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PREFACE

In this edition, Volume 28 No 4, 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:
“Characterization in the Egg Quality of Three Species of Phasianidae Bird”; “Influence of Moringa Leaf Meal As a Feed Additive on Antibody and Bursa Histomorphometry of Hybrid Ducklings”; “Quality of Bebek Ungkep as Affected by Natural Feed Additives”; “Quality of Bebek Ungkep as Affected by Natural Feed Additives”; “Influence of Region on Some Reproductive Parameters in Ouled Djellal Sheep”; “Comparison of Physicochemical, Microbiological, and Organoleptic Characteristics of Dali, Dangke, and Fresh Cheese from Goat Milk”; “Effect of Curcumin Enema Dosage Regimes on the Reduction of Giardia Cyst among Calve”; and “Molecular Characteristics and Evolutionary Relationships of Toll-Like Receptor (TLR21) of Indonesian KUB-1 Chicken”.

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, December 2023

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Influence of Region on Some Reproductive Parameters in Ouled Djellal Sheep

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ABSTRAK

Titaouine M, Gherissi DE, Chergui M, Mohamdi H. 2023. Pengaruh wilayah terhadap beberapa parameter reproduksi domba Ouled Djellal. JITV 28(4):220-226. DOI: http://dx.doi.org/10.14334/jitv.v28i4.3211.

Penelitian ini bertujuan untuk menilai kinerja reproduksi domba betina Ouled Djellal di empat lokasi Biskra yang berbeda: El Hadjeb, Sidi Okba, Ouled Djellal, dan Chaiba. Semua kelompok dipelihara secara ekstensif. Penelitia ini menggunakan 357 ekor domba betina Ouled Djellal yang sehat secara klinis dan tidak bunting. Perkawinan semua kelompok dilakukan secara bebas selama dua bulan: Mei dan Juni 2015 (61 hari). Selama periode beranak, jumlah total domba betina dan jumlah total domba di semua kelompok ditentukan. Dilakukan perbandingan tingkat kesuburan, fekunditas, dan proliferasi menggunakan uji $\chi 2$ untuk memverifikasi hubungan antara tingkat yang diukur dan empat lokasi, serta beberapa perbandingan yang menunjukkan perbedaan signifikan antarlokasi. Parameter yang dinilai dalam penelitian ini meliputi fekunditas, proliferasi, dan fertilitas. Nilai rata-rata keseluruhan yang diperoleh adalah 78% untuk kesuburan, 117% untuk produktivitas, dan 92% untuk kesuburan. Rata-rata ini ditemukan jauh lebih rendah dibandingkan dengan rata-rata yang diamati pada ternak yang dikelola secara intensif. Namun, angka terendah tercatat di lokasi 4 (Chaiba), dengan nilai 65% untuk kesuburan, 109% untuk produktivitas, dan 71% untuk fekunditas. Hasil penelitian ini jelas menunjukkan bahwa wilayah mempunyai pengaruh yang signifikan terhadap kesuburan (P= 0,001) dan fekunditas (P= 0,0001), namun tidak berpengaruh signifikan terhadap tingkat produktivitas (P= 0,074).

Kata Kunci: Domba Betina, Fekunditas, Fertilitas, Profikasi

ABSTRACT

Titaouine M, Gherissi DE, Chergui M, Mohamdi H. 2023. Influence of region on some reproductive parameters in Ouled Djellal sheep. JITV 28(4):220-226. DOI: http://dx.doi.org/10.14334/jitv.v28i4.3211.

The present study aims to assess the reproductive performance of Ouled Djellal ewes at four different Biskra locations: El Hadjeb, SidiOkba, Ouled Djellal, and Chaiba. All flocks were kept under extensive management. For this study, 357 clinically healthy and non-pregnant Ouled Djellal ewes have been used. Mating was practiced in an accessible mode, conducted over two months: May and June 2015 (61 days) for all herds. The total number of lambing ewes and lambs in all flocks was determined during the lambing period. We compared fertility, fecundity, and prolificacy rates using the $\chi 2$ test to verify the relationshipbetween the measured rates and the four sites and multiple comparisons that revealed significant differences. The variables assessed in this study encompassed fecundity, prolificacy, and fertility. The overall mean values obtained were 78% for fecundity, 117% for prolificacy, and 92% for fertility. These averages were significantly lower than those observed in intensively managed herds. However, the lowest rates were recorded at site 4 (Chaiba), with65% for fertility, 109% for prolificacy, and 71% for fecundity. The results of this study clearly show that the region has a significant effect on fertility (P= 0.001) and fecundity (P= 0.0001) but no significant effect on the prolificacy rate (P= 0.074).

Key Words: Ewes, Fecundity, Fertility, Prolificacy

INTRODUCTION

In Algeria, the sheep herd represents the most significant animal resource, estimated at more than 27 million heads, 80% of which are breeding sheep (Titaouine and Meziane 2015). Despite its economic and social importance, sheep farming needs better management concerning technical planning , production systems (Benyounes et al. 2013a), environmental conditions, and reproductive management,which cause low productivity. Improving productivity in sheep production must go hand in hand with controlling reproduction and increasing performance. It allows the choice of breeding period and the reduction of unproductive periods (Castonguay 2018). According to Benyounes et al. (2013b), the alignment of genetic baggage and ensuring good nutritional level before, during, and shortly after mating, as well as at the end of gestation, ensures the survival rate of ovulation and embryos, which consequently contributes to increasing fertility and fecundity.

In the Biskra department, sheep breeding is one of the most essential activities. Since the Ouled Djellal breed represents the majority of the flocks in the region, the Ouled Djellal breed has been the subject of several studies on production and reproduction performances. The latter is perfectly adapted to the extreme conditions of an arid environment. Therefore, this study aims to update and evaluate some reproductive performances of the Ouled Djellal breed in different regions of Biskra, , namely El Hadjeb, Sidi Okba, Ouled Djellal, and Chaiba. The goal is to calculate fertility, reproduction rate, and fecundity rate to determine the impact of the region on these parameters.

MATERIALS AND METHODS

Study area

The present study was conducted in the department of Biskra, located in central-eastern Algeria, at the gateway to the Algerian Sahara. It is a real buffer zone between north and south, about 400 km from the southeast of the capital, Algiers (Aissaoui et al. 2019). Biskra comprises four different geomorphological elements: the mountains, the plains, the plateaus, and the depressions (Makhlouf et al., 2020). The selection of the sites is based on the geographical distribution and characteristics of the areas (landforms and altitude). We selected four locations (Figure 1): El Hadjeb, SidiOkba, Ouled Djellal, and Chaïba, respectively referred to as Site 1, Site 2, Site 3, and 4. The first two sites have a flat landform, the third is a plateau, and the fourth site is a mountainous region.

Climate data

According to the National Meteorological Office, the study area is characterized by significant diurnal heat amplitudes. Thus, the temperature, which reaches very high values in the shade in summer, drops to as much as 50% at night. The variation of daily thermal amplitude is significant in all months of the year. The maximum temperature is reached in July: 49°C, but in January, it varies by 5°C, especially at night. The amount of precipitation is low and varies from month to month; depending on the season, the amount of precipitation increases, especially in autumn and winter. Relative humidity varies considerably depending on the season. It drops to 25% in summer due to high evaporation, especially in July. In winter, however, it rises to as high as 60%. Winds blow throughout the year, and in general, northwest winds dominate. The southerly winds are generally cold and dry in winter. In summer, they are hot and dehydrated: Sirocco. It increases the evaporation and, therefore, the dryness.

Studied flocks of sheep

The selected Ouled Djellal ewes are clinically healthy and non-pregnant, belonging to four flocks. The animals are extensively bred, kept outdoors most of the year during the day, and only wholly indoors for a limited period, depending on the weather. , All experimental animals are kept exclusively on steppe-type pastures, including Halfa (Stipa tenacissima), Diss (Ampelodesmos tenax), and white sagebrush (Artemisia herba alba), and on annual meadows, consisting of various grasses (mainly Cynodon dactylon), and legumes (especially Melilotus sulcata and Vicia monantha). Nutritional supplements are provided only for the breeding rams and the antennas in the breeding program, which are kept in pens. Males are entirely isolated from the females and are added to the flock only during the mating season (1 male for 30 to 40 ewes (1 ram in site 1, 2 rams for sites 2 and 3, and 5 rams for Site 4). The mating mode is accessible, and it was conducted for two months: May to June 2015 (61 days) for all herds. Reproduction parameters were determined five months later (October - November).

Studied parameters

During the lambing period from October to November 2015, we recorded the total number of lambing ewes and the total number of born lambs in each flock. The following reproduction performances (fertility, prolificacy, and fecundity) were evaluated in each flock:Fertility rate= (number of ewes lambing/number of ewes mated) x 100, Prolificacy rate= (number of lambs born/number of ewes lambing) x 100, Fecundity rate= (number of lambs born / number of ewes mated) x 100,All these parameters were calculated according (Dursun 2019).

Analysis method

Statistical analysis of the reproductive variables was conducted using SPSS Inc.'s "IBM SPSS Statistics 20" software, Chicago, Illinois, USA. We compared the means of fertility values, fecundity, and prolificacy rates of the four sites (El Hadjeb, Sidi Okba, Ouled Djellal, and Chaiba). We used the $\chi 2$ test to verify theassociation between the measured rates and the four sites. In addition to multiple comparisons, which revealed sites with significant differences.

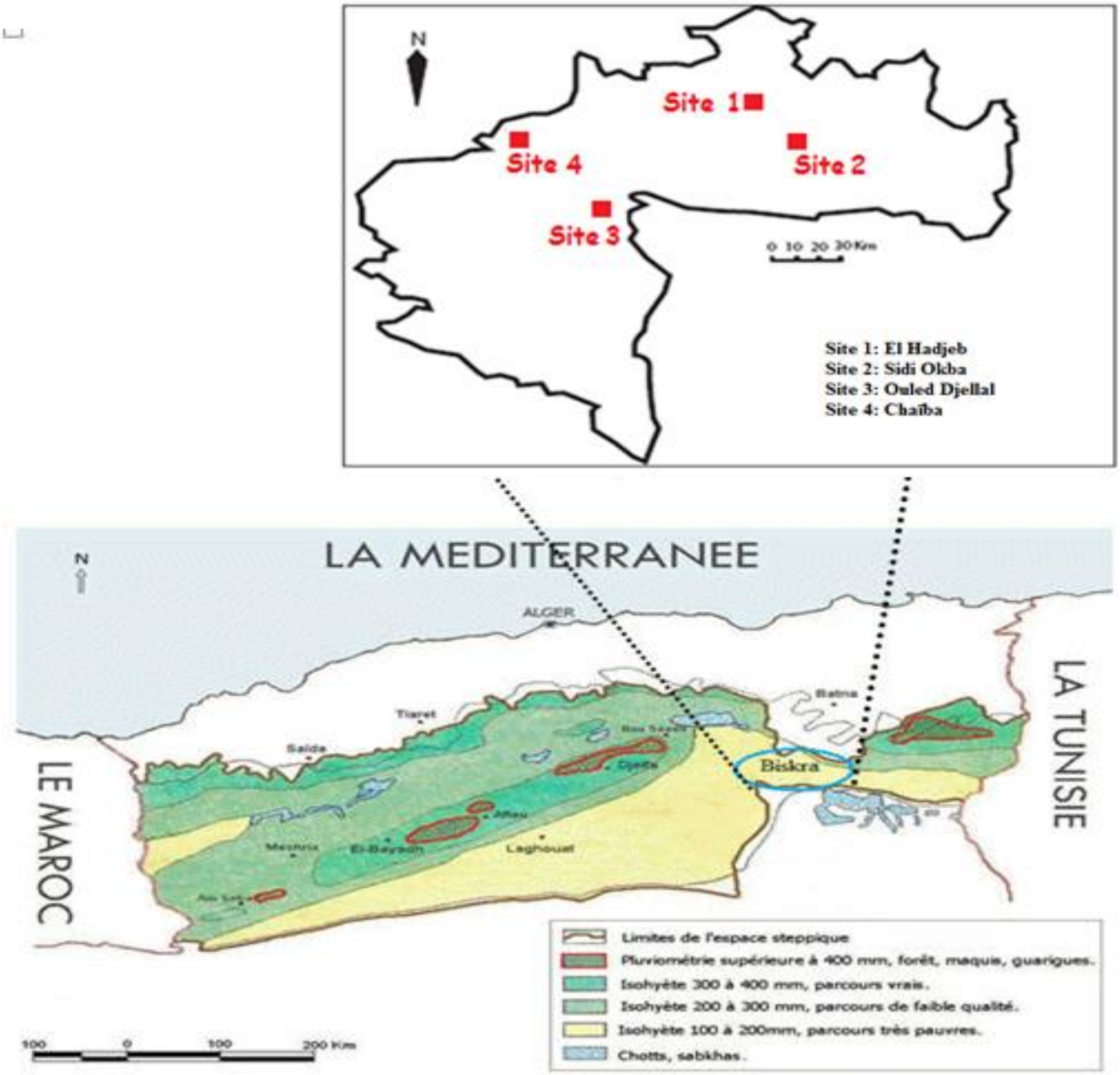

Figure 1. Geographical distribution of the studied sites in Biskra department (Senoussi et al. 2014)

