.1 M Trichloroacetic acid (5%) was introduced, followed by a subsequent incubation at 37°C for 10 minutes.
4. The resulting mixture was then subjected to centrifugation at 10,000 revolutions per minute (rpm) for a period of 5 minutes.
5. A volume of 0.75 milliliters of the supernatant was combined with 2.5 milliliters of 0.4 M Na2CO3 and 0.5 milliliters of Folin Ciocalteau reagent in a 1:4 ratio. This new mixture was incubated at 37°C for 20 minutes to induce color development.
6. Subsequently, the absorbance of the solution was measured at a wavelength of 578 nanometers.
7. For quantitative analysis, a standard curve was established using tyrosine as a reference. One unit of enzyme activity was defined as the amount of enzyme capable of liberating 1 millimole of tyrosine within a span of 1 minute, as per the generated tyrosine standard curve.

The determination of cellulase activity was executed in accordance with the methodology outlined by Camassola and J.P. Dillon (2012). The procedure is detailed as follows:

1. A circular piece of Whatman paper No. 1, measuring 25 millimeters in diameter, was positioned atop 0.25 milliliters of Maggot Protein Extract (MPE).
2. Subsequently, the setup was supplemented with 0.5 milliliters of diverse pH buffers, including 50 millimolar (mM) citrate-phosphate buffers at pH 5.0 and 6.0, 50 mM Tris-Cl buffers at pH 7.0, 8.0, and 9.0, and 50 mM Borate-NaOH buffers at pH 10 and 11.0.
3. The resulting solution was subjected to an incubation period lasting 60 minutes, maintained at a temperature of 40°C.

4. To arrest the enzymatic reactions, 1.5 milliliters of DNS (3,5-dinitrosalicylic acid) solution, prepared in a 1:4 ratio, was added.

5. The reaction was brought to a halt by boiling the reaction tube for 5 minutes and then immediately immersing it in cold water to rapidly cool it down.

6. For quantitative analysis, a standard curve was constructed using glucose as the reference. In this context, one unit of enzyme activity was defined as the amount of enzyme capable of liberating 1 millimole of glucose within a time frame of 1 minute, as determined by the generated glucose standard curve.

Statistical Analysis

For each sample, a comprehensive analysis was conducted, with every measurement carried out in triplicate to ensure the reliability of the results. The data is subsequently presented in the form of means ± standard error of the mean (SEM). The SEM, or Standard Error of the Mean, serves as a valuable indicator of the variability present within the dataset, and it is frequently employed to convey the precision associated with the calculated mean value.

RESULTS AND DISCUSSION

The Nutrient Profile of maggot meal

Protein profiles were analyzed using the NATIVE-PAGE discontinuous system on a 12% gel for separation. Protein samples were prepared by mixing protein levels and sample buffers at a ratio of 20 µl to 80 µl. Electrophoresis was carried out at a voltage of 100 volts with a constant current of 80 mA for 95 minutes. The distribution of bands was determined by staining silver-stained gels (Khoiriyah and Fatchiyah, 2013) using the Vivantis Protein Ladder (Tricolor Broad Range Prestained Protein Ladder) as a marker. The results of protein electrophoresis from three maggot sources showed the presence of distinct protein bands with estimated molecular weights of 95 kDa and 36 kDa, along with varying enzyme activity.

A thorough examination of the nutrient composition, as illustrated in Table 1, revealed discernible distinctions among three distinct types of maggots raised on varying substrates. This table presents a comprehensive overview of the nutritional content, highlighting the following key findings: Crude Protein: The maggots reared in layer manure exhibited the highest crude protein content, boasting a substantial 45.36%. In contrast, those cultivated on kitchen waste substrates displayed the lowest crude protein content, measuring in at 30.91%. Fat Content: The highest fat content, standing at 31.97%, was identified in maggots nurtured in kitchen waste. Conversely, the lowest fat content, accounting for 18.44%, was observed in maggots originating from layer manure. Crude Fiber: The analysis revealed a notably high crude fiber content, representing the cell wall components, in maggot meal sourced from larvae raised in restaurant waste (19.80%) and rejected milk substrates (19.62%). In contrast, the lowest crude fiber content, amounting to 10.14%, was derived from maggots reared on kitchen waste. Mineral Content: When considering mineral content, maggot meal obtained from kitchen waste exhibited the highest mineral concentration, reaching 21.80%. In stark contrast, the lowest mineral content, totaling 14.04%, was associated with maggot meal sourced from restaurant waste and rejected milk substrates. Nitrogen-Free Extract: The nitrogen-free extract content, which reflects carbohydrates, showcased relatively similar levels across the different maggot samples, indicating minimal variability in this particular nutritional aspect.

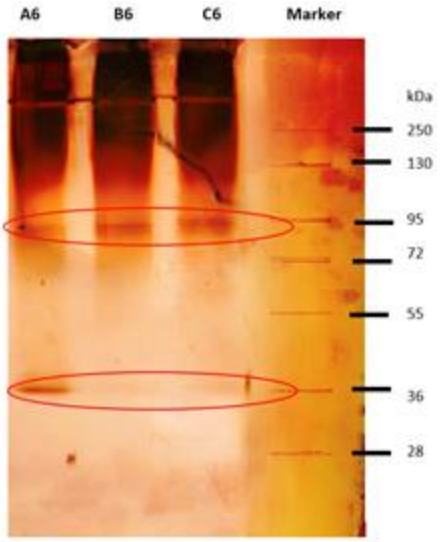

Figure 1 - Protein profile of maggot from three source of growing media (Marker contains proteins with BM 28 to 250 kDa) (A 6: Maggot Protein Concentrate (Restaurant waste and rejected milk at 60% Ammonium sulfate saturation); B 6: Maggot Protein Concentrate (Layers manure at 60% Ammonium sulfate saturation); C 6: Maggot Protein Concentrate (Kitchen waste at 60% Ammonium sulfate saturation) (C))

Table 1 - The nutrient content of maggot meal from rearing various substrates

Nutrient \ Maggot substrates	Restaurant waste and rejected milk (A)	Layers manure (B)	Kitchen waste (C)
Dry Matter (%)	96.11 ± 0.05	95.37 ± 0.06	95.38 ± 0.11
Crude Protein (%)	43.33 ± 0.08	45.36 ± 0.25	30.91 ± 0.63
Crude Fat (%)	22.29 ± 0.06	18.44 ± 0.11	31.97 ± 0.35
Crude Fiber (%)	19.80 ± 0.14	19.62 ± 0.11	10.14 ± 4.97
Ash (%)	14.04 ± 0.03	16.15 ± 0,.14	21.80 ± 0.71
NFE (%)	0.54 ± 0.10	0.42 ± 0.12	0.56 ± 0.11

NFE= Nitrogen-Free Extract: A measure of the carbohydrate content in a sample.

Variations in protein content and other nutrients closely reflect the nutrient availability of maggot-growing substrates. The layer manure substrates showed a high level of consistency compared to the others, which was attributed to the fact that the nutrient composition of commercial layer feed did not change during maintenance. The feed conversion ability of maggots is highly dependent on the availability of nutrient-rich substrates for growth. As elucidated by Gobbi et al. (2013) and Makkar et al. (2014), it has been established that the characteristics of the rearing medium play a pivotal role in determining both the nutritional quality and quantity of the body constituents in fly larvae at various developmental stages. Furthermore, these factors exert a considerable influence on larval survival during each instar and their subsequent progression through the metamorphic stages. Discrepancies observed in the nutrient content of rearing substrates, with particular attention to variations in carbohydrate and protein concentrations and their interplay, have the potential to induce notable adaptations in the behavior and physiological responses of insects (Simpson et al., 2015). The adaptability of the midgut in *H. illucens* larvae enables them to thrive on a multitude of feeding substrates by effectively adjusting to varying nutrient compositions (Bonelli et al., 2020). The same patterns of amino acid levels were observed in three types of maggot meal derived from larvae grown in different sources of media. In this investigation, a set of vital amino acids, encompassing valine, leucine, isoleucine, phenylalanine, methionine, threonine, histidine, lysine, and arginine, were discerned and acknowledged. Conversely, among the amino acids analyzed, aspartic acid, glutamic acid, serine, alanine, and tyrosine were identified as non-essential. The six amino acids with the highest levels in maggot flour were glutamate, aspartate, alanine, valine, leucine, and isoleucine. Meanwhile, the highest essential amino acid was leucine, and the lowest was methionine.

Essential branched-chain amino acids, commonly referred to as BCAAs, are a subgroup of indispensable amino acids, specifically isoleucine, leucine, and valine, that play a pivotal role in various biological processes, particularly in the context of livestock nutrition. These BCAAs are noteworthy for their unique characteristics, including being synthesized primarily within muscle tissues, a process integral to their function. One of their prominent functions in livestock is to act as safeguards against muscle tissue damage. When animals engage in activities that can exert significant stress on their muscles, such as physical exercise or growth, these BCAAs are essential in preventing the breakdown of muscle tissue. They do so by serving as a readily available energy source during periods of heightened demand, reducing the need for the body to break down muscle protein for energy. In essence, BCAAs act as a protective barrier, preserving the structural integrity of muscle tissue and preventing unwarranted muscle loss.

Table 2 - Amino acids of maggot meal rearing on various substrates

Maggot substrates / Amino acid (%)	Restaurant waste and rejected milk (A)	Layers manure (B)	Kitchen waste (C)
Aspartic acid	3.35	3.29	3.57
Glutamic acid	4.56	3.5	11.50
Serine	1.40	1.59	1.50
Histidine	0.66	0.82	1.00
Glycine	1.82	3.49	1.80
Threonine	1.72	1.38	1.43
Arginine	1.90	1.56	1.76
Alanine	2.37	2.43	3.11
Tyrosine	2.02	2.17	2.00
Methionine	0.26	0.13	0.05
Valine	2.29	2.40	2.46
Pheylalanine	1.57	1.52	1.65
l-Leucine	1.74	1.72	2.03
Leucine	2.56	2.56	2.65
Lysine	1.86	1.66	1.55
Total Amino Acid	30.07	30.20	38.06

Additionally, BCAAs are utilized to balance hormone release and brain function (Chasanah et al., 2015). The highest non-essential amino acid is glutamic acid, which, according to Winarno (2004) it was determined that the most abundant among the non-essential amino acids is glutamic acid. This particular amino acid has been noted for its significant role in supporting and enhancing various aspects of brain function. Notably, glutamic acid is associated with the facilitation of learning processes and the enhancement of memory, underscoring its importance in cognitive functioning. Glutamic acid also promotes an increase in muscle mass. Both glutamic acid and glutamine are interconvertible (Dutta et al., 2013), while among the non-essential amino acids, only glutamic acid is classified within the major group of neurotransmitters (Shih et al., 2005). Glutamic acid serves as a conjugate due to its capacity to enhance the effectiveness of an anti-cancer

medication while concurrently reducing its harmful impact on healthy cells. Dutta et al. (2013) highlighted the critical role of glutamine in maintaining optimal immune functionality. They emphasized that glutamine is essential for supporting the proliferation of lymphocytes and cytokines, crucial elements in immune system regulation. Furthermore, glutamine enhances the effectiveness of macrophages, which are substantial immune cells responsible for the ingestion and breakdown of various foreign materials, encompassing microbes and even inorganic compounds.

Elwert et al. (2010) compared the amino acid patterns of fish meals with reduced-fat BSF meal (BSF-37) and reported a relatively similar pattern. Through an examination of amino acid profiles relative to lysine, it was apparent that the levels of isoleucine, leucine, threonine, valine, phenylalanine, and arginine were notably more abundant in black soldier fly (BSF) meal when compared to fish meal. However, a significant contrast was noted in the histidine content, with black soldier fly (BSF) flour exhibiting a relatively lower concentration of methionine in comparison to that found in fish meals. This difference in amino acid composition underscores the distinct nutritional attributes of BSF meal in relation to its histidine and methionine content when contrasted with traditional fish meal.

