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PREFACE

In this edition, Volume 29 No 1, we proudly present articles from animal and veterinary sciences including genetic, reproduction; animal physiology; and veterinary from scientist all over the world. The articles published in this edition are:
“Determination of Growth and Survival Rates of Saanen Lamb in UniSZA Dairy Goat Farm”; “Estimate the effect of non-genetic factors on the reproductive traits of Afec-Assaf strain in Bani Naim Farm, Palestine”; “Innovation of Standardized Extract of Yam Bean Seed and Red Betel Leaf as Bioinsecticide for Myiasis Treatment”; “Comparison of Nutritional Composition of Black Soldier Fly Bred on Organic Waste and Bred on Commercial Pellet mixed with Rice Bran”; “Effect of Replacing Grass Silage with Hydroponic Maize Fodder on Nutrient Intake and Digestibility, Rumen Parameters and Blood Metabolites of Weaned Ongole x Brahman Calves”; and “Morphometric Diversity and Polymorphism of Melanocortin-4 Receptor (MC4R) Gene in Red Kedu and Kampung Chickens”.

We extent high appreciation to all peer reviewers who make this journal academically high value. Hopefully, these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from scientists to contribute their papers to the Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor

Bogor, March 2024

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LIST OF CONTENT

Determination of Growth and Survival Rates of Saanen kid in UniSZA Dairy Goat Farm

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ABSTRAK

Anuar NI, Ahamad N, Rosli NSA, Ahmad K, Azmi NS, Mustaffa NDAN, Ibrahim NS. 2024. Penentuan tingkat pertumbuhan dan kelangsungan hidup anak kambing Saanen di peternakan kambing susu UniSZA. JITV 29(1):1-8. DOI: http://dx.doi.org/10.14334/jitv.v29.i1.3297.

Berat badan hidup merupakan salah satu parameter dalam mengevaluasi produksi susu dan daging hewan ternak. Berat badan hidup menjadi indikator utama untuk meningkatkan karakteristik produksi. Studi ini dilakukan untuk mengukur kinerja pertumbuhan berdasarkan dua faktor; perbedaan jenis kelamin dan jumlah anak dalam satu kandungan serta mengamati tingkat kelangsungan hidup antara kedua faktor tersebut selama periode pengambilan sampel. Data dari 22 anak kambing Saanen dicatat selama 6 minggu. Hasil penelitian menunjukkan anak jantan memiliki berat badan hidup yang lebih tinggi daripada anak betina (P>0,05), dengan perbedaan 3,3% dalam persentase kenaikan berat badan hidup selama 6 minggu pengambilan sampel. Pertambahan berat badan 0,68 kg/hari dan 0,48 kg/hari untuk anak kambing betina. Studi ini juga menunjukkan anak kembar memiliki berat badan hidup rata-rata lebih tinggi daripada anak tunggal (P>0,05). Ukuran rata-rata anak kambing Saanen yang lahir tunggal berbeda secara tidak signifikan dengan yang lahir kembar (P>0,05). Anak kambing Saanen yang lahir kembar menunjukkan peningkatan berat badan hidup sebesar 9,9% dalam 6 minggu dibandingkan dengan anak tunggal. Kadar kelangsungan hidup adalah 86,4%, dengan anak tunggal melaporkan semua kematian. Kelangsungan hidup anak sebelum disapih dalam penelitian in tidak dipengaruhi oleh jenis kelamin atau jumlah anak dalam satu kandungan, tetapi dipengaruhi oleh usia induk. Faktor lain seperti kekurangan pakan harus diperhatikan karena akan memengaruhi produksi hewan secara keseluruhan. Data yang dikumpulkan selama penelitian ini berpotensi sebagai acuan penilaian nilai pemuliaan yang lebih akurat, serta perbaikan dalam pemberian pakan dan pengelolaan.

Kata Kunci: Anak Kambing, Pertumbuhan, Saanen, Daya Tahan Hidup

ABSTRACT

Anuar NI, Ahamad N, Rosli NSA, Ahmad K, Azmi NS, Mustaffa NDAN, Ibrahim NS. 2024. Determination of growth and survival rates of Saanen goat kid in UniSZA dairy goat farm. JITV 29(1):1-8. DOI: http://dx.doi.org/10.14334/jitv.v29.i1.3297.

Live weight is one of the parameters for evaluating milk and meat production of livestock. Live weight is an important indicator for improving production traits. The present study was conducted to measure growth performances based on two factors; sex differences and litter size and observation of survival between these factors during the sample period. Data from 22 Saanen kid were recorded for 6 weeks. Results showed that male kid had higher live weight than female kid (P>0.05), with a difference of 3.3% in the percentage of live weight gained over 6 weeks of sampling. This resulted in a body gain of 0.68 kg/day for male kid and 0.48 kg/day for female kid. The current study also showed that twin kid had a higher average live weight than single kid (P>0.05). The average size of single-born Saanen kid was not significantly different from twin-born Saanen goat kid (P>0.05). Twin-born Saanen kid gained 9.9% more live weight in 6 weeks than singleton- born. Survival was 86.4%, with all deaths attributable to singleton-born kid. In this study, preweaning kid survival was not affected by sex or litter size, but was affected by maternal age. Other factors such as pellet deficiency should be considered as they affect the overall production of the animals. The data collected in this study could potentially lead to more accurate evaluation of breeding value, and improvements in feeding and management.

Key Words: Goat Kid, Growth, Saanen, Survival Rates

INTRODUCTION

In Malaysia, the dairy goat industry is a relatively small component of the overall livestock sector, and there is no locally bred dairy goat. Instead, imported dairy goat breeds such as British Alpine, Saanen, and Toggenburg are used for milk production (Mohsin et al. 2019). In particular, the Saanen breed is known for its high milk production and adaptability to new environments, which has prompted the Malaysian government to import large number of Saanen goats. This trend is driven by the increasing popularity of goat milk consumption in Malaysia, due to its health benefits, therapeutic properties, and adherence to Islamic religious values (Nasir et al. 2018). However, goat milk production is still low, largely due to its management by smallholder farmers and the absence of modern technology to improve livestock productivity (Shahudin et al. 2018).

The Saanen dairy goat breed is named after its place of origin, the Saanen Valley in Switzerland (Silva et al. 2006). They have a rapid development process, gradually widening from the chest to the back. The Saanen breed is one of the large-framed dairy goat breeds that can reach withers heights of up to 80 cm and a full-grown live weights of up to 55 kg (Akdağ et al. 2011). In Malaysia, Saanen dairy goats are one of the most common dairy breeds, alongside other breeds such as Anglo Nubian and Jamnapari, which are recognized as dual-purpose goats.

There are two types of goat farming in Malaysia. Commercial farmers produce on a large-scale,while local and small farmers keep goats for meat and milk production as a source of income (Ibrahim and Jalil 2022). Goat farming can be profitable if animal management is focused on improving production traits, such as milk yield, growth rate, litter size, and reducing the mortality rates of kid and young adults. Live weight is a critical factor in determining potential milk production and traits of livestock, especially those with economic values (Khandoker et al. 2018; Moroz and Samorukov 2020). In additionbody size and live weight are two major factors that can be used to evaluate goat growth performance and are critical indicators for improving goat production (Kari et al. 2019; Fonseca et al. 2021).

Production qualities can be influenced by a combination of genetic and environmental factors, such as management practices, climate, nutrition, and health (McManus et al. 2008; Joy et al. 2020; Cheng et al. 2022). Recent research found that litter size, birth weight, and survival rates in kids and goat were significantly affected (Assan 2020; Habtegiorgis et al. 2022). In Malaysia, tropical conditions, including high temperatures and humidity, and fluctuating feed supply and quality posechallenges, and fluctuating in ruminant production (Khandoker et al. 2018).

During the weaning period, live weight is a critical trait for animal welfare, profitability, and production (Al-Dawood et al. 2020). However, pre-weaning kid mortality remains a major challenge for breeders, with kid' mortality being an important factor affecting goat production (Tesema et al. 2017). To achieve high market weight for goat kid, one approach is to improve their growth performance and survival rate by increasing their growth potential, which can be achieved through selection and improvement of environmental conditions (McManus et al. 2008; Tajonar et al. 2022).

The current study showed that Saanen goat production can be maximised by improving growth development and survival rate of weaned kid, which can have a a significant impact on the profitability of the goat industry. In addition, using weaned kid as breeding stock can improve overall quality and milk production.These results highlight the importance of focusing on growth performance, litter size, and survivability of weaned kid to ensure profitability in the goat industry. Moreover, the rapid growth during the pre-weaning period can reduce rearing costs and ultimately increase farmer profits. . Therefore, the knowledge and data obtained in this study can be used to increase the production of Saanen goats and ultimately improve the economic viability of the goat industry. The growth curve analysis presented in this study can have significant implications for the breeding, feeding, and management practices in the goat industry. Specifically, the knowledge gained from this analysis may allow for more accurate evaluation of breeding value, and information on more effective feeding and management strategies.

MATERIALS AND METHODS

Ethics approval

The UniSZA Animal and Plant Research Ethics Committee (UAPREC) approved the sampling method and experimental procedures in this study (Reference number: UAPREC/007/032).

Animal management

The present study was conducted at the UniSZA Dairy Goat Farm, Besut, Terengganu, from March to April 2022 in an intensive housing system. A total of 22 Saanen kid (15 females and 7 males) were selected based on their date of birth between March 9 and 15, 2022 and were 4-5 days old (0 weeks) at the beginning of the study. The kid were marked for identification according to their dam, and the experiment did not change the standard management and milking procedures for Saanen kid before weaning.All animals received colostrum directly from their dams up to seven days and were fed a standard diet consisting of pellets equal to 3% of their body weight in in the morning and fresh Brachiaria humidicola ad libitum in the afternoon, with free access to water until the end of sampling period. The live weight of each kid was recorded weekly for six weeks using a bucket to restrain any movement. Measurementswere taken and documented twice weekly, along with other information on feeding and age of the dam.The survival rate of each kid was observed weekly and statistically analyzed using the recorded data. These results may help to accurately assess breeding value and improved feeding and management of Saanen goat kid.

Statistical analysis

In this study, the growth performance and litter size of Saanen kid were subjected to statistical analysis using the T-test. Specifically, the T-test was used to examine the mean values of live weight ± standard error (SE) and mean live weight of litter size±SE, with statistical significance set at $P<0.05$. In additionthe relationship between sexr and litter size was examined using correlation and linear regression analysis. The linear regression model was used to develop the optimal equation for estimatinglive weight based on a regression line.

At the endof the study period, the survival rate (%) of the kid was determined by dividing the total number of live kid by the original number of kid at the beginning of the study. The resulting quotient was then multiplied by 100 to obtain the percent survival rate survivability, this method described by Al-Dawood et al. (2020).

RESULTS AND DISCUSSION

Growth influenced by sex

The objective of the current study was to determine the sexes and live weight of Saanen goat kid during the period before weaning, which is a crucial developmental stage for the growth and survival of these animals. During a six-week experimental period, the live weights of Saanen aanen kidlings, regardless of sex, was closely monitored and recorded to analyze their growth patterns. The inclusion of both male and female Saanen kidlets in the study provided a comprehensive understanding of potential sex differences in growth performance.This information may prove very valuable in the context of livestock management and has the potential to provide information on breeding practices, nutritional requirements, and general herd management strategies.

Figure 1 shows the results of the analysis of the average live weight of of Saanen kidlings before weaning by sex (female and male). The graph shows a consistent upward trend ($P>0.05$) in the live weight of male kidlets compared to female. The present study aligns with the previous study, indicating that there is no significant difference in the weaning of Saanen kidlets(Teke et al. 2009). In the second week the average live weight of female Saanen kid was higher than that of male Saanen kid. However, from the 4th week onwards, the graph shows a significant increase in the live weight of the male Saanen kid in contrast to that of their female counterparts. These findings contribute to a comprehensive understanding of the growth performance of Saanen goat kid, which is of critical for the development of optimal livestock management strategies.

Analysis of the average live weight of pre- weaned Saanen kid at the sixth week of sampling showed that male pre-weaned Saanen kid had the highest average live weight of 6.40±2.85 kg. These result indicate the body weight of pre-weaned male Saanen kid increased consistently from the first week to the sixth week of sampling, indicating good growth performance.

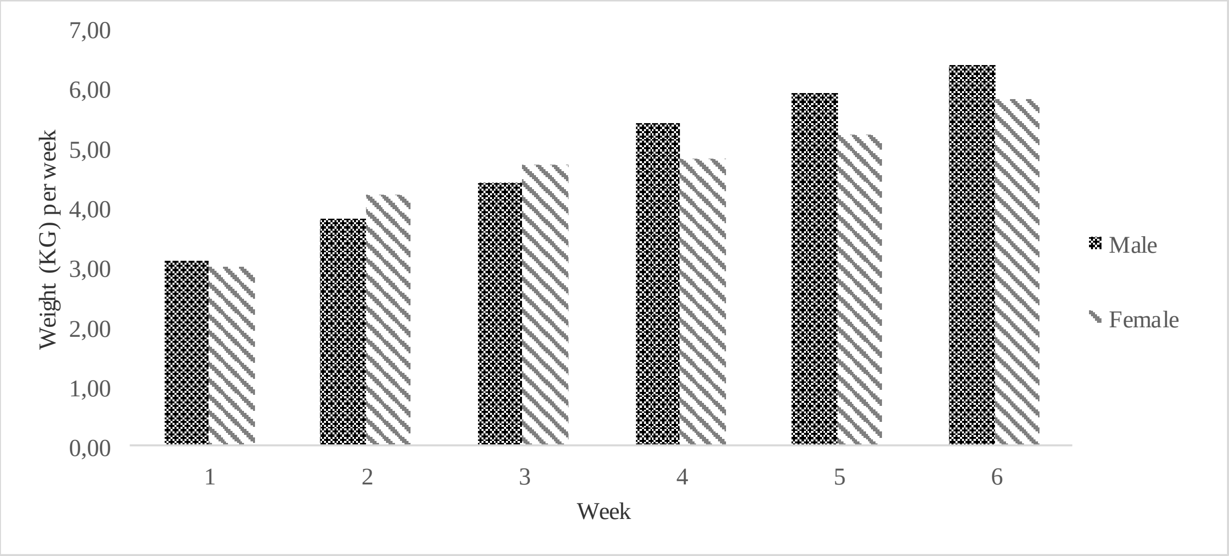


Figure1. Average live weight of Saanen goat kid before weaning, by sex

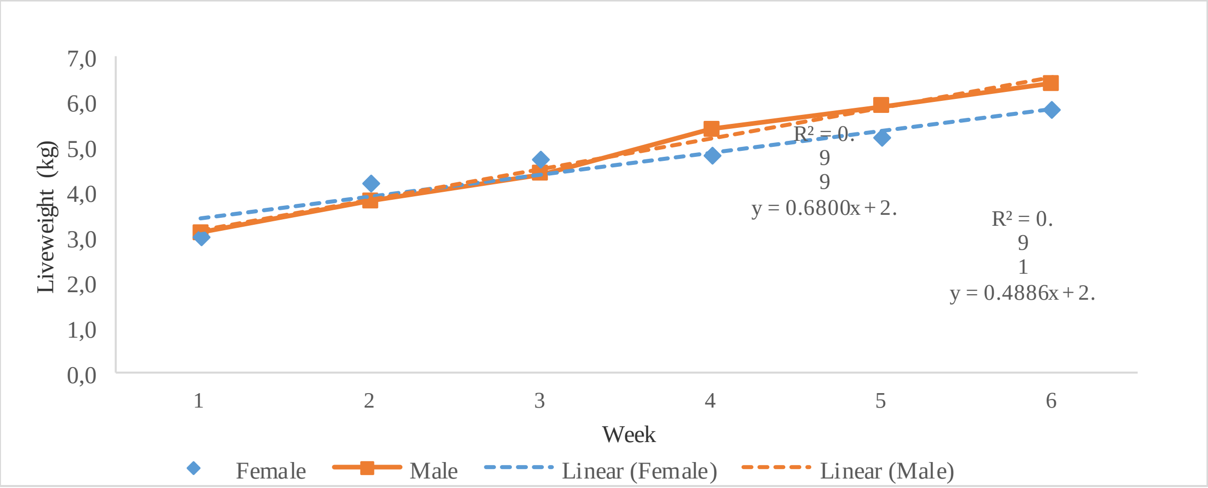
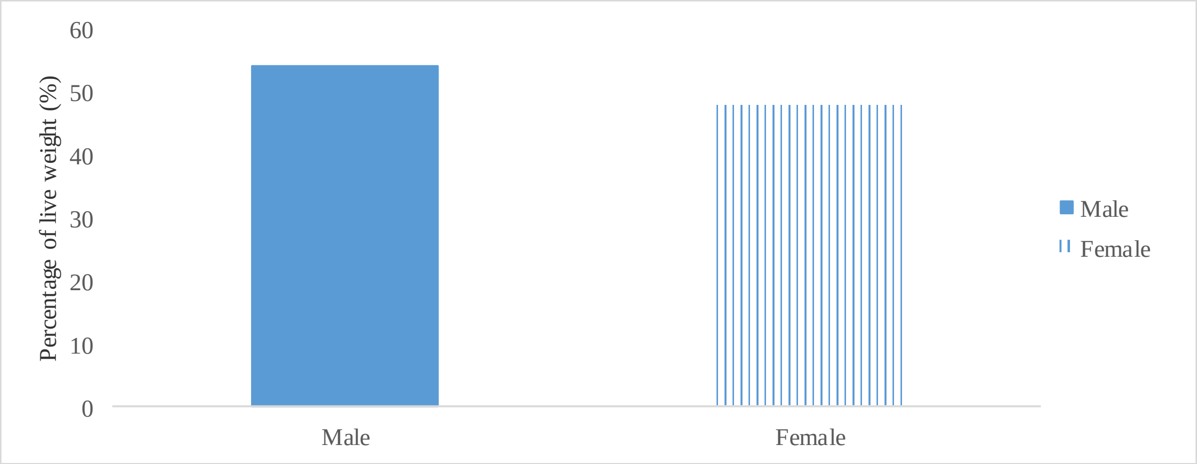


Figure 2. Regression plot of live weight grouped by sex



Fiure 3. Percentage of live weight gain of Saanen goat kid grouped bu sex before weaning during the six weeks

Similarly, female Saanen kid had the highest mean live weight of 5.80±2.48 kg pre-weaning at the sixth week of sampling, indicating a consistent increase in body weight from week one to week six and growth performance.. No significant difference (P>0.05) were observed between male and female Saanen kid. This current study are in the aggrement with the previous study where no significant sifference has been recorded in weaning Saanen goat kidlets (Teke et al. 2009). However, during in the second and third weeks of sampling, the mean live weight of pre-weaning female Saanen kid before weaning was higher than the mean live weight of male Saanen goats before weaning in the same week.

Data collected during live weight monitoring were then used to determine the sex of the Saanen kidlets and analyze their growth patterns. It is expected that the result of this research will increase knowledge of the factors affecting the growth and development of Saanen kidlings before weaning.

In the present study, the regression curve and the equation for live weight as a function of sex were shown in Figure 2.The male Saanen kid had a higher coefficient of determination (R^2) value of 0.9900 compared to the female Saanen kid, which had a value of 0.9144. The tightly clustered distribution of data points with no outliers resulted in a linear regression line for both male and female kid. The high R-value indicates a positive correlation between live weight and sex. The regression line for sex showed that male Saanen kid had a daily weight gain of 0.68 kg/day, while female Saanen kid had a daily weight gain of 0.48 kg/day. This information is useful in predicting the live weight of Saanen goat kid based on their sex. At the end of a six-week growth experiment, the proportion of live weight gain in male and female Saanen goat kid was determined.

Figure 3 showed the percentage of live weight gain of Saanen goat kid before weaning during the six weeks of the study. The data show that male Saanen kid had a higher percentage of live weight gain compared to their female counterparts, with a difference of 3.3%. However, statistical analysis showed that there was no significant difference (P>0.05) between male and female Saanen kidlets in terms of the percentage of live weight gained over the six-week period.

Growth influenced by litter size

The second factor under consideration pertained to ascertaining the impact of litter size on the growth and survival rates of Saanen goat kid. During the sampling period, the study recorded 16 individual kid (n=16) and three sets of twin kid (n=6). The recorded goat kid underwent weight measurement and the incidence of kid' mortality was documented between the first and the sixth week of life.

Figure 4 shows the mean body weight of Saanen goat kid before weaning in different litter sizes over a six-week sampling period. The observed trend for single pre-weaning kid shows a gradual increase in body weight from the first week of life up to the sixth week. Conversely, pre-weaning twin kid demonstrate a notably accelerated rate of body weight gain throughout the six-week sampling period, characterized by a rapid weight gain during six weeks period of study.

At week 6, the single-born Saanen kid reached their maximum average live weight of 5.60±2.86 kg. Thus, the variations in body weight over the six-week sampling period indicate differences in growth performance. Conversely, the mean live weight of twin-born Saanen kid remained comparable over the six weeks of sampling, with no significant variation detected (P>0.05). The highest mean live weight of the twin-born Saanen infants was recorded at week 6 at 7.10±0.58 kg. 7.10±0.58 kg. Discrepancies in body weight during the six-week period, therefore, indicatedifferent growth rates in the twin-born kid.

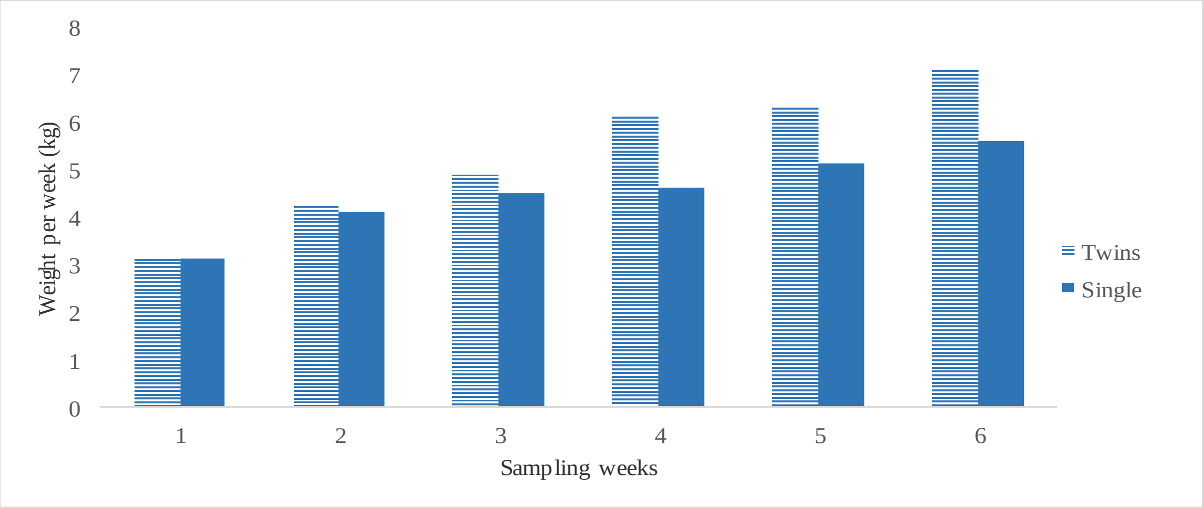


Figure 4. Average live weight of Saanen goat kid before weaning according to litter size (singles and twins)

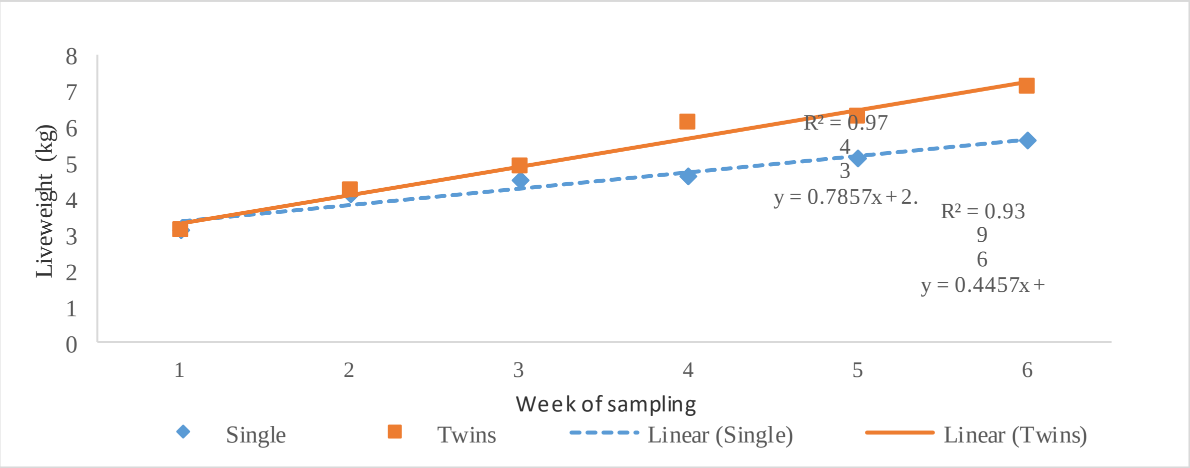


Figure 5. Regression curve of live weight on litter size

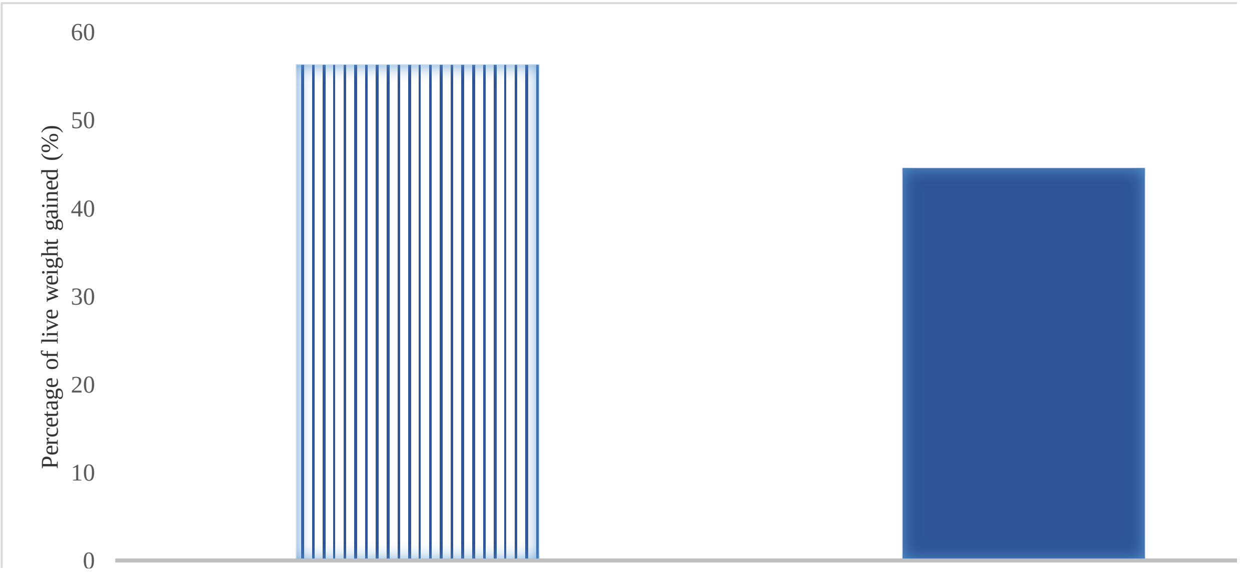


Figure 6. Percentage (%) live weight gained (litter size) over 6 weeks of sampling

After recording live weight, Saanen kidlings were evaluated for the effects of litter size on their growth patterns. The results of this study will improve our understanding of the factors affecting the growth and development of Saanen goat fawns in the pre-weaning period.