RESULTS AND DISCUSSION

Fertility

Table 1 summarizes the distribution of ewes in each of the four sites, while Table 2 shows how the region and its components affect the various reproductive parameters. Table 2 shows the overall average values of reproductiveperformances of the studied flocks regardless of their location. Basedon these results, it can be stated that the average fertility of ewes is 78%, i.e., 78 lambings and 22 non-lambing (infertile), the average prolificacy of the studied ewes is 117%, i.e., 17 more lambs for 100 lambs, and the average fecundity is 92%. The average fertility scores for flocks at Sites 1 and 4 were lower than the average at 0.7 and 0.65, respectively. In contrast, the average fertility values for the flocks at Sites 2 and 3 were higher than the overall average at 0.9 and 0.87, respectively. For prolificacy, we found that ewes at sites 1, 2, and 3 averaged above the overall average of 1.17 and above the average for the flock at Site 4, which was 1.09. Fecundity was below the overall average. The flocks at sites 1 and 4 were 0.83 and 0.71, respectively. On the other hand, the averages of the flocks at sites 2 and 3 were above the overall average, which was 1.06 and 1.07, respectively.

The total fertility rate in this study was lower compared to that of (Chellig 1992) in a similar sheep breed (100%), with a difference of 22% compared to our results and the rates recorded in Ouled Djellal breed described by (Dekhili, 2004) in Setif region, where the average was 92% and those of (Arbouche et al. 2013) which was 88% with a difference of 14% and 10% respectively.Our findings are consistent with the 75% fertility rate reported by (Zidane et al. 2021) in the Ouled Djellal sheep breed in the Chlef region of the semi-arid western Algerian Highlands. On the other hand, (Mefti Korteby et al. 2017) reported a fertility rate of 83% with a difference of 5%. In certain Moroccan breeds described by (Chikhi and Boujenane 2003) as the Boujaâd, Sardi, Timahdite and Beni Guil breeds, the fertility rates varied from 98%, 85% to 92%, 77% to 95%, and 82 to 87% respectively with differences of 20%, 7% to 14%, 1 % to17%, and 4 to 9%, respectively compared to our results. The lower values in the sheep flocks studied can be explained, on the one hand, by the breeding mode, especially the food supply during the mating season (No flushing technique), and, on the other hand, by the drought-induced nutritional conditions in the study area. Several authors, including(Ayele and Urge 2019) and (Desmarchais et al. 2022), reported that good feeding quality during the breeding period improves ewe fertility. Thus, fertility is influenced by the season and mating month (Catalano et al. 2015;Thompson et al. 2019).The best fertility results were obtained when mating occurred with abundant and high-quality forage. In this context (Nechifor et al. 2022) showed that a daily decrease in nutrient absorption decreased endometrial sensitivity to progesterone and affected embryo survival. From the results ($\chi2 = 22.829$, $p = 0.001$), it can be concluded that the region has a significant effect on fertility rate. Animals from sites 2 and 3 exhibited the best fertility rates: 90% and 87% ($p = 0.589$), compared to 70% and 65% ($p = 0.611$) for sites 1 and 4, respectively. Comparison of fecundity rates revealed a significant difference between sites 1 and 2 ($P= 0.016$), between sites 1 and 3 ($P= 0.041$), and a significant difference between sites 2 and 4 ($P= 0.0001$).

Prolificacy

The average prolificacy of Ouled Djellal ewes was 117%. Our results are lower than those obtained by Dekhili (2004) and Dekhili (2014) for Ouled Djellal sheep, which were 130% and 126%, respectively, with a difference of 13% and 9%. Moreover, the results obtained are lower than those of Chellig (1992) described in D'Man breed sheep, ranging from 185 to 200% with differences from 68% to 83%. Closer results were obtained by Dekhili (2002) in a similarly studied sheep breed (110% with a 7% difference) and in Rembi sheep (110% with a 7% difference) by Chellig (1992). Our results are similar to those of the Hamra and Moroccan Sardi breed, which ranged from 110 to 120% and 107 to 121%, with a difference of 3% and 4%, respectively (Chellig 1992). Finally, Dekhili & Aggoun (2007) found a slightly higher fertility rate in the Ouled Djellal breed in the Sétif region (109%, a difference of 8% from our results). Chikhi & Boujenane (2003) found higher average fertility in Beni Guil and Timahdite sheep breeds, which were 108% and 105%, with differences of 9% and 12%, respectively, compared tothe results in the Ouled Djellal sheep studied. The prolificacy rate is influenced by the feeding of ewes during estrus (Abd El-Hamid et al. 2016).Taherti and Rachid (2018) reported that the distribution of supplementary feed to ewes before and during the mating period improved the ovulation rate and, consequently, the prolificacy rate. Similarly, Sitaresmi et al. (2020) found that adequate and high-quality feeding during the mating season may favor twin births. Animals from site 3 had the best prolificacy rate (123% vs. 119%, 118%, and 109% for sites 1, 2, and 4, respectively). The results of the comparative test of prolificacy rates according to the region (site 1, site 2,site 3, and site 4) are presented in Table 3. Based on the obtained results ($\chi2= 6.933$, $P= 0.074$), we can say that the region has no significant effect on prolificacy.

Table 1. The numbers registered on different sites

Sites	Ewes number	Lambing ewes	Lambs number	Simple pregnancy number	Double pregnancy number
Site 1 (El Hadjeb)	30	21	25	17	4
Site 2 (SidiOkba)	60	54	64	44	10
Site 3 (Ouled Djellal)	70	61	75	47	14
Site 4 (Chaiba)	197	128	140	116	12
Total	357	264	304	224	40

Table 2. Effect of region on fertility, prolificacy, and fecundity in Ouled Djellal ewes reared under semi-arid conditions

Sites	Fertility rate, %	Prolificacy rate, %	Fecundity rate, %
Site1	70a	119	83a,b
Site2	90b	118	106a
Site3	87b,c	123	107a
Site 4	65a	109	71b
Mean±Standard Deviation	78±12	117±6	92±18
Pearson’s Chi-square	22,829	6,933	29,963
P value (Site 1 vs 2 vs 3 vs 4)	0.001	0.074	0.0001
P value (Site1 vs Site 2)	0.016	/	0.056
P value (Site 1 vs Site 3)	0.041	/	0.116
P value (Site 1 vs Site 4)	0.589	/	0.341
P value (Site 2 vs Site 3)	0.611	/	0.741
P value (Site 2 vs Site 4)	0.0001	/	0.0001
P value (Site 3 vs Site 4)	0.0001	/	0.0001

Values with different letters within columns are significantly different (P<0.05)

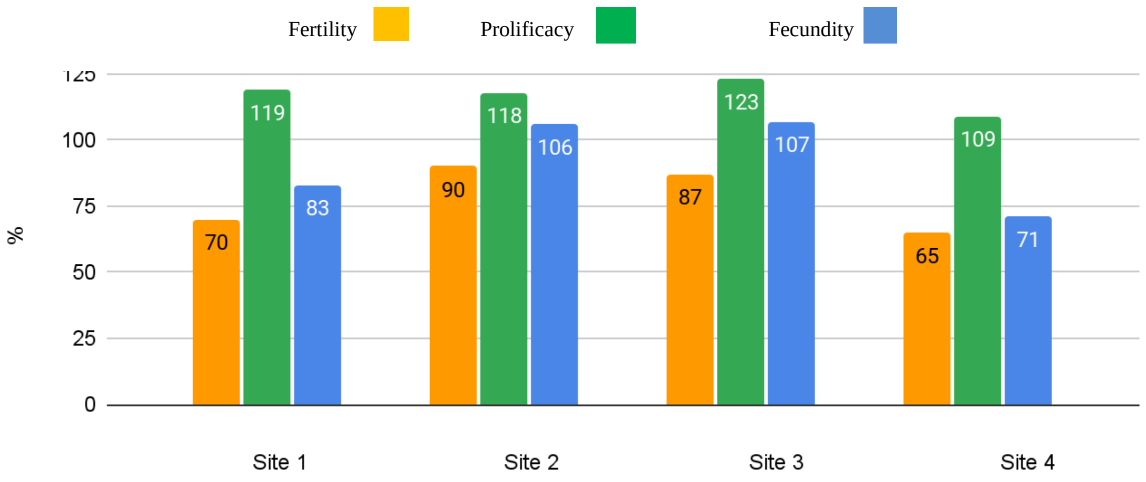

Figure 2: Results of the reproduction parameters refer to the 4 sites. Site 1 (El Hadjeb) and Site 2 (SidiOkba): flat landform regions; Site 3 (Ouled Djellal): plateau relief; Site 4 (Chaiba): mountainous region

Fecundity

The average fecundity rate of the studied Ouled Djallal ewes was 92%. This rate was comparable to that of other breeds of sheep, n, namely the Rembi and Barbarine breeds, which was 95%, with a difference of 3% (Chellig 1992). For the similar breed of ewes, our results were lower than those of Dekhili (2002) and Dekhili (2004), 110% and 128%, with differences of 18% and 36%, respectively. In addition,it was lower than those reported by Narimane et al. (2016), which ranged from 146% to 176%. However, our fecundity value was higher than that of Merghem (2009), which was 70% for the Ouled Djellal breed in the Setif region.The differences between the results obtained in the present study and those of several authors can be explained by racial factors, the rearing systems, and the type of feeding, especially during the control periods. In this study, the herds were reared extensively with inadequate feeding that only met maintenance needs. This malnutrition, especially during the control period, harmed the reproductive potential of the ewes studied. Sheep flocks at sites 2 and 3 had the best fecundity rates, 106% and 107%, versus 83%and 71% for sites 1and 4, respectively. The results of the comparative test of fecundity rate by region (site 1, site 2, site 3, and site 4) are reported in Table 3. We conclude that the region significantly affects fecundity based on the obtained results ($\chi 2= 29.963$, $P= 0.0001$). Pairwise comparisons of fecundity rates show a significant difference between site 2 and site 4 and between site 3 and site 4 ($P= 0.0001$), no significant difference of fecundity rates between site 1 and site 3 ($P= 0.116$), neither between site 1 and site 4 ($P= 0.341$) nor between the site 2 with site 3 ($P= 0.741$).