Table 3 - Fatty acids of maggot meal rearing on various substrates

Maggot substrates / Fatty Acid (%/%, w/w)	Restaurant waste and rejected milk (A)	Layers manure (B)	Kitchen waste (C)
Butyric acid	-	0.05	-
Caproic acid, C6:0	0.18	-	-
Caprilic acid, C8:0	0.65	0.07	-
Capric acid, C10:0	0.47	0.90	0.58
Lauric acid, C12:0	22.94	32.18	33.79
Myristic acid, C14:0	5.4	4.56	7.73
Myristoleic acid, C14:1	0.05	0.06	0.12
Tridecanoic acid, C 13:0	-	-	0.06
Pentadecanoic acid, C15:0	0.11	0.28	0.52
Palmitic acid, C16:0	19.70	9.68	13.92
Palmitoleic acid, C16:1	1.16	3.11	-
Heptadecanoic acid, C17:0	0.08	0.17	0.21
Cis-10-Heptadecanoic acid, C17:1	0.05	0.18	0.32
Stearic acid, C18:0	1.76	1.39	2.47
Elaidic acid, C18:1, n9c	0.15	0.42	0.14
Oleic acid, C18:1, n9c	12.58	15.13	10.33
Linoleic acid, C18:2, n6c	0.09	0.05	6.23
Arachidic acid: C20:2	0.35	8.35	0.07
Gama-linolenic C18:3, n6	0.09	0.06	0.11
Linolenic acid C18:3, n3	0.03	-	0.51
Heneicosanoic acid, C21:0	0.23	0.15	-
Cis 11,14 Eicosedienoic acid, C20:2	0.15	0.03	0.13
Cis-11-Eicosenoic acid, C20:1	-	-	0.16
Behenic acid, C22:0	0.03	-	-
Cis 11, 14, 17-Eicosatrienoic, C20:3, n6	0.02	0.02	0.20
Cis-5,8,11,14,17-Eicosapentaenoic acid, C20:5, n3	-	-	0.38
Cis-4,710,13,16,19-Docosahexaenoic acid, C22:6, n3	-	-	0.13
Arachidonic acid, C20:4, n6	-	0.17	0.40
Lignoceric acid C24:0	-	0.01	0.02
Nervonic acid (C24:1, n-9)	0.02	0.09	-
Total Fatty acid	66.30	77.13	-

As illustrated in Table 3, an in-depth analysis of the composition of major fatty acids within maggot meal derived from a range of substrates unveils several notable components. These prominent fatty acids encompass lauric acid (C12:0), known for its distinct characteristics, as well as palmitic acid (C16:0), oleic acid (C18:1), palmitoleic acid (C20:1), and stearic acid (C18:0), each playing a distinct role in the overall lipid profile of the maggot meal. Linoleic acid (C18:2) was only found in maggot meal from kitchen waste (C), while arachidic acid (C20:2) was observed only in maggot meal from layer manure (B). It's apparent that lauric acid stands out as the predominant fatty acid present in all three variations of maggots cultivated on diverse substrates. The lowest concentration of lauric acid was observed in maggot meal from restaurant waste and rejected milk (A), while the highest was in maggot meal from kitchen waste (C).

Apart from the presence of medium-chain saturated fatty acids, it's worth noting that maggot oil also exhibited relatively high concentrations of unsaturated fatty acids, particularly oleic acid. The percentage of oleic acid in maggot oil

ranged from 10.33% to 15.13%, underscoring the diversity of fatty acid components within this oil. The highest concentration of oleic acid was found in maggot meal derived from layer manure (B), making it a potentially better source of fatty acids than others. It is noteworthy to recognize that the fatty acid content within the larvae is not solely an intrinsic characteristic but, rather, is subject to partial modulation by the specific fatty acid composition present in the substrate on which these larvae are nurtured. This interaction between the dietary source and the resultant fatty acid content in the larvae underscores the complex and interconnected relationship between the nutritional environment and the composition of the organisms that develop within it. Lauric acid may also exhibit potential therapeutic properties to boost the body's immune system. When enzymes in the body hydrolyze lauric acids, they produce a bioactive compound known as monolaurin. Monolaurin has been the subject of scientific studies for its potential antifungal, antibacterial, and antiviral properties, suggesting that maggots could be a valuable source of antimicrobial agents. Numerous studies conducted by various researchers have explored fatty acids encompassing carbon chains ranging from 6 to 18 carbons, as well as select derivatives featuring diverse functionalized headgroups. Through this extensive investigation, it was conclusively determined that lauric acid (LA) with the molecular notation C12:0 emerged as the most potent antimicrobial lipid, effectively impeding the growth of Gram-positive bacteria (Schlievert et al., 1992; Subroto and Indiarto, 2020; Yoon et al., 2018).

Oleic acid, known as omega-9 fatty acids, is not an essential fatty acid and can be synthesized in the human body from stearic acid through a reaction catalyzed by D9-desaturase, unlike omega-3 and omega-6 fatty acids (Delgado et al., 2017). Studies by Schwingshackl and Hoffmann (2014) reported that dietary monounsaturated fatty acids, including oleic, gondoic, and nervonic acid, reduced the overall risk of all-cause mortality (11%), cardiovascular mortality (12%), cardiovascular events (9%), and stroke (9%).

Protein concentration and activity of protease and cellulase of MPE

The Bradford protein assay finds application in the quantification of total protein concentration within a given sample. The fundamental principle governing this assay is based on the interaction between protein molecules and Coomassie dye in an acidic environment, leading to a discernible color transition from brown to blue. The protein concentration of MPE ranged from 0.56 mg/ml to 0.601 mg/ml under 60% (w/v) ammonium sulfate precipitation, and from 0.555 mg/ml to 0.609 mg/ml under 70% (w/v) ammonium sulfate precipitation. The concentration of MPE protein appeared to be relatively consistent between both 60% and 70% ammonium sulfate precipitation. However, MPE derived from restaurant waste and rejected milk exhibited the lowest protein concentration.

Table 4 - Protein concentration of mpe from various source

Protein (mg/ml) / Substrates	60% (w/v) ammonium sulphate saturation	70% (w/v) ammonium sulphate saturation
Restaurant waste and rejected milk (A)	0.560 ± 0.0009	0.555 ± 0.0014
Layers manure (B)	0.601 ± 0.0185	0.609 ± 0.0002
Kitchen waste (C)	0.601 ± 0.0023	0.609 ± 0.0173

Protease, an enzyme with extensive applications, is commonly harnessed within the food industry to expedite the hydrolysis of peptide bonds present in protein molecules. This enzymatic action contributes to the enhancement of product quality and augments the nutraceutical value (Palsaniya et al., 2012). The diversity of proteases has implications for the molecular weight (MW or Molecular Weight: The average mass of molecules in a sample) and the amino acid sequence of the peptides they produce, resulting in different biological activities. The digestive system of *Hermetia illucens* larvae is equipped with protease enzymes, which empower them to effectively break down a wide range of organic materials and subsequently convert them into valuable protein resources (Kim et al., 2011).

The microbiome residing within the gut of Black Soldier Fly Larvae (BSFL) and the external bacterial communities associated with them demonstrate the capacity to generate and release microbial enzymes, including proteases, cellulases, lipases, xylanases, and pectinases. These enzymes play a crucial role in the breakdown of organic compounds found in animal manure. It seems that the majority of proteins and other organic substances within animal waste are degraded through the action of these digestive enzymes. These findings imply that the richness and diversity of microorganisms in animal waste play a substantial role in influencing the BSFL's proficiency in decomposing various types of organic waste (De Smet et al., 2018).

Measuring protease activity at various pH and a temperature of 40°C produced the following results. The optimum protease activity of MPE obtained from restaurant waste and rejected milk (A) was shown at pH 5.0, both at 60% and 70% ammonium sulfate precipitation, namely 0.451 U/mg and 0.656 U/mg, respectively. The optimum protease activity

of MPE (0.748 U/mg) from layer manure was observed at pH 7.0 (precipitation of 60% ammonium sulfate), and at 70% deposition of ammonium sulfate, the optimum activity was shown at pH 11.0, namely 0.774 U/mg. Meanwhile, the activity of protease MPE from kitchen waste showed optimal activity at pH 10.0 at either 60% or 70% ammonium sulfate precipitation, namely 0.617 and 0.821 U/mg, respectively (Figure 2).

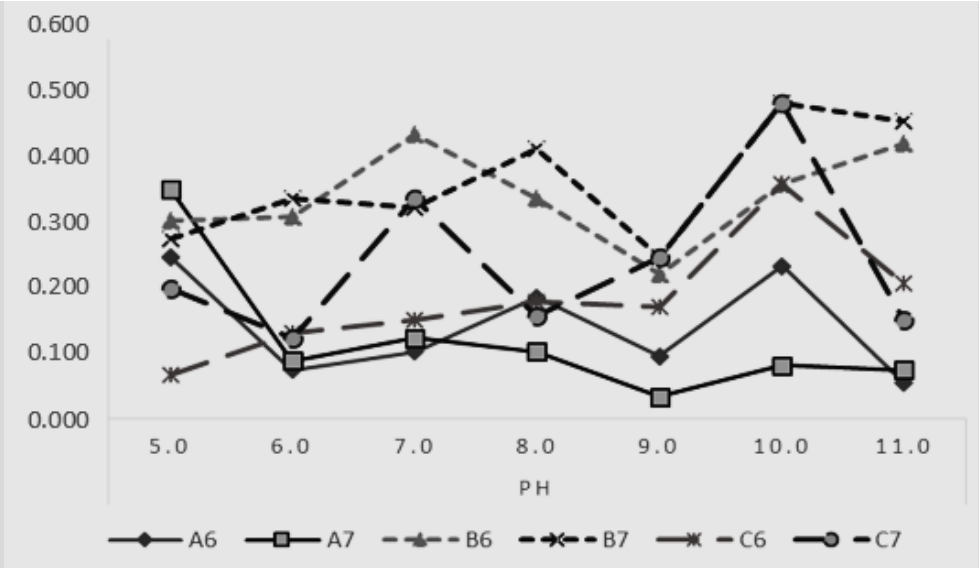

Figure 2 - Protease Activity of Maggot Protein Extract (MPE) From Various Substrates on various pH at 40° C. (A, MPE from restaurant waste and rejected milk; B, MPE from layer manure; C, MPE from kitchen waste; numbers 6 and 7 refer to 60% and 70% ammonium precipitation).

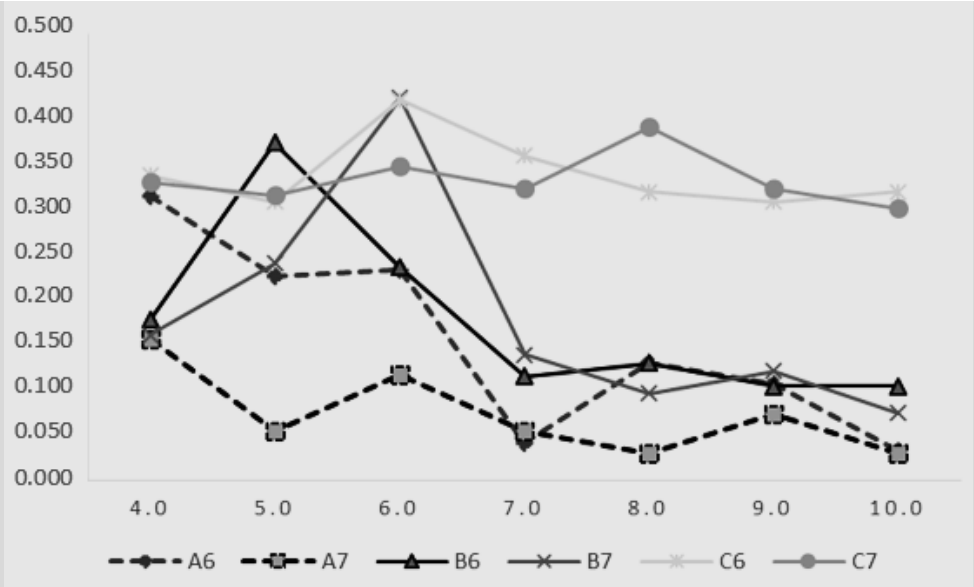

Figure 3 - Cellulase Activity of Maggot Protein Extract (MPE) From Various Substrates on various pH at 40° C. (A, MPE from restaurant waste and rejected milk; B, MPE from layer manure; C, MPE from kitchen waste; numbers 6 and 7 refer to 60% and 70% ammonium precipitation).