Figure 5 shows a regression plot of live weight against litter size that allows prediction of live weight using a linear line. The plot shows a dense clustering of data points with no significant outliers, resulting in a straight regression line for both single and twin kid. The R^2 value, which measures the goodness of fit of the regression line, was higher for twin kid (0.9743) than for single kid (0.9396), indicating a stronger correlation between live weight and litter size in the former. This means that twin kid gained 0.79 kg/day and single kid 0.45 kg/day during 6 weeks sampling period. The high R-value confirms a positive relationship between litter size and live weight, indicating that larger litter sizes contribute to better growth and development of Saanen goat kid in the pre-weaning period. At the completion of a six-week growth experiment, the percentage of live weight gained for twin and single-born Saanen goat kid was calculated.

Figure 6 shows that twin-born Saanen kid had a higher percentage of live weight gained over 6 weeks of sampling in compared to single-born goats, with an estimated difference of 9.9% This finding suggests that twins born prior to weaning Saanen kid in this specific farm had a greater rate of live weight gain than ingly born goats.The objective of the last analysis was to determine the survivaland mortality rate of Saanen goat kid. For this purpose, data on birth and death of Saanen goat fawns were collected over a period of six weeks. Figure 7 demonstrated that the survival rate of 22 Saanen kid was significantly greater than the mortality rate, exhibiting a difference of 72.8%.

It was observed that male kid had higher live weight than female kid. This result is consistent with previous studies(Kari et al. 2019; Al-Dawood et al. 2020; Erdem et al. 2021). The differences in growth of the kid are caused by differences in sex chromosomes, possibly in the position of growth-related genes,, physiological traits and the difference in endocrinal system (Sodiq 2012). Rapid physical development of male kid' is associated with higher live weight and the presence of androgenic hormones, which have a significant impact onon the growth process (Tesema et al. 2017). However, in the second week, the live weight of male kid is slightly lower than female kid. This could be related to the condition of the male kid in the second week, where 4 of the 7 male kid had diarrhea before the treatment.For twin kid, as the litter size increases, individual birthweight decreases because the mother does not have the physiological capacity to adequately supply the fetuses with metabolic substrates (Teke et al. 2009). This statement contradicts current research as the average live weight of twin kid is higher compared to single kid. However,this is in agreement with a previous study (Teke et al. 2009), which found that mode of delivery had little effect on the live weight of kid goats during the weaning period, such that twins were weaned from birth to 45 weeks of age.According to a study by Kari eat al., (2019), these differences could be related to differences in maternal mothering ability,, as well as feed intake and farm management practices. All these factors could affect the weight of the kid after weaning. In the present studies, this situation could be related to the fact that the farm management more focused on twin kid. This is also true for the twin kid that were provided with

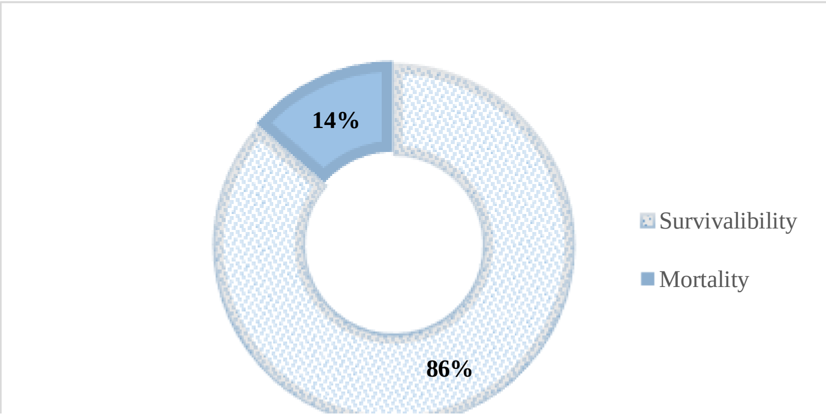


Figure 7. The survival rates of Saanen goat kid during the 6- weeks experimental period

multivitamins by the workers in the second week of sampling. The live weight of kid increased with the age of the dam, while in this study, the age of the dam in twin kid was 5 to 6-year-old and older than age of the dam in single-born kid. This statement agrees with Sodiq (2012), who claimed that the weights of young goat at birth and weaning were lower than those of older goats. The better development of mother's uterus with increasing parity and age might explain the parity of the mother effect, which has a great influence on growth before weaning (Vonnahme et al. 2018).

The mortality rate of kid before weaning has a massive impact on goat production. The survival rate of kid in this study was 86.4%, which was higher than in previous studies, that reported 79% (Ince 2010). However, the percentage of kid mortality in the current study was 13.6%, which is slightly higher than the 12.58% reported in a previous study in Bangladesh (Faruque et al. 2017). Previous studies have found that environmental factor in addition to disease have an impact on mortality rate (Khandoker et al. 2018; Chauhan et al. 2019). Farm management, coccidiosis, and weather conditionsduring the first week after birth could contribute to the high mortality rate of kid (Yıldırır et al. 2019).

In the present study, 2 of 3 mortality case were from female kid, and 1 case was from male kid (p>0.05). Tesema and colleagues found that sex and litter size did not effect kid survival but kid survival rate before weaning increased with maternal age (Tesema et al. 2017). This is consistent with the present study, whereas all 3 deaths were from kid of young mothers. In addition, the inconsistent pellet supply at this farm should be considered This situation might have caused nutritional stress and lead to a loss of the maternal body weight and a decrease in milk yield.

CONCLUSION

The growth of goat kid before weaning is influenced by their sex and the age of their mother. The survival rate of kid before weaning is not affected by their sex or litter size, but it is influenced by the age of the mother. Male kid tend to have higher average live weights over six weeks compared to female kid. It is important to improve feeding and management, especially for larger litters, in order to maintain body weight. Additionally, when managing Saanen goats, the difficulty of accurately measuring live weight under severe pellet deficiency conditions should be considered to improve production.

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Estimate the effect of non-genetic factors on the reproductive traits of Afec-Assaf strain in Bani Naim Farm, Palestine

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ABSTRAK

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Tujuan dari penelitian ini adalah untuk mengevaluasi penampilan reproduksi pada galur Afec-Assaf di peternakan intensif. Penelitian dilakukan di Bani Naim, kota Hebron, Palestina. Data yang dikumpulkan dari 450 betina dan 1660 domba yang lahir dari 2019 hingga 2022, digunakan untuk menyusun data untuk penelitian ini. Model linier dengan efek tetap digunakan untuk mengestimasi pengaruh tahun kelahiran dan paritas terhadap ukuran serasah lahir hidup (LS), ukuran serasah saat penyapihan (LSAW), dan interval beranak (LI). Komponen yang paling berpengaruh pada LS adalah paritas yang menghasilkan rata-rata 1,81 ± 0,9. Terdapat perbedaan yang nyata antara ukuran anakan yang lahir pada paritas pertama dan keempat, dengan anakan terkecil lahir pada paritas pertama (1,75±0,04) dan yang terbesar lahir pada paritas keempat (2,25±0,14). LSAW rata-rata 1,75±0,82 domba. LSAW secara signifikan dipengaruhi oleh paritas ($P<0,05$). LI adalah rata-rata 250,60±77,59 hari. Tidak ada hubungan yang signifikan ($P>0,05$) antara paritas dan LI. LI terpanjang diamati pada paritas ketiga (251,56±6,15 hari). Menurut temuan, jenis kelahiran anak domba secara signifikan dipengaruhi oleh paritas. Variabel non-genetik berdampak besar pada keragaman sifat reproduksi di Afec-Assaf.

Kata Kunci: Afec-Assaf, Litter Size, Lambing Interval, Parity, Domba

ABSTRACT

Abuzahra M, Wijayanti D, Effendi MH, Mustofa I, Eid LA. 2024. Estimate the effect of non-genetic factors on the reproductive traits of Afec-Assaf strain in Bani Naim Farm, Palestine. JITV 29(1):9-15. DOI: http://dx.doi.org/10.14334/jitv.v29.i1.3213.

The aim of the study was to evaluate the reproductive performance of the Afec-Assaf strain in intensive farming. The research was carried out in Bani Naim, Hebron city, Palestine. Data collected from 450 ewes and 1660 lambs born over the period 2019 to 2022 were used to compile the data for this study. Linear models with fixed effects were used to estimate the influence of year of birth and parity on litter size born alive (LS), litter size at weaning (LSAW), and lambing interval (LI). The most influential component in LS was parity, which resulted in an average of 1.81 ± 0.9. There was a significant difference between the size of litters born in the first and fourth parities, with the smallest litters born in the first parity (1.75±0.04) and the largest born in the fourth (2.25±0.14). LSAW lamb averaged 1.75±0.82. The LSAW was significantly influenced by parity ($P<0.05$). LI was 250.60±77.59 days on average. There was no significant ($P>0.05$) relationship between parity and LI. The longest LI was observed at third parity (251.56±6.15 days). According to the findings, the birth type of a lamb is significantly affected by the parity. Non-genetic variables have a substantial impact on the diversity of reproductive traits in Afec-Assaf.

Key Words: Afec-Assaf, Litter Size, Lambing Interval, Parity, Sheep

INTRODUCTION

Sheep are one of the most important animals because their high production and reproduction rates have a direct impact on the economy and other industries in different regions (Jalal et al. 2015; Gebreselassie et al. 2020; Hameed et al. 2022). In Palestine, one of the most common methods of earning a living from the livestock industry is raising sheep. Sheep are an important source of revenue and food for rural people, which allows them to maintain their standard of living (meat and milk).

As of October 1, 2021, the total number of sheep in Palestine had reached 771,168 heads. In Palestine, the sheep were divided up according to the different strains. There were 363,759 heads counted under the category of "local" or "Awassi", 283,651 heads counted under the category of "Asaf", 118,150 heads counted under the category of "hybrid" Afec-Awassi and Afec-Assaf, and

5,608 heads counted under the category of "other." There was a total of 322,239 heads raised using an intensive method (41.8%), while 448,929 heads were raised using a semi-intensive method (58.2%). Regarding the distribution of sheep in Palestine according to the primary reason for their raising, there were 88,592 heads (11.5%) only for meat, 99,132 heads (12.8%) mainly for meat, with some milk, 99,436 heads (12.9%) only for milk, 484,008 heads (62.8%) mainly for milk with some meat (Palestinian Central Bureau of Statistics 2022). There has not been enough research done to determine the reproductive capacity of sheep in Palestine, despite the importance of sheep in the region.

Breeding of the highly prolific Afec-Assaf strain began in 1986 when the Booroola mutation (B) at the BMPR1B gene was introduced into the Assaf breed by combining Assaf ewes with Booroola Merino (Gootwine 2014). This resulted in the introduction of the Booroola mutation into the Assaf breed. Because the B allele is present in the Afec-Assaf strain, it is inherited in a nearly dominant form, which contributes to the strain's exceptionally high rate of prolificacy (Seroussi et al. 2017). The average prolificacy of Awassi sheep is 1.30 LB/EL, while that of Assaf sheep is 1.65 LB/EL. To identify Booroola mutation carriers during backcrossing and intercrossing, researchers first tracked the frequency of forced ovulation in sheep lambs and then used direct genotyping of the FecB locus. This was carried out throughout the time that the mutation was being transmitted from one generation to the next. The breeding efforts resulted in the creation of the very productive Afec Awassi and Afec Assaf strains. Both of these strains carry the Booroola mutation and typically yield around 1.9 and 2.5 LB per embryo litter, respectively (Gootwine 2014; Gootwine 2020).

Sheep's "litter size" is one of their most valuable economic characteristics (Schmidová et al. 2014; Al-Thuwaini et al. 2020; Farrell et al. 2022; Abuzahra et al. 2023), which means the average number of lambs born to a single ewe during lambing season (Ziadi et al. 2021; Tao et al. 2021) Prolificity, also known as litter size, is the indication of sheep's reproductive efficiency that has been subjected to the most research. Prolificity is defined as the average number of lambs born from each pregnancy (Ahlawat et al. 2016; Ayele and Urge 2019; Hernández et al. 2019; Medina-Montes et al. 2021).

Sheep litter sizes also differ by breed, with the Texel and Suffolk having single births and the Booroola Merino having multiple births per litter (Ajafar Majeed Hameed et al. 2022). In contrast to more prolific breeds like the Finnsheep and the Romanov, which frequently give birth to triplets, Awassi breeds are mono ovulatory (Iber & Geyter 2013) and have very low incidences of twinning (Kridli et al. 2007). Sheep litter sizes can be affected by both genetic and environmental factors (Kumar et al. 2017).

The age of the dam has a significant impact on the amount of the litter. Once the animal reaches five years of age or reaches its fourth parity, the litter size should begin to decline (Amer & Hazzaa 2009). Peak prolificacy is typically reached between the ages of 4 and 8 years old, although other studies find an increase in litter size with increasing parity and greater litter size at the fifth parity (Assan 2020; Canché et al. 2016). Larger litter sizes are a direct effect of genetic improvement, which leads to higher meat production per flock (Assan 2020). The Afec Assaf is known as one of the most prolific strain, along with Finnsheep, Booroola Merino and Barbados Blackbelly (Kutluca Korkmaz & Emsen 2016; Mohamed et al. 2016). A Assaf breed sheep with Booroola srtain has been practiced in Middle East to increase prolificacy (Ahmed & Abdallah, 2013; Gootwine 2014; Gootwine et al. 2008; Reicher et al. 2012; Seroussi et al. 2017).

Litter size, lamb survival and lambing interval have been the objective of many studies. (Canché et al. 2016; Deribe et al. 2021; Tera et al. 2021; Kabalin et al. 2022), most notably in regions where traditional methods of husbandry are still widely used. Therefore, the aim of this study is to evaluate the reproductive performance of the Afec-Assaf strain, specifically focusing on traits such as litter size, litter size at weaning, and lambing interval. By examining these parameters, the study aimed to contribute to the understanding of the reproductive capacity and efficiency of the Afec-Assaf sheep starin, which is of significant importance in Palestine's livestock industry.

MATERIALS AND METHODS

Animal management

The research was carried out at a fattening Afec-Assaf farm in Bani Naim-Palestine, located at latitude 31° 30' 53.51 N and longitude 35° 9' 55.23 E. The flock was maintained using an intensive production technique that featured fast lambing and constant mating of the animals. One of the objectives of the breeding program was to increase the number of rams and ewe lambs suitable for breeding, in addition to the production of lambs for slaughter with a final body weight of approximately 45–60 kg. Ewe lambs were combined with rams into breeding groups when they reached the age of six months or a body weight of 45 kilograms. These groups consisted of 20–30 females and one male. During the whole course of observation, this breeding arrangement, which consisted of the same group of ewes and rams, was preserved. The ewes, lambs, and rams were selected at random, which resulted in the development of four distinct breeding groups. The sheep spent the daytime hours housed within vast barnyard pens. During the night, sheep were kept in separate pens according to their respective breeding groups. The ewes were split into smaller individual pens before the beginning of the lambing process. After a few days, the ewes and their newborn lambs were brought back to the breeding group. At the age of sixty days, the lambs were weaned. Every single sheep received a parasite prevention vaccine and was treated for parasites regularly. Because there was no grazing, all animals were given 300 grams of barley and 1.5 kilograms of hay ad libitum. During the final stage of pregnancy, they were gradually given 1.5 kg of concentrated feed, salt stones, and molasses until birth. The farms tried to achieve an average lambing interval of eight months, which would be equivalent to having 1.5 lambs per ewe per year, by having three lambs produces per ewe every two years.

Recording and the definition of variables

Individual records of 450 Afec-Assaf ewe and 1660 of lambing were used for this study from 2019 to 2022. The database was consulted to ascertain the birth year, the lambing parity, and the number of offspring produced. Immediately after the birth of the lambs, the number of newborns in the litter was counted. The lambing interval (LI) that followed each parity was determined by counting the number of days that had gone by since the previous set of lamb births. It was determined that the litter size at weaning (LSAW) should be taken into account when calculating, the LI (LI after the first parity is the number of days from the date of the first lambing to the date of the second lambing). For this study, the following categories of factors were specified: the year of birth (2019–2022), the parity of lambing (1-4), and the litter type (singleton– >4).

Statistical analysis Linear regression models with fixed effects were used to estimate the effect of non-genetic factors on the LS, LSAW and LI. Analyses were performed using IBM SPSS in the statistical program version.

The model used to analyze LS was:

$$Y_{ijkl} = \mu + A_i + P_i + e_{ij}$$

where Y_{ijkl} is observation on litter size, μ is overall population mean, A_i is fixed effect of the i^{th} year of lambing, P_j is fixed effect of the lth parity and e_{ij} is residual error.

The model for LI was:

$$Y_{ij} = \mu + A_i + P_i + e_{ij}$$

where Yij is observation on lambing interval, μ is overall population mean, Ai is fixed effect of the i^{th} year of lambing, P_j is the effect of j^{th} parity (j=1,2,3 and 4) and e_{ij} is residual error.

Because the ram and the season did not have a substantial impact on the phenotypic value of growth traits, we did not include them in our analysis. The data were provided with the mean together with the standard error (SE), with a significance level of $P<0.05$ for statistical significance and $P<0.001$ for extremely significant significance. An ANOVA was carried out to determine whether or not fixed factors had a significant influence, and the reproductive performance of ewes with different litter sizes and parities was analyzed using means and standard errors. On the SPPS spreadsheet, the obtained information was loaded and saved after it was obtained. After that, the data were examined by utilizing the SPSS application. Tukey's HSD was used to do the comparison between the means. Statistically significant values were P<0.01 and P<0.05.

RESULTS AND DISCUSSION

During this study, the reproductive performance of the Afec-Assaf strain in Palestine was evaluated, as well as the impact of non-genetic factors on these characteristics has been calculated and analyzed. Table 1 provides a presentation of descriptive statistical parameters on reproductive characteristics for the entire population. The overall mean ± and standard deviation for LS and LSAW were (1.81±0.9, 1.75±0.82) respectively. This study found that the average LI was 250.60±77.59 days. On the other hand, the absolute variability of LI was significantly lower (SD =59.3 days), in comparison to the current study findings (SD =77.59 days) in Table 1.

The year of lambing and parity were found to have a significant influence on the LS (P<0.05) in Table 2. It is commonly known that LS rises as ewe parity advances. Both the parity of the ewes and the year in which they lambed had a significant influence (P<0.001) on LSAW in Table 2. The current study found that the litter sizes of ewes that were having their first lambing had the lowest average. On the other hand, ewes that were in their fourth parity were shown to have the largest litter in Table 1. The effect of parity was favourable to dams in their earlier parities, which suggests that the characteristics necessary for consecutive litter bearing may have been well developed in such a dam. The results of the Chi-square test on the relationship between parity and litter size, as well as the relationship between parity and type of birth, are reported in Tables 3 and 4, respectively.

A greater mean and standard deviation in LS was obtained by the Afec-Assaf strain and recorded by (Gootwine 2014) (2.55±0.05), (Seroussi et al. 2017) (2.50±1.00), (Reicher et al. 2012) (1.92) and (Gootwine et al. 2008)(1.68± 0.06) in genotype ≥31/32 Assaf, however for litter size born alive (Gootwine et al. 2008) found that the LS were (1.58±0.06, 2.05±0.04 and 1.98±0.07) for genotypes ++, B+ and BB respectively, The similarities between these findings and our own are striking. (Gootwine et al. 2008)reported claim that the LS of the Afec-Assaf strain is 2.5, which is a larger number

Table 1. Descriptive statistics for litter size, litter size a weaning and lambing interval in Afec-Assaf strain

Reproductive trait	Descriptive statistics				
	N	Means	SD	Min.	Max.
LS (born alive)	913	1.81	0.9	1.00	5.00
LSAW	913	1.75	0.82	1.00	5.00
LI (days)	458	250.60	77.59	145	750

LS= Litter size, LSAW= Litter size at weaning, LI= lambing interval, SD= standard deviation

Table 2. Variance analysis of litter size, litter size a weaning and lambing interval in Afec-Assaf strain

Variable	Fixed factor	Degree of freedom	Mean square	F value	P value
LS	Year of lambing	3	5.53	4.028	<0.001
	Parity	3	4.346	5.435	<0.001
LSAW	Year of lambing	3	7.539	6.366	<0.001
	Parity	3	1.783	2.226	<0.05
LI	Year of lambing	-	-	-	-
	Parity	2	4029.501	0.749	<0.474

LS= Litter size, LSAW= Litter size at weaning, LI= lambing interval, SD= standard deviation, F Values= Mean±SE, SE= Standard Error of mean.

Table 3. Least square means ± standard error by the factor for litter size, litter size at weaning and lambing interval in Afec-Assaf strain

Fixed factor	Level of factor	LS		LSAW		LI	
		N	LSM±SE	N	LSM±SE	N	LSM±SE
Year of lambing	2019	63	1.63±0.13	63	1.46±0.09^{b}	-	-
	2020	187	1.62±0.08	187	1.53±0.07^{b}	-	-
	2021	327	1.81±0.06	327	1.77±0.06^{ab}	-	-
	2022	336	1.96±0.06	336	1.9±0.06^{a}	-	-
Parity	1	444	1.75±0.04^{b}	444	1.71±0.03^{b}	-	-
	2	272	1.80±0.05^{b}	272	1.74±0.05^{b}	279	250.80±4.5
	3	138	1.85±0.07^{b}	138	1.76±0.06^{b}	137	251.56±6.15
	4	59	2.25±0.14^{a}	59	2.03±0.12^{a}	42	236.57±9.58
	χ 2	22.843^{a}, P <0.05		29.783^{a}, P<0.01			

LS= Litter size, LSAW= Litter size at weaning, LI= lambing interval, N= Total lamb born, SD= standard deviation, Values= Mean±SE, SE= Standard Error of mean; ab superscript significant effect (P<0.05)

than what our research has revealed. This finding is comparable to those obtained by (Sodiq et al. 2011), in which the litter size of Batur sheep at the time of birth was 1.55 and 1.36 lambs after they were weaned.