In conclusion, the results of this study indicate the presence of differences in reproductive performances among the four herds raised in different regions. We consider herd 2 of SidiOkba region as the most fertile and herd 3 in the Ouled Djellal region as the most prolific and fertile, while herd 4 in the Chaiba region is considered the least fertile (0.65), the least fecundable (0.71), and the least prolific (1.09). It is known that the expression of its genotype determines the performance of an animal, the influence of the environment in which it develops, and the interaction between these two factors(Dekhili and Aggoun 2006). Therefore, the difference in reproductive performance among the four flocks is the first explanatory element of this response of Ouled Djellal ewes from one site to another. The poorer fertility, prolificacy, and fecundity rates at site 4 (Chaiba) can be explained by the decrease in progesterone levels in ewes at high altitudes(Barnett et al. 1978).

Given that (Chaiba), the highest region compared to other studied sites, has an altitude of 450 m; this variation can be attributed to its specific environmental and geographical characteristics. In light of the results obtained and the analysis of our experimental data, it is apparent that Ouled Djellal ewes do not show their best reproduction performances in high altitudes. Hence, the low rate of the latter explains the presence of a characteristic site effect, where it is expressed by a biological reaction of the ewes depending on the site. Moreover, Sutama et al. (2012) report that in ruminants,high blood progesterone levels can improve ovulation rates, embryo survival, and implantation of fertilized eggs in the uterus, and thus reproductive rates. Controlling livestock and improving food supply and its availability, which is uncertain in arid areas, can significantly improve breeding parameters.

Furthermore, considering the potential impact of climate change on reproductive performance in arid regions is an essential avenue for future research. Investigating how changing environmental conditions, such as temperature and precipitation patterns, may affect the reproductive capabilities of Ouled Djellal sheep could help develop adaptive strategies to mitigate potential adverse effects.

CONCLUSION

Our study concluded that the region significantly influenced fertility and fecundity; on the other hand, it had no influence on prolificacy. Further work is needed to complete this study, including determining the effects of age, season, rearing, feeding, and other climatic data on the reproduction and development of the sheep flock in Algeria. Continued research in these areas holds the potential to inform targeted breeding programs and management practices, ultimately enhancing the reproductive performance of Ouled Djellal sheep and other related breeds facing similar environmental conditions. .

REFERENCES

Abd El-Hamid IS, Nour El-Din ANM, Zaghloul AA, El-Bahrawy KA, Elshahawy II, Allam AM, EL-Zarkouny SZ, Hassan GA. 2016. Effects of calcium salts of fatty acids rich in palmitic and oleic fatty acids on reproduction and serum biochemistry in Barki ewes. Small Rumin Res. 144:113–118. DOI:10.1016/j.smallrumres.2016.08.001.

Aissaoui M, Deghnouche K, Boukhalfa HH, Saifi M. 2019. Growth performance of goat local breed reared in Southeastern Algeria. World J Environ Biosci. 8:59–66.

Arbouche R, Arbouche HS, Arbouche F, Arbouche Y. 2013. Facteurs influençant les paramètres de reproduction des brebis Ouled Djellal. Arch Zootec. 62:311–314. DOI:10.4321/S0004-05922013000200020.

Ayele S, Urge M. 2019. Productive and reproductive performances of indigenous sheep in Ethiopia: A

Review. Open J Anim Sci. 09:97–120. DOI:10.4236/ojas.2019.91009.

Barnett AR, Sandra DM, Howard JB. 1978. Plasma gonadotropins, prolactin and progesterone at the time of implantation in the mouse: Effects of hypoxia and restricted dietary intake. Biol Reprod. 19:558–565.

Benyounes A, Rezaiguia M, Lamrani F. 2013a. Reproductive performance of Ouled Djellal and Taâdmit ewes raised in the north-east of Algeria. Livest Res Rural Dev. 25.

Benyounes A, Rezaiguia M, Lamrani F. 2013b. Effet de la saison d'agnelage sur la mortalité des agneaux chez les races ovines Ouled Djellal et Taâdmit élevées dans le nord-est d'Algérie BENYOUNES. Rev Agric. 05:5–9.

Castonguay F. 2018. La reproduction chez les ovins. Mars 2018. Canada (USA): Université Laval.

Catalano R, Teruel M, González C, Williams S, Dorna IV, Callejas S. 2015. Reproductive performance of ewe lambs in non-breeding season exposed to hCG at day 12 post-mating. Small Rumin Res. 124:63–67. DOI:10.1016/j.smallrumres.2014.12.014.

Chellig R. 1992. Les races ovines Algériennes. Alger: Office des Publications Universitaires.

Chikhi A, Boujenane I. 2003. Zootechnical characterization of Sardi sheep in Morocco. Rev d'élevage médecine vétérinaire des pays Trop. 56:187. DOI:10.19182/remvt.9863.

Dekhili M. 2002. Performances reproductives des brebis Ouled-Djellal nées simples et doubles. Renc Rech Ruminants. 9:155.

Dekhili M. 2004. Etude de la productivité d'un troupeau de brebis de race Ouled-Djellal. 10ièmes Rencontres Autour des Recherches sur les Ruminants, INRA, 11, 234.

Dekhili M. 2014. Parametres phenotypiques et genetiques de la reproduction de la brebis ouled-djellal (Algérie). Arch Zootec. 63. DOI:10.21071/az.v63i242.543.

Dekhili M, Aggoun A. 2006. Paramètres génétiques de la productivité numérique des brebis Ouled-Djellal.

France(FRA): Institut de l'elevage. Rencontres autour des recherches sur les ruminants. p.221.

Dekhili M, Aggoun A. 2007. Performances reproductives de brebis de race ouled-djellal, dans deux milieux contrastés. Arch Zootec. 56:963–966.

Desmarchais A, Téteau O, Kasal-Hoc N, Cognié J, Lasserre O, Papillier P, Lacroix M, Vignault C, Jarrier-Gaillard P, Maillard V, et al. 2022. Chronic low BPS exposure through diet impairs in vitro embryo production parameters according to metabolic status in the ewe. Ecotoxicol Environ Saf. 229. DOI:10.1016/j.ecoenv.2021.113096.

Dursun. 2019. Effect of different short-term synchronization protocols on estrus and fertility in non-pregnant ewes during the breeding season. J Hell Vet Med Soc. 70:1461–1466. DOI:10.12681/jhvms.20813.

Makhlouf A, Titaouine M, Mohamdi H, Yakoub F. 2020. Effect of different altitude on reproductive performances and mineral assessment in Ouled Djellal ewes during the mating period. Trop Anim Health Prod. 52:3275–3283. DOI:10.1007/s11250-020-02358-8.

Mefti Korteby H, Koudri Z, Saadi MA. 2017. Caractérisation des performances de la race ovine Algérienne Ouled Djellal type Djellalia dans des conditions steppiques. Nat Technol. 17:01–05.

Merghem M. 2009. Caractérisation et paramètres zootechniques des ovins dans la région de Sétif. Setif (ALG): University of sétif -Algeria.

Narimane K, Lakhdara N, Benazouz H, Bensegueni A. 2016. Les paramètres zootechniques de reproduction chez les brebis Ouled Djellal après synchronisation et essais de deux doses d ' eCG. Options Méditerranéennes Ser A Mediterr Semin. 462:459–462.

Nechifor I, Florea MA, Radu-Rusu RM, Pascal C. 2022. Influence of Supplemental Feeding on Body Condition Score and Reproductive Performance Dynamics in Botosani Karakul Sheep. Agric. 12. DOI:10.3390/agriculture12122006.

Senoussi A, Hadbaoui I, Huguenin J. 2014. The pastoral space in the area of M'sila, Algeria: status and perspectives of rehabilitation. Livest Res Rural Dev. 26.

Sitaresmi PI, Widyobroto BP, Bintara S, Widayati DT. 2020. Effects of body condition score and estrus phase on blood metabolites and steroid hormones in Saanen goats in the tropics. Vet World. 13:833–839. DOI:10.14202/vetworld.2020.833-839.

Sutama I-K, Budiarsana I-G., Supriyati S, Hastono H. 2012. Perlakuan Progesteron eksogenus selama bunting untuk meningkatkan produksi susu dan pertumbuhan anak pada kambing Peranakan Etawah. JITV. 1:83–91.

Taherti M, Rachid K. 2018. Productivité de la brebis Ouled Djellal selon le mode de conduite de la reproduction. J Sci Liban. 19:47-58.

Thompson A, Bairstow C, Ferguson M, Kearney G, Macleay C, Thompson H, Paganoni B. 2019. Growth pattern to the end of the mating period influences the reproductive performance of merino ewe lambs mated at 7 to 8 months of age. Small Rumin Res. 179:1–6. DOI:10.1016/j.smallrumres.2019.08.007.

Titaouine M, Meziane T. 2015. The influence of altitude and landforms on some biochemical and hematological parameters in Ouled Djellal ewes from arid area of South East Algeria. Vet World. 8:130–134. DOI:10.14202/vetworld.2015.130-134.

Zidane A, Taherti M, Gadouche L, Metlef S, Ababou A. 2021. Seasonal variations in the reproductive performance of Ouled Djellal ewes in the Chlef region, Algeria. Rev d'Elevage Med Vet des Pays Trop. 74:193–198. DOI:10.19182/remvt.36801.

Comparison of Physicochemical, Microbiological, and Organoleptic Characteristics of Dali, Dangke, and Fresh Cheese from Goat Milk

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ABSTRAK

Wahyuningtyas AN, Taufik E, Soenarno MS, Sulfiar AET, Atmoko BA, Nugroho T. 2023. Perbandingan karakteristik fisikokimia, mikrobiologi dan organoleptik dali, dangke, dan keju segar asal susu kambing. JITV 28(4):227-236. DOI: http://dx.doi.org/10.14334/jitv.v28i4.3254.

Masyarakat Indonesia memanfaatkan susu kambing untuk diolah menjadi produk pangan secara tradisional. Pengolahan susu kambing dalam produk olahan susu diharapkan dapat menjadi salah satu alternatif untuk meningkatkan nilai konsumsi susu kambing tanpa mengurangi manfaat yang dikandungnya. Produk pangan yang diolah dari susu tersebut diantaranya adalah keju segar, dali (Sumatera Utara), dan dangke (Sulawesi Selatan). Tujuan penelitian ini adalah untuk mengkaji perbandingan karakteristik fisikokimia, mikrobiologi dan organoleptik dali, dangke, dan keju segar. Rancangan percobaan pada penelitian ini adalah Rancangan Acak Kelompok dengan 3 taraf perlakuan koagulan yang berbeda yaitu rennet, getah pepaya, dan daun Agave angustifolia sebagai enzim penggumpal kasein pada dali, dangke, dan keju segar, kemudian dilakukan uji ragam dan data non parametrik menggunakan uji Kruskal Wallis, jika ada perbedaan nyata digunakan uji banding berganda Tukey. Hasil penelitian menunjukkan bahwa nilai a_w, pH, dan kadar lemak antara dali, dangke, serta keju segar tidak berbeda, namun berbeda ($P<0.05$) pada nilai rendemen, kadar air, abu, protein, dan karbohidrat. Hasil uji mikrobiologi pada angka lempeng total (TPC) dan angka kapang khamir tidak berbeda nyata antar perlakuan. Hasil uji hedonik berpengaruh nyata terhadap produk keju segar. Keju segar memiliki nilai organoleptik yang lebih baik dibandingkan produk dangke dan dali.