Supriyatna andUkit (2015) reported that the activity of protease enzymes from intestinal extracts of *H. illucens* larvae grown on rice straw media and incubated at various temperatures of 30°C, 35°C, 40°C, 45°C, and 50°C were 0.57 U/ml, 0.67 U/ml, 0.87 U/ml, 0.96 U/ml, 0.77 U/ml, respectively. At 45°C, the protease enzyme reached the optimum temperature to hydrolyze the substrate with an activity value of 0.96 U/ml. The difference in protease activity in this study was attributed to the sampling process, which involved extracting not only the digestive tract (gut) but the entire internal organs of the whole body. The optimal activity of protease tended to be acidic in maggots grown in restaurant waste and rejected milk (A), neutral in layer manure (B), and basic in kitchen waste (C). Digestive enzymes in maggot extract are mainly derived from the salivary glands and gut (Kim et al., 2011). Therefore, the fact that MPE contains various types of proteases from the digestive tract with various properties is comprehensible. Bonelli et al. (2019) serine proteases, which

are endopeptidases optimized for alkaline pH conditions, assume a primary role in the initial stage of protein breakdown within the posterior segment of the black soldier fly (BSF) larval midgut. Insects commonly feature two primary serine proteases, known as trypsin and chymotrypsin (Walter R. Terra and Ferreira, 1994; Walter Ribeiro Terra et al., 1996; Bonelli et al., 2020) found in the posterior midgut of larvae (Bonelli et al., 2020). Lysozyme is an acidic protease generated within the luminal environment of the anterior and middle segments of the midgut tract. It potentially serves a significant function in eradicating pathogenic microorganisms that may be introduced into the digestive system through the ingested feeding substrate (Bonelli et al., 2019).

Maggot protein extract (MPE) obtained from various substrates exhibited cellulolytic activity, with the highest activity observed in kitchen waste substrates (C) at both 60% and 70% ammonium sulfate (Figure 3). While cellulase from C substrates appeared stable over a wide pH range of 5.0-11.0, that from B substrates (layer manure) was only optimal at pH 5.0 (60% ammonium sulfate) and 6.0 (70% ammonium sulfate). The lowest cellulase optimum activity was clearly observed in A substrates (restaurant waste and rejected milk) at pH 4.0 and 5.0. These varied results support the notion that rearing substrates affect cellulase activity and that kitchen waste contains higher amounts of crude fiber than layer manure, restaurant waste, and rejected milk.

The gut of Black Soldier Fly (BSF) larvae serves as a thriving ecosystem for a diverse array of cellulolytic bacteria, with cellulase production being primarily attributed to genera like *Bacillus* sp., *Bacillus thuringiensis*, *Ruminococcus* sp., and *Proteus* sp., (Supriyatna and Ukit, 2015). Among these, the *Ruminococcus* genus stands out, particularly certain species such as *R. albus* and *R. flavefaciens*, which exhibit the remarkable capability to ferment highly structured cellulose fibers and generate cellulase (Shweta, 2012).

Bacillus sp., a well-known genus of bacteria, is renowned for its capacity to secrete a wide spectrum of enzymes, including cellulolytic enzymes. The cellulases produced by Bacillus species are predominantly extracellular and soluble in nature. Notable strains within the Bacillus genus that are recognized for their cellulase secretion encompass *B. subtilis*, *B. polymyxa*, *B. licheniformis*, and *B. cereus*. This multifaceted enzymatic activity further underscores the significance of these cellulolytic microorganisms within the BSF larval gut environment.

CONCLUSION

In conclusion, this study investigated the nutrient profiles, protease, and cellulase activities of maggot protein extract (MPE) from *Hermetia illucens* larvae reared on different substrates. The key findings are as follows:

1. Nutrient profiles: The nutrient composition of maggot meal varied significantly depending on the rearing substrate. Maggot meal from layer manure had the highest protein content (45.36%) and the lowest fat content (18.44%). Amino acid profiles also differed among substrates, with maggot meal from kitchen waste containing high levels of glutamic acid and maggot meal from restaurant waste and rejected milk having notable lauric acid content.
2. Protein concentration: The protein concentration of MPE from different substrates showed a relatively consistent range, ranging from 0.555 mg/ml to 0.609 mg/ml, with layer manure-based MPE exhibiting the highest concentration.
3. Protease activity: The protease activity of MPE varied with pH and substrate source. MPE from layer manure had the highest protease activity at pH 7.0 and pH 11.0, while MPE from restaurant waste and rejected milk had its optimum activity at pH 5.0.
4. Cellulase activity: Kitchen waste-based MPE displayed the highest cellulase activity and remained stable over a wide pH range (pH 5.0-11.0), while MPE from other substrates showed varying pH optima for cellulase activity.

Suggestion

Future research in this field should prioritize several key areas. Firstly, the optimization of rearing substrates for maggot cultivation must be explored to identify the most suitable substrates for specific applications. A more detailed characterization of protease and cellulase enzymes in maggot protein extract (MPE) should be conducted to unlock their industrial potential. The development of innovative products and supplements using maggot-derived nutrients and enzymes should be explored for applications in animal feed and more. Investigating the role of *Hermetia illucens* larvae in waste decomposition and waste management systems is a promising avenue. Lastly, comprehensive safety and nutritional assessments of maggot-derived products are crucial for potential use in both animal and human diets, contributing to more sustainable food and waste management solutions.

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.

Ethics committee approval

This study was approved by the Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia in 2022, under Decision Letter (SK) number 2127/UN23/PT.01.02/2022.

Authors contribution

T. Widiyastuti was responsible for data collection, statistical analysis, contributing to result interpretation, and drafting the initial manuscript. S. Rahayu was the driving force behind formulating the original hypotheses, experiment design, result interpretation, and manuscript finalization. W. Suryapratama and F. M. Suhartati, both authors, have reviewed and given their approval for the completed manuscript.

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Consent for publication

All participants have consented to the submission of the review article to the journal.

Competing interests

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

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DETECTION AND PREVALENCE OF *Leucocytozoon* spp. IN LOCAL CHICKEN BREEDS IN AL MUTHANNA PROVINCE OF IRAQ

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ABSTRACT: *Leucocytozoon* species are avian haemoparasites with economic impacts on poultry production. The present study investigates the presence of *Leucocytozoon* in chickens of Al Muthanna province, Iraq. Eighty one blood samples were collected from chickens in Samawah, Rumaitha, Warkaa, and Kidhre regions to examine the prevalence of *Leucocytozoon*. An infection rate of 6.1% was found among chicken breeds. The study highlighted that the main symptoms of infection were decreased egg production, anemia, and loss of appetite. Notably, infection was more prevalent in the Rumaitha, Khidr and Samawah regions, while no cases were reported in Warka. Treatment methods included primaquine and pyrimethamine alongside care to manage the condition effectively. It is important to mention that the observed prevalence rate in chickens was lower compared to studies on birds in Iraq, where an overall blood parasite prevalence of 15% was documented. This difference could be attributed to factors like habitat variations, vector presence, or differing susceptibility among bird species. Our suggestion for future work can be the application of new programs for diagnosing and controlling parasites in chickens.

Keywords: Al-Muthanna Province, Avian health, Flocks, Hemoparasite, *Leucocytozoon* spp.

INTRODUCTION

Leucocytozoon belongs to a group of alveolates in the phylum *Apicomplexa*, which is also home to malaria parasites. These parasites are recognized for their life cycle involving blackflies (*Simulium* species) or biting midges as hosts, and birds as intermediate hosts. There have been more than 100 *Leucocytozoon* species identified worldwide, infecting avian hosts (Adler, 2019). In the life cycle of *Leucocytozoon*, gametocytes are present in the blood of hosts. They are acquired by female blackflies. The parasite undergoes a process of malaria. It does not produce hemozoin deposits, as *Plasmodium* does. Instead, merogony takes place in organs such as the liver, heart, and kidneys (Adler, 2019).

Pathogenic avian blood parasites can cause harm to poultry farming (Zhou et al., 2020). Infections by these blood-dwelling parasites can result in issues like anemia, weight loss, stunted growth, decreased egg production, and high mortality rates in poultry flocks (Adamu, 2017). This widespread presence of haemoparasites poses a risk to poultry due to exposure to insect vectors and environmental contamination (Wamboi et al., 2020).

Infections by haemoparasites can lead to changes in hematologic parameters in chickens, might affect their productivity (Wamboi et al., 2020). For example, *Haemoproteus* infections have been found to lower blood glucose levels in chickens, significantly likely because the parasites consume glucose for their metabolic needs (Wamboi et al., 2020). Moreover, these parasitic infections are often asymptomatic, which makes them challenging to identify and manage without monitoring. Helminth infections are widespread in free-range chickens, at levels leading to hidden illnesses that impact health and productivity (Sharma et al., 2018). Hence it is vital to establish controlling measures and regularly monitor these parasites to uphold poultry well-being and enhance production outcomes in backyard environments (Wamboi et al., 2020). Major avian haemosporidian genera include the potentially dangerous *Plasmodium spp.*, *Haemoproteus* spp., and *Leucoccytozoon* spp. (Bennett et al., 1993). Birds can acquire leucocytozoonosis from numerous species of the genus *Leucocytozoon* that spread via vectors. While *Leucocytozoon* is abundant, only a small subset of species is known to cause disease in birds (Forrester and Greiner, 2008). Waterfowls, pigeons, galliforms, raptors, and ostriches are all vulnerable to the phylum *Apicomplexa*, order *Haemospororina*, family *Plasmodiidae*, genus *Leucocytozoon* (Bennett et al., 1993). There are at least 67 identified species, with 66 infecting birds (Hsu et al. 1973). *Leucocytozoon* is birds' biggest and the most prevalent haemoparasite (Ahmadov et al., 2019). *Leucocytozoon* has two subgenera: *Akiba* and *Leucocytozoon* (Ahmadov et al., 2019). In Al-Muthanna, different epidemiological studies used

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physiological parameters to show more information on microbiological infections in animals (Hameed et al., 2022; Al-Yasari et al., 2024).

The aim of the present study was to compare our results with the previous epidemiological studies, including those carried out in Iraq, about *Leucocytozoon* in birds.

MATERIALS AND METHODS

Study area

The samples were collected from the Veterinary Teaching Hospital in four selected regions of Al-Muthanna Province, Iraq (Samawah, Rumaitha, Warkaa, and Kidhre).

Samples collection

Eighty one (5-13/month) blood samples were collected from local chicken flocks in different regions of Al-Muthanna Province (Samawah, Rumaitha, Warkaa, and Kidhre), Iraq. Fresh samples were transferred in sterile containers to the Protozoology Laboratory at the College of Veterinary Medicine/Al-Muthanna University and aliquoted into tubes with EDTA and without EDTA. The study period was nine months from October 2022 to June 2023. All the information about chickens including sex, region, date of collection, clinical signs, and treatment were recorded on the sample containers. Finally, the samples were evaluated by preparing thin and thick smears, stained with Giemsa, and examined under the light microscope.

Statistical analysis

After collecting the samples, the data, were recorded. These included clinical signs, sex, the main cities in Al-Muthanna Province, months of study, and treatment measurements (Graphs 1-4). These data were analyzed after the examination of samples. The analysis was done using GraphPad Prism 9, Chi-Square program ($P \leq 0.05$).

Ethical approval

This study was part of a bigger project that was technically approved by the Scientific Committee at the College of Veterinary Medicine at Al-Muthanna University (Registered code: REF-3-Iman K Alabadi).

RESULTS AND DISCUSSION

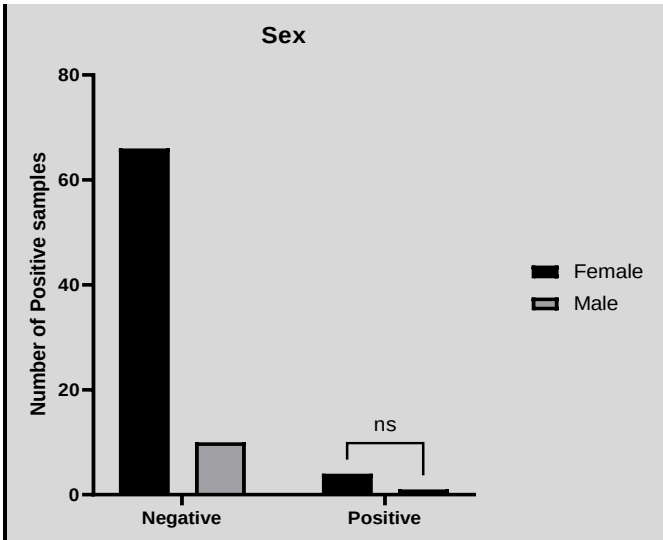
Epidemiology

Present results revealed that the total infection rate of *Leucocytozoonosis* was 6.1% in local Iraqi chicken breeds. Many host bird flocks have been infected with several *Leucocytozoon* species. *Leucocytozoon*'s gametogony takes place in leukocytes or erythrocytes, whereas its schizogony occurs in a wide variety of parenchymal and endothelial cells. *Leucocytozooon* gametocytes are pleomorphic, with certain species showing fusiform and exclusively spherical forms.