Vlahek et al. (2022) recorded an improvement in LS from 1.77 in 1st parity ewes to 2.21 in 5th parity ewes, whereas, in Afec-Awassi in Palestine, LS continually expanded gradually to 5th parity.(Ahmed & Abdallah 2013) Similar trends were observed in Romanov (Murphy et al. 2020), Batur (Sodiq et al. 2011), Rajshahi (Jalal et al. 2015), Awassi (Al-Najjar et al. 2022), Bonga (Tera et al. 2021), Pelibuey (Canché et al. 2016) and Romanov crossbred sheep (Freking & Murphy 2021). This is consistent with the findings to a certain extent, as LS was found to increase to fourth parity. (2.25±0.14) in Table 3.

The result demonstrated that the Afec-Assaf strain was substantially different P<0.05, Afec-Assaf strain increased as a result of increasing parities, and this indicated that the age of the sheep was significant. The findings of the chi-square test in Table 4 showed that the parity had a significant influence on the type of birth P<0.05. moreover, the relationship between litter size and its type of birth had a significant effect P<0.001 in Table 4. The physiological development of ewes and does results in an increase in litter size as parity increases.(Deribe et al. 2014) It is well recognized that ewes' reproductive effectiveness improves with age, which leads to larger litters. This improvement is due to an enhanced ovulation rate, larger uterine capacity, and more maternal features (Assan 2020).

Table 4. Litter size by type of birth and Parity of Afec-Assaf strain

Sources	Number of borns (n, %)
Overall	1660 (100%)
Type of birth	
Single	392 (24%)
Double	706 (43%)
Triplets	330 (20%)
Quadruplets	232 (14%)
$\chi^2 = 2447.212^a$, P<0.001	
Parity	
1	781 (47%)
2	490 (30%)
3	256 (15%)
4	133 (8%)
$\chi^2 = 24.229^a$, P<0·05	

n= number of borns. [a]superscript significant effect (P<0.05)

The reduced prolificacy of primiparous ewes may be attributed to immature reproductive traits required for successive litter bearing compared to multiparous does who had attained physiological maturity (Sodiq et al. 2011). The results for Afec-Awassi and Afec-Assaf in neighboring countries such as Jordan and Syria as well as the outcomes of our study indicate that there is a high potential for improvement of the prolificacy of these breeds through introgression of the mutation into sheep breeds in Palestine. This should be economically appealing given the significant increase in the price of lamb over the past few years. Models for LS analyses frequently incorporate lambing year (Canché et al. 2016; Al-Najjar et al. 2022; Vlahek et al. 2022). In the current investigation, the total impact of lambing season on LS was found to be statistically significant P<0.05 in Table 2. Ewes lambing in 2022 had statistically significant P <0.05 larger litters in Table 2, which may have been caused by the high percentage of ewes who were having their fourth lambing in that year.

During the lambing season of 2021, a greater number of LSAW were documented in Table 3. However, LSAW were found during 2019 at the commencement year of the selection program which was 1.46 ± 0.09^b litter/ewes. The percentage of LSAW outcomes that were similar to LS increased steadily throughout the selection. Similarly, (Habtegiorgis et al. 2022) stated a trend toward improvement in litter size characteristics as a result of the ongoing breeding of Bonga and Horro sheep. The influence of the lambing year on LSAW has also been documented by other researchers. (Hanford et al. 2005; Sodiq et al. 2011; Habtegiorgis et al. 2022) in Doyogena, Karacabey and Batur sheep, (Tesema et al. 2020) for Dorper x Tumele sheep breeds, and (Deribe et al. 2021) for Dorper crossbred lambs. (Habtegiorgis et al. 2022) stated the litter size at born 1.57 and weaning was 1.50. Other researchers stated the litter size at born and weaning were 1.422 and 1.35 respectively, for Karacabey Merino ewes, (Hanford et al. 2005) stated litter size at birth and weaning of Rambouillet sheep were 1.39 and 0.88 lambs, respectively. The current study of Afec-Assaf ewes obtained larger litter sizes at birth and weaning than the reports listed above on sheep, both when the lambs were born and when they were weaned.

The significant range in LI that was observed in this research might have been the result of intentional mating (continuous mating). The likelihood ratio (LI) was not significantly affected by any of the tested factor's P>0.05 in Table 2. After the second and third parities, the LI that was reported as being the longest (250.80±4.5 and 251.56±6.15 days) was significantly longer than the LI that was recorded after the fourth parities in Table 3. This is in agreement with the findings of (Vlahek et al. 2022) in Romanov sheep, (Ahmed & Abdallah 2013)in Afec-Awassi and improved Awassi, (Tera et al. 2021) in Bonga sheep and (Jalal et al. 2015) in Rajshashi sheep. (Ahmed & Abdallah 2013) informed non-significant P>0.05 differences in the first, second and third LI of Afec-Awassi ewes with different parities. same results were found by (Jalal et al. 2015). The average lambing interval for semi-intensive and intensive feeding systems was found to be 338 days (Ahmed and Abdallah 2013) but the current result was found to be shorter (250.60±77.59). Assuming a 60-day ideal post-lambing oestrus interval, a 90% first-service conception rate, and a gestational period of 150 days, the ideal time between lambing is approximately 210 days. It is possible to double the number of sheep produced in the country in just over one and a half years if the lambing interval can be shortened by optimizing the breed, age, parity, and management practices used in this study (Vlahek et al. 2022). It was hypothesized and discussed that a higher rate of milk production in multiparous ewes might be one of the factors contributing to the longer LI in ewes that have had more than one litter.(Ahmed and Abdallah 2013) reported milk production of 169.0 (43.7), 197.0 (61.3) and 196.7 (77.1) kg in total milk yield to 150 days in Afec-Awassi, Assaf and Awassi x Assaf sheep respectively.

CONCLUSION

The study found a significant increase in litter size at birth and weaning during the fourth parity. Parity did not have a significant impact on the lambing interval. Reproductive performance varied among the ewes, primarily due to non-genetic factors. Parity played a crucial role in determining litter size at birth and weaning. These findings are valuable for evaluating the reproductive efficiency of the Afec-Assaf breeding program and proposing breeding strategies for improvement. Further research on body weight during delivery and weaning, particularly considering different birth techniques, can provide economic insights for fattening farms. The introduction of the Booroola FecB gene resulted in increased prolificacy in the Assaf population, suggesting its potential to enhance the reproductive performance of sheep breeds in Palestine.

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Innovation of Standardized Extract of Yam Bean Seed and Red Betel Leaf as Bioinsecticide for Myiasis Treatment

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ABSTRAK

Mustika AA, Sutardi LN, Purwanto ES, Nefo F, Andriyanto, Wientarsih I, Sawitri DH, Wardhana AH. 2024. Inovasi ekstrak terstandar biji bengkuang dan daun sirih merah sebagai bioinsektisida miasis. JITV 29(1):16-28. DOI:http://dx.doi.org/10.14334/jitv.v29.i1.3158.

Miasis pada ternak menyebabkan kerugian yang signifikan secara ekonomi maupun produksi. Penggunaan bahan kimia untuk mencegah atau mengendalikan miasis pada ternak sering digunakan, namun penggunaan insektisida berbahan dasar kimia dalam jangka waktu tertentu dapat menimbulkan dampak negatif. Penelitian ini bertujuan untuk mengetahui khasiat kombinasi ekstrak etanol biji bengkuang dan daun sirih merah secara in vitro sebagai sediaan yang mampu membunuh semua stadium larva Chrysomya bezziana agen penyebab miasis. Penelitian ini terbagi menjadi 11 kelompok perlakuan. Masing-masing sebanyak 20 Larva instar 1 (L1), Larva instar 2 (L2), dan Larva instar 3 (L3) C.bezziana digunakan untuk pengujian in vitro menggunakan pot plastik yang berisi media larva dan ekstrak etanol biji bengkuang, daun sirih merah, serta kombinasi ekstrak etanol biji bengkuang dan daun sirih merah dengan konsentrasi bertingkat 0,5%, 1%, dan 2%. Asuntol dan akuades steril digunakan sebagai kontrol positif dan negatif. Hasil penelitian menunjukkan bahwa kombinasi ekstrak etanol biji bengkuang dan daun sirih merah pada konsentrasi 2% mampu menyebabkan 100% kematian larva dan 100% pupa tidak menetas. Pengujian L3 menunjukkan bahwa kombinasi ekstrak etanol biji bengkuang dan daun sirih merah mampu menyebabkan penurunan daya tetas pada semua konsentrasi. Pengujiaan L1 dan L2 untuk mengindikasikan efektivitas ekstrak sebagai racun perut, sedangkan pengujian pada L3 sebagai indikasi racun kontak. Biji bengkuang dan daun sirih merah memiliki daya larvasida terhadap beberapa jenis larva serangga Chrysomya bezziana.

Kata Kunci: Bioinsektisida, *Chrysomya bezziana*, Miasis, Biji Bengkuang, Daun Sirih Merah

ABSTRACT

Mustika AA, Sutardi LN, Purwanto ES, Nefo F, Andriyanto, Wientarsih I, Sawitri DH, Wardhana AH. 2024. Innovation of standardized extract of yam bean seed and red betel leaf as bioinsecticide for myiasis treatment. JITV 29(1):16-28. DOI:http://dx.doi.org/10.14334/jitv.v29.i1.3158.

Myiasis causes severe economic and production losses in animals. Chemicals are frequently employed to prevent or control myiasis in animals, however long-term exposure to chemical-based pesticides can have severe effects. This study aims to assess the effectiveness combination of ethanol extract of yam bean seeds and red betel leaf in vitro as a preparation capable of killing all larval stages of Chrysomya bezziana, the primary agent responsible for myiasis. This study consisted of eleven treatment groups. Twenty instar 1 (L1), 2 (L2), and 3 (L3) C. bezziana larvae were used for in vitro testing utilizing plastic pots containing larval media and ethanol extract of yam bean seeds, red betel leaf, and their combination as well as a combination of 0.5%, 1%, and 2% concentrations of ethanol extract of yam bean seeds and red betel leaf. Asuntol and sterile distilled water were utilized as positive and negative controls, rerspectively. The results demonstrated that the combination of 2% ethanol extract of yam bean seeds and red betel leaf was able to induce 100% larval death and 100% pupal failure. The L3 test demonstrated that the combination of yam bean seed ethanol extract and red betel leaf reduced hatchability at all doses. The L1 and L2 tests suggested that the extract was efficient as a stomach poison, whilst the L3 test indicated that it was also a contact poison. Yam bean seeds and red betel leaf show larvicidal efficacy against multiple species of Chrysomya bezziana insect larvae.

Key Words: Bioinsecticide, *Chrysomya bezziana*, Myiasis, Red Betel Leaf ,Yam Bean Seeds

INTRODUCTION

Myiasis is an infestation of fly larvae in living vertebrate tissues and organs, as well as in necrotic tissues. In endemic regions, the prevalence of myiasis reaches 95 %, affecting all animal species, including humans. Therefore, the World Organization for Animal Health (WOAH) categorizes this disease as list B, which is an infectious disease that has a socio-economic impact or a health importance value on a country in international trade associated to products of origin (Mustika et al. 2016a). The primary agents that cause myiasis are divided into three groups based on their distribution: the *Cochliomya hominivorax* fly (The New World Screwworm Fly) that is prevalent in the Americas, the *Wohlfahrtia magnifica* fly that is prevalent from Europe to China, and the *Chrysomya bezziana* fly that is prevalent in the tropics and parts of Africa (Wardhana and Diana 2014). This disease is caused by *Chrysomya bezziana*, an obligate parasite (Wulandari and Pemayun 2019).

Myiasis typically affects animals, including sheep, goats, cows, and other vertebrates (Zuleika 2015). Myiasis is prevalent in tropical regions, particularly among those from poor socioeconomic classes (Singh and Kaur 2019). The high incidence of myiasis in the tropics is caused by inadequate hygienic conditions and the increased aggressivity of myiasis-causing flies (Kristinawati et al. 2019). Myiasis has become a severe global issue due to the international spread of flies, particularly in tropical and subtropical settings. Cases of myiasis in ruminants in Iraq (Zhou et al. 2019), small animals in Australia (Welch et al. 2014), and both small animals and ruminants in South Africa (Mukandiwa et al. 2012) are examples of the rapid expansion of myiasis around the globe.

Myiasis causes significant economic losses, especially in areas where livestock is prevalent. Myiasis can be dangerous if not treated immediately; if left untreated for an extended period of time, it will target important organs and cause secondary infection. Due to myiasis, the condition of cattle will become weakened, with decreased hunger, fever, a drop in milk output and body weight, and even anemia (Susari et al. 2020).

Myiasis control is not yet excellent, despite the use of antibiotics and synthetic insecticides such as coumaphos, diazinon, fenthion, ivermectin, amitraz, enrofloxacin, and spiramycin by topical therapy (spraying) and dipping (Mustika et al. 2016a). The use of synthetic pesticides can result in adverse effects such as the emergence of resistant strains, threats to food safety, and environmental degradation (Ballweber and Baeten 2012). Therefore, alternate treatments, such as medicinal plant-based preparations, are required for the treatment of myiasis in the field.

Traditional and modern therapeutic compounds have been derived from medicinal plants (Khater 2012). Historically, plant-derived bioactive chemicals have been utilized as an effective parasiticide against resistant populations (Molento et al. 2020). In situations of myiasis, nutrient-rich vegetables might be utilized as an alternative treatment. Previous research has demonstrated that plants from the Meliaceae, Annonaceae, Asteraceae, Piperaceae, and Rutaceae families can be utilized as bioinsecticides, including for the treatment of myiasis (Wientarsih et al. 2017). Yam bean seeds (*Pachyrhizus erosus*) and red betel leaf (*Piper crocatum*) have the potential to be used as myiasis bioinsecticides.

Rotenon is the insecticide-potent active component of the yam plant. The rotenone chemicals in yam have been shown to be effective larvicides against Aedes aegypti and Musca domestica mosquito larvae. This chemical is said to impede insect metabolism as its mode of action (Oguh et al. 2019). The ethanol extract of yam bean seeds has been proven to be effective as a larvicide against the parasite *Chrysomya bezziana*, which causes myiasis in livestock (Mustika et al. 2016a). Essential oils, carvcrol, eugenol, chavicol, flavonoids, arecolin, and tannins are present in red betel leaf phytochemicals. Carvacrol is an antiseptic and disinfectant, but eugenol is a pain reliever. Red betel leaf essential oil contains chavicol and eugenol, which have antibacterial properties. When used to cure infections caused by harmful bacteria in the body, red betel leaf's antimicrobial qualities are quite effective (Kurniawati et al. 2014). Red betel leaf contains flavonoids, arecolin, and tannins, which promote wound healing and blood flow (Widhyari et al. 2018). In the treatment of myiasis, it is anticipated that the interaction between these two medicinal herbs will be synergistic. This study intends to establish *in vitro* whether a combination of ethanol extract of yam and red betel seeds is effective against all stages of the myiasis-causing parasite *Chrysomya bezziana*.

MATERIALS AND METHODS

This study was conducted in multiple phases. Using the Liquid Chromatography Mass Spectrometry (LCMS-MS) instrument, the active chemical is identified in the first step. In the second phase, L1, L2, and L3 *C. bezziana* larvae were examined *in vitro* for larvicidal activity.

Extraction of yam bean seeds and red betel leaf

The maceration procedure was used to extract dried simplicia yam bean seeds and red betel leaf. Three 24-hour macerations were performed using 96% ethanol as the solvent. The proportion of simplicia to solvent was 10 :1. The extract produced from maceration was then evaporated using a rotary evaporator at 40-50 °C and 50 rpm in a temperature range of 40-50 °C (Mustika et al. 2016a).

Identification of active compound

Using a Liquid Chromatography Mass Spectrometry (LCMS-MS) apparatus, the active components of yam bean seeds and red betel leaf will be identified. UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific with Accucore C18 column, 100 x 2.1 mm, 1.5 m was utilized as the LCMS (ThermoScientific).

In vitro test of the combination of yam extract and red betel leaf extract at L1 and L2

In vitro studies were conducted on two distinct media: meat-blood mixture (MBM) media for the first instar larval test (L1) and larval rearing media (LRM) media for the second instar larvae test (L2) (L2). MBM medium that has been combined with yam bean seed extract and red betel leaf at tiered concentrations of 0.5%, 1%, and 2% are placed in a plastic container measuring 18.5 x 4.5 x 4.5 cm. Twenty (L1) of each replication were placed on the media and maintained at a room temperature of 30–32°C. The larvae that survived until day 2 were transferred to new plastic containers and raised on LRM media until they developed into pupae and hatched into imago. L2's examination process is identical to L1's, but LRM media are employed (Wardhana et al. 2014).

In vitro test of the combination of yam extract and red betel leaf extract at L3

The test was conducted in a medicinal pot containing 0.5%, 1%, and 2% concentrations of yam bean extract and red betel leaf in stratified proportions. The larvae were immersed in the test solution for 10 seconds, 60 seconds, and 180 seconds as part of the contact toxic effect test. Twenty larvae per replication were submerged in 10 ml of each treatment solution for 10, 60, and 180 seconds. The larvae were incubated at 30-32 °C until they developed into pupae and hatched into imago (Spradbery 2002).

RESULTS AND DISCUSSION

Analysis of the active compound in yam bean seed

The LC-MS results for the ethanol extract of yam bean seeds revealed that the extract contains 168 active compounds. Figure 1 presents the chromatogram from the LC-MS analysis of the ethanol extract of red betel leaf

Rotenone, curcumin, and coumarin are the active compounds in the ethanol extract of yam bean seeds that have potential as insecticides (Figure 2). In addition, yam contains the active substances rotenone, coumarin, coumestan, isoflavone, isoflavanone, and other isoflavonoid groups, according to a review of the scientific literature (Basukriadi and Wilkins 2014; Mustika et al. 2016b).

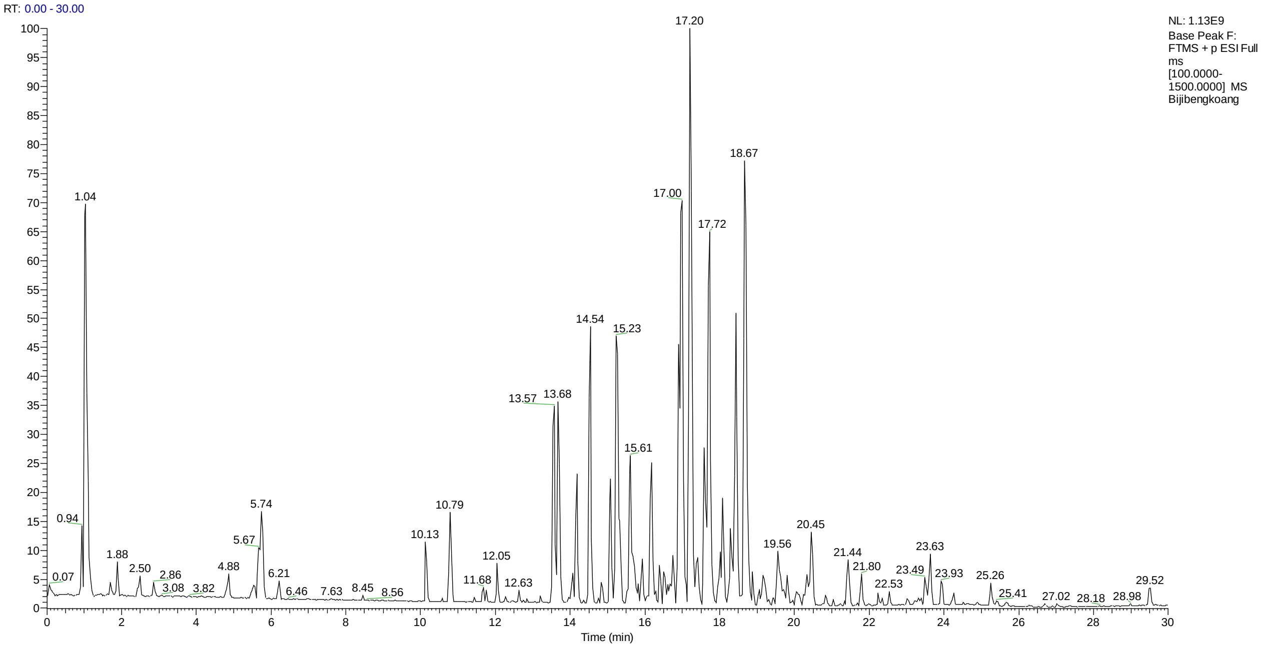
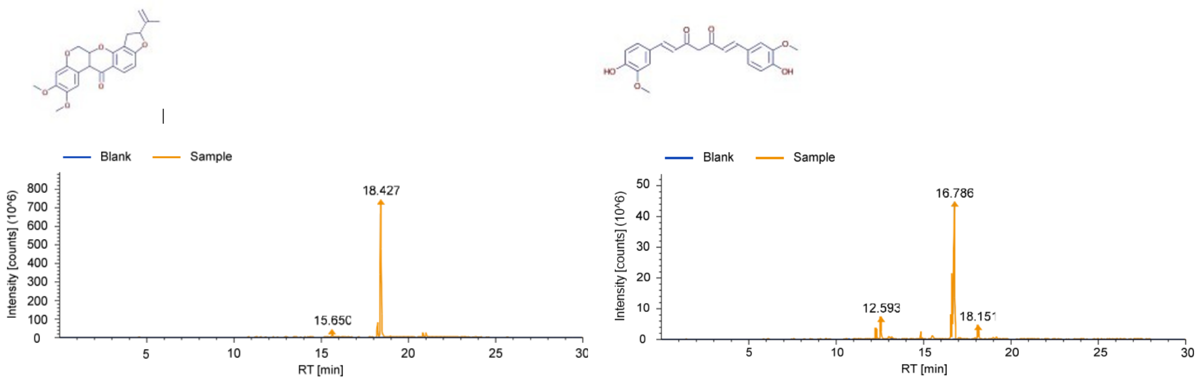
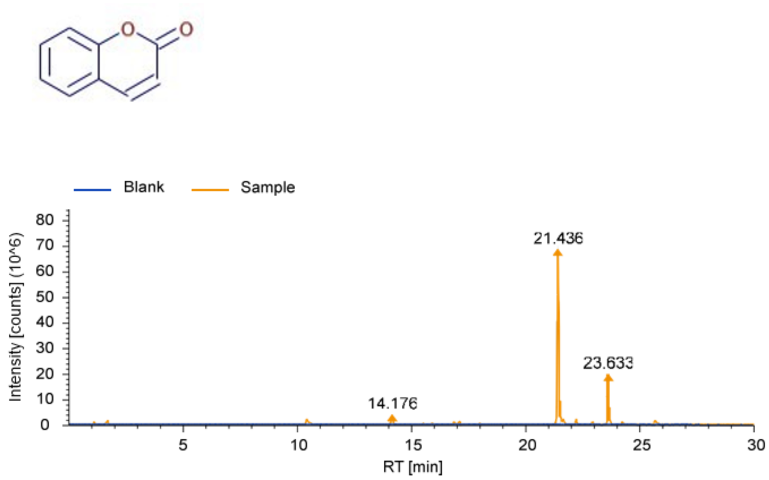


Figure 1. LC-MS chromatogram graph of yam bean seed extract



A. Rotenone

B. Curcumin



C. Coumarin

Figure 2. The results of the LC-MS analysis of yam bean seeds active compound (A: rotenone¸ B: curcumin, C: coumarin)

Rotenone ($C_{23}H_{22}O_6$) functions as a larvicide by inhibiting nicotinamide adenine dinucleotide CoQ reductase (complex 1) in the initial phase of mitochondrial electron transport (Barceloux 2008). Blocking mitochondrial electron transport prevents the generation of energy that will be sent to the cell and disrupts the physiological function of the cell, leading to the larvae's death (Ahdiyah and Purwani 2015). Curcumin ($C_{21}H_{20}O_6$) is an insoluble flavonoid molecule that is soluble in acetone, ethanol, and dimethylsulfoxide. Curcumin has a molecular weight of 368.37 g/mol and a boiling point of 183°C, according to (Sethi et al. 2009). Organophosphate and carbamate insecticides target the enzyme acetylcholinesterase, which plays a key role in synaptic transmission and is a primary molecular target (Matiadis et al. 2021). Curcumin is recognized to inhibit acetylcholinesterase enzyme activity similarly to pyridostigmine bromide and malathion (Rao et al. 2021). Coumarin ($C_9H_6O_2$) is a phenylpropanoid having a six-ring lactone structure and a 2H-1-benzopyran-2-one core (Isnawati et al. 2008). A number of the pharmacological effects of coumarine have been thoroughly examined. Coumarin can stimulate the generation of reactive oxygen species (ROS) and reduce the selectivity of cell membranes (Barbosa et al. 2018).