Kata Kunci: Dali, Dangke, Keju Segar, Susu Kambing

ABSTRACT

Wahyuningtyas AN, Taufik E, Soenarno MS, Sulfiar AET, Atmoko BA, Nugroho T. 2023. Comparison of physicochemical, microbiological and organoleptic characteristics of dali, dangke and fresh cheese from goat milk. JITV 28(4):227-236. DOI: http://dx.doi.org/10.14334/jitv.v28i4.3254.

Indonesian people utilize goat milk to be traditionally processed into food products. Goat milk processing into dairy products is expected to be an alternative way to increase the value of goat milk consumption without reducing the benefit. The traditional dairy products from goat milk are Dali from North Sumatra and Dangke from South Sulawesi. This study aimed to examine the physiochemical, microbiological, and organoleptic characteristics of dali with Agave angustifolia leaf extract coagulant (alo-alo leaf), dangke with papaya latex coagulant, and fresh cheese with rennet coagulant. The experimental design used a randomized block design with three different levels of coagulant treatment, namely Rennet, papaya latex, and Agave angustifolia leaves as casein coagulating enzymes for dali, dangke, and fresh cheese. The variance and non-parametric data test used the Kruskal-Wallis test, followed by Tukey's multiple comparison test. The results showed that the values of aw, pH, and fat between dali, dangke, and fresh cheese were not different. Meanwhile, there is a significant difference ($P<0.05$) for yield value, dry matter, ash, protein, and carbohydrate. The results showed that the total plate count, yeast, and mold count of fresh cheese, dali, and dangke were not significantly different. The results of the hedonic test showed that fresh cheese differs significantly from others ($P<0.05$). Fresh cheese had a better organoleptic value than dangke and dali.

Key Words: Dali, Dangke, Fresh Cheese, Goat Milk

INTRODUCTION

Goat milk is well known to have high nutritional value and good effects on human health. It contains highly conjugated linoleic acids essential in immune stimulation, growth, and disease prevention. Most of the goat milk protein component is similar to human milk. Thus, goat milk has more digestible nutrients than cow milk (Turkmen 2017). Moreover, goat milk has a lot of small-size fat globules and low lactose content

(Silanikove et al. 2010), which results in more digestible and can be consumed by an infant (Prosser 2021).

Goat milk processing was proposed to improve the shelf life, durability, and food variation without reducing the benefit (Van Leeuwen et al. 2020; Nayik et al. 2021). Goat milk cheese is one of the popular dairy goat products produced by several European countries. It is known to have distinctive textural, flavor, and aroma characteristics to cow milk (Medina & Nuñez 2017). In Indonesia, cheese consumption is very low (Susilawati & Wahyuningsih 2020). However, several cultures in Indonesia has dairy product, such as dadih (West Sumatra), dali ni horbo (North Sumatra), dangke (South Sulawesi), Cologanti, and Litsusu (Nusa Tenggara). Dali and dangke production concepts are similar to cheese processing because of the use of a coagulant (Surono 2015; Zakariah et al. 2022).

Dangke is one of the most popular dairy products in Sulawesi. Besides its distinctive taste, it has pretty high nutritional value because it is made from cow's or buffalo's milk, which is heated and then added papaya latex and then packaged using banana leaves (Zakariah et al. 2022). Dali is a traditional food for the people of North Sumatra, which is made by coagulating buffalo milk using pineapples (Pratiwi et al. 2019). In addition to pineapples, alo-alo leaf extract (*Agave angustifolia*) is a coagulant material for making dali with the hope that it will act as a milk coagulant and produce better curd. Therefore, this study aimed to examine the physiochemical, microbiological, and organoleptic characteristics of dali with *Agave angustifolia* leaf extract coagulant (alo-alo leaf), dangke with papaya latex coagulant, and fresh cheese with rennet coagulant.

MATERIALS AND METHODS

The research was conducted at the Animal Product Processing Laboratory, Faculty of Animal Husbandry, Division of Animal Product Technology, Bogor Agricultural University, and the Microbiology Laboratory, Faculty of Agricultural Technology, Bogor Agricultural University.

Experimental research

The materials used in the study were fresh cheese, dangke, and dali, which were then grown in an incubator at 37 °C for 48 hours for total plate count (TPC) and 25 °C for 72 hours for yeast mold numbers. The research method uses a randomized block design (RBD). The study was conducted using three different levels of coagulant treatment, namely Rennet, papaya latex, and *Agave angustifolia* leaves, each treatment having three replications.

Fresh cheese, dali, and Dangke processing

Fresh cheese processing carried out refers to the previous study (Permainy et al., 2013), which was modified, namely 1 liter of fresh goat's milk was pasteurized first at 70 °C, then cooled to 35 °C, then 0.2 g of Rennet was added while stirring evenly for 1 minute. During 30 minutes of standing, the coagulation results will separate the curd from the whey, then filtered, and the curd is taken. Dali processing from fresh goat milk, according to the previous study (Sirait, 1991) method, and formula, which is modified, namely Agave angustifolia leaves, are pounded and squeezed to obtain the juice. As much as 5.4% of the leaf extract is then put into 1 liter of goat's milk and heated at 70 °C until curd is formed. Dangke processing was based on a previous study (Mukhlisah et al., 2017) by heating 1 liter of fresh goat's milk to 70 ml and mixing 2% of papaya latex (papain enzyme). The milk and papaya latex solution is then allowed to stand until it forms curd.

Data collection

Fresh milk quality analysis

The milk quality tested was the total plate number (TPC) and the yeast mold number to determine the number of bacteria and yeast mold numbers (Maturin and Peeler, 2001) found in fresh goat's milk and pasteurized milk. Lactoscan is used for testing the quality of milk. The milk sample used for making curd is placed in a container, then quality levels are measured, including solid non-fat (SNF), density, fat content, lactose, salt, and protein.

Characteristics analysis of fresh chees, dali, and dangke

The characteristics of the samples tested in this study included physicochemical characteristics (aw, pH, yield, dry matter, ash, fat, protein, and carbohydrates) and organoleptic (taste, color, aroma, and texture), microbiological (Total et al. and yeast), and organoleptic (hedonic quality test and hedonic test).

Water activity analysis (a_w), pH, and yield

Water activity (aw) is the free water needed to grow microorganisms. Determination of the aw value of the product is measured using a measuring device, namely the aw meter. The instrument was calibrated before measuring the aw of the product with a saturated NaCl solution with an aw of around 0.7509. Several samples are placed into the aw meter. The start button is pressed when the tool is in the ready position. The aw value can be read if the tool is in the completed position (AOAC 2005). The pH value is measured with a Schott pH meter that has been turned on and stabilized for 15 to 30 minutes. The pH electrode was standardized with a buffer solution. The electrode was rinsed and dried with a tissue and then dipped into the sample until the pH meter showed a stable number. The yield is calculated based on the percentage of the resulting sample weight to the weight of the milk used (Irmayanti, 2016). The yield value was calculated based on the formula:

$$Yeald\ (\%) = \frac{Curd\ weight}{Milk\ weight}\ x\ 100\%$$

Dry matter analysis

The sample was weighed (1 g) in a cup. Samples were put in an oven at 105 °C for 8 hours, then cooled in a desiccator and weighed (AOAC 2005). dry matter was calculated according to the formula:

$$Dry\ matter\ (\%) = \frac{First\ weight - Final\ weight}{first\ weight}\ x\ 100\%$$

Ash content analysis

The sample was weighed (1 g) in a porcelain cup. Samples were fired or ashed in a kiln at 600 °C for 2 hours or until they were not smoking. The sample is weighed after being cooled in a desiccator (AOAC 2005). Ash content was calculated according to the formula:

$$Ash\ content\ (\%) = \frac{Ash\ weight}{sample\ weight}\ x\ 100\%$$

Fat content analysis

The sample was weighed (2 g) and spread on cotton lined with filter paper. The filter paper is rolled up to form a thimble and placed in a Soxhlet flask. Samples were extracted for 6 hours with 150 mL hexane solvent. The extracted fat was dried in an oven at 100 °C for 1 hour (AOAC 2005). The formula calculates fat content:

$$Fat\ content\ (\%) = \frac{Fat\ extraction\ weight}{sample\ weight}\ x\ 100\%$$

Protein content analysis

The Kjeldahl method was used in the analysis of the protein content of the sample, which is an analysis of the total N content. A total of 0.1 g of the sample was placed in a 100 mL Kjeldahl flask, and selenium was added in a ratio of 1:1 to the sample and 3 mL of concentrated H2SO4. The sample is digested until the solution becomes clear for about 1 hour, then the digestion flask is cooled, 50 mL of distilled water and 20 mL of 40% NaOH are added, then distilled. The distillation results were collected in an Erlenmeyer containing 10 mL of 2% H3BO3 solution and two drops of pink Methyl Red. After the volume of the distillate becomes 10 mL and the color is bluish-green, the distillation is stopped, and then the distillate is titrated with 0.1 N HCl until it is pink. The same treatment was also applied to blanks (AOAC 2005). Protein levels can be calculated using the formula:

$$Protein\ content\ (bb\%) = 6.25\ x\ \%\ nitrogen$$

Carbohydrate content analysis (bb%)

otal carbohydrate content was determined by the method of carbohydrate by difference (AOAC 2005). The percentage of carbohydrates is calculated using the formula:

$$bb\% = 100\% - (dry\ matter + ash\ content + protein\ content + fat\ content)$$

Total Plate Count (TPC) and Yeast

Parameters observed, namely total plate count (TPC) and yeast mold number (Maturin and Peeler 2001), were also tested for organoleptic taste, aroma, color, and texture (Setyaningsih et al. 2010).

TPC and yeast samples were prepared according to the Bacteriological Analytical Manual (BAM) (Maturin and Peeler 2001). Samples were weighed as much as 25 g and put in sterile plastic wrap. 225 mL of BPW diluent was added to the sample. The mixture was homogenized, and a tenth dilution (P^{-1}) was obtained. Furthermore, 1 mL of P^{-1} was pipetted and dissolved in 9 mL of BPW diluent to obtain P^{-2}, and so on in the same way until P^{-4}.

Fertilization was carried out for each desired dilution (P^{-1} to P^{-4}) by pipetting 1 mL of the dilution into a petri dish and adding 10-12 mL of PCA medium for TPC and PDA media for yeast added with chloramphenicol as much as 0.01%. The mixture is homogenized by moving it to form a number 8 and left until the agar hardens. The Petri dishes were then incubated at 37°C in an inverted position. The growing colonies were counted after 48 hours of incubation. The interval between the number of colonies in the TPC calculation is 25-250 colonies. Colony count was carried out after 48 hours based on the BAM method in units of CFU mL^{-1} (Maturin and Peeler 2001). The data for mold and yeast numbers are combined, and the formula for calculating TPC and yeast molds is as follows:

$$TPC: N = \frac{\sum C}{\left((1xn1) + (0.1xn2)\right)xd}$$

where N is number of colonies per mL or g of product, ∑C is the number of all colonies counted on all Petri dishes, n1is number of Petri dishes counted in the first dilution, n2 is number of Petri dishes counted in the second dilution, and d is the dilution in which the first petri dish was calculated.

Organoleptic

Organoleptic was carried out on hedonic test and hedonic quality test. Sample analysis was performed by 50 semi-trained panelists consisting of people who had studied organoleptic tests. Hedonic test attributes include color, taste, aroma, and texture, with a rating of very dislike (scale 7) to like very much (scale 1). Rating 1 = very like, 2 = like, 3 = somewhat like, 4 = neutral, 5 = somewhat dislike, 6 = do not like, and 7 = really dislike. The hedonic quality test attributes color (white to grayish white), bitter taste (very strong to very weak), milk aroma (very strong to very weak), and texture (hard to soft) (Setyaningsih et al. 2010).