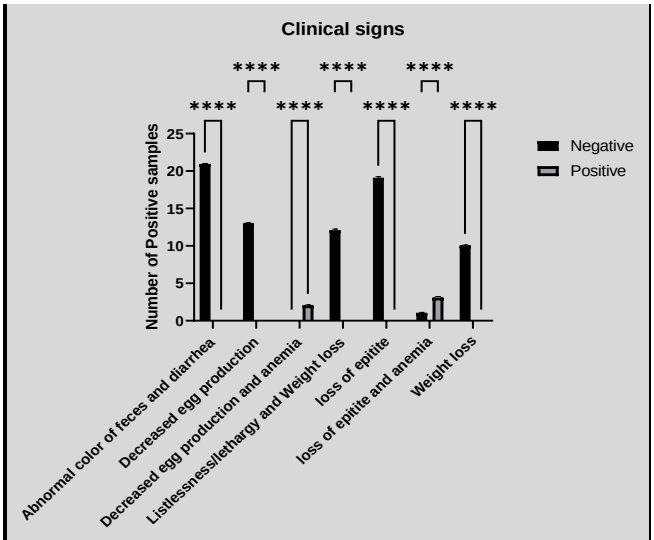
The life cycle of *Leucocytozoon* spp. involves two hosts. *Simuliid* black flies and *Culicoid* midges exhibit sporogony, after that sporozoites travel to the insect's salivary glands. Subsequently, the vertebrate host is infected, and internal organs such as the liver, brain, spleen, and lungs undergo schizogony. It is important to study intermediate hosts for both medicinal and veterinary purposes (Alkhuzaie et al., 2019 and Shaker et al., 2024), as they can infect many organs such as the nasal cavities and sinuses (Alhayali et al., 2022). Our results revealed no significant differences between males and females infected with *Leucocytozoon* (Graph 1). Al-Biatee (2014) recorded *Leucocytozoon* spp. infection rates of 10.52% in quail in Baghdad City. They found out that female quails had a greater infection rate than males.

Pathogenesis and clinical signs

From present recordings, decreased egg production, anemia, and loss of appetite were the only significant signs (Graph 2). Different clinical signs were recorded such as anorexia, weight loss, feed conversion drawbacks, anemia, green feces, and frequent mortality. These can result from parasitic infections with *Plasmodium* and *Leucocytozoon* spp. Infections with *Leucocytozoon* spp. cause severe anemia. Pneumonia, lung congestion, and the resulting occlusion of alveolar capillaries are also all potential outcomes in turkey. Moreover, liver necrosis, enlarged spleen, lymphocytic infiltration of the liver and heart, and hemosiderosis may be present (Atkinson and Van Riper, 1991). Illness and mortality in young ducks, both domestic and wild, can be caused by *Leucocytozoon simondi*. Infection can be more prevalent in flocks of ducks, especially those close to lakes. Recovering ducklings may be permanently dwarfed. Adult birds are sometimes severely impacted to the point of death. Most of the time, they can make a full recovery, however, they continue to carry the parasite in their blood and spread it to other birds, especially young ones (Wehr and Farr, 1956).



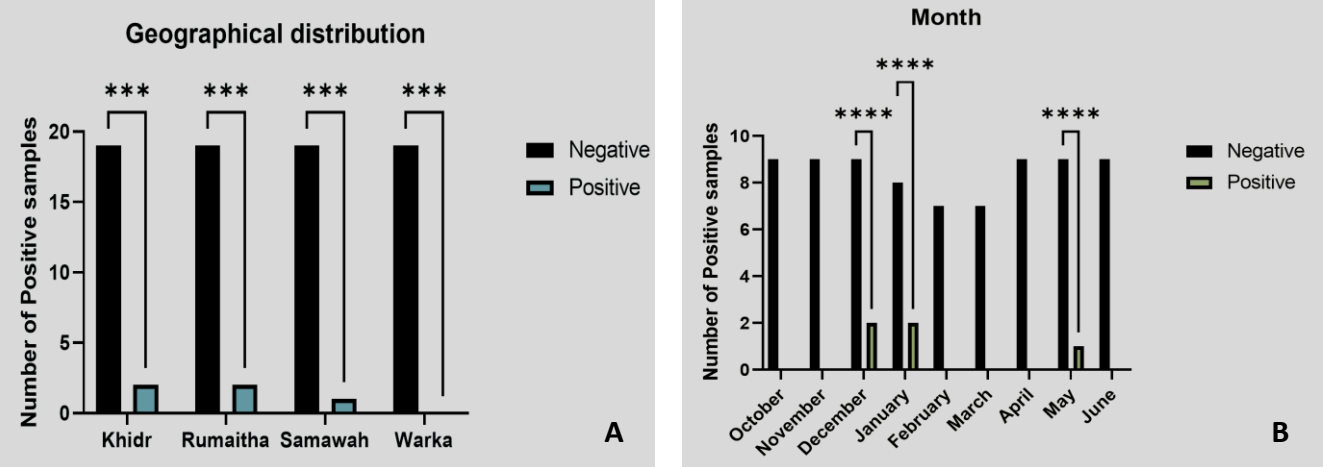
Graph 1 - Number of infected samples according to sex.



Graph 2 - Number of infected samples according to clinical signs.

Geographical distribution

The collected data showed that the infection was centered mainly in Rumaitha, then Khidr and Samawah. It is while there are no recorded positive results in Warka (Graph 3 A). These cities represent the main places in Al-Muthanna province. In addition, the researchers recorded positive results only in December, January, and May. This may be related to the nature of environmental conditions in Al-Muthanna, which could increase the growth rates of the transmission vector (Graph 3 B).



Graph 3 - Number of infected samples according to geographical distribution (A) and months (B).

Al-Shuaibi (2008) did not find the *Leucocytozoon* infection in chickens in Al Ramadi, about 110 kilometers west of Baghdad. *Leucocytozoon* spp. were the least common haemoparasite according to a study by Abdullah (2013), and their prevalence was low (13.5%), with no signs of sickness present among the chickens in the Qaradagh district of the Kurdistan region of Iraq, around 45 kilometers from Sulaimani Province. The prevalence of mixed hemoparasite infections in local chickens was 7.5% for *Plasmodium* spp. and Leucocytozoon spp., and 1.5% for *Leucocytozoon* spp. and *Haemoproteus spp.* (Abdullah, 2013). However, Hasson (2015) did not find any Leucocytozoon spp. record in adult chickens in Diyala. While mixed infection with the triple hemiparasites (*Plasmodium* spp., *Heamoproteus* spp., and *Leucocytozoon* spp.) was found in adult chickens at a 36.8% rate, *Leucocytozoon species* are widely dispersed in farmed chickens (*Gallus gallus domesticus*) in Baghdad city, with a higher infection rate of 30% (Ibrahim and Al-Rubaie, 2020). Additionally, mature chickens have a higher infection rate than young chickens, just as females compared to males. In Nineveh Villages, where geese were examined, Shamaun et al. (2007) found that the prevalence of *Leucytozoon simondi* was 33.33%. Al-Shuaibi (2008) reported a 10.7% infection rate of *Leucocytozoon* spp. in geese in different areas of Al Ramadi. This rate was reported as 5.37% and a mixed infection with *Plasmodium* spp. + *Leucocytozoon* spp., and 14.1% in geese at Sulaimani Province (Mohammed, 2014a). 14.2% was the rate reported in different areas of Mosul Governorate in northern Iraq (Mohammed, 2020).

In the marbled teal from Al-Tharthar Lake, Salahuddin Province, in the northern part of the middle region, Mohammad (2014b) observed triple infection with *Leucocytozoon simondi, Epomidiostomum uncinatum*, and *Diploposthe*

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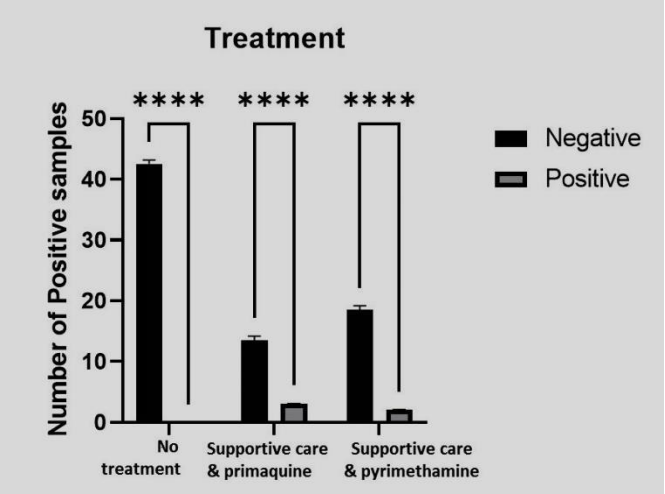
laevis with total infection rates of 3% and *Leucocytozoon simondi* was the rarest among parasites. According to Mohammad (2015), ferruginous ducks were infected with *Leucocytozoon* spp. at the Dalmaj Lake, Al-Diwaniya Province, where the infection rate was 9.09%. At a few neighborhood markets in Baghdad, the infection rate was 10%, whereas it was 5.56% in Ferruginous ducks at the center of Iraqi territory (Mohammad, 2016). AL-Zurfi and AL-Rubaie (2016) discovered that *Leucocytozoon simondi* in mallards was prevalent in local markets of Baghdad City at 16.66%. It was reported as 5.06% in the middle of Iraq by Mohammad (2016). Phasianid birds from various locations in the north, middle, and south of Iraq recorded free of *Leucocytozoon* infection (Mohammad et al., 2001).

Treatment and control

Our data revealed that the main therapies used to treat *Leucocytozoon* in chickens were Primaquine and pyrimethamine. In addition, the supportive care plays a significant role in treatment measurements (Graph 4).

Primaquine was found to be effective against *Leucocytozoon* spp. gametocytes, however treatment with pyrimethamine mixed with sulfadimethoxine was reported to be partially successful in treating avian leucocytozoonosis (Zhao et al., 2016). According to Chiang et al. (2022), daily treatment of 0.5 g of *Artemisia annua* powder in chickens boosted body weight gain and decreased *Leucocytozoon caulleryi* parasite concentration, which in turn decreased mortality, pale comb, and the production of green feces. Based on present work, it's suggested to use both laboratory and field efforts for the control of *L. caulleryi* by immunization with an oil-adjuvanted rR7 vaccine (Recombinant R7 protein from second-generation *L. caulleryi* schizonts). These measures have yielded encouraging results (Ito and Gotanda, 2004; Saeed et al., 2022).



Graph 4 - Number of infected samples according to treatment measurements.

CONCLUSION

In conclusion, a total of 81 blood samples were taken from chickens in different areas of Iraq, like Samawah, Rumaitha, Warkaa, and Kidhre to investigate the presence of *Leucocytozoon*. 6.1% was the infection rate among the chicken breeds. The study highlighted signs of infection such as decreased egg production, anemia and loss of appetite. Infections were more prevalent in regions like Rumaitha, Khidr, and Samawah compared to Warka region, where no cases were found. Notably the observed prevalence of *Leucocytozoon* in chickens was lower than similar studies on birds in Iraq, which reported an overall blood parasite prevalence of 15%. This difference could be attributed to factors like habitat variations, availability of vectors or varying susceptibility among bird species, etc. it is suggested to conduct studies mapping out the distribution of *Leucocytozoon* comprehensively in Iraq, as well as identifying haemosporidian parasites which can vary significantly across different regions and bird species.

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.

Author contributions

The designing of the study and writing the manuscript were done by Iman Aabadi. Sura Alkhuzei and Zahraa Abbas rewrote the article and revised the whole manuscript. HK revised the final version of the article.

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Competing interests
The authors state that there is no conflict of interest regarding the publication of this article.

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THE HEALTH AND ECONOMIC DIMENSIONS OF HONEY PRODUCTION IN IMO STATE, NIGERIA

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ABSTRACT: A study was conducted on honey production in Imo state of Nigeria, with a focus on the health and economic dimensions of the industry. The research was carried out using a multi-stage sampling procedure, and a sample size of 80 honey-producer respondents was selected. Data was collected through a well-structured questionnaire and analyzed using descriptive and inferential statistics. The study found that honey producers in the area had a mean age of 51 years, 11 years of education, 21 years of farming/bee-keeping experience, and a household size of 6 persons. The average annual household income was €709.10, with a farm size/number of hives kept of 72 hives per farmer and a quantity of honey produced per annum of 145 litres. The cost and returns analysis showed that the cost of production of honey per litre and profit per litre were €0.40 and €2.40, respectively. The study also determined the nutritional uses and health benefits of honey (e.g. healing wounds, treating ulcers, controlling sore throats and colds, boosting immunity, and as an antibacterial agent). Several factors, including uncontrolled bush burning, bee forage shortage, deforestation, theft of beehives, colony absconding, and poor agricultural practices which strongly constrain honey production has been observed. It is concluded that honey production is a very profitable venture with numerous uses and health benefits and venturing youths into honey production as a source of livelihood should be encouraged, and extension education should be tailored to technologies in beekeeping and the identification of genuine honey to minimize the success of adulteration, among others.