Analysis of the active compound in red betel leaf

The LC-MS study of red betel leaf ethanol extract revealed that the extract included 208 chemicals as active components. Figure 3 illustrates the chromatogram of the ethanolic extract of red betel leaf as determined by LC-MS.

Quassin and coumarin are the possible insecticides found in the ethanol extract of red betel leaf, according to a review of the relevant literature (Figure 4). Quassin ($C_{22}H_{28}O_6$) is the first member of the class C Tetracyclic C20-quassinoids to be isolated from *Picrasma*.

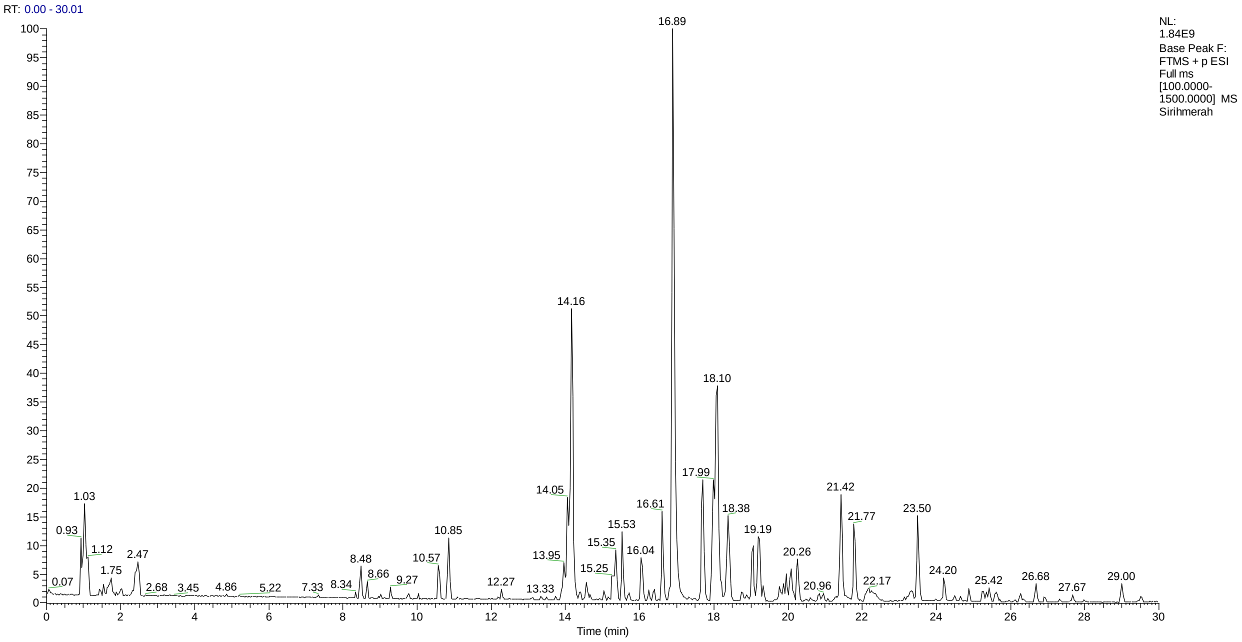
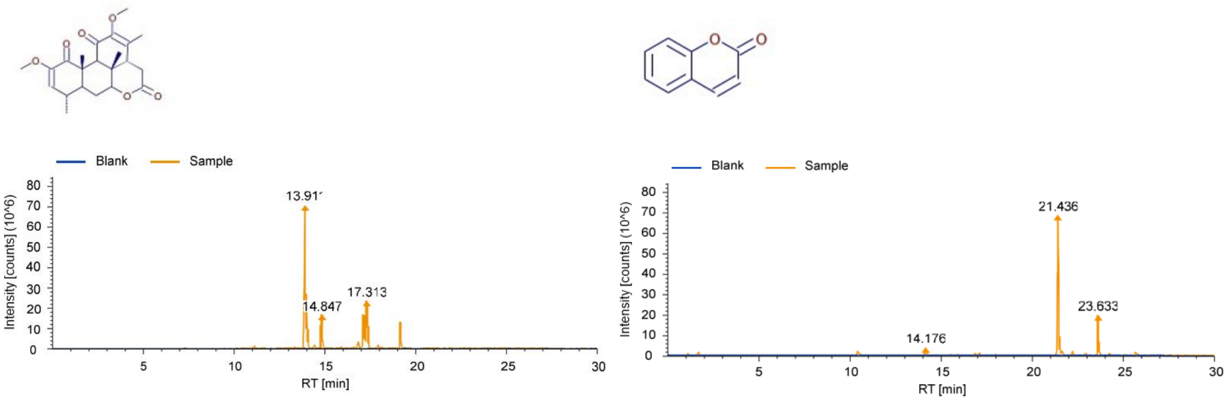


Figure 3. LC-MS chromatogram graph of red betel leaf extract



A. Quassin

B. Coumarin

Figure 4. The results of the LC-MS analysis of red betel leaf active compound (A: quassin¸ B: coumarin)

ailanthoids. C_{26}, C_{25}, C_{20}, C_{22}, C_{19}, and C_{18} are classed as six distinct classes of quassinoids (Duan et al. 2021). Quassinoids exhibit many biological effects, including anticancer, antimalarial, anti-inflammatory, antifeedant, insecticidal, amoebicidal, antiulcer, and herbicide properties, according to recent pharmacological and clinical investigations (Houël et al. 2013). Coumarin ($C_9H_6O_2$) is a phenylpropanoid containing a 2H-1-benzopyran-2-one core and a six-ring lactone ring (Isnawati et al. 2008). Several of coumarine's pharmacological activities have been thoroughly examined. Coumarin can stimulate reactive oxygen species (ROS) generation and result in a loss of cell membrane selectivity (Barbosa et al. 2018)

In vitro results

Mortality of larvae and imago, pupa weight, and hatchability were evaluated *in vitro*. Larvae weighing less than 30 mg are typically incapable of developing into pupae, but pupae weighing less than 23.5 mg do not typically hatch into adult flies (Wardhana and Muharsini 2004). Environmental variables can have an impact on pupa hatchability (Wardhana 2016). The interaction between pupa weight and hatchability can influence the mortality rate of larvae and imago.

Table 1 demonstrates that ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf are larvicidal against L1. This is shown by the fact that the larval death rate increases as the concentration of the extract increases. The administration of ethanol extract of yam bean seeds at varying dosages was able to kill all of the larvae, preventing their development into pupae. As a result, the larvae in this population never reached the imago stage.

Depending on the concentration of the preparation, red betel leaf ethanol extract was able to induce variable levels of larval mortality. The injection of increasing doses of an ethanolic extract of red betel leaf resulted in mortality rates of 80%, 82%, and 93% among L1 larvae. Larvae can develop into pupas, but as their weight and volume decrease, their hatchability diminishes. Larvae fed red betel leaf at concentrations of 1% and 2% were able to hatch into imago, however the imago's wing condition was so poor that they could not live long.

The combination of ethanol extract of yam bean seeds and red betel leaf induced 77% larval mortality at a concentration of 0.5%, whereas at concentrations of 1% and 2% it was capable of killing 100% of L1 larvae. The larvae were able to develop into pupae after receiving 0.5% ethanol extract of yam bean seeds and red betel leaf, but their weight and volume decreased, resulting in a decrease in hatchability. The larvae were unable to grow into pupae at doses of 1% and 2%. As a result, the larvae in this population never reached the imago stage.

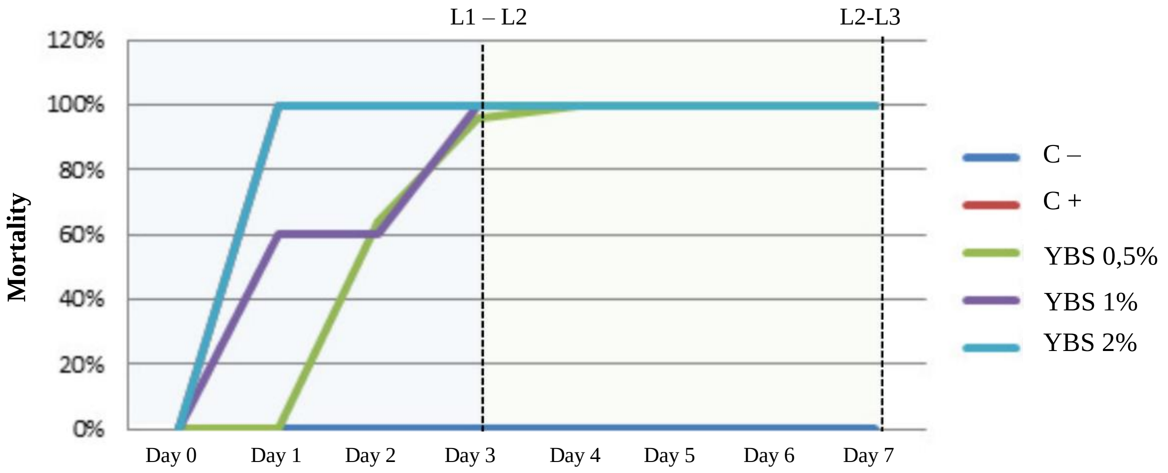
At all doses, the yam bean ethanol extract was able to eliminate all L1 larvae. Between the first and third day, larval mortality occurred (Figure 5). At concentrations of 0.5%, 1%, and 2%, the ethanol extract of red betel leaf killed L1 larvae by 80%, 82%, and 93%, respectively. From the third to the seventh day following administration of an ethanolic extract of red betel leaf, larvae died (Figure 5). At concentrations of 0,5% the ethanol extract of yam bean seeds and red betel leaf killed 77 percent of L1 larvae, while at a concentration of 1% and 2% it was able to kill 100 percent of L1 larvae. From the second to seventh day, larval mortality occurred (Figure 5).

Table 2 demonstrates that ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf has larvicidal activity against L2 and tend to have the same impact as L1. The administration of ethanol extract of yam bean seeds at varying dosages was able to kill all of the larvae, preventing their development into pupae. As a result, the larvae in this population never reached the imago stage. Depending on the concentration of the formulation, red betel leaf ethanol extract was able to induce variable levels of larval mortality. 7 %, 26 %, and 21 %, respectively, of L2 larvae were killed by increasing concentrations of ethanolic extract from red betel leaf. Larvae are able to develop into pupas, but their hatchability decreases as their weight and volume decrease. Larvae fed a 0.5% concentration of red betel leaf were able to hatch into imago, however the imago's wings were deformed, limiting its ability to survive. Different quantities of yam bean seed ethanol extract were able to kill 100 percent of L2 larvae, preventing their development into pupae. As a result, the larvae in this population never reached the imago stage.

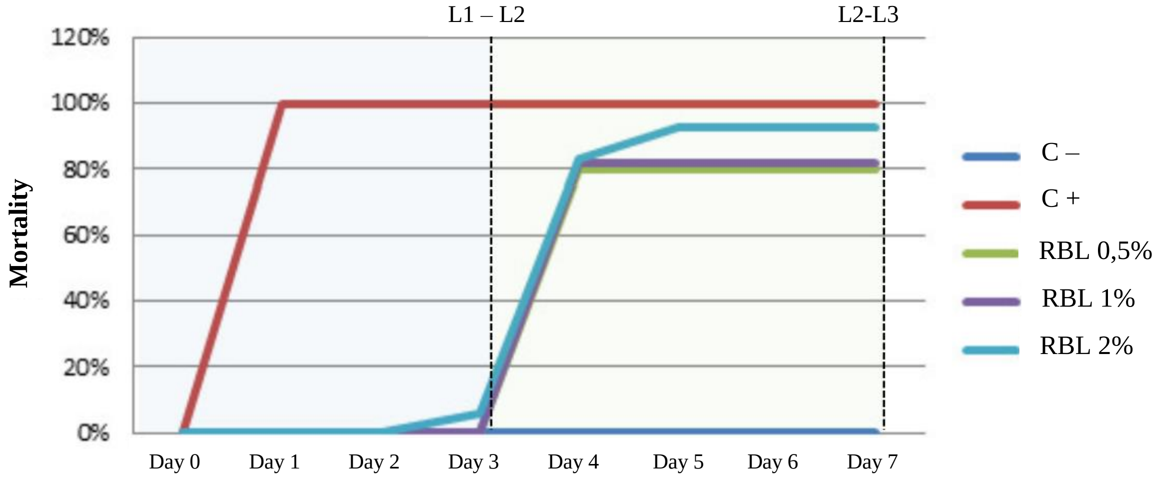
Table 1. The significance of larval mortality, average pupal weight, hatchability, and mortality of imago L1 based on *in vitro* tests using ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Treatment groups	Mortality of larvae (%)	Mean of pupal weight (g)	Hatchability rate (%)	Mortality of imago (%)
Yeam bean seed 0.5%	$100±0^{a}$	$0±0^{c}$	$0±0^{c}$	$100±0^{a}$
Yeam bean seed 1%	$100±0^{a}$	$0±0^{c}$	$0±0^{c}$	$100±0^{a}$
Yeam bean seed 2%	$100±0^{a}$	$0±0^{c}$	$0±0^{c}$	$100±0^{a}$
Red betel leaf 0.5%	$80±7.91^{b}$	$0.00589±0.00309^{c}$	$0±0^{c}$	$100±0^{a}$
Red betel leaf 1%	$82±13.51^{ab}$	$0.0073±0.01026^{c}$	$40±54.8^{b}$	$100±0^{a}$
Red betel leaf 2%	$93±13.04^{ab}$	$0.0064±0.00921^{c}$	$40±54.8^{b}$	$100±0^{a}$
Combination 0.5%	$77±24.9^{ab}$	$0.01761±0.01464^{b}$	$0±0^{c}$	$100±0^{a}$
Combination 1%	$100±0^{a}$	$0±0^{c}$	$0±0^{c}$	$100±0^{a}$
Combination 2%	$100±0^{a}$	$0±0^{c}$	$0±0^{c}$	$100±0^{a}$
Control -	$0±0^{c}$	$0.03431±0.000741^{a}$	$86±7.42^{a}$	$14±7.42^{b}$
Control + (Asuntol)	$100±0^{a}$	$0±0^{c}$	$0±0^{c}$	$100±0^{a}$

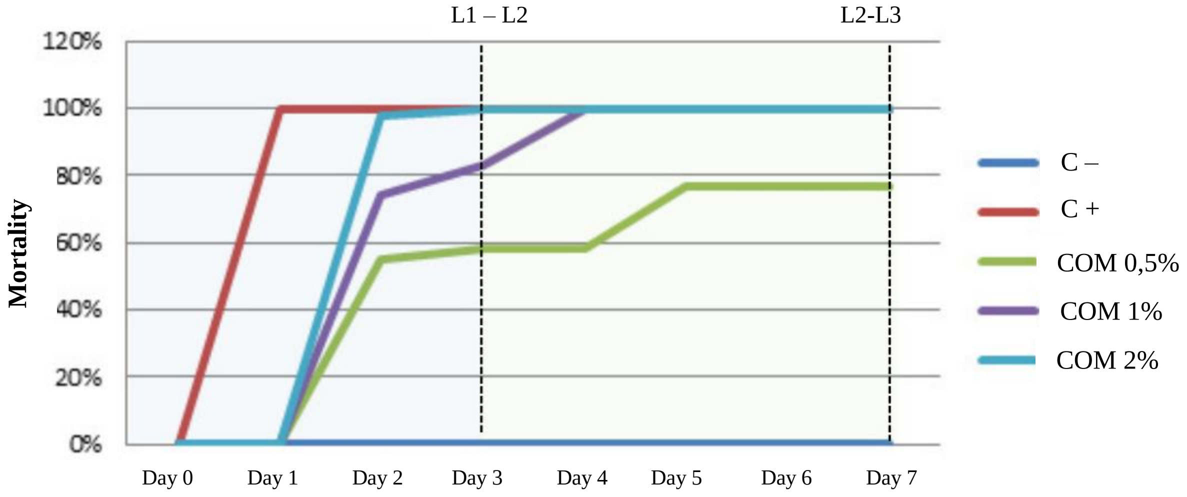
Different superscript letters on the same line showed significant differences (P<0.05)



A. Mortality time graph of L1 ethanol extract of yam bean seed



B. Mortality time graph of L1 ethanol extract of red betel leaf



C. Mortality time graph of L1 the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Figure 5. Mortality time graph of L1 ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Table 2. The significance of larval mortality, average pupal weight, hatchability, and mortality of imago L2 based on *in vitro* tests using ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Treatment groups	Mortality of larvae (%)	Mean of pupal weight (g)	Hatchability rate (%)	Mortality of imago (%)
Yam bean seed 0.5%	100 ± 0^{a}	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Yam bean seed 1%	100 ± 0^{a}	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Yam bean seed 2%	100 ± 0^{a}	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Red betel leaf 0.5%	$7\pm6,.71^{cd}$	0.03665 ± 0.00322^{a}	$67,1\pm23,2^{b}$	90 ± 14.58^{a}
Read betel leaf 1%	26 ± 19.49^{b}	0.01831 ± 0.00835^{b}	0 ± 0^{a}	100 ± 0^{a}
Read betel leaf 2%	21 ± 1.884^{bc}	0.012678 ± 0.00132^{b}	0 ± 0^{a}	100 ± 0^{a}
Combination 0.5%	100 ± 0^{a}	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Combination 1%	100 ± 0^{a}	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Combinantion 2%	100 ± 0^{a}	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Control -	0 ± 0^{d}	0.0396 ± 0.001493^{a}	$59\pm13,42^{b}$	59 ± 13.42^{b}
Control + (Asuntol)	100 ± 0^{a}	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}

Different superscript letters on the same line showed significant differences ($p<0.05$)

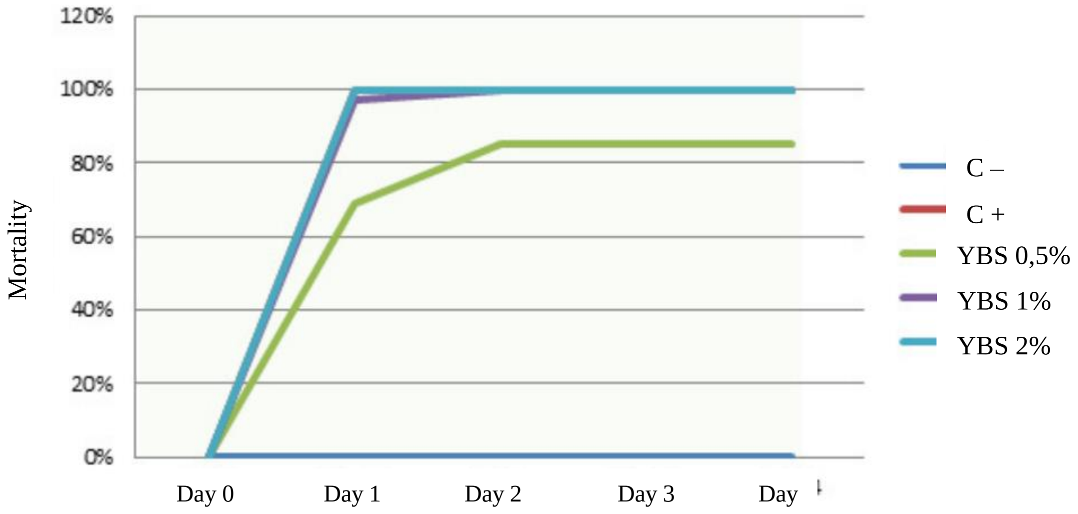
At all doses, the ethanol extract of yam bean was able to kill 100 percent of L2 larvae. Death of larvae happened between the first and fourth day (Figure 6). At concentrations of 0.5%, 1%, and 2%, red betel leaf ethanol extract was able to kill L1 larvae by 7%, 26%, and 21%, respectively. Larvae administered an ethanolic extract of red betel leaf perished between the first and fourth day (Figure 6). At all doses, the combination of ethanol extract of yam bean seeds and red betel leaf killed 100 percent of L2 larvae. Death of larvae occurred between the first and fourth days (Figure 6).

Table 3 demonstrates that the ethanol extract of yam bean seeds, the red betel leaf, and the combination of the ethanol extract of yam bean seeds and the red betel leaf have larvicidal action against L3 and tend to have the same impact as L1 and L2. The L3 larvicidal test revealed that all treatment groups (ethanol extract of yam bean seeds, red betel leaf, and a combination of ethanol extract of yam bean seeds and red betel leaf) at 10, 60, and 180 seconds of immersion resulted in smaller changes in pupa weight and were significantly different from the group negative control, but not from the group positive control.

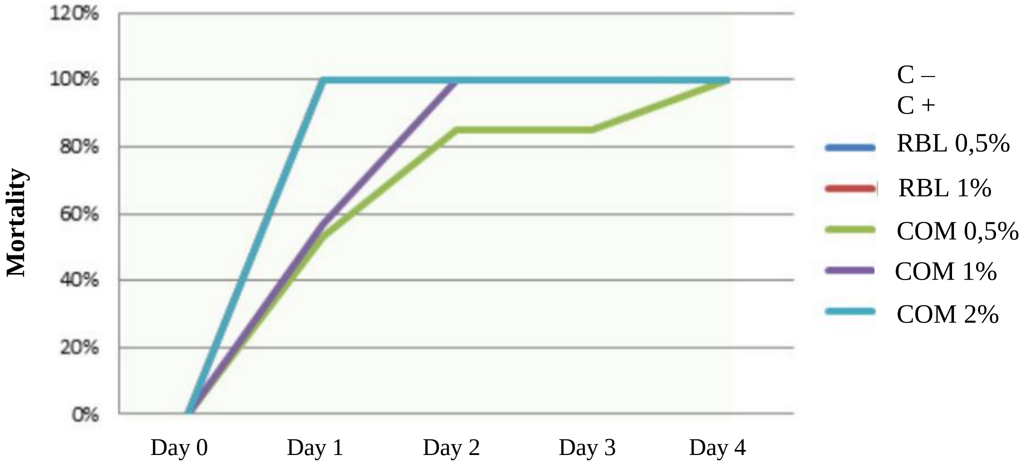
All treatment groups were able to lower hatchability at 10 seconds of immersion, based on hatchability measurements. This is evidenced by the preparation's 0% hatchability, which indicates that these three preparations did not differ significantly from the positive control. In contrast, only red betel leaf ethanol extract with a 0.5% concentration showed a 67.1% hatchability. This means that there is no substantial difference between the preparation and the negative control. The imago mortality rate at 10 seconds of immersion indicated that all preparations were able to raise imago mortality. This is evidenced by the fact that the mortality rate of imago is 100 percent in all preparations, except for the 0.5% ethanol extract of red betel leaf, where it is 90 percent (Table 3).

Table 3 demonstrates that the hatchability and mortality of imago after 60 seconds of immersion varied based on the concentration of the preparation. At 60 seconds of immersion, the concentration of 2% ethanol extract of yam bean extract and red betel leaf, which was 8%, showed the lowest hatchability. The difference between this activity and the positive control was not statistically significant. All preparations were able to raise imago mortality after 60 seconds of immersion. This is evidenced by the fact that the mortality rate of imago in each preparation was not substantially different from that of the positive control. Immersion for 60 seconds also revealed that 2% ethanol extract of yam bean extract and 2% ethanol extract of yam bean seeds and red betel leaf produced the highest imago mortality rate.

The hatchability and mortality of imago during 180 seconds of immersion varied with the concentration of the solution. The combination of ethanol extract of yam bean seeds with a concentration of 2% had the lowest hatchability after 180 seconds of immersion, at 9% (Table 3). This shows that the preparation does not differ significantly from the control sample. In general, the imago mortality rate at 180 seconds of immersion demonstrated that all preparations were able to raise imago mortality. This is evidenced by the fact that the



A. Mortality time graph of L2 ethanol extract of yam bean seed



B. Mortality time graph of L2 the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Figure 6. Mortality time graph of L2 ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

imago mortality rate of each preparation did not differ significantly from the positive control. Immersion for 180 seconds also revealed that a 2% mixture of ethanol extract of yam and red betel leaf extract had the highest mortality rate among imago.

Chemicals are frequently used to prevent or control pest infestations since they are the simplest and most cost-effective method (Hidalgo et al. 1998). However, prolonged use of chemical-based insecticides can lead to vector resistance if the use is not targeted, the dose is not appropriate, the application is not timely, and it has a negative effect on the environment and non-target organisms (Kementerian Kesehatan RI 2018). Therefore, a safe alternative to traditional insecticides is required. Plant products have been traditionally employed as pesticides throughout the world for decades. Compared to synthetic pesticides, vegetable insecticides are safer for the environment, cheaper, and easier to use by farms and small businesses (Belmain et al. 2001). This is due to the fact that bioinsecticides are effective against a restricted number of species, frequently biodegrade into non-toxic chemicals, and may be suited for use in integrated pest control, making them safer (Kim et al. 2003). Vegetable insecticides are natural substances derived from plants that contain bioactive secondary metabolites. These bioactive chemicals can be employed as synthetic pesticides.