Data analysis

ANOVA analyzed the data obtained to determine the effect of each treatment. Tukey's multiple comparison test is carried out if the treatment has a very significant effect. The data from the organoleptic test results were analyzed by non-parametric statistics using the Kruskall-Wallis test.

RESULTS AND DISCUSSION

Fresh goat milk quality

Milk quality is essential to pay attention to at the beginning of the production process. Milk is the main ingredient in cheese making, and milk components such as protein form texture and flavor. The quality of the milk tested is the total plate number (TPC) and the number of yeast molds to determine the number of bacteria and the number of yeast molds found in fresh goat's milk and pasteurized milk. Results of TPC and yeast in fresh goat's milk and milk pasteurization are presented in Table 1. Test results for the total plate number (TPC) and yeast mold count on fresh goat's milk, respectively 4.6x105 CFU mL-1 and <1.0x105 CFU mL-1. This value does not meet the standards set by the Thai Agricultural Standard for the total plate number (TPC) of fresh goat's milk, that is, a maximum of 2x105 CFU mL-1 (Thai Agricultural Standard 2008). The condition was presumably because of initial contamination during milking. Results from yeast mold numbers indicate that the value meets the specified standard value by the Thai Agricultural Standard for fresh goat's milk, i.e., none

(Thai Agricultural Standard 2008). The condition was presumably because of initial contamination during milking. Results from yeast mold numbers indicate that the value meets the specified standard value by the Thai Agricultural Standard for fresh goat's milk, i.e., none

(Torkar & Vengušt 2008).

According to the Indonesian Standard, milk is a liquid from the udder of cattle, healthy and clean milk obtained by the correct milking method by applicable regulations whose natural content is not reduced or added anything and has not received any treatment except the process cooling. Determination of the quality of milk must follow the quality standards determined by the National Standardization Agency for fresh milk (BSN 2011).

Chees processing using the pasteurization process. However, there is also cheese made without going through the process. Previous studies found that making processed food using pasteurized milk is better for food safety. Pasteurization is done by heating at 65 °C for 30 minutes (low-temperature long time/LTLT). This matter is required because it will kill bacteria that can affect quality cheese, such as coliform, which can spoil texture and taste prematurely (Fernandes 2009).

Test results for the total plate number (TPC) and yeast mold number of pasteurized milk, respectively, are 5.1x103 CFU mL-1 and 4.6x102 CFU mL-1. The value meets the requirements set by the Kenya Bureau of Standards for the total plate number (TPC) of pasteurized milk, which is <104 CFU mL-1 and does not meet the Kenya Bureau of Standards for pasteurized milk yeast mold numbers, namely 0 CFU mL-1. The quality of pasteurized goat milk meets the standards set by the Kenya Bureau of Standards so that goat's milk can be used for dairy products, especially soft cheese production (Kenya Bureau of Standards 2015).

Physical characteristics of fresh cheese, dali, dangke

The physical characteristics of fresh cheese, dali, and dangke, processed from goat milk, including water activity (aw), pH, and yield value, were presented in Table 2. The results of the analysis of variance showed that the water activity (aw) of dali, dangke, and fresh cheese was not significantly different (P>0.05). It was shown that the difference in coagulants does not affect the aw value of the resulting product. The aw values of dali, dangke, and fresh cheese were 0.82, 0.83, and 0.82, respectively. Water activity shows the amount of free water in food that microbes can use for growth (Walstra et al. 2005). The lower the aw value, the more bacterial growth decreases. At the lowest aw value, the bacteria will not grow (Walstra et al. 2005).

The analysis of variance showed that the pH between dali, dangke, and fresh cheese was not significantly different (P>0.05). Calculating the pH value of cheese is important because it affects the aroma

Table 1. TPC and yeast in fresh goat's milk and pasteurized milk

Milk type	P value (CFU mL^{-1})		Standard Value (CFU mL^{-1})	
	TPC	Yeast	TPC	Yeast
Fresh milk	$4.6x10^5$	$<1.0x10^5$	$2x10^{5a}$	-
Pasteurized milk	$5.1x10^3$	$4.6x10^2$	$<10^{4b}$	Nil^b

^{a} Source: Thai Agricultural Standard (2008).; ^{b} Kenya Bureau of Standard (2015)

Table 2. Physical characteristics of fresh cheese, dali, and dangke, which is processed from goat milk

Testing	Types of cheese		
	Fresh cheese	Dangke	Dali
a_w	0.82±0.01	0.83±0.01	0.82±0.03
pH	6.34±0.05	6.2±0.19	6.23±0.12
Yield value	21.38±0.76^b	24.52±3.09^{ab}	27.40±1.87^a

Difference of superscripts in the same row showed significant differences (P<0.05).

Table 3. Chemical characteristics of fresh cheese, dali, and dangke, which is processed from goat milk

Variable	Types of cheese		
	Fresh cheese	Dangke	Dali
Dry matter	57.58±2.38^b	72.78±0.67^a	68.81±4.49^a
Ash	2.02±0.375^a	1.27±0.45^b	1.63±0.10^{ab}
Fat content	18.73±1.39	13.17±2.40	13.87±3.56
Protein content	14.28±2.89^b	8.11±1.49^b	11.28±1.58^{ab}
Carbohydrate content	7.39±0.80^b	4.66±1.58^b	4.41±0.27^b

Difference of superscripts in the same row showed significant differences (P<0.05)

and ripening of cheese (Jamilatun 2009). The pH values of dali, dangke, and fresh cheese were 6.23, 6.2, and 6.34, respectively. Based on the data in Table 1, the higher the pH value of the product, the lower the yield. The relationship between pH and the yield shows that the pH in the coagulation process affects the curd yield in this study. Casein clumping can occur at a pH of 4.2 to 5. The lower the pH value, the faster the clumping process will occur (Walstra et al. 2006).

The analysis of variance showed that the yield of dali and fresh cheese was significantly different (P<0.05). The yield between dali, dangke, and fresh cheese is the same. The average yields of dali, dangke, and fresh cheese were 27.40%, 24.52%, and 21.38%, respectively. A previous study found that curd obtained from the coagulation process can reach 10% to 30% of milk volume (Walstra et al. 2006). Yield is the percentage ratio of the weight of the curd produced to the weight of the milk used. The higher yield value produced indicates that the weight of the curd produced is more and more (Irmayanti 2016). Factors that cause different yield values in this study are due to differences in the coagulants used. The curd formation occurs due to agglomeration and isoelectric activity (Walstra et al. 2006). Casein coagulates into the curd at its isoelectric point at pH 4.6.

Chemical characteristics of fresh cheese, dali, dangke

The chemical characteristics of fresh cheese, dali, and dangke, processed from goat milk, including dry matter, ash, fat, protein, and carbohydrate content, were presented in Table 3.

The analysis of variance showed that the water content of dali, dangke, and fresh cheese was significantly different (P<0.05). The water content between dali and dangke does not differ, but the two products have a different water content than fresh cheese. Fresh cheese in this study had the lowest water content based on the average value and the results of multiple comparison tests (Table 3). The high water content of dali and dangke is thought to be due to the coagulant used, which has weak enzyme activity, so the whey is still bound in the curd. Compared to fresh cheese made with commercial Rennet, the whey separates more from the curd. It is shown that coagulation activity in fresh cheese is more optimal than in dali and dangke. According to Yuniwati et al. (2008), the optimal addition of enzymes will produce yield values that are not so high with low water content due to a better coagulation process so that the whey from the curd formed is easily separated. Low water content is considered good because the less water content, the longer it lasts (Pardede et al. 2013). Dali, dangke, and fresh cheese are classified as soft cheeses based on their water content. Soft cheese has a water content ranging from 50% -80% (Kongo and Malcata 2015).

The analysis of variance showed that the ash content of dali, dangke, and fresh cheese was significantly different ($P<0.05$). Intermediate ash content and dangke based on further test results are different. The ash content of fresh cheese is higher than that of dangke. The ash content in dali did not differ from that in dangke and fresh cheese (Table 3). The ash content indicates the mineral content contained in the food (Pardede et al., 2013). Milk minerals such as calcium, phosphorus, and magnesium are concentrated in the curd, forming during coagulation (Juniawati et al. 2015).

The analysis of variance showed that the fat content contained in dali, dangke, and fresh cheese was not significantly different ($P>0.05$). The fat content, which was not significantly different, was thought to be due to the absence of influence between the heating temperature of the milk and the coagulant used in each product manufactured on the resulting curd fat. The result was similar to the previous study in which there was no real interaction between the heating temperature and the concentration of papain (coagulant) in making dangke (Mukhlisah et al. 2017). Classification of cheese based on its fat content according to the general standard of Codex Cheese (1978) consists of high fat (>60%), full fat (40% to 60%), medium fat (25% to 45%), low fat (10% to 25%) %). Based on the fat content in Table 3, dali, dangke, and fresh cheese are classified as low-fat cheese.

This study's protein levels in dali, dangke, and fresh cheese were 11.28, 8.11, and 14.28%. The analysis of variance showed that the protein content of dali, dangke, and fresh cheese was significantly different ($P<0.05$). Fresh cheese's protein content in this study differed from that of dangke. Dali has a protein content that is not different from dangke and fresh cheese (Table 3). The protein levels in curd are influenced by water content. The higher the water content in the curd, the lower the protein content would be because the protein components were increasingly dissolved in water. The more protein dissolves in water, the lower the protein content (Mukhlisah et al. 2017). The results of this study follow the statement. Based on the average value, the protein content of dangke was the lowest, with the highest water content when compared to the average protein content and water content in dali and fresh cheese (Table 3). The protein and water content of dangke in this study (8.11% and 72.78%) differed from the results in the previous study (Mukhlisah et al. 2017), which were 16.03% and 59.60%. This significant difference is influenced by the concentration of papain in the papaya latex used in the two studies. Enzyme concentration affects the process of curd formation. The amount of enzyme that is insufficient in coagulation will reduce the activity of the enzyme for the coagulation reaction to occur; conversely, if the enzyme is added too much, it will allow the available media to be inadequate for the needs of the enzyme activity, so that the protein content in the product decreases when the enzyme is added excessively (Pardede et al. 2013).

The test results in Table 3 show that the carbohydrate content between dali and dangke is significantly different from that of fresh cheese ($P>0.05$). The carbohydrate content in fresh cheese was 7.39%, significantly higher than dali and dangke (Table 3). The carbohydrate content in this study was calculated using the carbohydrate by difference method (AOAC 2005): 100% - (moisture content + ash + protein + fat). The difference in carbohydrate content between dali and dangke and fresh cheese is thought to be influenced by the different coagulant agents used to manufacture these products. Based on Table 3, water content is the most significant chemical component in curd, so it is suspected that the water content in the product also affects the carbohydrate content in the three products. The water content between dali and dangke, significantly different from fresh cheese, was inversely proportional to the carbohydrate content. Fresh cheese has the lowest water content and the highest carbohydrate content compared to dali and dangke. The result was in line with another study, which states that the carbohydrate content is inversely proportional to the water content (Wulandari 2003).

Microbiological characteristics of fresh cheese, dangke, and dali

The microbiological characteristics tested on fresh cheese, dangke, and dali were the total plate count (TPC). to determine the number of bacteria and yeasts in fresh cheese, dangke, and dali. Sampling was done thrice (week 1, week 2, and week 3). Microbiological characteristics of fresh, dangke, and dali cheese are presented in Table 4.