Keywords: Economic, Forage shortage, beekeeping, Honey, Health Benefits, Natural products

INTRODUCTION

Based on available studies, Obianefo et al. (2019) and FAO (2021) highlighted that the agricultural sector is the largest employer in Nigeria, engaging over 70% of the population, with honey production being a significant contributor to agricultural gross domestic product (GDP) and employment. However, the share of agriculture in Nigeria's GDP has been on a decline, from about 90% before independence to around 22.35% in early 2021 (CBN, 2014). Given this concerning trend, there is a need to explore underutilized agricultural sectors like beekeeping (Muktar, 2018).

Beekeeping offers low land requirements, cost-effectiveness, and substantial economic benefits as it promotes crop pollination while creating job opportunities in both rural and urban areas (Ogunola et al., 2019; Degrandi-Hoffman et al., 2019). Nigeria's honey production is currently at only 15,000 tons annually, well below its potential (Ayodele, 2017). Smallholder farmers dominate this sector, but despite the favorable conditions for honey production, the industry largely remains untapped and outdated in its methods. Nevertheless, rising interest and investment in the sector suggest a growth potential.

Honey's therapeutic properties extend to wound healing and other health conditions, boasting numerous benefits such as antioxidant, antimicrobial, and anti-inflammatory effects (Samarghandian et al., 2017; Medhi et al., 2008). Moreover, honey is a healthier alternative to refined sugars, being lower on the glycemic index (Babacan and Rand, 2007; Pataca et al., 2007). However, despite its profitability, with returns of €0.50 per litre of honey (Babatunde et al., 2008) and an average net income for beekeepers, production levels remain insufficient to meet domestic demand, leading to significant imports (Muktar, 2018).

Issues of honey adulteration further complicate the market, as adulterants like starch and inverted syrup are commonly used to increase profits (Aliaño-González et al., 2020; Arroyo-Manzanares et al., 2019). While recent techniques have been developed for quality control, knowledge gaps about the economics and health benefits of honey production in Imo State persist. This study aims to address these gaps by exploring farmers' perceptions of honey's health benefits, assessing the economic returns for beekeepers, and addressing the challenges faced in production.

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MATERIALS AND METHODS

The study was conducted in Imo State, which is divided into three agricultural zones: Owerri, Orlu, and Okigwe. The state lies within latitude 4°45'N and 7°15'N and latitude 6°50'E and 7°25'E. The state is bounded in the east by Abia state, in the west by River Niger and Delta state, in the north by Anambara state, while Rivers state lies in the south. Imo state covers an area of about 5,100sq/km and a population of 3,934,899 (National Population Commission, (NPC, 2006). Rainfall distribution is bimodal with peaks in July and September and a two-week break in August. The rainy season begins in March and lasts till November. The high temperature and humidity experienced in the state favour the luxuriant vegetation of tropical rainforests. This also favors honey production as there is an abundant supply of nectar from flowers for their consumption.

Multi-stage sampling procedure was used in sampling. Stage one involved random selection of Owerri agriculture from the three zones because the state has the same type of vegetation all over the zones Owerri zone has eleven LGAs. Stage two involved a purposive selection of two Local Government Areas, Aboh Mbaise and Ahiazu Mbaise based on their intense honey production activities as reported by extension agents in Imo State, Agricultural Development Programs (ADP). Stage three involved random selection of five Communities each from the 2 selected LGAs giving (10) Communities. Thereafter two villages were selected randomly from each of 10 communities selected in stage three above, giving 20 villages for the study. Finally, in stage 4, four households that engage in honey production were selected purposively from each of the 20 villages giving 80 respondents.

Data for the study were gathered through a well-structured questionnaire administered to primary data sources. Data collected included those on the farmers' socio-economic characteristics, the nutritional/health benefit of honey as food to farmers, the cost and revenue components of honey production and the real and perceived uses of honey in the study area amongst others. Data were analyzed using descriptive and inferential statistics and net return model as appropriate The Likert type measurement scale was used extensively in this study because it has the feature of transforming a respondent's subjectivity into an objective reality. The net return model specified below was used to determine the net return accruing to honey producers.

$NI = TR - TC$ ... eqn (I)

TC = Total Variable Cost + Total Fixed Cost ...equ (II)

$NI = TR - (TVC + TFC)$ ...eqn (III)

Where; NI= Net income/Return, TR= Total Revenue, TC = Total Cost

RESULTS AND DISCUSSION

Socioeconomic characteristics of respondents

Table 1 displays how the respondents are distributed based on their socioeconomic characteristics. The results show that the mean age of honey producers is 51 years. This implies that the respondents are mature men who are at the prime and productive time of their lives. They are expected to have gotten reasonable experience in honey production given their ages. This finding agrees with that of Ogunola, et al. (2019) who found that most honey producers in their study area are between the ages of 41-50 years and are able-bodied men strong enough to produce effectively and efficiently. The mean number of years spent in formal education is 11 years. This implies that the respondents are mostly literate farmers having acquired secondary education and therefore can read and write. This standard of education implies that the crop of beekeepers can learn and be in a position to adopt modern technologies involved in honey production. They should also be produced efficiently and in large quantities. This finding is in tandem with that of Bifarin et al. (2008) who found that the majority (100%) of the honey producers were literate and married while 98% of them were males. The mean level of experience is 21 years. This of course shows that respondents have very good knowledge of honey production and should be producing efficiently. They are also in the best position to know the best methods of keeping bees and harvesting honey. However, this finding does not agree with Oluwaseyi, (2019) who opined that most honey producers in Kwara state had experience of between 6-10 years only and made use of modern bee-keeping technology.

The average household size in the area is six persons, which conforms to the international standard of one man, one woman, and four children. This finding agreed with the United Nations Database of Household Size and Composition (2017) which revealed that the household size within Europe and Northern America is fewer than three persons whereas in Africa and the Middle East, the average household size is five or more persons. The mean annual household income is €709.10. This implies that the per capita household annual income is €118.18 and daily income of €0.32 which is below one dollar per day at the current exchange rate of about €0.43/dollar (Degrandi-Hoffman et al, 2019). It also implies that the farmers in the study area belong to the poverty-ridden class who survive on less than one dollar per day. However, following the new international poverty line as set (Wakagri and Yigezu, 2021) at $2.15 using 2017 prices, people who survive on less than $2.15 a day are living in extreme poverty. About 648 million people globally were in this situation in 2019. Farmers in this area are living in more than extreme poverty and require special intervention to escape from the poverty level where they are.

Table 1 - Socioeconomic characteristics

Socioeconomic variable	Mean
Age	51 years
Household size	6 persons
Farming experience	21 years
Level of education	11 years
Annual household income	€709.10
Farm size	72 hives
Quantity of honey produced	145 litres/annum
Sex	100% male

Source: Field survey data, 2023

Nutritional and health usefulness of honey

Table 2 shows the nutritional and health benefits of honey as perceived by respondents in the study area. According to Table 2, out of the 13 factors a priori expected and perceived to be among the nutritional and health usefulness of honey, only 11 are significant given the five points Likert scale measurement system where those whose mean is greater or equal to (Mean ≥ 3.0) are adjudged to be significant. The above result reveals that honey is used to improve the taste of food with a mean of 4.8, in confectionaries with a mean of 4.49, in cosmetics with a mean of 4.49, controlling cough 4.42, and in traditional medicines 4.13. Other important uses and health usefulness of honey include in facilitation of healing wounds, treatment of ulcers, control of sore throats, and colds, as an immune booster, and as an antibacterial agent. The other expected healthy benefits of honey as shown in Table 3.2 may not have been significant amongst the respondents due to ignorance of their uses in the activities or conditions by the respondents in the study area. These findings are in tandem with the observations of Samarghandian et al. (2017) who said that traditionally, honey has been used in the treatment of various health conditions such as eye. diseases, bronchial asthma, throat infections, tuberculosis, thirst, hiccups, fatigue, dizziness, hepatitis, constipation, worm infestation, piles, eczema, and for healing ulcers and wounds. It is also used as a nutritious supplement. Moreover, honey is known to have ingredients that exhibit antioxidant, antimicrobial, anti-inflammatory, antiproliferative, anticancer, and antimetastatic effects. Also, Medhi et al. (2008) and Kumari and Nishteswar (2012) found that honey accelerates the healing of wounds; this agreed with the finding of this study with a mean value of 3.67.

Net returns from honey production

Table 3 shows the cost and return analysis of honey production in the study area. According to Table 3, the total cost of producing one litre of honey in the study area is €0.40. On the other hand, the profit or net return per litre of honey sold is €2.40. This indicates that honey production is a highly profitable business. This finding is consistent with the research conducted by Babatunde et al. (2008), which suggests that honey production is a lucrative venture. The study found that beekeepers produced an average of 313 litres of honey per annum and earned a gross income of €0.50 per litre.

Qualities and mode of identification of original/Genuine honey

Table 4 shows the perception of respondents on the qualities and mode of identification of genuine honey. The study found that all the factors except crystallization are significant in determining the authenticity of honey. Among the identified factors, the viscosity of honey is the most significant, followed by the thumb test, heat/matchstick test, water test and vinegar test. Other tests like infrared-based spectroscopy, Raman spectroscopy, Nuclear magnetic resonance spectroscopy and Isotope ratio mass spectrometer are also significant. Still, most of the respondents showed poor knowledge of their applicability. A study by Khalil et al. (2015) found that honey is rich in antioxidants, flavonoids, phenolic acids, organic acids, amino acids and proteins. These antioxidants have several preventative effects against various diseases, making honey a popular source of antioxidants. Additionally, honey has healing effects and antibacterial properties, making it useful in treating ulcers and wounds. Honey also has a hygroscopic feature that allows it to absorb moisture when exposed to air, which is a useful quality test. The study also revealed that the respondents were aware of the healing effects of honey and its antibacterial properties, as well as its hygroscopic feature. However, they showed ignorance of the applicability of other factors like the antioxidant feature and the vinegar test. Studies like Chen et al. (2011), Özbalci et al. (2013), Ribeiro et al. (2014), and Salvador et al. (2019), and have used various spectroscopy techniques to identify the components present in honey and detect adulteration.

Constraints to commercial production of honey in the study area

Table 5 shows the constraints to large-scale/commercial honey production in the study area. The results indicated that uncontrolled bush burning, shortage of bee forage, and deforestation were the most significant challenges to honey production in the study area. Other obstacles like theft of beehives, colony absconding and poor agricultural practices were also found to have a strong impact on honey production. Moreover, drought, extreme temperatures, pests and diseases, and relative humidity were also observed to affect honey production in the study area. These findings are consistent with those of Wakagri and Yigezu's (2021) review paper, where they identified that extreme temperatures, relative humidity, drought, deforestation, poor apicultural practices, unsafe pesticide utilization, and pests were among the factors that limit honey production.