The belief that red betel leaves and yam bean seeds are effective insecticides is backed by the fact that both plants contain naturally occurring compounds believed to be effective as insecticides. The chromatographic study of yam bean seeds revealed the presence of chemicals with rotenone and curcumin. Rotenone is an isoflavonoid with contact and gastrointestinal toxicities.

Table 3. The significance of average pupal weight, hatchability, and mortality of imago L3 based on *in vitro* tests using ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Treatment groups	Mean of pupal weight (g)	Hatchability rate (%)	Mortality of imago (%)
Immersed for 10 seconds			
Yam bean seed 0.5%	0±0^{c}	0±0^{a}	100±0^{a}
Yam bean seed 1%	0±0^{c}	0±0^{a}	100±0^{a}
Yam bean seed 2%	0±0^{c}	0±0^{a}	100±0^{a}
Red betel leaf 0.5%	0.03665±0.00322^{a}	67.1±23.2^{b}	90±14.58^{a}
Red betel leaf 1%	0.01831±0.00835^{b}	0±0^{a}	100±0^{a}
Red betel leaf 2%	0.012678±0.00132^{b}	0±0^{a}	100±0^{a}
Combination 0.5%	0±0^{c}	0±0^{a}	100±0^{a}
Combination 1%	0±0^{c}	0±0^{a}	100±0^{a}
Combination 2%	0±0^{c}	0±0^{a}	100±0^{a}
Control -	0.0396±0.001493^{a}	59±13.42^{b}	59±13.42^{b}
Control + (Asuntol)	0±0^{c}	0±0^{a}	100±0^{a}
Immersed for 60 seconds			
Yam bean seed 0.5%	0.02449±0.00276^{bc}	35±17.68^{cde}	100±0^{a}
Yam bean seed 1%	0.02741±0.001989^{b}	69±6.52^{abc}	98±4.47^{a}
Yam bean seed 2%	0.02281±0.00396^{bc}	30±14.58^{de}	100±0^{a}
Red betel leaf 0.5%	0.02546±0.0032^{bc}	75±11.73^{ab}	90±7.91^{a}
Red betel leaf 1%	0.02559±0.000521^{bc}	64±14.75^{bcd}	98±4.47^{a}
Red betel leaf 2%	0.02316±0.00252^{bc}	65±10.61^{abcd}	95±5^{a}
Combination 0.5%	0.02138±0.00498^{bc}	39±25.3^{cde}	93±8.37^{a}
Combination 1%	0.02343±0.002222^{c}	52±28.9^{bcd}	94±8.22^{a}
Combination 2%	0.02124±0.001984^{c}	8±8.37^{e}	100±0^{a}
Control -	0.03694±0.000659^{a}	100±0^{a}	0±0^{b}
Control + (Asuntol)	0.02414±0.002198^{bc}	33±19.24^{de}	100±0^{a}
Immersed for 180 seconds			
Yam bean seed 0.5%	0.020738±0.001731^{b}	55±7.91^{bcde}	96±6.52^{a}
Yam bean seed 1%	0.02089±0.00322^{b}	46±14.32^{de}	97±6.71^{a}
Yam bean seed 2%	0.02052±0.001098^{b}	9±12.45^{f}	99±2.24^{a}
Red betel leaf 0.5%	0.02264±0.00214^{b}	72±13.04^{bcd}	88±10.37^{ab}
Red betel leaf 1%	0.02289±0.001962^{b}	73±7.58^{bc}	99±2.24^{a}
Red betel leaf 2%	0.02296±0.00337^{b}	80±11.73^{ab}	77±15.65^{b}
Combination 0.5%	0.02143±0.00394^{b}	68±10.95^{bcd}	92±10.37^{ab}
Combination 1%	0.02167±0.002161^{b}	60±15^{bcd}	89±9.62^{ab}
Combination 2%	0.02199±0.00242^{b}	50±12.75^{cde}	100±0^{a}
Control -	0.03818±0.001015^{a}	100±0^{a}	0±0^{c}
Control + (Asuntol)	0.0195±0.00274^{b}	33±19.24^{ef}	100±0^{a}

Different superscript letters on the same line showed significant differences ($P<0.05$)

Rotenone is a broad-spectrum cytotoxin because it can impede the mitochondrial electron transport chain. Rotenone is a respiratory enzyme inhibitor, hence it can cause respiratory failure (Ware and Whitacre 2004). Curcumin in yam bean seeds possesses insecticidal properties by suppressing nervous system enzymes (Prasad and Muralidhara 2014) and cell proliferation (Cui et al. 2016). According to the findings of the study (Sethuraman et al. 2017), curcumin compounds exhibited a cytotoxic effect on insect cell lines.

Quassin is the possible pesticide present in the ethanolic extract of red betel leaf, according to a review of the scientific literature. Quassin is a moderate pesticide with multiple modes of action, primarily preventing feeding, stomach poisoning, nervous system diseases, and contact poisoning (Jababu et al. 2019). Quassin is larvicidal against *Culex quinquefasciatus* mosquitoes by inhibiting tyrosinase activity, which impacts cuticle growth (Evans and Kaleysa 1992). Enzyme inhibition mechanism and cytotoxic activities are also present in Quassin. (Hertel et al. 2006) found that at relatively high doses, quassin had an effect on the insect circulatory system.

Chromatographic study of red betel leaf and yam bean seeds revealed the presence of additional insecticide-potential active components, particularly coumarin. As pesticides, coumarin chemicals immobilize insects through their method of action (Nicholson and Zhang 1995). The insect will undergo a slow-developing paralysis that will ultimately result in death. Disruption of muscle bioenergy has been identified as the primary mechanism underpinning surangin B's insecticidal activity (Zheng et al. 1998). In addition, homology models and docking studies reveal that coumarins and other terpene chemicals can inhibit acetylcholinesterase and block the octopamine receptor pathway, rendering them hazardous to insects (Khanikor et al. 2013). In addition to causing mortality, coumarins can have other deleterious impacts on insect populations, such as a reduction in reproductive capacity.

The synergistic impact of the active chemicals in yam bean seeds and red betel leaf as myiasis insecticides is connected. The identification of active substances revealed that the chemicals found in yam bean seeds and red betel leaf can increase imago mortality, decrease pupa weight, diminish hatchability, and reduce pupa weight. Based on the findings of this study, the most effective myiasis bioinsecticide is a 2% concentration mixture of yam bean seed ethanol extract and red betel leaf. Based on the observed parameters of larval mortality, average pupal weight, hatchability, and imago mortality, it was determined that the concentration was more effective than the control group.

CONCLUSION

According to the findings of this study, yam bean seeds contain 168 chemicals, but red betel leaf has 208. In yam bean seeds, rotenone, curcumin, and coumarin have the ability to operate as myiasis bioinsecticides, while in red betel leaf, quassin and coumarin have this capacity. The treatment's efficacy as a larvicide against myiasis *in vitro* has been demonstrated. The most efficient larvicidal action against *Chrysomya bezziana* larvae was demonstrated by the combination of ethanol extract of yam bean seeds and red betel leaf at a concentration of 2%.

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Comparison of The Nutritional Composition of Black Soldier Fly Bred on Organic Waste and Bred on Commercial Pellet mixed with Rice Bran

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ABSTRAK

Ningrum SG, Ilham MZ, Yulianto AB, Nussa ORPA, Purnamasari K. 2024. Perbandingan komposisi nutrisi Lalat Tentara Hitam yang dikembangbiakkan pada sampah organik dengan yang dikembangkanbiakan pada pakan campuran pelet komersial dengan dedak padi. JITV 29(1):29-35. DOI:http://dx.doi.org/10.14334/jitv.v29.i1.3357.

Lalat tentara hitam (BSF, *Hermetia illucens*) merupakan serangga yang sering digunakan sebagai pakan ternak yang mudah, murah, dan cepat berkembang biak. Namun, lalat ini belum pernah digunakan untuk makanan unggas. Penelitian ini bertujuan untuk membandingkan kandungan nutrisi lalat tentara hitam, larva-instar ketiga, dan pupa yang diberi pakan limbah organik dan yang diberi pakan campuran pelet ayam komersial dengan dedak padi. Metode yang digunakan dalam penelitian ini menggunakan analisis proksimat kandungan nutrisi lalat prajurit hitam untuk bahan kering, abu, protein kasar, lemak kasar, serat kasar, kalsium, ekstrak bebas nitrogen, dan energi bruto. Hasil penelitian ini dapat digunakan sebagai pakan alternatif unggas di Indonesia di masa mendatang.

Kata Kunci: Nutrisi Ternak, Lalat Tentara Hitam, Diptera, Walet

ABSTRACT

Ningrum SG, Ilham MZ, Yulianto AB, Nussa ORPA, Purnamasari K. 2024. Comparison of the nutritional composition of Black Soldier Fly Bred on organic waste and bred on commercial pellet mixed with rice bran. JITV 29(1):29-35. DOI:http://dx.doi.org/10.14334/jitv.v29.i1.3357.

The black soldier fly (BSF, *Hermetia illucens*) is an insect often used as animal feed that is easy, inexpensive, and fast to breed. However, these flies have never been used for the poultry diet. This study compares the nutritional content of black soldier flies, third-instar larvae, and pupae fed with organic waste and a mixture of commercial chicken pellets with rice bran. The method used in this study uses proximate analysis of the nutritional content of black soldier flies for dry matter, ash, crude protein, crude fat, crude fiber, calcium, nitrogen-free extract, and gross energy.

Key Words: Animal Nutrition, Black Soldier Fly, Dipterans, Swiftlet

INTRODUCTION

The escalating demand for sustainable and nutritionally balanced animal feed has led to exploring unconventional protein sources (Hawkey et al. 2021; Sajid et al. 2023). Among these, the Black Soldier Fly (BSF), renowned for its efficient conversion of organic waste into nutrient-rich biomass (Raksasat et al. 2020; Rehman et al. 2022; Surendra et al. 2020), emerges as a promising candidate. This research explores the innovative process of creating nutrients obtained from BSF-derived ingredients. It focuses on optimizing poultry nutrition and investigating their potential inclusion in the formulation for breeding swiftlets, particularly those intended for edible nest production.

As global populations burgeon, the poultry industry faces the challenge of meeting the escalating demand for high-quality, protein-rich animal products (Guiné et al. 2021; Scanes 2018). Simultaneously, the swiftlet breeding industry strives for innovative solutions to enhance the nutritional content of their birds (Looi & Omar 2016), ultimately influencing the quality of edible nests. The study delves into the meticulous preparation of BSF-derived ingredients, aiming to unlock their full potential as a sustainable protein source for poultry and a supplementary component in the breeding of swiftlets.

We are facing a problem: swiftlets can not be cultivated because of their wild nature, so commercial feed production often fails to be applied (Ammartsena & Ditthapan 2023). Edible-nest swiftlet is an insectivore that eats flying insects from its environments, such as bees, beetles, mosquitoes and flies (Mursidah et al. 2020). Although swiftlets do not need to choose high-nutrition feeds, some ingredients such as carbohydrates, fats, and proteins are necessary for their body's metabolism (Ahmad et al. 2019). In addition, the nutritional content of EBN, namely protein and minerals, can also be affected by the feed consumed by edible-nest swiftlet (Benjakul & Chantakun 2022). Black soldier fly (BSF; *H. illucens*) is a fly (Diptera) generally used as animal feed. These flies originate from the American continent but are now widely bred in countries with tropical and temperate climates worldwide. BSF is an alternative protein with many benefits compared to other farmed insect species (Wang & Shelomi 2017). Using environmentally friendly natural biodiversity wealth such as BSF (Cattaneo et al. 2023; Rifai & Permata 2023), this research is expected to help swiftlet farmers avoid economic losses. Also, this research can enhance the future edible-nest swiftlets population by providing alternative feed for swiftlets.

This research addresses the need for novel, sustainable protein sources in poultry diets and explores the uncharted territory of utilizing BSF-derived ingredients to enhance the nutritional profile of swiftlets. By unraveling the intricacies of preparation methods, we aspire to contribute valuable insights that may revolutionize how we formulate animal feeds, promoting ecological sustainability and improving avian breeding practices.

MATERIALS AND METHODS

Preparation of BSF (*H. Illucens)* samples

This research method refers to previous studies with modifications. The life stages of BSF used in this study included third-instar larvae and pupae. BSF collected from the Magot Breeding Center in Surabaya, East Java, Indonesia. BSF colonies were maintained in polypropylene containers (10 L, with a capacity for 100 individuals) covered with muslin cloth at 25°C.

Preparation of feeding

The breeding materials were obtained from organic (mixed vegetable) waste and a mixture of commercial chicken feed and rice bran (G-11, Comfeed, Japfa, Jakarta, Indonesia). The materials were mixed with water (ratio 1:2) in a container. BSF adults were allowed to mate and fed each 25 g of the proposed diet. After breeding and hatching, half of the population of the third-instar larvae was collected for analysis. The other larvae were allowed to grow until pupation. Pupae were collected for further analysis.

Nutrient analysis of BSF

About 100 g of each of the third-instar larvae and pupae of BSF were collected, washed first, and stored in the freezer at -20 °C for 24 hours before being analyzed. A slightly modified SNI 01-2891-1992 (Badan Standardisasi Nasional 1992) was applied to the samples with three replicates. Nutritional levels were examined on dry matter, ash, crude protein, crude fat, crude fiber, calcium, nitrogen-free extract (NFE), and gross energy.

Dry matter content (DM) was measured by sampling 2 g of each sample, aliquoted and dried in a forced-f convection drying oven at 105°C for 3 h, and then cooled in an excicator. Moisture content was calculated using the formula (original weight of the sample: the dried weight of the sample) x 100%. DM was the difference between 100% (sample) and moisture content.

Total ash was determined by burning 3 g of sample to be perfect ash in an electric furnace at a maximum temperature of 550°C, and then samples were cooled in an excicator. Ash content was calculated using the formula: (weight of the sample and cup after burned – empty cup weight/original weight of the sample) x 100%. The difference between DM and total ash was the amount of organic matter.

Crude protein was estimated using the Kjeldahl method (18-8-31/MU/SMM-SIG, Kjeltec). Approximately 0.51 g of sample was first digested with 2 g of selen mix (2.5 g of SeO_2, 100g of K_2SO_4, and 20 g of $CuSO_45H_2O$) and 25 mL of concentrated H_2SO_4. After the mixture was cooled at room temperature, 5 mL of NaOH 30% was added to the flask. The flask was then placed in a distillation connection unit, and the distillate was mixed with 2% boric acid and a few drops of methyl red. The distillate mixture was titrated with 0.01 N HCl, and protein concentration was calculated using the formula: ((V1-V1) x N x 0.014 x f.k x fp): w. The difference between organic matter and crude protein was the amount of nitrogen-free organic.

Crude fat was measured using the Weibull method in petroleum ether by calculating the formula: ((w1 - w2): w) x 100%. About 2 g of sample was added with 30 mL HCl 25% and 20 mL water. The mixture was boiled for 15 min and filtered with filter paper. The filter paper and the content were dried at 105°C and extracted in petroleum ether for 3 h at 80°C. The ether part was removed and dried in an oven at 105°C. The lipid content was calculated as the difference between fat flask weight after extraction (g) and fat flask weight before extraction (g) divided by the original weight of the sample (g) x 100%. Total sugar was determined by calculating nitrogen-free organic and lipid (Marrubini et al., 2017).

The extracted sample with H_2SO_4 and NaOH determined the crude fiber. A total of 4 g of sample was added by 50 mL of 1.25% H_2SO_4 and boiled for 30 min. After that, the boiled sample was added 50 mL of 3.25% NaOH and boiled again for 30 min. The mixture was filtered and rinsed with 1.25% H_2SO_4, hot water, and 96% ethanol. The residue weight was dried at 105°C. The dried residue (w2) was weighed and heated in an electric furnace at 550°C to form ash (w1). The fiber was the difference between w2 and w1 divided by the original weight of the sample x 100%. NFE was determined by calculating the total sugar and crude fiber (Ningrum 2021). Gross energy was calculated using the formula: (9.11 kcal/g x % fat + 5.86 kcal/g x % crude protein + 3.95 kcal/g x % total sugar): 100 (Garcia et al. 2016). ICP-OES 18-13-1/MU/SMM-SIG method (Chasanah et al. 2020) was used to determine the calcium content.

Statistical analysis

The nutritional composition among the life stages and the breeding materials were compared by one-way analysis of variance (ANOVA) followed by Duncan's post hoc test in SPSS 25.0 ($P<0.05$). Boxplots expressed a comparison of the nutritional value of third-instar larvae and pupae of BSF for different breeding materials.

RESULTS AND DISCUSSION

Significant differences in nutritional content were found based on comparing the nutritional value of each BSF stage (third-instar larvae and pupae) with different breeding materials (Table 1). The dry matter of third-instar larvae and pupae bred on chicken pellet with bran was significantly higher (97%) than that of the third-instar larvae (96%) and pupae (85%) bred on organic waste ($F=1604.306$, $df=1$, $P<0.05$). For ash content, pupae bred on chicken pellets with bran showed the highest quantity (26%) compared with the others ($F=3241.252$, $df=1$, $P<0.05$).

The third-instar larvae bred on mixed chicken pellet with bran has the highest crude protein (32%) ($F=415.944$, $df=1$, $P<0.05$), crude fat (14%) ($F=496.382$, $df=1$, $P<0.05$), and gross energy contents (2703 KJ/g) ($F=392.192$, $df=1$, $P<0.05$). At the same time, pupae showed the highest content for crude fiber (28%) ($F=140.803$, $df=1$, $P<0.05$) and calcium (5%) ($F=3241.252$, $df=1$, $P<0.05$). The third-instar larvae bred on organic waste showed the highest NFE content ($F=25.957$, $df=1$, $P<0.05$).

Based on the results of this study, the two growth stages of this species provide different information on nutrient content; this is due to the different nutrient requirements at each stage of the life of this species. For example, larvae need much energy to make the pupation process successful (Dzepe et al. 2020; Kim et al. 2021; Stadler & Takáč 2022). In this study, the larvae were shown to have a high crude protein and crude fat content, so the energy produced was also more significant than the pupae. However, pupae have a higher mineral content than larvae. It is normal because the pupae have undergone chitinization. In the results of a comparative analysis of the BSF stages developed in each breeding material, the results show that the third-instar larvae developed using a mixture of commercial chicken pellets and bran provide a higher nutritional content compared to the larval or pupal stages bred with organic waste; this is due to the nutritional content of commercial chicken pellet mixtures with bran containing more fat, protein, and minerals.

On the other hand, organic waste provides a higher crude fiber content in the pupae but a high NFE in the larval stage. This result shows that the pupae grown on organic waste have lower nutrient content than the larvae and pupae produced on commercial chicken pellet mixture material with bran. Despite their low digestibility, our results show that pupae grown on commercial chicken pellets mixed with bran have a very

Table 1. Nutritional value (means±SD) of black soldier fly (BSF, *H. Illucens)* for different breeding diets materials and different growth stages

Breeding Diets Materials	Growth Stage	Dry matter (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	NFE$^{1)}$ (%)	Gross energy (KJ/g)	Calcium (g/100g)
Organic waste	Larvae	96.89±0.08$^{b2)}$	10.56±0.06^{d}	27.53±0.33^{d}	5.37±0.32^{d}	26.21±0.06^{b}	27.20±0.70^{a}	2367.79±8.20^{b}	2.85±0.12^{d}
	Pupae	85.36±0.38^{c}	11.51±0.28^{c}	30.49±0.10^{b}	13.95±0.29^{b}	28.64±0.21^{a}	0.74±0.69^{c}	2166.99±6.26^{c}	3.35±0.15^{c}
Chicken pellet + rice bran	Larvae	97.44±0.26^{a}	15.11±0.12^{b}	32.42±0.32^{a}	14.85±0.63^{a}	21.40±0.01^{c}	13.65±0.74^{b}	2703.39±25.82^{a}	3.72±0.10^{b}
	Pupae	97.13±0.09^{ab}	26.42±0.00^{a}	29.67±0.10^{c}	12.39±0.36^{c}	28.09±0.58^{a}	0.55 ± 0.31^{c}	1968.58±31.91^{d}	5.22±0.24^{a}

NFE= Nitrogen-free extract. Different superscripts in each row in the same column indicate that they significantly differ for the nutritional value at $P<0.05$. All testing was performed with three replicates

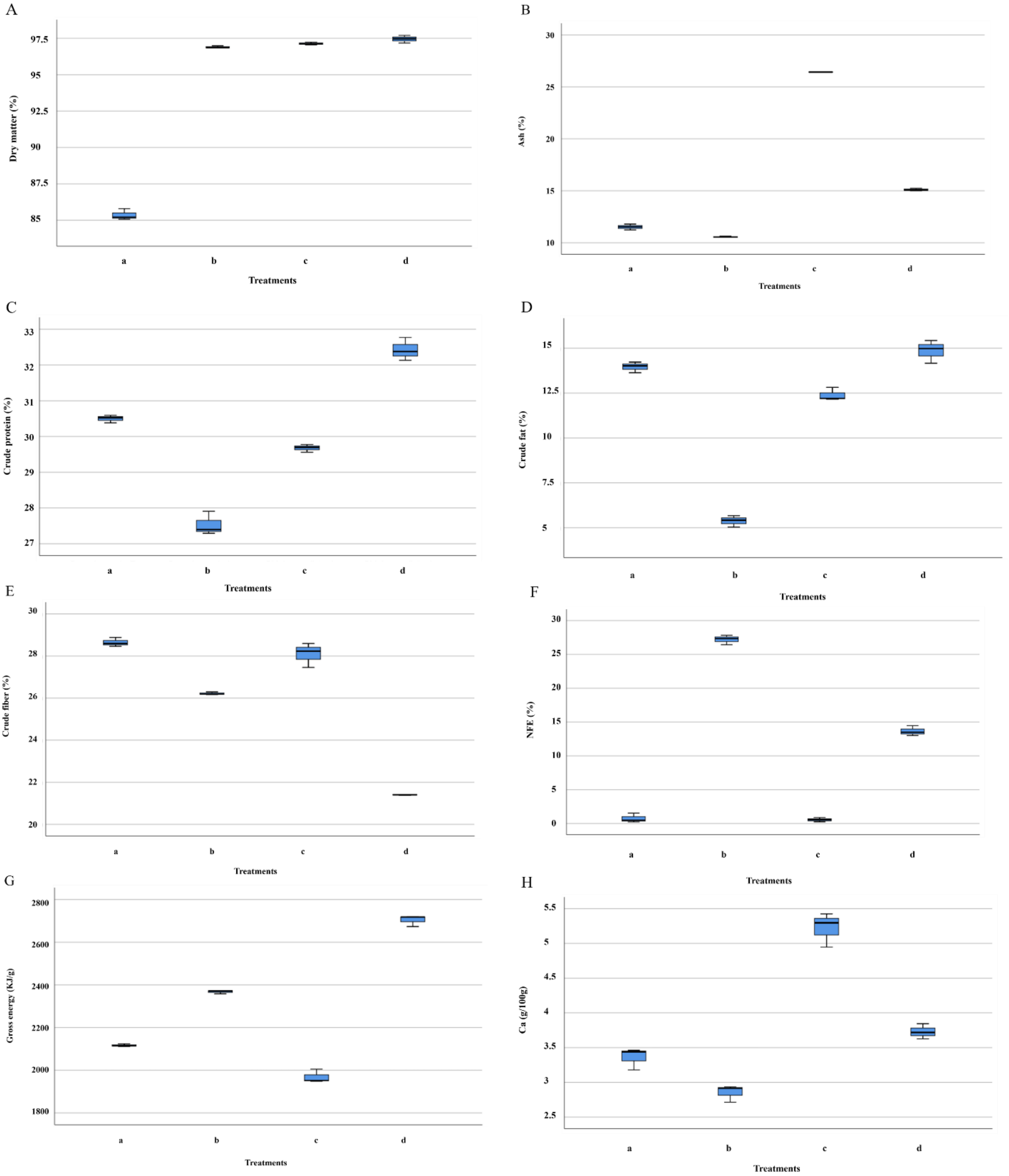

Figure 1. Nine groups of boxplots correspond to each of the treatments. A: Dry Matter, B: Ash, C: Crude protein, D: Crude fat, E: Crude fiber, F: Nitrogen-free extract (NFE), G: Gross energy, H: Calcium. a: organic waste-pupae, b: organic waste-third-instar larvae, c: chicken pellet with rice bran-pupae, d: chicken pellet with rice bran-third instar larvae

high content. This result indicates that pupae grown on commercial chicken pellet mixtures with bran suit the swiftlet egg production process. Even so, providing immobile feed such as pupae as swallow feed is a challenge for swallow breeders. To the best of our knowledge, this study is the first to report a comparison of the nutritional content of BSF (*H. Illucens)* third-instar larval stages and pupae grown in a mixture of commercial chicken pellets with bran and organic waste. There were significant differences between the types of BSF breeding diet materials used.