The results of the variance analysis showed that the treatment of coagulant use did not have a difference in influence on the total plate count (TPC) of fresh cheese, dangke, and dali (Table 2). The test results of the total plate count (TPC) of fresh, dangke, and dali cheeses were 6.73×10^3 CFU g^{-1}, 4.27×10^3 CFU g^{-1}, and 6.80×10^3 CFU g^{-1}, respectively. This figure tends to be high from the

Table 4. Microbiological characteristics of fresh cheese, dali, and dangke, which is processed from goat milk

Testing	Types of cheese (CFU g^{-1})			P value	Standard Value (CFU g^{-1})
	Fresh cheese	Dangke	Dali		
TPC	6.73x10^{3}	4.27x10^{3}	6.80x10^{3}	>0.05	<10 ^{a}
Yeast	1.65x10^{3}	2.77x10^{3}	8.93x10^{2}	>0.05	<50 ^{b}

^{a} Source: Army Regulation Medical Services (USDA 2009); ^{b} USDA Foreign Agricultural Service (Baych 2021)

Table 5. The hedonic quality tests of fresh cheese, dali, and dangke, which is processed from goat milk

Testing	Cheese Type		
	Fresh cheese	Dangke	Dali
Color	1.26±0.75^{a}	1.64±0.85^{b}	3.92±0.44^{c}
Milk aroma	3.50±0.93^{b}	3.20±0.90^{b}	2.60±1.21^{a}
Bitter taste	3.88±1.04^{a}	2.32±1.27^{b}	3.36±1.02^{c}
Texture	2.02±0.79^{a}	2.38±0.69^{a}	3.72±0.92^{b}

Difference of superscripts in the same row showed significant differences ($P<0.05$). Hedonic quality (product quality based on panelist assessment); color: white (1), cloudy white (2), yellowish white (3), greenish-white (4), grayish white (5); cheese aroma: very strong (1), strong (2), moderately strong (3), weak (4), very weak (5); bitter taste: very strong (1), strong (2), slightly strong (3), weak (4), none (5); texture: hard (1), slightly hard (2), soft (3), slightly soft (4), very soft (5)

maximum limit of total bacteria in processed cheese set by the Army Regulation Medical Services regarding the maximum limit of contaminant fungi in dairy products (solid and semi-solid), which does not exceed 10 CFU g^{-1} (USDA 2009). The contamination was possible because the production process was not aseptic. The sources of bacterial contamination in dairy and processed products are due to the sanitation of processing equipment, workers, and raw materials used (Velázquez-Ordoñez et al. 2019).

The various analysis results showed that the coagulant treatment did not have a difference in the influence on the number of yeast in fresh cheeses (Table 4). The yeast molds from fresh cheese, dangke, and dali were 1.65x10^{3} CFU g^{-1}, 2.77x10^{3} CFU g^{-1}, and 8.93x10^{2} CFU g^{-1}. respectively. This result does not match the USDA Foreign Agricultural Service stipulates that it does not exceed 50 CFU g^{-1} (Baych 2021). The contamination was thought to be due to the pre-production process, such as the coagulants used were not sterilized first. The contamination of yeast found on canteen serving and dinner plates comes from the air around the canteen environment. Moreover, air conditioning and production chambers are the main sources of yeast (Damayanthi et al. 2008).

Organoleptic test

Organoleptic testing is divided into 2: the hedonic and the hedonic quality tests. Organoleptic testing collected data from 50 semi-trained panelists on color, aroma, taste, and texture. The samples used in the organoleptic test were fresh cheese, dangke, and dali.

Hedonic quality test

The hedonic quality test aims to see the good and bad impressions of the product being tested. Tests observed included color, milk aroma, bitter taste, and texture. Presentation of hedonic quality test data is presented in Table 5. Based on the hedonic quality test results, different coagulants showed very different results on the color of fresh cheese, dangke, and dali. The colors of fresh cheese, dangke, and dali are 1.26, 1.64, and 3.92, respectively, which are rounded off to 1 (white), 2 (turbid white), and 4 (greenish white), respectively. Fresh cheese, dangke, and dali are made with the basic ingredients of fresh goat's milk, so following Indonesian standards, the color of processed milk is white. The result was similar to the results of a study by Nasution and Marya, which stated that soft cheese made from the milk of the Peranakan Etawah, Saanen, and Crosses PE-Saanen goats has a whiter color than cheese made from slightly yellow cow's milk (Nasution and Marya 2021). Dali produces a greenish-white color because the coagulant in the manufacture of dali uses Agave angustifolia leaf extract.

The results of the hedonic quality test, using different coagulants, showed very different results for milk aroma. The milk aroma values of fresh cheese, dangke, and dali were 3.50, 3.20, and 2.60, respectively, which were rounded off to 4 (weak) and 3 (moderatly strong); this shows that the aroma of milk in fresh cheese and dangke produces a weak aroma of milk, while dali produces a moderatly strong aroma of milk. The aroma of milk is reduced due to the process of separating the curd from whey and the heating process in pasteurization.

Table 6. Results of hedonic tests on fresh cheese, dangke, and dali

Testing	Cheese type		
	Fresh Cheese	Dangke	Dali
Color	2.10±0.97a	2.28±0.88a	4.24±1.58b
Cheese aroma	3.20±1.19ab	3.06±1.15a	3.70±1.78b
Bitter taste	3.94±1.47a	4.72±1.65b	4.44±1.47ab
Texture	3.20±1.38	3.32±1.28	3.38±1.42

Numbers accompanied by different lowercase letters in the same row indicate significant differences ($P<0.05$). Hedonic test (liking power); Color: really like (1), like (2), like a bit (3), neutral (4), rather dislike (5), do not like (6), really do not like (7); Aroma: really like (1), like (2), like a bit (3), neutral (4), rather dislike (5), do not like (6), really do not like (7); Taste: really like (1), like (2), rather like (3), neutral (4), somewhat dislike (5), dislike (6), really dislike (7); Texture: really like (1), like (2), rather like (3), neutral (4), somewhat dislike (5), dislike (6), really dislike (7)

The assessment of bitter taste in fresh cheese, dangke, and dali products was significantly different statistically. The bitter taste of fresh cheese, dangke, and dali are 3.88, 2.32, and 3.36, respectively, which are rounded off to 4 (weak), 2 (strong), and 3 (rather strong); this shows that dangke products have a bitter taste, dali has a slightly bitter taste and fresh cheese does not have a bitter taste. The bitter taste produced in dangke and dali products is slightly bitter because the coagulant used comes from papaya latex for dangke and dali using *Agave angustifolia* leaf extract; this follows the previous study that the bitter taste produced bytaste is caused by the content of karpain alkaloid compounds (Dewa et al. 2018). Moreover, the bitter taste of dangke is closely related to its levels of nutrients; presumably, apart from the effect of papain, it is also due to the proteolysis process (Arini et al. 2016).

The results of the texture hedonic quality test were statistically very different. The textures of fresh cheese, dangke, and dali are 2.02, 2.38, and 3.72, respectively, which are rounded off to 2 (hard), 2 (hard), and 4 (soft). Fresh cheese and dangke produce a slightly firm texture, while dali produces a slightly softer texture. The soft consistency produced in dali is probably due to the low percentage of coagulant in *Agave angustifolia* leaf extract, so the resulting curd becomes less compact. This result was similar to the previous study that stated that administering enzymes at low concentrations causes imperfect clumping because the enzyme activity is not optimal while administering enzymes at high concentrations also allows low enzyme activity because the available substrate is insufficient for the enzymes needed to work (Musra et al. 2021).

Hedonic test

Hedonic tests also carried out organoleptic testing. The hedonic test determines the panelist's level of preference for the product. The tests observed included color, aroma, taste, and texture. The samples tested were fresh cheese, dangke, and dali. The hedonic test results can be seen in Table 6. Color is the primary determinant of whether consumers like a product because the physiological response and the objective stimulus of the sense of sight are more reflexive in judging (Schiano et al. 2017). Color ratings on products are statistically different. The panelists' acceptance of the colors on fresh, dangke, and dali cheeses were 2.10, 2.28, and 4.24, respectively, with scores of two preferred and four neutral. The colors of cheese and dangke were similar.

Meanwhile, both of them were different from Dali. The color difference was due to the coagulant used in dali, the juice of *Agave angustifolia* leaves. The leaves of *Agave angustifolia* are green with thin stripes of bright white color (Verloove et al. 2019), so the dali becomes greenish-white.

Fresh cheese, dangke, and dali flavors are 3.94, 4.72, and 4.44, respectively, rounded to 4 (neutral) and 5 (somewhat disliked). Taste assessments on products differ statistically. The results of the hedonic test showed that the panelists' difficulty level on the three products was neutral for fresh cheese and dali products, and the panelists somewhat disliked the taste of dangke products. The flavors of cheese and dangke are significantly different, while the flavors of the two with dali are not significantly different. The difference in flavor is because the dangke is made using papaya sap coagulant, so the resulting taste is bitter.

Volatile compounds found in the product were assessed using a sense of smell tool by capturing volatile compounds (Aprea 2020). The results of the scent hedonic test are statistically different. The hedonic test results showed that the aromas the panelists somewhat preferred were fresh cheese and dangke products, with average values of 3.20 and 3.06. The aroma of dali is neutral, with an average value of 3.70. The aromas of dangke and dali are noticeably different. The aroma resulted from the coagulants in dangke using papaya sap. While in Dali, using coagulant leaves of *Agave angustifolia* juice.

The textures of fresh, dangke, and dali cheeses are 3.20, 3.32, and 3.38, respectively (somewhat like). Results show that the textures of fresh, dangke, and dali cheese products are no different. The resulting texture in fresh cheese, dangke, and dali products has the same level of liking, which is somewhat like.

CONCLUSION

It was concluded that fresh cheese, dali, and dangke have similar TPC and yeast. However, fresh cheese had a better organoleptic value than dangke and dali. Aw, pH, and fat values between dali, dangke, and fresh cheese were similar, meanwhile, there were significant differences in yield value, dry matter, ash, protein, and carbohydrate. The preference level for fresh cheese was preferable compared to dali and dangke.

REFERENCES

AOAC. 2005. Official methods of analysis of tAOAC International. 18th Ed. Horwitz W, Latimer Jr GW, Editor. Washington (USA): AOAC International.

Aprea E. 2020. Volatile Compounds and smell chemicals (Odor and aroma) of Food. Molecules. 25:1–4. DOI:10.3390/molecules25173811.

Arini N, Sudarwanto MB, Sudirman I, Indrawati A. 2016. Improvement efforts of sensory quality and preservation cow’s milk dangke with addition cow’s milk fat and *Lactobacillus plantarum* supernatant. Int J Dairy Sci. 11:20–27. DOI:10.3923/ijds.2016.20.27.

Baych A. 2021. China notifies cheese standard. Washington DC (USA) United States Department of Agriculture.

BSN. 2011. SNI 3141.1:2011 Susu segar-Bagian 1: Sapi. Jakarta (Indones): Badan Standardisasi Nasional.

Damayanthi E, Yuliati LN, Suprapti VY, Sari F. 2008. Aspek sanitasi dan higiene di kantin asrama tingkat persiapan bersama (TPB) Institut Pertanian Bogor. J Gizi dan Pangan. 3:22. DOI:10.25182/jgp.2008.3.1.22-29.

Dewa FAT, Sirajuddin S, Hendrayati. 2018. Pengaruh konsentrasi getah pepaya terhadap kualitas dangke dan daya terima masyarakat. J Teknol Pangan. 1:0–14.

Fernandes R. 2009. Microbiology handbook: Dairy products. Cambridge (UK): Leatherhead Food International Ltd.

Irmayanti. 2016. Nilai rendemen dan karakteristik organoleptik dangke berbahan dasar susu segar dan susu bubuk komersial (Thesis). Makassar (indones): Hasanuddin University.

Jamilatun M. 2009. Optimalisasi fermentasi pembentukan curd dan analisis kualitas keju mentah yang terbentuk (Thesis). Surakarta (Indones): Sebelas Maret University.