Table 2 - Nutritional and health benefits of honey as perceived by respondents in the study area

Perceived nutritional/health usefulness of honey	SA		A		UND		D		SD		Mean	Rank
	F	%	F	%	F	%	F	%	F	%		
Improve Taste of food	65.00	81.00	14.00	18.00	1.00	1.00	0.00	0.00	0.00	0.00	4.80	1st*
Healing of wound	40.00	50.00	20.00	25.00	10.00	12.50	5.00	6.25	5.00	6.25	3.67	5th*
Control cough	50.00	62.50	20.00	25.00	5.00	6.25	3.00	3.80	2.00	2.50	4.42	3rd*
Treating of ulcer	20.00	25.00	20.00	25.00	30.00	37.30	5.00	6.25	5.00	6.50	3.56	6th*
Control cold	15.00	18.80	20.00	25.00	30.00	37.30	12.00	15.00	3.00	3.80	3.40	8th*
Control Hypertension	15.00	18.80	10.00	12.50	20.00	25.00	29.00	36.30	6.00	7.50	2.99	10th
Control burn	5.00	6.25	10.00	12.50	30.00	37.30	20.00	25.00	15.00	18.80	2.62	12th
Control of sores throat	18.00	22.50	10.00	12.50	40.00	50.00	10.00	12.50	2.00	2.50	3.40	8th*
Immune booster	30.00	37.50	10.00	12.50	20.00	25.00	5.00	5.25	15.00	18.80	3.44	7th*
Use in Confectionaries	50.00	62.50	25.00	31.30	1.00	1.25	2.00	2.50	2.00	2.50	4.49	2nd*
Beer making	5.00	6.25	10.00	12.50	35.00	43.80	25.00	31.30	5.00	6.50	2.81	11th
Tobacco making	5.00	6.25	10.00	12.50	40.00	50.00	15.00	18.80	10.00	12.50	2.81	11th
Traditional medicine/herb mixture	40.00	50.00	15.00	18.80	20.00	25.00	5.00	6.25	0.00	0.00	4.13	4th*
Cosmetics/cream/soap	50.00	62.50	20.00	25.00	9.00	11.30	1.00	1.25	0.00	0.00	4.49	2nd*
Antioxidant qualities	1.00	1.25	5.00	6.25	50.00	52.50	20.00	25.00	4.00	5.00	2.06	13th
Antibacterial agent	20.00	25.00	10.00	12.50	25.00	31.30	20.00	25.00	5.00	5.25	3.25	9th*

*Source: Field survey data, 2023 SA = Strongly Agreed, A = Agreed, UND = Undecided, D = Disagreed, SD = Strongly disagreed. Mean value ≥ 3.0, Significant Rank depicts the position of significant.

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Table 3 - Cost and return analysis of honey production in the study area.

Annual cost / Expenditure items		Annual benefit/Income items	
Variable costs		(F) Total output of honey	11,570 L
Packaging container	€1778.42		
Labour cost	€1287.23	(G) Average unit price of honey	€2.81/L
Transportation	€465.78		
Processing cost	€372.62	(H) Total revenue from honey (F*G)	€32480.07
Miscellaneous cost	€124.21		
(A) Total variable Cost (TVC)	€4028.25	(I) Mean revenue from honey	€406.00
Fixed cost components			
Depreciation of items	€547.64	(J) Net return (H - C)	€27825.14
Rent on Farm Office	€67.75		
Utilities	€11.29	(K) Mean net return (J/80)	€347.81
(B) Total fixed cost (TFC)	€626.68	(L) Net return/litre (J/F)	€2.40
(C) Total cost (A+B)	€4654.93		
(D) Mean cost of production	€58.19	Profit/litre	€2.40
(E) Quantity of honey produced	11,570 L		
Cost of Production/litre (TC/E)	€0.40/L		

Source: Field survey data, 2023. Variables in parentheses are the (A) Total Variable Cost, which is the cost incurred in the production processes of honey; (B) Total Fixed Cost, which is the combination of the cost of equipment, and other fixed inputs used in the production; (C) Total Cost, which is the sum of the total variable cost and total fixed cost; (D) Mean Cost of production, which is the average cost of production by the total sample size; (E) Quantity of honey produced, which is the volume of honey extracted from the numbers of hives kept; (F) Total Output of honey, which is the volume of honey extracted from the numbers of hives kept; (G) Average unit price of honey, which is the average price sold by the total respondents; (H) Total revenue from honey (Price × Quantity of honey produced); (I) Mean revenue from honey which is the average revenue earned by the respondents; (J) Net Return, which is the return earned after cost have been deducted; (K) Mean net return, which is the return earned after cost have been deducted from the individual respondents, and (L) Net Return per litre, which is the return earned from the sales of a litre of honey after the cost of producing a litre of honey is deducted.

Table 4 - Perception of respondents on qualities and identification of genuine honey

Qualities and identification of genuine honey	SA		A		UND		D		SD		Mean	Rank
	F	%	F	%	F	%	F	%	F	%		
Hygroscopic	10.00	12.50	20.00	25.00	20.00	25.00	20.00	25.00	10.00	12.50	3.00*	7th
Antibacterial/Healing	20.00	25.00	20.00	25.00	20.00	25.00	10.00	12.50	10.00	12.50	3.40*	4th
Antioxidant effects	10.00	12.50	10.00	12.50	50.00	62.50	5.00	6.25	5.00	6.25	3.20*	5th
Treating of ulcer	20.00	25.00	20.00	25.00	20.00	25.00	5.00	6.25	5.00	6.25	3.20*	5th
Use of infrared-based spectroscopy	5.00	6.25	10.00	12.50	60.00	75.00	2.00	2.50	3.00	3.75	3.20*	5th
Use of Raman spectroscopy	10.00	12.50	10.00	12.50	50.00	62.50	5.00	6.25	5.00	6.25	3.20*	5th
Nuclear magnetic Resonance Spectroscopy	5.00	6.25	5.00	6.25	65.00	81.30	4.00	5.00	1.00	1.25	3.10*	6th
Isotope Ratio Mass Spectrometry	5.00	6.25	10.00	12.50	60.00	75.00	3.00	3.75	2.00	2.50	3.20*	5th
Heat testing (Matchstick test)	30.00	37.50	20.00	25.00	20.00	25.00	5.00	6.50	5.00	6.50	3.80*	2nd
Water test	20.00	25.00	30.00	37.70	20.00	25.00	3.00	3.75	7.00	8.75	3.70*	3rd
Thumb test	25.30	1.25	25.00	31.25	20.00	25.00	6.00	7.50	4.00	5.00	3.80*	2nd
Vinegar test	10.00	12.50	5.00	6.50	50.00	62.50	10.00	12.50	5.00	6.50	3.10*	6th
Crystallization	10.00	12.50	30.00	37.50	20.00	25.00	10.00	12.50	10.00	12.50	2.70	8th
Viscosity	30.00	37.50	30.00	37.50	20.00	25.00	0.00	0.00	0.00	0.00	4.10*	1st

*Source: Field Survey Data, 2023; SA= Strongly Agreed, A= Agreed, UND= Undecided, D= Disagreed, SD= Strongly Disagreed. Mean value ≥ 3.0, Significant rank depicts the position of significant

Table 5 - Constraints to commercial honey production in the study area.

Constraints to commercial honey production	SA		A		UND		D		SD		Mean	Rank
	F	%	F	%	F	%	F	%	F	%		
Extreme temperatures	20.00	25.00	30.00	37.50	10.00	12.50	10.00	12.50	10.00	12.50	3.50	5th
Pest and diseases	20.00	25.00	20.00	25.00	20.00	25.00	10.00	12.50	10.00	12.50	3.40	6th
Relative humidity	10.00	12.50	20.00	25.00	40.00	50.00	5.00	6.25	5.00	6.25	3.30	7th
Drought	20.00	25.00	30.00	37.50	10.00	12.50	15.00	18.70	5.00	6.25	3.60	4th
Deforestation	30.00	37.50	40.00	50.00	5.00	6.25	2.00	2.50	3.00	3.75	4.20	1st
Poor agricultural practices	30.00	37.50	30.00	37.50	10.00	12.50	8.00	0.10	2.00	2.50	4.00	2nd
Colony absconding	30.00	37.50	30.00	37.50	10.00	12.50	10.00	12.50	0.00	0.00	4.00	2nd
Poor attitude to pesticide usage	20.00	25.00	30.00	37.50	20.00	25.00	5.00	6.25	5.00	6. 25	3.70	3rd
Shortage of bee forage	35.00	43.80	30.00	37.50	10.00	12.50	5.00	6.25	0.00	0.00	4.20	1st
Theft of beehives	30.00	37.50	30.00	37.50	15.00	18.70	3.00	3.75	2.00	2.50	4.00	2nd
Uncontrolled bush burning	30.00	37.50	40.00	50.00	5.00	6.25	3.00	3.75	2.00	2.50	4.20	1st

Source: Field Survey Data, 2023; SA = Strongly Agreed; A = Agreed; UND = Undecided; D = Disagreed; SD = Strongly Disagreed. Mean Value ≥ 3.0; Significant Rank depicts the position of significant factors @ ≥ 3.0.

CONCLUSION AND RECOMMENDATIONS

Based on the findings of this study, producing honey as a farm business can be very profitable, with a net return of €2.40 per litre of honey sold. Findings showed that by producing a litre of honey, the farmer made a profit of €0.10. This can improve the economic well-being of the farmers if done repeatedly. Honey is known to be a healthy food for humans and has many beneficial properties, such as being an antioxidant and antibacterial agent. It can also be used as a prophylactic substance in medicine. Honey is a better alternative to refined sugar, especially due to its low glycemic index value.

Based on the findings of the study, the following recommendations were made;

1. The government are required to encourage youths to engage in honey production as a profitable enterprise by providing soft loans specifically for honey production to interested farmers.

2. The study suggested adequate extension education on honey production, emphasizing the importance of honey as a healthy food and medicine. This will also bring about the teaching of better technologies for honey production, making it attractive to both the highly educated and others.

3. Extension education are encouraged to focus on the scientific methods of identifying genuine honey, as most of the farmers interviewed do not have an idea of the modern and scientific methods of identifying genuine honey.

4. Farmers are encouraged to be trained on appropriate farming practices to avoid destroying the eco-support systems that protect and preserve the lives of bees and other flora and fauna useful for honey production.

5. Controlled grazing and preservation of our forests, as well as adequate security in our fields, should be ensured to avoid observed theft and destruction suffered by farmers.

6. The government are suggested to provide poverty alleviation facilities such as health care and education in the area to reduce the level of poverty observed in the area.

DECLARATIONS

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Authors' contribution

I.U.O.Nwaiwu, F.A.Kadiri, M.N.Osuji, I.I.Ukoha, and K.H.Anyiam: Conceptualization, Methodology design, Models Design, Data Analysis, Section writing and proofreading.

U.G.Anyanwu, F.O.Nwosu, I.O.Oshaji, and E.U.Nwachukwu: Questionnaire design, data collection, section writing and grammar check.

O.C.Enoch, I.G.Isaiah, M.B.Bala, and A.C.Obasi: Data collection, Data sorting and data entering.

J.A.Madu, and E.I.Nnorom = Data coding, Data curation, Data Processing.

Ethical consideration

There is no direct contact with the bees, and the study is based on analytical findings.

Consent to publish

All the authors consented to publish the article.

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Competing interests

There is no existence of conflict of interest among the authors.

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EFFECTS OF SUPPLEMENTING CULTURED *Cordyceps militaris* MUSHROOM MYCELIA IN THE PREGNANT SOW'S DIET ON THE HEALTH AND PERFORMANCE OF THE MOTHERS AND THEIR SUCKLING PIGLETS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Present study aimed to evaluate the effects of supplementing cultured Cordyceps mushroom mycelia (CMM) in the diets of pregnant sows on the productivity of the mothers and their suckling piglets during their first week of age. A total of 30 pregnant F1 (Landrace x Yorkshire) sows were randomly allocated to 5 dietary treatments with 6 replicates each: Control (sows fed the basal diet), and T30, T50, T100, and T200, where sows were fed the basal diet supplemented with 30, 50, 100, and 200 g of dried CMM, respectively. The animals were individually housed and fed twice daily. The performance and health status of the sows and their piglets were recorded accordingly. The results showed that the inclusion of CMM in the diets of pregnant and lactating sows affected the performance and health status of both the mothers and their piglets. For the piglets, the total number of piglets born and alive was higher in the T50, T100, and T200 groups compared to the control and T30 groups, but there was no effect on the survival rate at 7 days old. Daily gains per piglet were higher in the T30, T50, and T100 groups compared to T200 ($P<0.05$). For the sows, daily feed intake was lower in the T30 group compared to the other treatments ($P<0.05$). The values of gross energy in the milk produced by the sows were higher in the control, T30, T50, and T100 groups compared to T200 ($P<0.05$). Both the piglets and the sows on diets supplemented with CMM experienced fewer health problems than those on the control diet ($P<0.05$). In conclusion, the supplementation of 50 and 100 g of CMM per day in the diets of pregnant and lactating sows improved litter size and health status but did not affect the performance of either the mothers or their piglets.

Keywords: Cordyceps mushroom mycelia, Health status, Pregnant sows, Suckling piglets, Weight gain.