Since the scarcity of food sources for edible-nest swiftlet can threaten the swiftlet population in Indonesia, commercial feed for edible-nest swiftlet is highly desirable, and providing swiftlet feed is a challenge for edible-nest swiftlet breeders in the future. Edible nest swiftlet feed is expected to provide complete nutrition for the growth of this bird species. Even though it is given manually, edible nest swiftlet feed should have the property of "moving" because these birds eat insects that fly or move. These birds commonly consume insects from the orders Hymenoptera, such as ants, wasps, and bees (Tong et al. 2021). Also, edible-nest swiftlets consume aphids, plant lice, mayflies, beetles, booklice, butterflies, grasshoppers, caddisflies, thrips, mites, ticks, true bugs, termites, and spiders. However, ants and wasps consume edible nest swiftlets most (Fujita & Leh 2020). These birds prefer to prey while flying (Yaacob et al. 2021) and can still stick to a vertical surface when preying (Duerr & Gage 2020). In addition to this bird feed, which must live, edible nest swiftlet feed should be economical and easy to produce. BSF maggot is often used as an alternative feed for poultry (Ahmed et al. 2023; Edea et al. 2022; Khan 2018) and fish (Sari et al. 2021; Shah et al. 2022; Sinansari & Fahmi 2020), but has never been used for feeding edible nest swiftlets. This study compared and determined the difference in nutritional content between the pupae and the third instar BSF larvae bred on organic waste with those bred using a mixture of chicken pellets and bran. From this study, we aim to make it easier for swiftlet breeders to develop an edible nest of swiftlet live food. The results suggest combining commercial chicken pellets with bran is more nutritious than organic waste for BSF third-instar larvae and pupae stages. Thus, the third-instar larval of BSF could be challenged as an edible nest swiftlet's diet.

The utilization of specific diet materials in swiftlet breeding, mainly focusing on comparing organic waste and a combination of commercial chicken pellets with bran, presents a crucial consideration for swiftlet breeders. Analyzing the cost-benefit aspects of these diet materials becomes imperative to guide breeders in making informed decisions. Utilizing organic waste for breeding BSF larvae may be cost-effective due to its availability. However, the nutritional content may vary, and the long-term sustainability of this approach needs assessment. While the mixture of commercial chicken pellets with bran might incur additional costs, the potential increase in nutritional value could justify the investment. The improved nutritional profile may contribute to swiftlet health and overall breeding success. Although organic waste is a natural and sustainable resource, the present study suggests that the nutritional content of BSF third-instar larvae and pupae bred on this substrate is inferior to those bred on a combination of chicken pellets and bran. The results indicate that combining chicken pellets with bran enhances the nutritional content of BSF larvae and pupae; this implies potential benefits in improved swiftlet health, reproductive success, and the quality of edible nests. While organic waste may provide a low-cost option, its sustainability and consistent nutritional quality need scrutiny. Fluctuations in nutrient availability may impact the long-term success of swiftlet breeding operations. Investing in a more nutritious diet may contribute to the long-term viability of swiftlet breeding programs. The potential improvement in swiftlet health and reproductive outcomes could outweigh the initial cost considerations.

This study hints at the possibility of using third-instar larvae of BSF bred on a diet of chicken pellets and bran as an edible nest swiftlet's diet. This potential shift in dietary practice could offer additional benefits, such as enhanced nest quality and increased swiftlet population. In conclusion, while the initial costs of utilizing a mixture of chicken pellets with bran may be higher, the potential long-term benefits of enhanced nutritional content and breeding success outweigh the expenses. Swiftlet breeders should carefully evaluate the cost-effectiveness and sustainability of their chosen diet materials, considering both short-term and long-term implications for the health and productivity of their swiftlets and the quality of the edible nests produced.

CONCLUSION

Black soldier fly (BSF*, H. Illucens)* third-instar larval stage fed with a mixture of commercial chicken pellets and rice bran had the highest crude protein, fat, and energy content. Pupae fed with a mixture of commercial chicken pellets and bran had the highest mineral and calcium content. As consideration for breeding material, commercial chicken pellet mixtures can increase the nutritional content of BSF in the third-instar larvae and pupae stages.

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Effect of Replacing Grass Silage with Hydroponic Maize Fodder on Nutrient Intake and Digestibility, Rumen Parameters and Blood Metabolites of Weaned Ongole x Brahman Calves

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ABSTRAK

Benu I, Jelantik IGN, Penu CL, Laut MM. 2024. Pengaruh substitusi silase rumput dengan fodder jagung hidroponik terhadap konsumsi nutrient, kecernaan nutrient, parameter rumen dan metabolit darah pedet sapi Ongole x Brahman lepas sapih. JITV 29(1):36-44, DOI: http://dx.doi.org/10.14334/jitv.v29i13331.

Fodder jagung hidroponik (FJH) dapat menggantikan silase rumput sebagai alternatif hijauan ternak ruminansia selama musim kemarau. Percobaan in vivo dilakukan untuk mengevaluasi pengaruh penggantian silase rumput dengan FJH terhadap konsumsi nutrient, kecernaan nutrient, parameter rumen dan parameter darah pedet sapi lepas sapih. Sebanyak 4 ekor pedet sapi jantan persilangan Ongole x Brahman (BB 127±17,45 kg) lepas sapih diberikan pakan 70% silase rumput + 30% konsentrat (G); 35% silase rumput + 35% FJH + 30% konsentrat (GCF1); 17,5% silase rumput + 52,5% FJH + 30% konsentrat (GCF2); dan 70% FJH + 30% konsentrat dalam percobaan bujur sangkar latin 4x4. Pedet sapi yang diberikan FJH dengan level berbeda memiliki lebih sedikit (P<0,05) total konsumsi bahan kering (BK), total konsumsi bahan organik (BO), dan total konsumsi serat kasar (SK) dibandingkan pedet yang hanya mendapat silase rumput saja. Tidak terdapat perbedaan antar perlakuan (P>0,05) untuk konsumsi protein kasar, kecernaan BK, kecernaan PK, kecernaan SK, konsumsi serat kasar tercerna, konsumsi protein kasar tercerna dan pH rumen. Pedet sapi yang mengkonsumsi FJH memiliki konsentrasi ammonia-nitrogen (NH3-N) dan total volatile fatty acids (TVFA) lebih tinggi (P<0,05) dibandingkan pedet sapi yang hanya mendapat silase rumput saja. Tidak ada perbedaan antar perlakuan (P>0,05) untuk konsentrasi asam asetat, propionate, butirat, iso-butirat, iso valerat, glukosa darah dan urea darah. Dengan demikian, dapat disimpulkan bahwa FJH dapat digunakan sebagai pengganti silase untuk meningkatkan fermentasi rumen pedet sapi jantan Ongole x Brahman lepas sapih, walaupun mungkin dapat menurunkan konsumsi bahan kering.

Kata kunci: Hijauan Jagung Hidroponik, Intek Nutrien, Kecernaan Nutrien, Pedet Sapi Persilangan Ongole Brahman

ABSTRACT

Benu I, Jelantik IGN, Penu CL, Laut MM. 2024. The effect of replacing grass silage with hydroponic maize fodder on nutrient intake, nutrient digestibility, rumen parameters and blood metabolites of weaned Ongole x Brahman calves. JITV 29(1):36-44. DOI: http://dx.doi.org/10.14334/jitv.v29i13331.

Hydroponic maize fodder (HMF) may replace grass silage as an alternative green forage for ruminants during dry seasons. An *in vivo* experiment was conducted to investigate the effect of replacing grass silage with HMF on nutrient intake, nutrient digestibility, rumen parameters and blood metabolites of weaned calves. Four weaned Ongole x Brahman calves (BW= 127±17.45 kg) were fed 70% grass silage + 30% concentrates (G; control), 35% grass silage + 35% HMF + 30% concentrates (GCF1), 17,5% grass silage + 52,5% HMF + 30% concentrates (GCF2), and 70% HMF + 30% concentrates (GCF3) in a 4 x 4 Latin Square Design experiment. Calves fed HMF had lesser total dry matter intake (DMI) (P<0.05), total organic matter intake (OMI), and total crude fibre intake (CFI) compared with calves fed only grass silage. There were no differences (P>0.05) between treatments for total crude protein (CP) intake, dry matter digestibility (DMD), crude protein digestibility (CPD), crude fibre digestibility (CFD), digestible crude fibre intake (DCFI), digestible crude protein intake (DCPI) and ruminal pH. Calves provided HMF in their diet had the greatest (P < 0.05) ammonia-nitrogen (NH3-N) and total volatile fatty acids (TVFA) concentration compared with calves fed only grass silage. There were no differences between treatments (P> 0.05) for the concentration of acetate, propionate, butyrate, iso-butyrate, iso-valerate, blood glucose or blood urea of calves. Hence, hydroponic maize fodder could be used as a replacement for silage to improve the rumen fermentation of weaned Ongole x Brahman calves, although it might decrease the total dry matter intake.

Keywords: Hydroponic Maize Fodder, Nutrient Intake, Nutrient Digestibility, Ongole X Brahman Calves

INTRODUCTION

Sufficient growth of weaned Ongole x Brahman calves is required for higher beef production. Providing adequate nutrients at the early age of calves is necessarily important to prepare for the success of weaning (Fischer et al. 2019) as well as to facilitate the animal to express the genetic potential to maximize the intramuscular fat production or marbling during finishing (Xuan et al. 2018) and thus to achieve a higher beef production (Tian et al. 2019). On the other hand, insufficient nutrition during the early life of calves may impair growth performance and production (Vendramini & Moriel, 2018). Early weaned calves, however, are generally inefficient in utilizing nutrients due to their undeveloped both structure and rumen function as compared to adult ones (McCoard et al. 2019; van Niekerk et al. 2021). In addition, early weaning programs also induced stress to the weaned animal (Ungerfeld et al. 2016; Diao et al. 2019). Therefore, the provision of high protein and energy feeds is necessary for newly weaned calves (Fischer et al. 2019). Good quality fresh forages including young leafy grass and forage legumes are required to ensure a high growth rate of newly weaned calves. In the seasonally dry tropics of west Timor-Indonesia, the availability and quality of fresh forages throughout the year is problematic. During the rainy season, the availability of good quality forages is abundant but becomes scarce during the dry season. The lack of high-quality forages during the dry seasons has been reported to adversely affect the body weight gain of weaned Bali calves (Mullik & Jelantik 2009). Hence, providing grass silage during the dry season is one important option and it has gained a lot of success. However, for newly weaned crossbred calves, grass silage alone may not be sufficient in terms of nutrient availability (McCoard et al. 2019). Indeed, newly weaned calves commonly have low intake due to stress and undeveloped rumen function (Rajkumar et al. 2018). Therefore, it may require a higher quality of forages which has high digestibility but provide a certain amount of fibre. Fibre is also required for both rumen health and rumen development (Diao et al. 2019). In addition, smallholder farmers are unable to maintain grass silage for long time during the dry season due to difficulty in grass conservation. It is therefore crucial for finding an alternative green forage as a replacement for grass silage in tropical areas.

Hydroponic maize fodder offers an alternative feed to grass or green forage particularly during the dry season where high-quality forage is limited. In addition, hydroponic maize fodder also can be used as an alternative feed when there are no other basic feeds for the animal. This is due to its flexibility to produce at any time of the year with minimum investment. Furthermore, hydroponic maize fodder production is efficient in water utilization and small land utilization, especially for those with marginal land and limited resources. Maize fodder is produced from maize grains that are germinated and grown for a short period (Arif et al. 2023). Hydroponic maize fodder can be grown and harvested within a week and fed to cattle (Naik et al. 2016). There is limited information, however, on the effects of the rate of substitution of maize fodder in a grass-based diet for calves in West Timor. Therefore, this study was designed to investigate the effect of increasing concentrations of hydroponic maize fodder in the diet on the dry matter intake and rumen parameters of weaned Ongole x Brahman calves.

MATERIALS AND METHODS

Location of the study

The present study was carried out at the dry land laboratory (-10.1761° S, 123.3793° E) of Nusa Cendana University Kupang, West Timor, Indonesia.

Hydroponic maize fodder production

The maize grain used in this study was purchased from the local market. Five kilograms of maize were washed in tap water to remove debris. The maize was then washed with antifungal solution (Antracol; Bayer Indonesia; Jakarta) at 2 ml/l (v/v) concentration to minimize fungi contamination and then stored in a dark area to allow the grains to steep for 12 hours (overnight). The maize was then soaked in fresh tap water for 24 h before distributing maize evenly into the planting trays. The trays were manually watered two or three times per day depending on the weather. The sprouted maize seeds were grown for 7 days before harvesting and fed to the animals. A sample of the harvested sprouted maize seeds was taken for every tray and then dried in the oven at 60^0C for 48-72 h before the representative samples were taken for further proximate analyses following the method of AOAC (2005).

Animals, diets, and experimental design

The experiment used four males weaned Ongole x Brahman calves (127±17.45 kg) in a four 21-day periods x four diets in a Latin Square design experiment. Each period consisted of a 14-day dietary adaptation followed by 7 days of sample collection. The four diets offered were 70% grass silage +30% concentrates (G), 35% grass silage +35% hydroponic maize fodder +30% concentrates (GCF1), 17.5% grass silage +52.5% hydroponic maize fodder +30% concentrates (GCF2) and 70% hydroponic maize fodder +30% concentrates

Table 1. Ingredient and chemical composition of experimental diets

	Treatments			
	$G^{1)}$	GFC1	GFC2	GFC3
Ingredients				
Grass silage (% DM)	70	35	17,5	0
Hydroponic maize fodder (% DM)	0	35	52,5	70
Ground Corn (% DM)	16	16	16	16
Rice bran (% DM)	10	10	10	10
Fishmeal (% DM)	3	3	3	3
Mineral (% DM)	1	1	1	1
Total	100	100	100	100
Chemical composition				
DM (% DM)$^{2)}$	93.12	90.67	91.54	92.85
OM (% DM)	81.75	87.64	88.48	88.35
CP (% DM)	5.35	12.67	12.64	12.49
CF (% DM)	35.34	18.17	16.62	14.86
Ash (% DM)	11.38	3.04	3.06	2.89

G= 70% grass silage + 30% concentrates; GFC-1= 35% grass silage + 35% hydroponic maize fodder + 30% concentrates; GFC-2= 17.5% grass silage + 52.5% hydroponic maize fodder + 30% concentrates; GFC-3= 70% hydroponic maize fodder + 30% concentrates; DM= Dry matter; OM= Organic matter; CP= Crude protein; CF= Crude fibre

(GCF3). The animals were familiarized with handling and sampling procedures before the start of the experiment. Throughout the experiment, the animals were assigned to individual pens (2 x 1 m) within a cattle housing facility. The animals were fed their dietary treatments (Table 1) twice per day. Water was offered ad libitum.

Parameters measured

To determine the dry matter intake of the animals, orts were weighed following each meal for concentrates, grass silage, and hydroponic maize fodder. Daily intake was calculated as diet allowance – orts= daily intake. Total dry matter intake (DMI) was calculated as (daily intake x %DM= DMI) for concentrate, grass silage, and maize fodder. Total nutrient intake was also calculated for DMI grass silage as (DMI grass silage x % grass silage nutrient profile= grass silage nutrient intake), for DMI maize fodder as (DMI maize fodder x % maize fodder nutrient profile= maize fodder nutrient intake), and for DMI concentrate as (DMI concentrate x % concentrate nutrient profile= concentrate nutrient intake).

At the end of the digestibility trial, samples of rumen fluid were collected from each calf using a stomach tube. The rumen liquor samples were then filtered using four layers of cheesecloth before measurement of pH using a digital pH meter. Ammonia concentration was measured using micro diffusion plates following the method of Conway (1947). Total volatile fatty acids (VFA) were determined using the steam distillation method while the partial VFA was assessed by gas-liquid chromatography. Blood samples from each calf were collected at the end of each period of the study via jugular venepuncture and using vacutainer tubes containing EDTA 4 hours after the morning meal. The samples were immediately placed on ice and brought to the laboratory for later analysis.

Statistical analysis

Data obtained in this study were statistically analyzed using General Linear Model (GLM) procedure adapted by IBM SPSS Statistic for Windows, version 21 (IBM Corp Armonk, N.Y USA) for user’s guide with ANOVA. Duncan’s multiple range tests within the SPSS program were conducted to examine the degree of significance among means. The significant difference was set at $P<0.05$ and $0.05<P<0.10$ was assigned as a tendency to be significant.

RESULTS AND DISCUSSION

Intake and digestibility of nutrients

The voluntary feed intake and nutrient digestibility of calves fed grass silage alone or with an increasing substitution rate of maize fodder are presented in Table

2. Calves fed grass silage alone had greater dry matter intakes compared with calves fed various substitution rates of the maize fodder (P= 0.02). As the substitution rate of maize fodder increased, the dry matter intake of calves decreased, with the lowest intake in the GCF3 treatment. Similarly, the increasing substitution rate of maize fodder in the diet of calves caused a decrease in their total OM intake (P= 0.02) and total CF intake (P= 0.02) compared with intake of calves fed grass silage only. There were no differences, however, between treatments for total CP intake (P= 0.53). There were also no differences between treatments for DMD (P= 0.67), CPD (0.56), CFD (P= 0.47). In addition, there were no differences between treatments for DCFI (P= 0.05) and DCPI (P= 0.72).

Total dry matter, as well as organic matter intake, were linearly decreased in the present experiment with an increasing substitution rate of hydroponic maize fodder. This decline was unexpected and not in line with our hypothesis that feeding a diet with an increasing substitution rate of maize fodder would increase DM intake. The dietary CP was increased and CF was reduced with an increasing level of substitution. Increasing dietary CP commonly results in an increasing rate of ruminal digestion which, therefore, stimulates dry matter intake (Tian et al. 2019). Similarly, diets with lower crude fibre content commonly have higher digestibility (Fustini et al. 2017). Moreover, the concentration of TVFA in rumen liquid as an indication of rumen carbohydrate fermentation (Liu et al. 2020) was also significantly increased in the present experiment. Previous studies also demonstrated that hydroponic maize fodder was palatable to ruminant animals and it contains no substance that may reduce intake. Kide et al. (2015) reported that goats consumed all parts of maize fodder (leaf, stems, and roots), showing that it is considered palatable to goats. Moreover, dry matter intake (DMI) has been demonstrated to be increased when maize fodder is included in the diets formulated for cattle. Rajkumar et al. (2018) also showed that the DMI of calves was increased with increasing substitution rates of hydroponic maize fodder in the diet.

The decline of DMI in the present experiment might have resulted from the high-water content in hydroponic maize fodder. Previous results showed that feeding excessively high moisture feeds lessened dry matter intake. The intracellular or inexpressible water might increase the bulk of feedstuffs. In the previous reports that demonstrated negative effects of high feed moisture content on intake, it was commonly argued that water intake contributes to rumen fill (Havekes et al. 2020). This was caused by intracellular or inexpresible water of increased bulk of feedstuffs particularly when forage proportion in the diet increases. The contained water will be released when the cellular structures in feedstuffs are broken through mastication or fermentation and

Table. 2. The intake and digestibility of dietary treatments of weaned Ongole x Brahman male calves

Parameters	Treatments				Standard Error of Mean	P-Values
	G[1)]	GCF1	GCF2	GCF3		
Daily intake						
Dry matter, (g/d)	4132.11$^{a2)}$	3301.66^{b}	2859.34^{b}	1557.63^{b}	275.78	0.02
Organic matter, (g/d)	3015.82^{a}	2613.83^{b}	2456.42^{b}	1497.49^{b}	205.25	0.04
Crude protein, (g/d)	201.23	225.58	258.47	212.95	25.82	0.53
Crude fibre, (g/d)	1293.95^{a}	886.35^{ab}	661.55^{bc}	299.88^{cd}	104.38	0.02
Nutrient digestibility						
DMD, (%)[3)]	70.16	62.57	62.26	66.15	4.84	0.67
CPD, (%)	48.52	48.39	52.25	69.86	11.08	0.56
CFD, (%)	72.65	60.01	56.94	55.95	7.33	0.47
DDMI, (g/d)	2989.82^{a}	2099.17^{ab}	1752.98^{ab}	1063.27^{c}	333.51	0.09
DOMI, (g/d)	2071.73	1634.07	1550.54	1089.52	252.80	0.23
DCPI, (g/d)	108.44	107.85	141.56	150.29	31.64	0.72
DCFI, (g/d)	978.68	555.11	365.77	197.42	116.08	0.05

G= 70% silage grass + 30% concentrates; GFC-1= 35% silage grass + 35% hydroponic maize fodder + 30% concentrates; GFC-2= 17.5% silage grass + 52.5% hydroponic maize fodder + 30% concentrates; GFC-3= 70% hydroponic maize fodder + 30% concentrates. Values in the same row with different superscripts are significantly different (P< 0.05). DMD= Digestibility of dry matter; CPD= Crude Protein Digestibility; CFD= Crude fibre digestibility; DDMI= Digestible Dry matter intake; DOMI= Digestible Organic matter intake; DCPI= Digestible Crude protein Intake; DCFI= Digestible Crude fibre intake

Table 3. Ruminal fermentation of weaned Ongole x Brahman male calves fed with experimental diets

Parameters	Treatments				Standard Error of Mean	P-values
	G$^{1)}$	GCF1	GCF2	GCF3		
pH	7.79	7.36	7.41	7.39	0.26	0.68
NH_3-N, (mg/L)	$64.96^{a2)}$	84.24^{a}	202.87^{b}	210.47^{b}	19.49	0.02
Acetate, (mM)	19.65^{a}	22.29^{ab}	25.10^{ab}	25.55^{b}	1.24	0.11
Propionate. (mmol/L)	5.71^{a}	7.09^{a}	8.83^{ab}	11.04^{b}	1.03	0.11
Iso-butyrate, (mmol/L)	1.16	1.12	1.69	2.55	0.64	0.49
N-butyrate, (mmol/L)	2.57^{a}	4.49^{a}	5.25^{ab}	6.95^{b}	0.60	0.05
Iso-valerate, (mmol/L)	0.76	1.03	1.68	1.49	0.17	0.09
N-valerate, (mmol/L)	0.35	1.05	1.04	1.26	0.22	0.19
TVFA, (mmol/l)$^{3)}$	30.22^{a}	37.09^{ab}	43.61^{bc}	48.86^{c}	2.29	0.03
Molar proportion						
Acetate, (%)	64.02^{a}	60.52^{b}	57.47^{ab}	53.68^{b}	1.69	0.07
Propionate, (%)	19.04	18.61	20.41	22.58	1.53	0.41
Butyrate, (%)	12.96	15.06	15.96	18.49	1.97	0.41
Valerate, (%)	2.59	2.98	3.79	2.85	0.38	0.33
Iso-valerate, (%)	1.37	2.82	2.35	2.38	0.47	0.34
Iso-Butyrate, (%)	3.73^{a}	5.61^{a}	6.95^{ab}	9.50^{b}	1.03	0.10
Ratio	0.24	0.25	0.27	0.31	0.02	0.33

G= 70% silage grass + 30% concentrates; GFC-1= 35% silage grass + 35% hydroponic maize fodder + 30% concentrates; GFC-2= 17.5% silage grass + 52.5% hydroponic maize fodder + 30% concentrates; GFC-3= 70% hydroponic maize fodder + 30% concentrates. Values in the same row with different superscripts are significantly difference, (P<0.05). TVFA= Total Volatile Fatty Acids

potentially increase the satiety of the animals thus decreasing DMI (Hafla et al. 2014). Moreover, increased intake of water could be greater than the ability to transport water from the rumen, therefore limiting intake due to rumen fill (Havekes et al. 2020). Decreased DMI was similarly reported by Farghaly et al. (2019) when calves were fed barley fodder compared to that fed roughage as the control diet. The decrease in DMI found in the present study, as well as in other studies, could be due to the high-water content of hydroponic maize fodders. NRC (2001) indicated that a decrease in DMI does not occur until the moisture content of the diet increases above 50%. Moreover, Khan et al. (2014) observed a decline in DMI of cows when greater amounts of water were added to a high-moisture total mixed ration (TMR). In contrast, a study by Denißen et al. (2021) found a significant effect on DMI when cows were fed a diet with addition of water to total mixed ration (TMR) with a DM content of 42-47%. In addition, cows that were fed a diet with added water were reported to have greater DMI compared with the control group (Havekes et al. 2020). Results of the present study demonstrated that reduced DMI by the animals was caused by the high-water content of hydroponic maize fodders.