Juniawati, Usmiati S, Damayanthi E. 2015. Karakteristik Fisik Kimia Keju Rendah lemak dari berbagai Bahan Baku Susu Modifikasi. J Penelit Pascapanen Pertan. 12:28–36.

Kenya Bureau of Standard. 2015. Kenya Standard Pasteurized goat milk — Specification. Nairobi (KEN): Kenya Bureau of Standard.

Kongo JM, Malcata FX. 2015. Cheese: types of cheeses - soft. 1st Ed.: Elsevier Ltd. DOI:10.1016/B978-0-12-384947-2.00132-X.

Van Leeuwen SS, Te Poele EM, Chatziioannou AC, Benjamins E, Haandrikman A, Dijkhuizen L. 2020. Goat milk oligosaccharides: their diversity, quantity, and functional properties in comparison to human milk oligosaccharides. J Agric Food Chem. 68:13469–13485. DOI:10.1021/acs.jafc.0c03766.

Maturin L, Peeler JT. 2001. BAM Chapter 3: Aerobic plate count FDA. Maryland (USA): Food and Drug Administration.

Medina M, Nuñez M. 2017. Cheeses From Ewe and Goat Milk. Fourth Edi. Elsevier Ltd. DOI:10.1016/B978-0-12-417012-4.00041-7.

Mukhlisah AN, Arief II, Taufik E. 2017. Physical, microbial, and chemical qualities of dangke produced by different temperatures and papain concentrations. Media Peternak. 40:63–70. DOI:10.5398/medpet.2017.40.1.63.

Musra NI, Yasni S, Syamsir E. 2021. Karakterisasi keju dangke menggunakan enzim papain komersial dan perubahan fisik selama penyimpanan. J Teknol Indust Pangan. 32:27–35. DOI:10.6066/jtip.2021.32.1.27.

Nasution Z, Marya DT. 2021. Uji organoleptik terhadap keju lunak dari susu kambing Peranakan Etawah (PE) Saanen dan persilangannya (PESA). J Green Swarnadwipa. 10:337–341.

Nayik GA, Jagdale YD, Gaikwad SA, Devkatte AN, Dar AH, Dezmirean DS, Bobis O, Ranjha MMAN, Ansari MJ, Hemeg HA, Alotaibi SS. 2021. Recent insights into processing approaches and potential health benefits of goat milk and its products: A review. Front Nutr. 8:1–16. DOI:10.3389/fnut.2021.789117.

Pardede BE, Adhitiyawarman, Arreneuz S. 2013. Pemanfaatan enzim papain dari getah buah pepaya (*Carica papaya* L) dalam pembuatan keju cottage *Lactobacillus bulgaricus*. JKK. 2:163–168.

Permainy A, Wasito S, Widayaka K. 2013. Effect of Differences Rennet of protein and fat content in soft cheese of dairy milk. J Ilm Peternak. 1:208–213.

Pratiwi MB, Sinaga H, Julianti E. 2019. The influence of coagulants and cooking period on the quality of dali ni horbo. IOP Conf Ser Earth Environ Sci. 260:012098. DOI:10.1088/1755-1315/260/1/012098.

Prosser CG. 2021. Compositional and functional characteristics of goat milk and relevance as a base for infant formula. J Food Sci. 86:257–265. DOI:10.1111/1750-3841.15574.

Setyaningsih D, Apriyantono A, Sari MP. 2010. Analisis Sensori untuk industri pangan dan agro. Bogor (Indones): IPB Press.

Silanikove N, Leitner G, Merin U, Prosser CG. 2010. Recent advances in exploiting goat's milk: Quality, safety and production aspects. Small Rumin Res. 89:110–124. DOI:10.1016/j.smallrumres.2009.12.033.

Sirait CH. 1991. Penggunaan susu sapi Fries Holland Untuk pembuatan dali suatu produk susu olahan tradisional Sumatera Utara. Bogor (Indones): IPB University.

Surono IS. 2015. Traditional Indonesian dairy foods. Asia Pac J Clin Nutr. 24:S26–S30. DOI:10.6133/apjcn.2015.24.s1.05.

Susilawati E, Wahyuningsih S. 2020. Statistics of Food Consumption 2020. Jakarta (Indones): Pusdatin Kementan RI.

Thai Agricultural Standard. 2008. Raw goat milk. Bangkok (THA): National Bureau of Agricultural Commodity and Food Standards.

Torkar KG, Vengušt A. 2008. The presence of yeasts, molds, and aflatoxin M1 in raw milk and cheese in Slovenia. Food Control. 19:570–577. DOI:10.1016/j.foodcont.2007.06.008.

Turkmen N. 2017. The Nutritional Value and Health Benefits of Goat Milk Components. Elsevier Inc. DOI:10.1016/B978-0-12-809762-5.00035-8.

USDA. 2009. Appendix O of the Department of Defense food safety and quality assurance action level: dairy (other than cheese and milk products: solid and semi-solid dairy products). Washington DC (USA): United States Department of Agriculture.

Velázquez-Ordoñez V, Valladares-Carranza B, Tenorio-Borroto E, Talavera-Rojas M, Antonio Varela-Guerrero J, Acosta-Dibarrat J, Puigvert F, Grille L, González Revello Á, Pareja L. 2019. Microbial contamination in milk quality and health risk of the consumers of raw milk and dairy products. In: Mózsik G, Figler M, Editors. Nutr Heal Dis - Our Challenges Now Forthcom Time. Rijeka (CRO): IntechOpen; p. 1–25. DOI:10.5772/intechopen.86182.

Verloove F, Thiede J, Rodríguez ÁM, Salas-Pascual M, Reyes-Betancort JA, Ojeda-Land E, Smith GF. 2019. A synopsis of feral agave and furcraea (agavaceae, asparagaceae s. lat.) in the Canary Islands (Spain). Plant Ecol Evol. 152:470–498. DOI:10.5091/plecevo.2019.1634.

Walstra P, Wouters JTM, Geurts TJ. 2006. Dairy Science and Technology, 2nd Edition. Abingdon (UK): Taylor & Francis.

Wulandari BRD. 2003. Kombinasi penggunaan getah biduri (Calototropisgigantea) dan bakteri asam laktat pada pembuatan keju lunak (Thesis). Bogor (Indones): IPB University.

Zakariah MA, Malaka R, Laga A, Ako A, Zakariah M, Mauliah FU. 2022. Quality and storage time of traditional dangke cheese inoculated with indigenous lactic acid bacteria isolated from Enrekang District, South Sulawesi, Indonesia. Biodiversitas. 23:3270–3276. DOI:10.13057/biodiv/d230656.

Effect of Curcumin Enema Dosage Regimes on the Reduction of Giardia Cyst among Calves

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ABSTRAK

Ural K, Erdoğan H, Ural DA, Paşa S, Erdoğan S, Ayan A, Gültekin M, Balikçi C. 2023. Pengaruh dosis curcumin Enema terhadap pengurangan giardia cyst pada sapi pedet. JITV 28(4):237-243. DOI:http://dx.doi.org/10.14334/jitv.v28i4.3276.

Giardiasis, mungkin salah satu serangan parasit yang paling signifikan dan terabaikan pada anak sapi, yang menyebabkan infeksi usus. Agen protozoa ini memperoleh kepentingan diagnostik dan terapeutik karena potensi zoonosisnya sehingga intervensi terapeutik alternatif/pengganti baru menjadi cukup penting. Hal ini karena obat antiparasit yang biasa digunakan untuk pengendalian dan pemberantasan untuk melawan infeksi ini, biasanya menimbulkan efek samping. Oleh karena itu, proyek ini mengevaluasi aktivitas anti-protozoa kurkumin, pada dosis berbeda yang digunakan melalui rute rektal, konstituen utama kunyit. Kelompok enema rektal [Kelompok I: 2 gr/kg, Kelompok II: 80 mg/kg dan Kelompok III: 10 mg/kg] dan pengobatan oral Kelompok IV: 2 gr/betis terdiri dari pengobatan kurkumin dengan dosis yang sesuai ditunjukkan di sini sebagai kontrol kelompok V tidak diobati. Semua kelompok perlakuan menunjukkan penurunan jumlah kista yang signifikan secara statistik pada hari ke 10, dibandingkan dengan nilai hari ke 10 pada kelompok kontrol (p<0,005). Di antara kelompok perlakuan, pengurangan kista tertinggi terlihat pada 99,86% pada kurkumin 10 mg/kg enema rektal yang diberikan pada anak sapi (Kelompok III), yang menerima dosis penelitian terendah. Kurkumin pada semua dosis, namun terutama digunakan melalui rektal dengan rejimen dosis terendah 10 mg/kg, dapat mempercepat kemanjuran klinis dan parasitologis melalui kemungkinan penghambatan proliferasi dan adhesi Giardia, seperti yang dijelaskan sebelumnya. Modalitas pengobatan melalui rute rektal ini mampu mengubah keadaan, menyoroti protokol pengobatan baru.

Kata Kunci: Kurkumin, Giardia, Oral, Rektal, Perlakuan

ABSTRACT

Ural K, Erdoğan H, Ural DA, Paşa S, Erdoğan S, Ayan A, Gültekin M, Balikçi C. 2023. Effect of curcumin enema dosage regimes on reducing giardia cyst among calves. JITV 28(4):237-243. DOI:http://dx.doi.org/10.14334.jitv.v28i4.3276.

Giardiasis is one of the most significant and neglected parasitic infestations among calves, resulting in intestinal infections. This protozoan agent gained diagnostic and therapeutical importance because of its zoonotic potential, in which novel alternative/substitute therapeutical interventions are important, and this is because commonly used antiparasitic drugs for control and eradication for battling this infection commonly presented side effects. Therefore, the present project evaluated the anti-protozoan activity of curcumin at different dose regimes used via the rectal route, the main constituent of turmeric. Rectal enema groups [Group I: 2 gr/kg, Group II: 80 mg/kg, and Group III: 10 mg/kg] and oral treatment Group IV: 2 gr/calf consisted of curcumin treatment at appropriate dosages showed herein in which the control group V were untreated. All treatment groups showed a statistically significant decrease in cyst counts on day 10, compared to day 10 values of the control group (p<0.005). Among the treatment groups, the highest cyst reduction was evident at 99.86% in curcumin 10 mg/kg rectal enema-administered calves (Group III), which received the lowest dose of the study. Curcumin at all dosages, primarily used in the rectal route at the lowest dosage regimen of 10 mg/kg, could have hastened clinical and parasitological efficacy via probable inhibition of Giardia proliferation and adhesion, as described previously. This treatment modality via the rectal route can change the game, highlighting novel treatment protocols.

Key Words: Curcumin, Giardia, Oral, Rectal, Treatment

INTRODUCTION

Giardia duodenalis, a well-recognized protozoal parasite, is frequently detected worldwide among production animals. Subclinical infection in cattle is common, whereas giardiasis prone calves to diarrhea and diminished weight gain (Geurden et al. 2009). The emergence and spread of giardiasis among calves population are increasing problems in our country (Ural et al. 2022; Ural et al. 2022). For the treatment of giardiasis, several drugs are preferred. Table 1 presented anti-giardia treatment options (Pérez-Arriaga et al. 2006), with the vast majority related to side effects. Unexpected, however, well-recognized side effects and therapeutical failures in treatment are known (Darlan et al. 2020). Besides, the parasite might be resistant to drugs, indicating the requirement for novel substitute therapy approaches, natural in origin if possible. Given the significance of natural plants as new antiparasitic compounds, several might be promising.