INTRODUCTION

In Vietnam, "Dong Trung Ha Thao" in Vietnamese and "Winter Worm Summer Grass" in English have been used as traditional folk medicine for hundreds of years (Thanh et al., 2018; Lu, 2023). Cordyceps is a phenomenon in which worms of the *Hepialus* genus in the *Lepidoptera* family are parasitized by a fungus with the scientific name *Cordyceps sinensis* (Berk.) of the *Ascomycetes* family (Kobayashi, 1982). Cordyceps belongs to the fungus family and is parasitic on the bodies of insects (Kumar et al., 2015). Under natural conditions in the winter, the fungi parasitize insects, develop into fungal mycelium (the asexual stage), use nutrients from the insect's body, and kill the insect. In the summer, the asexual fungal mycelium changes to the sexual stage, forming a mushroom—a structure that contains sexual spores and emerges from the ground, although the root is still attached to the stem (Figure 1A). Cordyceps has long been used in traditional Chinese medicine with the belief that it can treat various diseases. Under artificial conditions, *Cordyceps militaris* easily formed fruiting bodies in the culture environment (Figure 1B).

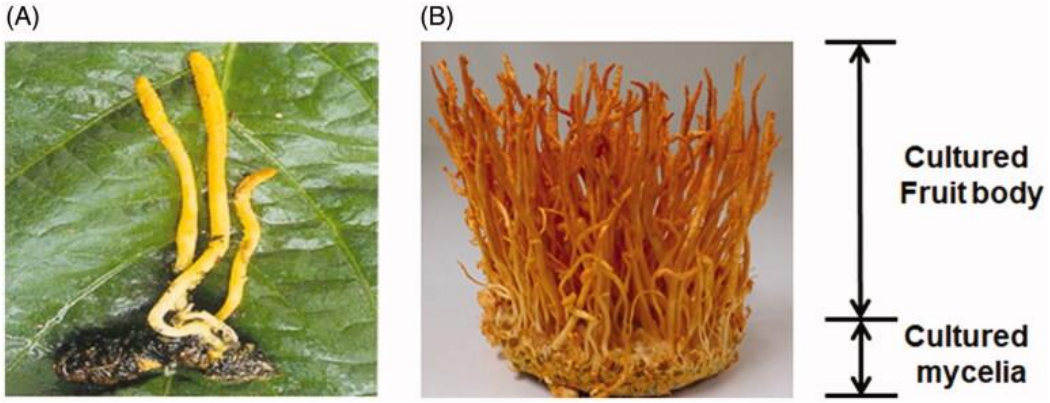

Figure 1 - Natural (A) and cultured (B) *Cordyceps militariris* (Adapted from Chou et al., 2024)

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The fruiting body of *Cordyceps* is collected from infected pupae or larvae, while the mycelia or corpus is considered a by-product, referred to as "Cordyceps mushroom mycelia – CMM (Sharma et al., 2024) ". It is believed that the fruiting body and the mycelium or corpus of *C. militaris* have different functions due to the former growing above ground and the latter existing underground (Hong et al., 2007). According to Hur (2008), the *Cordyceps* corpus contains most of the essential amino acids and essential fatty acids, such as linoleic acid (n-6) and alpha-linolenic acid (n-3), as well as biologically active compounds like adenosine and cordycepin. Additionally, Boontiam et al. (2020a,b) and Omthonglang et al. (2021) described the *Cordyceps* spent mushroom substrate (CMM) as including the mushroom corpus and the substrates from the culture medium of *Cordyceps* mushrooms. In this study, the CMM was used, and its bioactive compounds, as well as the concentrations of amino acids and fatty acids, were analyzed.

Koh et al. (2003) found that *Cordyceps* mycelium can serve as an alternative antibiotic growth promoter to improve weight gain and immunity in broiler chickens. Additionally, the inclusion of 1 g/kg of fermented *C. militaris* significantly increased weight gain in broiler chickens (Han et al., 2015). In weaning pigs, diets supplemented with 1,000 µg/kg fermented *Cordyceps* promoted growth performance and cell-mediated immunity (Cheng et al., 2016). In growing pigs, supplementation with CMM at 2 g/kg of diet increased growth performance, immunoglobulin secretion, and antioxidant capacity, while lowering leukocyte percentage, cholesterol, and MDA concentrations (Boontiam et al., 2020a). Ahtwichai et al. (2019) supplemented 2.45-4.9 mg cordycepin in diets for pregnant sows until weaning and reported that *C. militaris* supplementation affected reproductive performance, altered oxidative status, and reduced the fecal score in suckling piglets. Therefore, supplementing feed with *Cordyceps* substrates might provide an alternative approach in livestock production, improving both animal health and performance.

To our knowledge, there are few or no published reports on using *Cordyceps militaris* mushroom mycelia as a feed additive for sows and their suckling piglets. We hypothesized that the presence of biologically active components in *Cordyceps militaris* mushroom mycelia in gestation and lactation diets may affect the performance and health of sows and their piglets. This study, therefore, aimed to evaluate the effects of supplementing *Cordyceps* mushroom mycelia in the diets of pregnant sows on the productivity of the sows and their suckling piglets at the first week of age.

MATERIALS AND METHODS

The experiment was carried out at the Pig's Farmer Farm in Tien Giang province, Viet Nam during March-July 2023.

Ethical regulation

The precent study was approved by the Scientific Comittee of the Faculty of Veterinary Medince & Animal Sciences, HUTECH University; Date: October 25, 2023.

Experimental design

Total pregnant 30 F1 (Landrace × Yorkshire) sows at 2-3 litters were randomly allocated to one of 5 dietary treatments, namely Control, in which the sows were fed the basal diet (Table 1); and T30, T50, T100 and T200, in which, animals were fed a basal diet, and supplemented with 30, 50, 100 and 200 g *Cordyceps militaris* spent mushroom substrates (CMM), respectively. The pregnant sows were individually kept in pens with water and feed supplying systems. They were fed regularly according to the farm procedure.

The cultured *Cordyceps* mushroom mycelia (CMM) used in this study originated from VINABIOMUSH Vietnam Biological Mushroom Limited Company. The CMM comprised the corpus and substrates, which included ingredients such as brown rice, bean sprouts, coconut juice, and silkworm pupae. These ingredients, added to the culture medium, provided nutrients for the *Cordyceps* fungus to grow. After harvesting the fruiting bodies, the CMM was dried and crushed into small particles prior to mixing with feed ingredients. The contents of amino acids, fatty acids, and bioactive compounds in the CMM are presented in Table 2.

Measurements

Performance

In the piglets, total piglet number born, born alive and stunted (less 800 g/pig) piglets; total litter weight at birth and at 7-day old, average daily weight gain of total litter and an individual piglet at the first week of age. In the sows: Daily feed intake during the experiment was recorded. Energy released from milk of the sow was calculated using National Research Council (2012): GE (kcal/kg) = (4.19 x ADG) – (90 x L)

Which, ADG: average daily gain of the litter (g); L: heads of piglets in the litter

Health status

In the piglets, diarrheal rate is total days that piglet got diarrhea incidence divided by total days that piglets raising; Rates of arthritis and painful hoof are proportion between number of piglets got arthritis or painful hoof and total piglet number. In the sows, main health problems such as metritis, missing fetus or remaining placenta, hoof pain, poor milking, mastitis, diarrhea and some disorders without known cause were daily recorded.

Chemical analysis

Samples of *Cordyceps* spent mushroom substrates were collected from VINABIOMUSH, and analyzed at the Quality Assurance & Testing Centre 3 (QUATES 3), District 1, HCMC, Viet Nam. Amino acid composition was analyzed by Performic Acid Oxidation with Acid Hydrolysis–Sodium Metabisulfite Method (AOAC 994.12; 1997). Fatty acid compositions were

analyzed by Gas Chromatography of Fatty Acid Methyl Esters Method (ISO 2017). Adenosine and cordycepin concentrations were analyzed by HPLC and detection was performed with a variable-wavelength UV detector at 260 nm.

Statistical analysis

Data were presented in the form of the mean (M). The data were statistically processed by analysis of variance (ANOVA) by General Linear Model in Minitab version 17.2. The difference between the mean values was determined by the Tukey method at a confidence level of 95%. Statistical model: $Y_{ij} = \mu + T_i + e_{ij}$

Where: μ is the average value; T_i is the effect of dietary treatments; e_{ij} is the experimental error.

Table 1 – Ingredients and nutritive values of diets for the sow

Parameters \ Types of animals	Gestation	Lactation
Ingredients (%)		
Maize meal	50.0	46.4
Rice bran	33.4	28.5
Soybean meal	8.0	15.0
Fishmeal	5.0	5.0
Oil	2.0	3.5
Lysine	0.08	0.08
Methionine	0.02	0.02
Premix minerals	0.5	0.5
Premix vitamins	0.5	0.5
Salt	0.5	0.5
Nutritive value (%) *		
Dry matter	89.2	89.4
ME (kcal/kg)	3,040	3,190
Crude protein	14.5	16.5
Crude fibre	10	6
Ca	0.9	1.0
P	0.6	0.8
Lysine	0.8	0.9
Methionine + Cysteine	0.6	0.6

*= Calculated

Table 2 - Amino acid and main fatty acid composition of Cordyceps mushroom mycelia*

Amino acids	mg/g DM	Fatty acids	% as total fatty acids
Histidine	2.15	Palmitic acid (C16:0)	22.4
Arginine	4.47	Oleic acid (C18:3)	37.7
Threonine	3.66	Stearic acid (C18:0)	4.36
Valine	5.32	Linoleic acid (C18:2 n-6)	31.6
Methionine	2.38	Alpha-linoleic acid (ALA, C18:3 n-3).	0.96
Lysine	2.80	Cis-11-Eicosenoic acid (C20:1)	0.39
Isoleucine	6.75	Arachidic acid (C20:0)	0.81
Aspartic acid	8.11	Behenic acid (C22:0)	0.34
Phenylalanine	3.89	Arachidonic acid (C20:4)	0.22
Glutamic acid	13.80		
Alanine	5.09		
Glycine	4.36		
Tyrosine	2.66	Bioactive coumpounds	mg/kg
Proline	4.75	Adenosine	2.60
Serine	3.75	Cordycepin	7.27
Total amino acid	77.70		

*Analysed by QUATEST 3

RESULTS

Performance

Total piglets born and alive were affected by diets (Table 3). The number of piglets born in the Control and T30 was lower than in T50 and T100 ($p<0.05$) but not significant different with T200 ($p>0.05$). The stunted rates were higher in the T100 and T200 than in the Control and T30; however, no stunted piglet born was found in the T50. Number of 7-day old piglets and the survival rate at 7th day old wasn't different among treatments ($p>0.05$).

In Table 4, total litter live weight at birth, at 7th day old and average daily gain (ADG) of the litter were not significantly different between treatments ($p>0.05$). However, the ADG per piglet at the first week of age were higher in T30, T50 and T100 than in T200 ($p<0.05$). In the sows, daily feed intake was significantly lower in T30 than in the other treatments ($p<0.05$). Additionally, the values of GE in milk produced by the sows were significantly higher in Control, T30, T50 and T100 than in T200 ($p<0.05$).

Health status

Piglets

The ratio of suckling piglets got diarrhea was highest in the Control and T30 and lowest in the T100 and T200 ($p<0.05$), and ratio of arthritis was higher in the Control than in T30, T100 and T200 ($p<0.05$).

Lactating sows

In Table 6, the sows got significantly some health problems after farrowing among different treatments. The sows in the Control got more the symptoms of metritis, missed fetus, pain in hoof, matitis, diarhea and fever with un-known cause, meanwhile the sows in the T30, T50, T100 and T200 got less the symptoms than in the Control, excep for painful hoof in T30 and fever and loss of appetite with unknown cause in T30, T50 and T100.