Rumen parameters

Ruminal pH did not differ between treatments (P= 0.26; Table.3). Ruminal ammonia-N concentration increases significantly with increasing substitution rates of maize fodder in the complete diet (P= 0.02). Total Volatile Fatty Acids (TVFA) concentration increased significantly with increasing substitution rates of maize fodder in the complete diet (P= 0.03). However, the molar proportion (as a percentage of TVFA) of acetic acid (P= 0.11), propionic acid (P= 0.11), butyrate acid (P= 0.05), iso-butyrate (P= 0.49) and iso-valerate (P= 0.09) did not differ between treatments.

Soaking grains has been reported to increase moisture content and various enzymes activity (Thakur et al. 2021). This leads to the breakdown of storage compounds into more simple and digestible fractions, such as starch to sugars or protein to amino acids (Sharma & Gujral 2020). Moreover, the germination of seeds to sprouts has been reported to increase the hydrolysis of nutrient reserves stored in the seed, allowing for the release of soluble compounds and, increasing the nutrients available for the rumen microflora (Lei et al. 2021). In addition, sprouted maize fodder supplies vitamins and enzymes which function as biocatalysts to support feed metabolism and release of energy from the feed (Farghaly et al. 2019). Providing the animal with a concentrates mixture and sprouted maize fodder caused a significant increase in both ruminal ammonia N and TVFA, presumably due to increased micro-organism activity (De Oliveira Franco et al. 2017). The same results were demonstrated by a previous study performed with goats, where ammonia N concentration increased as a result of sprouted barley feeding (Helal 2015). Based on those results, we hypothesized that feeding a diet with an increasing substitution rate of maize fodder would increase rumen fermentation, as well as DM and nutrient digestibility. The results of the present experiment, however, revealed that there were no differences between treatments for dry matter and nutrient digestibility. Hence, it is not in line with the majority of the previous studies where hydroponic fodder feeding increases nutrient digestibility compared with a control diet. Rather, the result of the present study was similar to those reported by Fazaeli et al. (2021) and Alharthi et al. (2023) that there were no significant effects of sprouted grains feeding on nutrient digestibility for dairy cows and lambs.

The absence of increasing nutrient digestibility with increasing the substation rate of maize fodder for grass silage that we observed in this experiment was somewhat difficult to explain particularly when the ruminal data was considered. The ruminal concentration of TVFA as well as ammonia were increased with the substitution. Since rumen ammonia and VFA concentrations are products of rumen fermentation, their concentrations would indicate the rate and extent of rumen degradation of both carbohydrate and protein (Alharthi et al. 2023). Therefore, rumen fermentation was possibly improved when grass silage was substituted with maize fodder, the DM and nutrient digestibility were expected to increase. According to Allen et al. (2019) DM digestibility is prominently dependent upon the rate and extent of rumen degradation. There was, however, a situation when improved rumen degradation did not follow an improvement in the total tract DM digestibility, i.e., when the improved rate of rumen degradation is, at the same time, followed by an increase of ruminal outflow rate (Jelantik et al. 2011). This perhaps occurred in the present experiment with diet composed of increasing proportion of maize fodder as a replacement of grass silage. With higher water content, the functional specific gravity (FSG) of those diets was increased and diet with a higher FSG had a higher ruminal passage rate (Teimouri Yansari 2017).

Another reason for the absence of treatment deference was that maize fodder contained a large amount of root. Although the total content of crude fibre declined with increasing substitution rate of grass silage with maize fodder, most fibre might be coming from the root which had a lower digestibility than leaf and stem. In addition, Al-zubiadi (2016) reported that the roots of fodder contained husk of the seeds that kept the endosperm at both seed formation and maturity (Dung et al. 2010) and had greater fibre which was made up of the cell wall polysaccharides and thus are more resistant to digestion compared to the young succulent shoots of the fodder (Dung et al. 2010).

Blood parameters

Increasing the substitution rate of hydroponic maize fodder in the diet did not affect (P= 0.189) plasma glucose concentration. Similarly, there was no difference between treatments (P= 0.065) for blood urea.

Blood urea is associated with the uptake and utilization of nitrogenous substances in the feed (Hristov et al. 2019). Feeding a diet with an increasing substitution rate of hydroponic maize fodder tended to increase (P = 0.06) blood urea of calves. This finding was in line with our hypothesis that increasing the substitution rate of hydroponic maize fodder will increase blood urea nitrogen as a result of increasing protein consumption. This might be due to the ammonia produced from protein degradation in the rumen being efficiently used for microbial protein synthesis..

Blood glucose concentration is an indication of carbohydrate digestion, absorption, and metabolism (Harmon & Swanson 2020). There was no difference between treatments on blood glucose concentration (P= 0.18) observed in this study. Glucose concentration was expected to increase with an increasing substitution rate of maize fodder in the diet of calves. When rumen fermentation was limited by nitrogen availability in the rumen, supplementation of protein-rich feed commonly improved rumen fermentation (Ma et al. 2021). A higher rate of rumen fermentation stimulated higher propionate concentration (Zeidali-Nejad et al. 2018; Castillo-Umaña et al. 2020). In addition, a higher rate of rumen fermentation stimulated higher microbial protein synthesis which resulted in increased amino acids absorption (Ma et al. 2021). Since propionate and amino acids are the important precursors for glucose synthesis

Table. 4. Blood urea nitrogen and blood glucose concentrations of weaned Ongole x Brahman male calves fed with increasing substitution rates of maize fodder and concentrates

Parameters	Treatments				Standard Error of Mean	P-values
	$G^{1)}$	GCF1	GCF2	GCF3		
Blood urea, (mg/dL)	$14.53^{a2)}$	17.58^{ab}	23.60^{b}	21.37^{b}	1.43	0.06
Blood glucose, (mg/dL)	121.77	80.58	120.73	80.54	13.15	0.18

G= 70% silage grass + 30% concentrates; GFC-1= 35% silage grass + 35% hydroponic maize fodder + 30% concentrates; GFC-2= 17.5% silage grass + 52.5% hydroponic maize fodder + 30% concentrates; GFC-3= 70% hydroponic maize fodder + 30% concentrates. Values in the same row with different superscripts are significantly difference, ($P<0.05$)

in the liver (Castillo-Umaña et al. 2020), increasing the substitution rate of maize fodder in the diet of calves was expected to increase blood glucose concentration. The factor responsible for the failure of the increasing substitution rate of maize fodder in the diet in increasing blood glucose concentration in the present study might be related to the degradable protein from concentrate in the basal diet (about 30%) which provides sufficient amino acids for microbial synthesis and therefore the blood glucose level decreased. Another reason for lower blood glucose concentration was probably due to the role of the liver which is to control the uptake of nutrients from the digestive tract and to detect the rates of metabolites uptake such as glucose and amino acids to keep more stable levels for the general circulation (Castillo-Umaña et al. 2020).

CONCLUSION

Feeding hydroponic maize fodder as a substitute of grass silage to weaned Ongole x Brahman calves resulted in a decrease in the feed intake and nutrient digestibility. However, rumen parameters including TVFA and NH_3-N increased as a result of increasing the substitution rate of maize fodder for grass silage. Hence, hydroponic maize fodder could be used as a replacement for silage to improve the rumen fermentation of weaned Ongole x Brahman calves, although it might decrease total dry matter intake.

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Morphometric Diversity and Polymorphism of Melanocortin-4 Receptor (MC4R) Gene in Red Kedu and Kampung Chickens

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ABSTRAK

Faizah AU, Ismoyowati, Purwantini D, Rosidi, Susanto A, Sulistyawan IH. 2024. Keragaman morfometrik dan polimorfisme gen melanocortin-4 receptor (MC4R) pada Ayam Kedu Merah dan Kampung. JITV 29(1):45-55. DOI: http://dx.doi.org/10/14334/jitv.v29i13307.

Penelitian bertujuan untuk membandingkan perbedaan morfometrik dan mengetahui adanya polimorfisme gen MC4R pada ayam Kedu Merah dan Kampung. Materi penelitian adalah ayam Kedu Merah dan Kampung sebanyak 98 ekor. Metode yang digunakan yaitu eksperimental dengan pengukuran morfometrik pada ayam berumur 37 minggu. PCR menggunakan sepasang primer MC4R berdasarkan kode akses GenBank AB01221 untuk mengamplifikasi target PCR sepanjang 221 base pair. Analisis data menggunakan uji-t untuk membandingkan morfometrik antara ayam Kedu Merah dan Kampung, kemudian perhitungan frekuensi genotipe, frekuensi gen, heterozigositas, dan jarak genetik untuk mengetahui adanya polimorfisme. Analisis variansi untuk mengetahui pengaruh genotipe terhadap bobot badan dan Panjang shank. Hasil penelitian menunjukan bahwa terdapat perbedaan nyata (P<0.05) antara ayam Kedu Merah dan Kampung baik jantan dan betina pada beberapa parameter morfometrik. Sekuensing produk PCR ditemukan SNP pada base pair 54G>C. Nilai frekuensi genotipe GC dan GG pada ayam Kedu Merah sebesar 0.51 dan 0.49, sedangkan pada ayam Kampung sebesar 0.32, 0.50, dan genotipe CC 0.18. Nilai frekuensi alel G dan C pada ayam Kedu Merah dan Kampung, masing-masing sebesar 0.74, 0.26, dan 0.66, 0.34. Nilai heterozigositas sebesar 38% dan 45%. Jarak genetik pada ayam Kedu Merah dan Kampung memiliki hubungan kekerabatan dekat yakni 0.42. Gen MC4R berpengaruh tidak nyata (P>0.05) pada bobot badan dan panjang shank sehingga gen MC4R tidak dapat digunakan sebagai kandidat marker assisted selection.

Kata Kunci: Ayam Kampung, Gen MC4R, Morfometrik, Polimorfisme, Ayam Kedu Merah

ABSTRACT

Faizah AU, Ismoyowati, Purwantini D, Rosidi, Susanto A, Sulistyawan IH. 2024. Morphometric diversity and polymorphism of melanocortin-4 receptor (MC4R) gene in Red Kedu and Kampung Chickens. JITV 29(1):45-55. DOI: http://dx.doi.org/10/14334/jitv.v29i13307.

The aim of this research was to compare morphometric differences and to determine the presence of MC4R gene polymorphisms in Red Kedu and Kampung chickens. This research used a total of 98 Red Kedu and Kampung chickens. The 37-week-old chickens were subjected to experimental study with morphometric measurements. PCR used a pair of MC4R primers based on GenBank access number AB01221 to amplify the PCR targets 221 base pairs long. Data analysis used the t-test to compare the morphometrics between Red Kedu and Kampung chickens. The genotype frequency, gene frequency, heterozygosity, and genetic distances determine the presence of polymorphisms. Analysis of variance to determine the effect of genotype on body weight and shank length. The results showed significant differences (P<0.05) between male and female Red Kedu and Kampung chickens in terms of body morphometric parameters. Sequencing of the PCR product found SNP in base pair 54G>C. GC and GG genotype frequencies of Red Kedu chicken were 0.51 and 0.49, while those of Kampung chicken were 0.32, 0.50, and the CC genotype was 0.18. Allele frequency for G and C of Red Kedu and Kampung chickens were 0.74 vs. 0.26 and 0.66 vs. 0.34, respectively, and the heterozygosity was 38% and 45%, respectively. The genetic distance between Red Kedu and Kampung chickens showed a close kinship of 0.42. Conclusively, the association of the MC4R gene had no significant effect (P>0.05) on body weight and shank length, and therefore, the MC4R gene could not be used as a marker assisted selection.

Key Words: Kampung Chickens, MC4R Gene, Morphometric, Polymorphism, Red Kedu Chickens

INTRODUCTION

Local chickens that spread across Indonesia have positively influenced the diversity of local poultry genetic resources. Efforts to preserve local chickens are carried out by zoning the original habitat (the maintenance system) to maintain the population in a genetic program. Local chickens are common source of animal protein derived from poultry, and local chicken meat is very popular in Indonesia. Local chicken meat production has continued to increase in recent years. The local chicken population in 2021 showed a 0.31% increase, amounting to 306.4 million (DJPKH 2022). In other words, local chicken production increases with local chicken population and people's purchasing power in Indonesia (Zandos et al. 2021). As a genetic source, local chickens produce meat and eggs and contribute to the ecology and the socio-economic and culture of village communities (Partasasmita et al. 2017).

Free-range chicken, despite its low production, makes a major contribution to the community's economy, increases food security, and potentially provides financial profits and market outreach (Zandos et al. 2021). The advantages of free-range chickens are high adaptability to the new environments, good feed modifiers, and resistance to disease, parasites, and tropical climate stress (Sumantri et al. 2020). The distinctive characteristics of free-range chickens are their non-specific feather colors (yellow, red, black, white, and a mixture of black), medium and small body size, but strong and tough muscle structure which that so that native chickens are also called fighting cocks (Partasasmita et al. 2017).

Identification of Kedu chickens, is very important for sustaining genetic quality improvement program in Indonesia. Descriptively, the qualitative characteristics of Kedu chickens include feather color, shank color, skin color, comb color, and comb shape. These qualitative characteristics can influence the production of kedu chickens (Untari et al., 2013). Kedu chicken can produce up to 124 eggs per year (Telnoni et al. 2021).

One of the efforts to increase the productivity of local chickens is through selections. The first step before selecting local chickens is characterization which, according to Saputra et al. (2021), is the basis for breeding livestock by identifying the morphometrics. The quantitative characteristics of chickens can be measured from its various body parts in order to find the correlations or associations in estimating the body weight. Estimating the value of this correlation is the first important step in the selection (Djegho & Kihe, 2020).

Another way to carry out selections on the measurement of local chickens is using genetic markers by identifying candidate genes coding for economic traits. The economic characteristics of livestock are polygenic traits which are mostly controlled by the number of genes. The diversity of traits is influenced by two factors: genetic and environmental factors. The value of genetic parameters in a population can be used as a guide for improving genetic quality. One of the heritability values or inheritance rates that is often used in local chickens is body weight (Saputra et al. 2021).

The Melanocortin-4 Receptor (MC4R) gene is one of the most influential genes on the growth traits of livestock that have economic value. Melanocortin-4 Receptor gene is responsible for regulating feed intake, energy balance, body weight and bone development of chickens. Melanocortin-4 receptor genes play an important role in regulating food intake, energy expenditure, body weight, metabolism, obesity and energy balance (Zhang et al. 2017). The association between the MC4R gene and body weight has a significant effect on body weight of chickens at 2, 4, and 10 weeks of age. There is a significant relationship between MC4R and livestock body growth because the MC4R gene can affect the activity or function of a protein related to body weight of chickens (Kubota et al. 2019). This study aims to determine differences in morphometric characteristics, identify MC4R gene polymorphisms and their association with body weight and shank length in Kampung and Kedu chickens.

MATERIALS AND METHODS

Animals and experimental design

All procedures in this study were approved by the Research Ethics Commission, Faculty of Veterinary Medicine, Gadjah Mada University (Record no. 047/EC-FKH/Eks./2022). This experimental study used 98 Red Kedu and Kampung chickens, comprising 9 male and 40 female of each strain. Phase 1 research was carried out when the chickens were 20-37 weeks old, kept in battery cages, and fed continuously with rations composed of 40% corn, 30% bran, and 30% laying hen concentrate (18.23% crude protein, 5.83% crude fat, 5.68 % crude fiber, 2825 kcal/kg metabolic energy (ME), 1.09% lysine, 0.35% methionine, 3.60% calcium (Ca), and 0.92% phosphorus (P)). Drinking water was provided ad libitum.

In Phase 2, the materials used were chicken blood samples, EDTA as an anticoagulant, 70% alcohol, DNA isolation kit materials, proteinase K, PCR core kit materials, 2 pairs of primers, TBE 10x buffer, ethanol absolute, fluoroVue gel stain, DNA stain Leadder, 16% acrylamide, 3% glycerol, 10% APS, TEMED, formamide, and aquabidest. Blood collection and measurement were undertaken using caliper, metline, stationery, camera, digital scale, disposable syringe, ice flask, and vacutainer tube. Equipment for DNA isolation consisted of a set of DNA isolation kits, micropipette, centrifuge, waterbath, thermocycler machine, duoplate, collection tubes (white top, yellow top, and blue top). Tools for PCR were PCR machines, 0.2 ml PCR tubes, and a set of PCR kits, while the tools for electrophoresis were measuring cups, gloves, Erlenmeyer flasks, submarine electrophoresis, gel pen glass, comb, and UV visualization.

Body weight and morphometric measurement

Morphometric measurements were carried out on Red Kedu and Kampung chickens aged 37 weeks. Body measurements included body weight using a digital scale, while beak length and wattle length were measured using a caliper. Measurements of chest circumference,

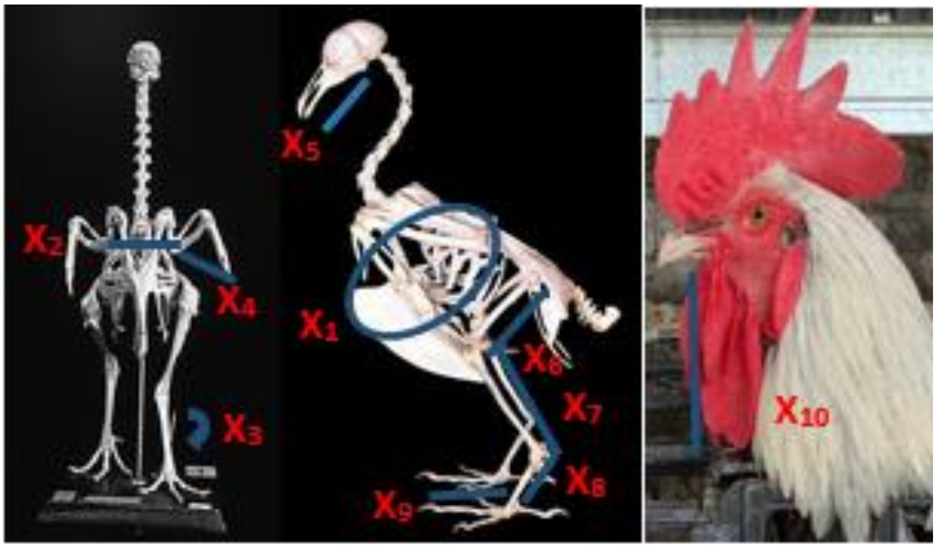

Figure 1. Local chicken body size. Description: X_1 : chest circumference, X_2 : chest width, X_3 : shank circumference, X_4 : wing length, X_5 : beak length, X_6 : femur length, X_7 : tibia length , X_8 : shank length, X_9 : 3rd digiti femur length, and X_{10} : wattle length

wing length, chest width, femur length, tibia length, shank length, shank circumference, and 3rd digit femur were measured using metline. Measurement: X1. The measurement of the chest circumference, namely the circumference from the sternum front to the back (mm); X2. The width of the chest was measured between the anterior and posterior limits on the sternum (mm) (Ismoyowati et al. 2018); X3. Shank circumference is measured by circling the center of the shank (mm) (Sophian et al. 2021); X4. Wing length is measured from the base of the humerus to the tip of the wing bone (mm); X5. Beak length was measured from the base to the tip of the beak (mm); X6. The measurement of the femur was measured from the base to the tip of the femur (mm); X7. Tibia length measurement from the patella to the tip of the tibia (mm); X8. The length of the shank was measured following the length of the tarsometatarsus (mm); X9. The length of the third digit was measured from the base to the tip of the third finger (mm); X10.The length of the wattle from the length of the base of the wattle to the tip of the wattle (mm) (Ismoyowati et al. 2018). Qualitative observations included feather color, feather pattern color, plumage, shank color, comb type, comb color, earlobe color, and eye color

Blood sample and DNA isolation

Three ml of blood were drawn from the subcutaneous area of each chicken (n=98 samples), then put into a vacutainer tube containing EDTA, then transferred 50 µl of it into a 1.5 ml Eppendorf tube and stored in a refrigerator. DNA isolation was carried out following the procedure of FavorPrepTM Plant Genomic DNA Extraction Mini Kit Protocol by Favorgen. DNA isolation was carried out in several stages including cell isolation, cell wall and membrane lysis, DNA extraction, DNA purification, and precipitation.

Primer design and amplification of DNA fragments with PCR

The procedure for preparing the MC4R primer solution in which the PCR primers (MC4R1 F 5'-GAA TTT CAC CCA GCA TCG-3', MC4R1 R 5'-GAG GTT CTT GTT TTG GCT AT-3') match the MC4R DNA sequence (accession number AB012211) (Li & Li 2006) 0.5 µl each, PCR mix 2x powerpoll 6.25 µl, ddH_2O 3.25 µl, and DNA sample 2 µl. The PCR cycle conditions were pre-denaturation at 94˚C for 7 minutes, denaturation at 94˚C for 30 seconds, and annealing at 55˚C for 30 seconds. Elongation occurred when PCR reaction stopped at 72˚C for 45 seconds, then post elongation completed DNA elongation for 10 minutes at 72˚C. The results of the PCR reaction were repeated 35 times to get maximum results. The DNA fragments produced from PCR products were then subjected to electrophoretic tests using acrylamide gel at the SSCP stage.

PCR SSCP and DNA sequencing

At the Single Strand Conformation Polymorphism (SSCP) stage, 12.5 µl PCR product was mixed with 16 µl loading buffer (95% formamide and 5% glycerol) then denatured at 98ºC for 10 minutes. After that, the sample was placed on ice for 5 minutes, then electrophoresed for 17 hours at 10 V/cm in 16% acrylamide gel 3.99 ml, 3% glycerol 0.26 ml, 10x TBE 0.38 ml, aquabidest 2.41 ml, 10% APS 0.075 ml, and TEMED 0.01 ml. The silver stain method was developed to show the bands. The individual band patterns of the PCR-SSCP were determined under UV visualization (Li & Li 2006).

PCR sequencing was carried out by the Integrated Research and Testing Laboratory at Gadjah Mada University, resulting in nucleotide sequences. The electropherogram graph is marked by different colors of nitrogenous bases, namely green for A nucleotides (Adenine), black for G nucleotides (Guanine), blue for C nucleotides (Cytosine), and red for T nucleotides (Timine) (Ismoyowati et al. 2018). The product sequenced in the MC4R gene sample was read using MEGA 11 Software and the BioEdit program to see the Single Nucleotide Polymorphism (SNP) genotyping by aligning the product sequence according to GenBank access number AB012211.

Statistical analysis

The t-test was used to determine differences in phenotypic characteristics and to compare morphometrics between Red Kedu and Kampung chickens. Data analysis used the t-test (Chernick & Friis, 2003), with the following formula:

$$t = \frac{\overline{Y_1} - \overline{Y_2}}{\sqrt{\frac{(N_1 - 1)Sd_1^2 + (N_2 - 1)Sd_2^2}{N_1 + N_2 - 2}\sqrt{\frac{N_1 + N_2}{N_1\, x\, N_2}}}}$$

where, $\overline{Y_1}$= Mean phenotypic characteristics of Kampung chickens; $\overline{Y_2}$= Mean phenotypic characteristics of Red Kedu chickens; Sd_1= Kampung chickens' variance; Sd_2= Red Kedu chickens' variance; N_1= number of samples of Kampung chickens; N_2= number of samples of Red Kedu chickens.

Calculation of allele frequency, genotype Frequency, and heterozygosity value determined using (Hamillton, 2021). Allele frequencies are calculated using formula (1):

$$xi = \frac{2nii + \sum nij}{2N}$$

where xi= allele frequency ii, nii= Number of individuals with genotype ii, nij= Number of samples with genotype ij, N= Number of individual samples.

Genotype frequencies are calculated using formula (2):

$$xij = \frac{nii}{N} x100\%$$

where xii= frequency of homozygous genotypes (ii), xij= frequency of heterozygous genotypes (ij), nii= number of individuals of genotype ii, nij= number of individuals of genotype ij, N= number of individual samples.

Heterozygosity values in this study were calculated using formula (3):

$$He = 1\sum_{i=1}^{n} (pi)^2$$

where He = heterozygosity, n = number of alleles, i = alleles, Pi = allele frequency i.

Genetic distance was calculated using formula (4):

$$D = -ln[Gxy/\sqrt{}GxGy]$$

$$Gx = \sum (pi)^2$$

$$Gy = \sum (qi)^2$$

$$Gxy = \sum piqi$$

Kinship relationship determined the kinship between Red Kedu and Kampung chickens and analyzed using MEGA11 software based on their genetic distance. The correlation analysis was carried out to determine the kinship between genotypes and body weight and shank length of local chickens. The correlation value is calculated using the mathematical model of (Chernick & Friis, 2003):

$$rxy = \frac{\mathrm{n}\Sigma\mathrm{xy} - (\Sigma\mathrm{x})(\Sigma\mathrm{y})}{\sqrt{[\mathrm{n}\Sigma\mathrm{x}^2 - (\Sigma\mathrm{x})^2\ ][\mathrm{n}\Sigma\mathrm{y}^2 - (\Sigma\mathrm{y})^2]}}$$

where, r = correlation coefficient; X = genotype; Y = body weight and shank length of local chickens; n = the number of repetitions.