Table 3 - Number of piglets at born and at 7th day of age

Items \ Treatments	Control	T30	T50	T100	T200	p-value
Total piglet born (head)	14.1^{b}	13.6^{b}	15.8^{a}	17.6^{a}	15.2^{ab}	0.049
Piglet born alive (head)	13.1^{ab}	11.9^{b}	14.6^{ab}	15.9^{b}	14.0^{ab}	0.045
Stunted piglets (head)	0.2	0.2	0	0.58	1.0	0,065
Stunted piglet rate (%)	1.12^{b}	1.67^{b}	0	4.93^{a}	3.42^{a}	0.021
Total piglets at 7th day (head)	11.9	10.4	12.5	13.3	12.7	0.059
Survival rate at 7th day (%)	92.47	90.79	87.13	88.53	94.03	0.575

*a,b; Means within a column with different superscripts differ significantly ($P<0.05$)

Table 4 - Total litter weight and average daily gain (ADG), and estimated milk gross energy (GE) produced by the sow at 1st lactation week

Items \ Treatments	Control	T30	T50	T100	T200	p-value
Total litter birth weight (kg)	21.57	17.83	19.51	22.03	20.70	0.318
Total litter weight at 7th day old (kg)	36.42	32.43	34.13	37.41	31.05	0.233
ADG 0-7 (kg/litter)	2.12	2.09	2.09	2.20	1.48	0.742
ADG 0-7 (g/piglet)	180^{ab}	202,9^{a}	200^{a}	195,7^{a}	135,7^{b}	0,002
Daily feed intake (kg/sow)	3.21^{a}	2.27^{b}	3.43^{a}	3.47^{a}	2.71^{ab}	0.046
Milk GE 0-7 (kcal/day)#	7815^{a}	7821^{a}	7631^{a}	8022^{a}	5061^{b}	0.037

*a,b: Means within a column with different superscripts differ significantly ($P<0.05$). Gross Energy: (4.19 x ADG x 1000) – (90 x L), in which L: number of piglets, #: according National Research Council (2012)

Table 5 - Piglets suffering from diarrhea, arthritis and painful hoof (% as total)

Items \ Treatments	Control	T30	T50	T100	T200	p-value
Diarrhea	0.20^{a}	0.20^{a}	0.04^{b}	0.08^{c}	0.09^{c}	0.012
Arthritis	5.05^{a}	2.88^{b}	3.40^{b}	2.15^{b}	2.36^{b}	0.026
Painful hoof	8.43	5.77	6.81	7.53	10.16	0.462

*a,b; Means within a column with different superscripts differ significantly ($P<0.05$)

Table 6 – Sows suffering from diseases after farrowing (% as total)

Items \ Treatments	Control	T30	T50	T100	T200	p-value
Metritis	33.4[a]	16.7[b]	0	0	0	Sig
Missed fetus/remaining placenta	16.7	16.7	0	0	0	NS
Painful hoof	33.4	33.4	0	0	0	NS
Poor milk lactating	16.7	0	0	0	0	Sig
Mastitis	16.7	0	0	0	0	Sig
Diarrhea	16.7	0	0	0	0	Sig
Fever, loss of appetite without known cause	50.1[a]	50.1[a]	50.1[a]	50.1[a]	16.7[b]	Sig

*a,b: Means within a column with different superscripts differ significantly (P<0.05).

DISCUSSION

Chemical composition and bioactive compounds

In recent years, knowledge of the full chemical composition, bioactive compounds and nutritive values of cultured *C. militaris* has been studied (Ji et al., 2020; Sharma et al., 2024; Trung et al., 2024). In the framework of research of Hur (2008) and Chan et al. (2015), the proximate composition and content of amino acids, fatty acids, elements, vitamins, and bioactive compounds of the fruiting body (FB) and mycelial biomass or corpus (MB) of *C. militaris* were studied. The authors found significant differences in some important parameters of the chemical composition between FB and MB. In general, the MB contained valuable nutrients 15-20% compared to the FB such as the contents of free amino acids, some fatty acids and bioactive compounds of the FB were much higher than in MB. In addition, the MB were much cheaper than the FB.

In this study, the content of total free amino acids in the CMM is 77.7 mg/g DM (Table 2), and the most abundant amino acids are glutamic acid, aspartic acid and isoleucine. Meanwhile, the contents of lysine and methionine are low as compared with other amino acids. The content of total free amino acids in the study is ranged between the previous once. In the previous reports, the content of free amino acids in the corpus was 14.03 mg/g (Hur, 2008) and in the mycelial biomass was 24.98% as DM or 249.8 mg/g DM (Chan et al., 2015). Furthermore, Hur (2008) reported also that the most abandon amino acids in the corpus were proline (2.99 mg/g DM), lysine (2.2 mg/g DM) and glutamic acid (1.4 mg/g DM). It is not in case of Chan et al. (2015), who has found the most abandon amino acids in the mycelial biomass were alanine (3.61%), glycine (3.12%) and arginine (2.76%), and low contents of glutamic acid (1.82%) and aspartic acid (1.92% as DM).

Regarding to the content of fatty acids, the most abandon fatty acids in the CMM in this study are palmitic acid (22.4%), oleic acid (37.7%) and linoleic acid (31.6%) and low alpha-linolenic acid (0.96%). In our study, the content of linoleic acid (LA) is higher than alpha-linolenic acid (ALA). This finding is agreement with the study of Hur (2008) and Chan et al. (2015). The contents of LA and ALA in the corpus were 33% and 20.6% as total fatty acid, respectively (Hur, 2008) and LA of 40.7% and ALA 0.9% as total fatty acid in the mycelium (Chan et al., 2015).

The contents of bioactive compounds as adenosine and cordycepin in this study were 2.60 mg/g DM and 7.27 mg/g DM, respectively (Table 2). Cronstein et al. (1994) indicated that adenosine inhibits some functions of neutrophils, which are a type of inflammatory cell in human being. Cordycepin is an adenosine derivative, and was shown to possess diverse pharmacological properties, in addition to its well-known antioxidant effects (He et al., 2013; Olatunji et al., 2016), including antiviral activity (Ryu et al., 2014), inhibition of lipopolysaccharide (LPS)-induced inflammation (Kim et al 2006), reduction of blood lipids (Guo et al 2010), inhibition of platelet aggregation (Cho et al., 2007), and induction of apoptosis in neuroblastoma and melanoma cells (Baik et al., 2007) in human being. In the previous study, Hur (2008) shown the contents of adenosine and cordycepin in the corpus were 0.06% (0.6 mg/g) and 0.36% (3.6 mg/g), respectively. In the mycelial biomass, the content of cordycepin was 0.182% or 1.82 mg/g (Chan et al., 2015) and 1.74 mg/g (Cohen et al., 2014). Therefore, the content of cordycepin in our recent study was higher than those mentioned above. Some studies reported that the concentration and distribution of bioactive compounds is not uniform event in the fruiting bodies. Additionally, the optimal drying temperature for *C. militaris* is 60°C and higher temperature causes a loss of the content of cordycepin and phenolic compounds (Wu et al., 2019).

Performance and health status

In this study, the supplementation of the CMM in diets for pregnant and lactating sows improved the litter size, the health of suckling piglets and the sows, and didn't affect growth rate of piglets and milk energy released from the sows except when the sow got 200 g CMM/day. As calculation, the sows in T30, T50, T100 and T200 got 2.18; 3.64; 7.27 and 14.54 mg cordycepin per day, respectively. Ahtwichai et al. (2019) reported that supplementing 2.45 mg and 4.9 mg of

cordycepin per day in the diets of gestation and lactating sows didn't affect the sow's productivity and piglet's production parameters but affected litter fecal score and wean to estrus interval.

In human being, the inclusion of *C. militaris* extract in the diet can have several health benefits. However, a single substance may have a less therapeutic effect and may not show synergistic effects with other compounds present in mushroom. Among all bioactive substances mentioned in the studies, the greatest therapeutic potential is associated with cordycepin (Jedrejko et al., 2021). Additionally, understanding the production modes and metabolite yields of *C. militaris* is crucial for realizing its full potential in medicinal and industrial applications (Chou et al., 2024).

According to Boontiam et al. (2020b), suckling piglets received a diet supplemented with cordyceps, had secrete more IgA and IgG. In addition, piglets received IgG through colostrum periodically through an intestinal transport mechanism that operated specifically during the first 24 hours after birth allowing the delivery of maternal IgG into the intestine via the circulatory system. In addition, this stimulation may be that cordyceps, originating from the Thai rice medium, contained large amounts of γ-oryzanol, which has been shown to have a potential effect on immunity, by way to activate IgA production (Yang et al., 2014; Henderson et al., 2012).

Cordyceps militaris is effective in reducing the amount of pathogenic *E. coli* negative gram bacteria and increasing the amount of probiotic *Lactobacillus* spp. may be due to the abundance of polysaccharides (Yu et al., 2004). Galactomanna is the main polysaccharide antigen (mannooligosaccharides: MOS) in *C. militaris* and is useful for prebiotic production. A study by Kudoh et al. (1999) on mice, IgA levels increased in the feces of mice supplemented with MOS, IgA secretion can be regulated by the presence of bacterial antigens in the intestine and IgA plays a role in important for mucosal immunity because it inhibits bacterial adhesion and invasion, which further reduces bacterial colonization. In addition, according to Farmer and Quesnel (2009), IgA titers increased in sow milk. Therefore, the difference in the rate of piglet diarrhea days can be explained that adding *Cordyceps* to the diet helps reduce the rate of piglet diarrhea, which is in agreement with the study of Omthonglang et al. (2021). In addition, the results reported by Boontiam et al. (2020b), *C. militaris* can be used to control diarrhea because it has the ability to reduce the amount of *E. coli* in feces in weaned pigs. Additionally, serum immunoglobulin is an important index to determine humoral immunity in pigs. IgA and IgG play an important role in protecting against invading pathogens, so changes in the levels of these proteins can affect the growth performance and immunity of pigs (Hedegaard et al., 2016). Cordycepin and polysaccharides in fermented *Cordyceps* corpus are considered prebiotics in animal nutrition (Chuang et al., 2020; Sun et al., 2021). Prebiotics promote the growth of beneficial bacteria and enhance the absorption of nutrients, helping to reduce the amount of harmful metabolic products in the intestines and stimulating the growth of useful bacteria.

CONCLUSION

Supplementation of *Cordyceps* mushroom mycelia in the diets for the pregnant and lactating sows affected:

- Total piglets born and alive, average daily gains per piglet at the first week of age but not the survival rate of 7-day old piglets;
- Daily feed intake and the values of gross energy in milk produced by the sows;
- The health of both piglets and their mothers.

In conclusion, the supplementation of 50-100 g dried *Cordyceps* mushroom mycelia in the pregnant and lactating sows' diets improved performance and health status of both the mothers and their piglets.

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.

Author contributions

Nguyen Vu Thuy Hong LOAN and Do Ngoc Yen PHUONG conceived, designed the experiments; Do Ngoc Yen PHUONG performed the experiments; Nguyen Vu Thuy Hong LOAN analysed the data; Nguyen Vu Thuy Hong LOAN and wrote the paper; all authors reviewed and approved the final manuscript.

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Competing interests

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

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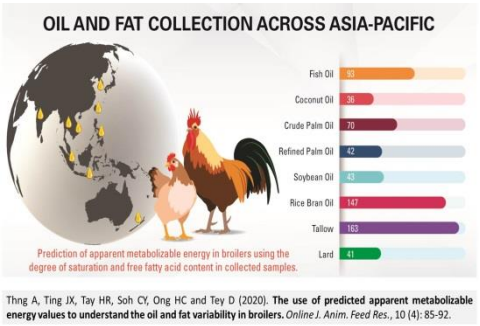

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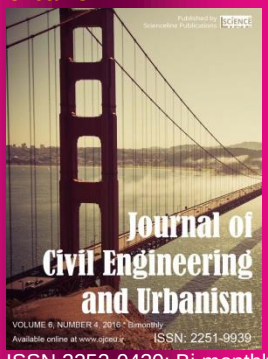

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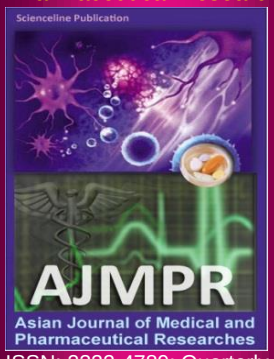

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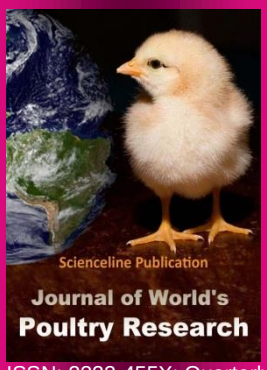

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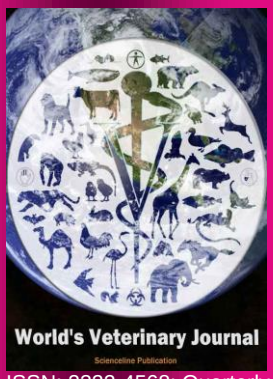

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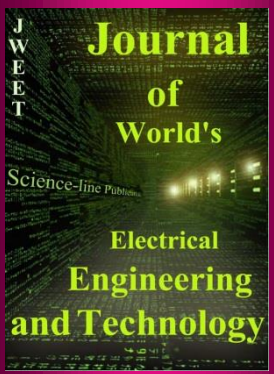

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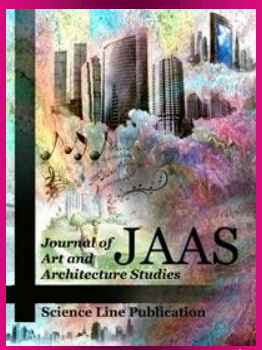

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