Analysis of variance was carried out to determine the effect of genotype on body weight and shank length. Replications used individual chickens identified by genotype and morphometrics, thus producing unequal replicates. If the genotype had a significant effect on the measured variables, the MC4R gene had an association with body weight and shank length.

RESULTS AND DISCUSSION

Qualitative characteristics of Red and Kampung Kedu chickens

Qualitative characteristics of local chickens included feather color, feather pattern color, feather glimmer, shank color, comb type, comb color, earlobe color, and eye color (see Table 1). Feather color is dominated by various colors and influenced by qi control genes or genotype Ii (Crawford 1990), while white fur marks the absence of melanin pigment (Roulin & Ducrest 2013). There are two main types of melanin responsible for feather pigments: eblack melanin produced by umelanin, red melanin produced by phaeomelanin, and white coloration due to some reflectivity on their surface. While most colors of bird feathers are produced by the presence of pigments (Stevens 1991), white feathers are very rare but very expensive in tropical countries, thus benefiting farmers (Brown et al. 2017).

This study identified that the dominant feather patterns were Columbian and black (Table 1). Three types of feather patterns of birds are Columbian, black, and wild, with distinctive color border on the head, chest, wings, and tail (Ismoyowati et al. 2018). Columbian (Co), as well as Mahogany (Mh) and Columbian Dark Brown (Db), belong to locus E which limits the distribution of eumelanin and affects the color of the primary feather. These three alleles will influence the color of the feathers on the back, wings, femur, and tail. The black gene (E) has black fur all over the body. The wild color gene (e+) has a black stripe on the back and the Colombian (e) has black feathers on the neck, wings, and tail. (Dako *et al*., 2020).

The color of the shank in this study was dominated by black or black and white. While pigments affect the diversity of shank colors in local chickens, the other contributing factors to varied shank color are the interaction of the main modifier genes (Salces et al. 2015), and differences between local free-range chickens in different regions, diverse pigments, and the genes responsible for determining color (Odah et al., 2019). Melanin pigment affects shank color and is found in the dermis and epidermis layers, so the absence of melanin in both layers results in white shank.

Various types of combs in local chickens are attributed to genetic factors. The comb types in this study – ranked from the most to the least dominant – were single, rose, and pea types. According to Brown et al. (2017), the low frequency of pea comb is due to irrelevance to the tropical climate because the adaptive nature of cold climates can reduce body heat. Odah et al. (2019), stated that chickens with a single comb type were significantly more resistant to the effects of heat than their female counterparts.

The colours of comb and earlobe of local chickens were dominated by red and blackish red, while eye color was dominated by orange and brown. When the chicken's eyes hatch, they will be dark in color and will be visible when the chicken is sexually mature, where the pigments melanin and carotene will be fully expressed. (Riyanti *et al*., 2023). The diversity of eye colors is caused by genes that affect blood supply, increased melanin pigment, and environmental effects (Orounladji et al. 2021). The characteristics of eye color depend on carotenoid pigments and blood circulation in the eye (Odah et al. 2019). In addition, eye color correlates with shank color, and can be modified by feather color genes (Salces et al. 2015).

Morphometric characteristics of Red Kedu and Kampung chickens

The results of the t-test showed different performance between male (Table 2) and female Red Kedu and Kampung chickens (Table 3) in terms of body weight, chest circumference, shank length, beak length, and femur length in male chickens, while body weight, wattle length, chest circumference, chest width, shank length, and third digit length in hens ($P<0.05$). Meanwhile, no significant differences ($P>0.05$) were observed on the size of the shank circumference, tibia length, wing length, beak length, and femur length in females, as well as wattle length, chest width, and third digit length in males. The average body weight of male Red Kedu was higher than that of Kampung chicken, namely 2.34 ± 0.36 and 2.2 ± 0.20 kg (Table 2). While varied body weights are due to genetic factors and uniform environmental influences (Henrik et al. 2018), low body weight is the result of poor management and diverse genetic composition (According to Odah et al. 2019). Male chickens had higher body weight than their female counterparts due to different hormones contained in body size. Similarly, gender affects rooster's body weight through androgenic hormones (Febrianto et al. 2018). Dimorphism in chickens is regulated by different genes and hormones (Salces et al. 2015). Chicken body weight is a common morphometric component to analyze species diversity in local chickens (Sophian et al. 2021). In addition to genetic factors, weight gain in chickens is strongly influenced by the quality of the feed given, where a more balanced nutritional component can improve the performance of poultry digestive organs (Utama et al. 2020). The rate of bone growth can be influenced by the protein consumed (Lukmanudin et al., 2018). Increasing the maximum growth rate can be supported by providing efficient feed and adequate nutrition (Hanafi et al., 2021).

The results of the t-test on chest circumference, chest width, femur length, shank length, and third digit length had significant differences ($P<0.05$) in body weight. According to Liyanage et al. (2015), chest circumference and shank length are the best predictors to determine live weight. The shank bone can be measured by the length of the shank and the circumference of the shank. Shank bone has the ability to support the body of livestock. A long shank size will affect body weight more significantly than a shorter shank size (Debes et al., 2015).

The mean chest circumferences of male and female Red Kedu chickens were not significantly different from that of Kampung chickens. According to Putranto et al. (2018), it can be assumed that local chickens are local species that potentially runs dual-purpose as the producers of meat and eggs. Meanwhile, differences in morphometric measurements are attributed to genetic and environmental factors (Rofii et al. 2018).

Table 3 shows that the coefficient of variation of shank length is higher in male than female because male shank is longer than the female. Intensive maintenance can result in a short shank and relatively large body. Meanwhile, the length of femur, length of tibia, and length of the third digit can be used to estimate the

Table 1. Differences in the qualitative characteristics of Red Kedu and Kampung chickens

Qualitative characteristics	Red Kedu chicken		Kampung chicken	
	Male	Female	Male	Female
Feather Colour	Coloured	Coloured	Coloured	Colour and White
Feather Pattern Colour	Black and Columbian	Black and Columbian	Columbian	Columbian and Wild
Feathers	Silver	Silver and Gold	Silver and Gold	Silver and Gold
Shanks colour	Black	Black and HP	Black and HP	White, Yellow, Black, and HP
Comb type	Single	Pea, Single, and Rose	Singles and Rose	Pea, Single, and Rose
Jigger colour	Red	Red and MH	Red	Red
Earlobe Colour	Red	Red and MH	Red and Black	Red and Black
Eye colour	Chocolate	Orange and Chocolate	Orange and Chocolate	Orange and Chocolate

HP= Black and white, MH= Red and black

Table 2. Mean, standard deviation, and coefficient of diversity of body morphometric characteristics of Red Kedu and Kampung male chickens

Parameter	Red Kedu chicken	Kampung chicken	CV (%)	
			Red Kedu chicken	Kampung chicken
Beak length (mm)	33.58 ± 2.87^{a}	38.26 ± 3.57^{b}	8.56	9.33
Wattle length (mm)	45.82 ± 6.81	34.95 ± 13.20	14.86	37.76
Chest circumference (mm)	350.56 ± 25.30^{b}	315.56 ± 20.68^{a}	7.22	6.55
Chest width (mm)	66.84 ± 8.94	67.14 ± 10.87	13.37	16.19
Wing length (mm)	206.11 ± 16.35	200.00 ± 29.15	7.93	14.58
Tibia length (mm)	147.78 ± 25.87	155.56 ± 10.14	17.51	6.52
Femur length (mm)	103.89 ± 9.28^{a}	116.67 ± 10.00^{b}	8.93	8.57
Shank length (mm)	105.56 ± 7.27^{b}	98.89 ± 7.82^{a}	6.88	7.91
Shank circumference (mm)	49.44 ± 1.67	51.10 ± 5.46	3.37	10.69
3rd Digiti length (mm)	60.56 ± 6.35	65.00 ± 6.12	10.48	9.42
Body weight (kg)	2.34 ± 0.36^{b}	2.20 ± 0.20^{a}	15.26	8.95

CV= Coefficient of variation. Different superscripts in the same line show significantly different

suitability of body shape and positively correlated with body weight (Febrianto et al. 2018). According to Abinawanto et al. (2021), the sternum length, shank circumference, shank length, and the third digit length have significant differences with body weight and have a higher diversity than other characters.

The body weights of male Red Kedu and Kampung chickens were significantly different (see Table 2). Red Kedu chickens have a higher body weight than Kampung chickens, even though the two chicken lines have the same origin. Red Kedu and Kampung chickens descend from the same ancestor, namely the Red Junglefowl (Gallus gallus) (Sulandari et al. 2008)

Based on the results of this study, chicken body size affected bone length. Febrianto et al. (2018) stated that differences in body size are caused by genetic, environmental, and feed factors. In addition, maintenance factors, treatment, and health conditions also contribute to differences in body size (Abinawanto et al. 2021). Phenotypic diversity is caused by differences in body shape and size through morphometric measurements (Febrianto et al. 2018). High diversity and geographic differentiation affect the phenotypic and morphometric characteristics of chickens (Otecko et al. 2019). The highest coefficient of diversity on Red Kedu chickens was found in the tibia length of males (17.51%), and the wattle length of females (29.61%). In both male and female Kampung chickens, the highest coefficient was found in the length of the wattle (Table 2). The interaction between genes is one of the factors for the emergence of new phenotypes through body weight and morphometric characteristics (Henrik et al. 2018).

Table 3. Mean, standard deviation, and coefficient of diversity of body morphometric characteristics of Red Kedu and Kampung female chickens

Parameter	Red Kedu chicken	Kampung chicken	CV (%)	
			Red Kedu chicken	Kampung chicken
Beak length (mm)	33.05 ± 2.42	34.18 ± 3.27	7.31	9.56
Wattle length (mm)	17.51 ± 5.19^b	14.38 ± 2.87^a	29.61	19.97
Chest circumference (mm)	320.98 ± 17.17^b	297.78 ± 25.19^a	5.35	8.46
Chest width (mm)	61.46 ± 6.65^b	55.04 ± 6.20^a	10.82	11.26
Wing length (mm)	168.03 ± 17.10	175.08 ± 20.38	10.18	11.64
Tibia length (mm)	126.70 ± 9.12	127.15 ± 12.72	7.20	10.01
Femur length (mm)	106.25 ± 15.47	104.30 ± 14.74	14.56	14.13
Shank length (mm)	92.63 ± 9.47^b	85.88 ± 8.84^a	10.23	10.29
Shank circumference (mm	43.95 ± 5.22	42.33 ± 4.65	11.88	11.00
3rd Digiti length (mm)	56.40 ± 6.03^a	58.43 ± 4.57^b	10.70	7.83
Body weight (kg)	1.93 ± 0.21^b	1.61 ± 0.24^a	10.66	15.21

CV= Coefficient of variation. Different superscripts in the same line show significantly different

MC4R gene polymorphism PCR-SSCP results

The PCR product obtained a target of 221 bp as expected, as in GenBank access number AB012211 (Figure 2). The successful PCR was evident from a clear band. PCR results that have a constant concentration and purity are considered as good because they are able to amplify and produce several products even in only one cycle.

The PCR-SSCP results revealed that the MC4R gene was polymorphic, meaning that 3 genotypes were found, namely GG, GC, and CC (Figure 3), which indicated the presence of a single nucleotide polymorphism (SNP) located on CDS (Coding DNA Sequens) at the 54th base sequence from G>C (Figure 4). According to Li & Li (2006), this base mutation leads to changes in Glutamine (Gln) to Histidine (His). Changes in protein configuration result in differences of biological functions between wild and mutants. The GG and CC genotypes are homozygous because individuals inherit alleles from their parents. The GC genotype is called the heterozygous which occurs when a pair of alleles at a locus are not identical, due to the influence of incomplete dominance or additive genes. The results of sequencing the samples obtained 36 GG genotypes, 30 GC genotypes, and 7 CC genotypes, and the rest did not show any genotype. This was because the DNA band did not appear nor smear.

Genetic diversity based on the MC4R gene locus

The results obtained on the alignment of the nucleotide base sequences indicated a mutation at the 54th base, namely G>C. The sequencing results showed a polymorphism due to a mutation of guanine (G) to cytosine (C) at base 54 with a length of 221 bp of PCR product. The values of genotype frequency, gene frequency and heterozygosity of the MC4R gene in Red Kedu and Kampung chickens are presented in Table 5.

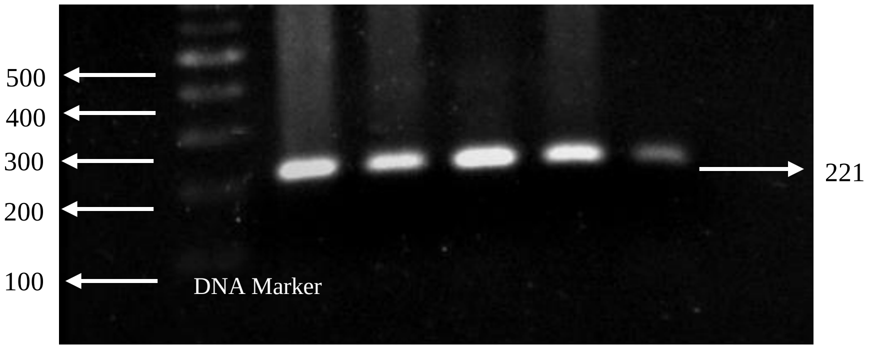

Figure 2. Visualization of 221 bp target DNA PCR products with a marker size of 100-3000 bp

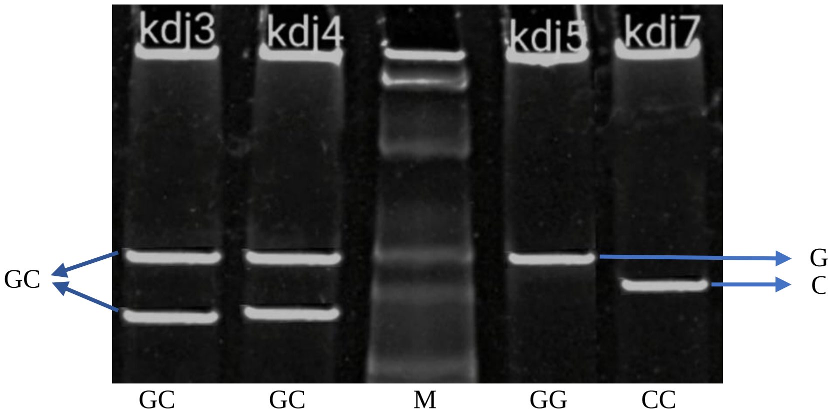

Figure 3. Visualization of the PCR-SSCP results of the MC4R gene with M: marker 100-3000 bp.

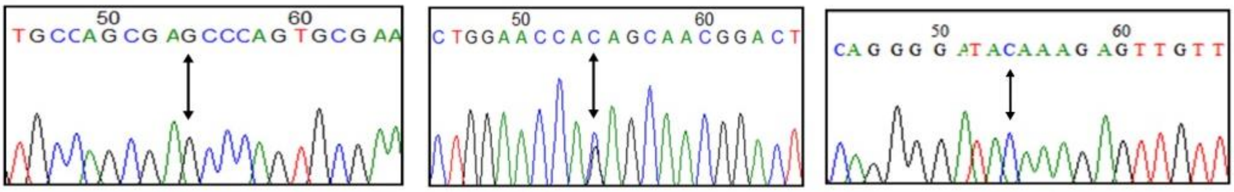

Figure 4. Electropherogram of the MC4R SNP at 54 bp (GG, GC, and CC genotype)

Table 5. Genotype frequency, gene frequency, and heterozygosity in the MC4R gene

Sample	Number of samples (N)	Genotype frequency			Gene frequency		Heterozygosity
		GC (N)	GG (N)	CC (N)	Allele G	Allele C	
Red Kedu chicken	35	0.51 (18)	0.49 (17)	0	0.74	0.26	0.38
Kampung chicken	38	0.32 (12)	0.50 (19)	0.18 (7)	0.66	0.34	0.45

Table 6. Genetic distance in several lines

Strain	*Gallus gallus*	Red Kedu chicken	Kampung chicken
Gallus gallus			
Red Kedu Chicken	0.71	-	
Kampung chicken	0.41	0.42	-

The calculation resulted in three genotypes, namely GC, GG, and CC with respective values of 0.51 and 0.49 (Red Kedu chicken) and 0.32, 0.50, and 0.18 (Kampung chicken). The frequencies of the G and C genes of Red Kedu chicken were 0.74 and 0.26, while in Kampung chicken the values were 0.66 and 0.34, respectively. According to Harahap et al., (2017), the allele frequency value ranges between 0.45-0.63 indicating polymorphism. A population is polymorphic if it has multiple alleles and the frequency value is below 0.99. At the same, it is monomorphic if the frequency value for one of the alleles reaches 1.00 at the same mutation point position.

Based on Table 2, the heterozygosity values for Red Kedu and Kampung chickens were 0.38 and 0.45. According to Serrote et al., (2020), The heterozygosity value ranges from 0 to 1 (if the number of alleles is high with the same frequency value). he genetic variation increases with the heterozygosity number. The heterozygosity in this study (0.50) was still relatively low, thus affecting a low level of genetic diversity (Tamzil & Indarsih 2020). According to Henrik et al. (2018), a low level of genetic diversity in a population indicates a very small possibility of genetic mutations.

Genetic distance and kinship of Red Kedu and Kampung chicken

The results of calculating genetic distance using MEGA11 software showed that the Red Junglefowl (*Gallus gallus*) had a close kinship with Kampung chickens with a genetic distance value of 0.41 (Table 6).

If the value of genetic distance is closer to 0.00, the kinship relationship between lines is closer. The genetic distance between Kedu and Kampung chickens is close (0.42) compared to that between the Red Junglefowl and Red Kedu chickens (0.71). It is in accordance with Abinawanto et al. (2021) that the genetic distance between Kampung chicken and Red Junglefowl is closer than with Green Junglefowl. Genetic distance is the level of gene diversity in a population, which is measured based on a numerical score and calculated based on its genetic frequency. Different genetic distances can be caused by differences in genes and analytical methods (Febrianto et al. 2018). Genetic distance values support the grouping and closeness of each individual in a population, between groups, and nucleotide differences (Abinawanto et al. 2021).

Genetic distance as the basis for the reconstruction of a phylogenic tree. Based on the results of the phylogenic tree, it was shown that the Red Junglefowl and Red Kedu chicken lines had a closer relationship than the Kedu chicken (Figure 6).

The phylogenetic relationship in the phylogenic tree shows that Red Junglefowl (*Gallus gallus*) has the same cluster as the Kampung chicken. That is, the kinship between Red Junglefowl (*Gallus gallus*) and Kampung chicken is very close because its branch length is 0.21. When compared with the Red Kedu chicken, it looks very far from the Red Junglefowl (*Gallus gallus*) with a branch length of 0.28. This may be due to the high genetic variation in the Red Junglefowl and Kampung chickens. According to Blanchette et al. (2012), the close distance in branch length between clusters is probably caused by the absence of genetic mutations. According to Abebe et al. (2015), uniform grouping in one cluster indicates the genome fraction in an individual has the same ancestor. The kinship between Red Kedu and Kampung chickens has a genetic distance value of 0.42 or is closely related. This can be expected because the Red Kedu chicken has undergone a process of genetic quality improvement. According to Febrianto et al. (2018), the closeness of kinship is due to a specific genetic composition for interactions in environmental conditions. It can be concluded that any effort made to improve genetic quality will affect kinship.

Association of the MC4R gene with body weight and shank length

The relationship between the MC4R gene on body weight and shank length was calculated using analysis of variance. The calculation results showed that the genotype had no significant effect on body weight and shank length (P>0.05). This is probably because the chickens in this study were not selected and the MC4R gene did not change the configuration of the protein base. Therefore, the MC4R gene cannot be used to detect body weight and shank length of local chickens. This is consistent with the findings of Molee et al. (2018) that the genotypes were not significantly different across all measured characteristics because the chickens came from relatively unselected populations and each genotype trait was largely varied; therefore, the observed differences were not significant. Based on the correlation value between the genotype and body weight of 0.171, which means the correlation value is low. According to Asmara et al. (2020), the value of the correlation coefficient is between -1 and +1, the +1 value indicates a perfect positive correlation, while the -1 value indicates a perfect negative correlation. Based on the analysis results, the correlation value between the genotype and the shank length is 0.043, indicating a very low correlation..

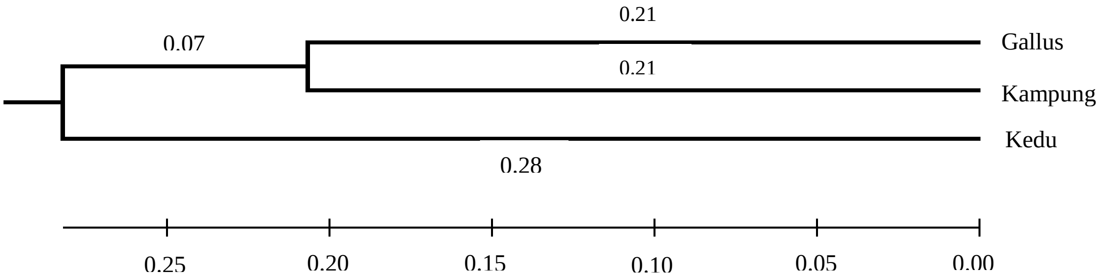

Figure 5. Phylogenic tree using MEGA11

Table 7. Genotype association on body weight and shank length of local chickens

Genotype	Number of samples (N)	Body weight (g)	Shank length (mm)
GG	36	1791.67 ± 343.41	90.28 ± 14.24
GC	30	1736.67 ± 291.53	90.03 ± 10.46
CC	7	1600.00 ± 152.75	88.00 ± 9.33

According to (Hastuti et al., 2021), shank length has the lowest coefficient compared to body weight. A strong relationship between body weight and shank length could possibly be used as a selection criterion, but the genes must be regulated by the same gene action. The results of this study were different from those of Li & Li (2006), which indicated the possibility of identifying the MC4R gene as a marker for selecting body weight and carcass weight. This can be seen in the effect of the MC4R gene on poultry, which contributed 12.02% and 26.97% to body weight and shank length, respectively.

CONCLUSION

There were significant differences in male and female Red Kedu and Kampung chickens in terms of body weight, chest circumference, shank length. The MC4R gene in Red Kedu and Kampung chickens was polymorphic, and the kinship relationship between the two strains was very close with a genetic distance value of 0.42. The MC4R gene was not associated with body weight and the shank length of Red Kedu and Kampung chickens, and therefore, not applicable as a Marker Assisted Selection.

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Book:

a. Alshelmani M, Abdalla E, Kaka U, Basit M. 2021. Advances in poultry nutrition research. In: Kumar Patra A, editor. Adv Poult Nutr Res. London (UK): IntechOpen; p. 19–32. DOI: 10.5772/intechopen.91547.
b. Reece W. 2015. Respiration in mammals. New Jersey (USA): Willey-Blackwell.
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Proceeding:

Damayanti R, Wiyono A, Dharmayanti N. 2021. Pathogenicity study of ducks infected with a local isolate of highly pathogenic avian influenza-H5N1-clade 2.3. . In: Inounu I, Priyanti A, Burrow H, Morris S, Min R, Suhubdy, Sutaryono Y, editors. Proc 4th Int Semin Livest Prod Vet Technol. Bogor (Indones): Indonesian Center for Animal Research and Development; p. 277–288.

Thesis:

Mwasame DB. 2020. Analysis of the socio-economic contribution of donkey ownership and use to houshold livelihoods in Kiambu country, Kenya (Thesis). Nairobi (KE). University of Nairobi

Electronic magazines:

Maranga B, Kagali R, Omolo K, Sagwe P. 2022. Effect of growth substrates on water quality, catfish (*Clarias gariepinus*) culture, and spinach (*Spinacia oleracea*) propagation under the aquaponic system. Livest Res Rural Dev.:82. http://www.lrrd.org/ lrrd34/9/3482mara.html.

Institution:

a. [PSA] Philippine Statistics Authority. 2016. Dairy Industry Performance Report, January – December 2015. Quezon City (Philiphine): Philippine Statistics Authority. P. 1-11
b. [FAO] Food and Agriculture Organization. 2021. Gateway to dairy production and products. Food Agric Organ United Nations. [accessed August 10, 2021]. https://www.fao.org/dairy-production-products/production/feed-resources/en/.

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