A famous plant, turmeric, best known as Curcuma longa, has traditional and scientifically therapeutical usage. The latter Asian tropical plant (Shahiduzzaman & Daugschies 2011) constituently involves yellow bioactive material obtained from the rhizomes of Curcuma are curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Dyab et al. 2016). Curcumin is the most significant bioactive ingredient related to the medicinal activity of Curcuma (Holt et al. 2005). It has anti-inflammatory, antitumoral, antioxidant, and antimicrobial efficacy, dedicated to its promising natural compound that might be beneficial against bacterial, fungal, and viral agents (Kumar et al. 2011). Writing down the state of the art regarding curcumin usage within literature data should be wise at the bottom lines.

Given the usage of curcumin, as was the subject of the present study, the antiparasitic efficacy of curcumin received arousing interest. For a better understanding of audiences, several researches were focused on *in vitro* usage of curcumin against giardiasis. Curcumin exhibited activity against several parasites (Pérez-Arriaga et al. 2006; Das et al. 2008; Shahiduzzaman et al. 2009; Pinlaor et al. 2010; Shahiduzzaman & Daugschies 2011). Given curcumin's cytotoxic efficacy on Giardia lamblia trophozoites (Pérez-Arriaga et al. 2006), this data must, at least herein, be an in-depth analyte. *In vitro*, research published in 2017 interpreted cytotoxic influence and morphological alterations of 50,000/mL Giardia parasites subjected to curcumin intervention. In that study, it was hypothesized that curcumin selectively caused inhibition of trophozoite adhesion/proliferation/growth. Abrupted morphology was dedicated to deranged membranes, dorsal/ventral surfaces, flagella, and ventral disk. It was also concluded that curcumin could bind to and interact thoroughly within the most profused protein of the giardia cytoskeleton, denoted as tubulins, establishing microtubules within the parasite. Consequently, microtubules were defragmented (Gutiérrez-Gutiérrez et al. 2017). Another exciting article denoted an 85% mortality rate for Giardia cysts after treatment with curcumin extract at a 50 mg/mL concentration after 60 min (Dyab et al. 2016). Furthermore, curcumin prescription at 20 mg/kg/day diminished cyst excretion and trophozoites (84.7%) within the fecal samples of treated ones (Dyab et al. 2016). Apart from those mentioned above *in vivo* studies, one previous field investigation searched the efficacy of 2 g/calf/day curcumin via rectal route for 10 days) compared to the control group of calves. There was 58.17% (P=0.125), 99.68% (P=0.001), and 100% (P=0.001) reduction on days 3, 7, and 10, respectively, among calves treated with curcumin enema against giardiasis and diarrhea (Ural et al. 2022). Apart from the last studies, the difference between the present research and other relevant ones was based on curcumin usage from a clinical perspective, as this was an *in vivo* model. As a novelty, different dose regimes were used in this study. With the purpose and wishes of searching for promising anti-giardial natural compounds, in the present work, *C. longa* (curcumin) was selected to evaluate the activity of clinical and parasitological efficacy against giardiasis among calves via rectal or oral route.

MATERIALS AND METHODS

Animals and housing

A sample size of 35 Holstein and Jersey calves aged 14 to 36 days was purchased from a commercial vendor in Aydin City, Turkey. Given that the incubation period of giardiasis usually takes 7 to 10 days before investigation, meaning before the treatment modality was applied, all calves were physically examined and determined to have varying degrees of diarrhea. During the allocation period (10 days), all calves were monitored daily at 12-hour intervals to confirm the presence/absence of G. duodenalis cysts within the fecal samples withdrawn. All calves were monitored for 6 months after therapy apart from the study period. No prior drug treatments were available, which was an exclusion criterion. During the trial, the housing prevented cross-contamination. Ethical guidelines were considered for enrollment into the groups, randomly performed by a coin toss.

This study was summarized partially from a national project funded by Adnan Menderes University, Research Projects Funding Unit, with Project number VTF-22027/2022. Along with the written owner consent of farm managers, ethical guidelines were followed: Aydın Adnan Menderes University HADYEK (Local Ethics Committee) with no: 64583101/2020/129 at 17/12/2020

Sub-classification to study groups

As denoted above, random selection by a coin toss permitted us to classify and denote the treatment groups as described in Fig. 1 below. Each group consisted of 10 calves enrolled. Curcumin was purchased from a commercially available pharmaceutical company with an ingredient of 95% pure curcumin powder available.

Table 1. Anti-giardia treatment options with mechanism of action anti-giardiasis compounds related to side effects

Antigiardiosic drugs		Mechanism of action
Nitroimidazoles	Metronidazole	induce DNA fragmentation influencing host cells contraindicated during pregnancy (Hausen et al. 2006; Benchimol et al. 2023)
Benzimidazoles	Albendazole	Effects on parasite cytoskeleton causing parasite detachment through intestinal epithelium (Harder et al. 2001)
Nitrofuran	Furazolidone	effects parasite adhesion capacity but not its viability (Hoyne et al. 1989)
Nitazoxanide	Tiazolidic derivative	Presented the same efficacy as metronidazole (Ponce-Macotela et al. 2001; Mehmood et al. 2022)

Source: Perez-Arriaga et al. 2006

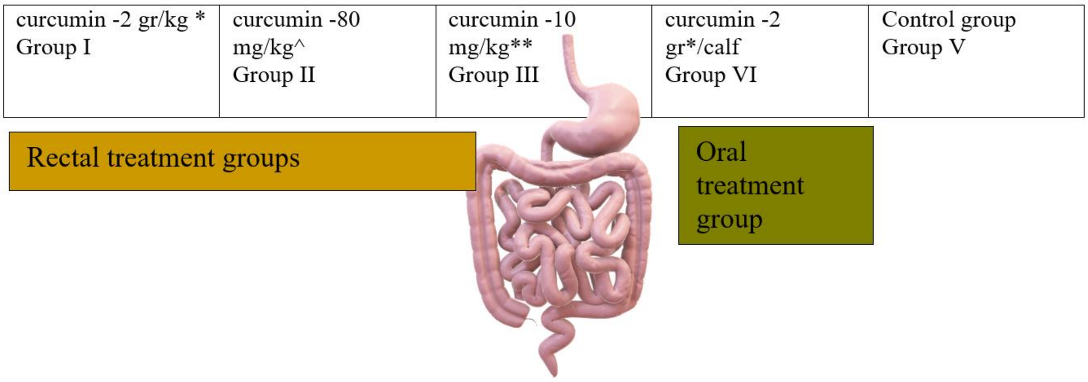

Figure 1. Classification of the groups and treatment decision tree methodology (*Oh et al. 2013; ^Hashemzadeh-Cigari et al. 2014; **Biswas et al. 2017). Rectal enema treatment groups (Groups I to III were administered curcumin per kg of calf body weight, whereas Group IV received 2 g per calf orally, and the control group received an equivalent volume of saline

Fecal flotation and microscopic examination of fecal samples

Pretreatment day 0 (d0) was designated as the start of the trial. Fecal samples from each calf were withdrawn in two sets by investigators in this study and were designated either d0 or day 10 (d10) (the last day of the treatment). Fecal samples (5-7 g) were withdrawn on d0 manually from the rectum of all calves that participated (Ural et al. 2014) and were submitted immediately to laboratory work for fecal flotation. A brief methodological explanation could be explained with previous work by some of the selected researcher groups (Ural et al. 2014) based on a prior description (Wilson & Hankenson 2010). Prepared slides were examined 40x power for the possible Giardia cysts. Microscopical fecal sample proof was based on (Escobedo et al. 2003).

Clinical observation of referring veterinarians

Data regarding clinical status (i.e., existence of diarrhea and several others) and fecal consistency were recorded before and after treatment. The fecal consistency was scored as previously described by Le Jambre et al. (2007) and closer to what has been described elsewhere (Geurden et al. 2011).

Assessment of efficacy of treatment

The therapeutical efficacy of curcumin, whether used by rectal route or orally in the present study, was assessed via microscopic examination of fecal samples collected on D0 and D10 (on the last day of treatment) and analysis based on the probable diminishment in cyst excretion for treated calves in comparison to those of control calves. Deduced cyst excretion was interpreted by use of the Henderson & Tilton's formula (Henderson & Tilton 1955), composing geometric mean cyst counts as described previously (Geurden et al. 2011):

$$100x[-\frac{Ta\ x\ Cb}{Tb\ x\ Ca}]$$

Ta and Tb represented the geometric mean cyst count in the secnidazole treatment group before and after treatment. In contrast, Ca and Cb were the geometric mean cyst count in the control animals before and after treatment (Presidente PJA. 1985).

Statistical analysis

Statistical analyses were performed using the SPSS statistical software package (version 13; SPSS), similar to what has been described previously (Ural et al. 2014).

RESULTS AND DISCUSSION

Statistical analysis along with cyst reduction to those of treatment (both rectal enema and oral) and control groups were shown in Table 2. All treatment groups showed a statistically significant decrease in cyst counts on day 10, compared to day 10 values of the control group (P<0.005). Among treatment groups, the highest cyst reduction was 99.86% in curcumin -10 mg/kg rectal enema-administered calves. Figure 3 shows groups I and III calves sampled. Given cyst counts, the geometric mean was shown in Figure. 4. There were no treatment side effects, and all calves accepted oral or rectal route usage of curcumin. Following 6 month period of monitorizatşon, no recurrence was observed.

At the initial planning of our study, we hypothesized that rectal enema curcumin usage would be beneficial for combatting giardiasis among calves in our country. As giardiasis is a common problem, reasonably priced, economical, and practical, thus natural, if possible, treatment is warranted. Given the antiparasitic efficacy of curcumin (Pérez-Arriaga et al. 2006; Das et al. 2008; Shahiduzzaman et al. 2009; Pinlaor et al. 2010; Shahiduzzaman & Daugschies 2011) and it is cytotoxic efficacy on Giardia lamblia trophozoites (Pérez-Arriaga et al. 2006), we herein at this study for the first time used different dose regimes of curcumin, comparatively by both oral and rectal enema routes, apart from the researches mentioned above. As a dissimilarity to *in vitro* studies of curcumin usage against giardiasis, we performed a clinical trial at this study.

In vitro, research published in 2017 interpreted cytotoxical influence and morphological alterations of 50,000/mL Giardia parasites subjected to curcumin intervention. In that study, it was hypothesized that curcumin selectively caused inhibition of trophozoite adhesion/proliferation/growth. Abrupted morphology was dedicated to deranged membranes, dorsal/ ventral surfaces, flagella, and ventral disk. It was also concluded that curcumin could bind to and interact thoroughly within the most profused protein of the giardia cytoskeleton, denoted as tubulins, establishing microtubules within the parasite. Consequently, microtubules were defragmented (Gutiérrez-Gutiérrez et al. 2017). Another exciting article denoted an 85% mortality rate for Giardia cysts after treatment with curcumin extract at a 50 mg/mL concentration after 60 min (Dyab et al. 2016).

Furthermore, curcumin prescription at 20 mg/kg/day diminished cyst excretion and trophozoites (84.7%) within the fecal samples of treated ones (Dyab et al. 2016). In the present study, as an *in vivo* model, the efficacy of both rectal enema and oral curcumin was detected among calves with giardiasis. Group I, II, and III of calves received rectal enema curcumin at different dosages, giving 99.84%, 99.68%, and 99.86% efficacy, respectively. The highest efficacy was received in group III of calves administered curcumin at the lowest dosage. A direct comparison among those studies is difficult as most were *In vitro* studies. However, comparatively, Ural et al. (2022) determined the efficacy of 2 g/calf/day curcumin via rectal route for 10 days compared to control ones. There was a 100% (P=0.001) reduction on day 10 among calves with giardiasis. In the present study, as mentioned above, we received 99.86% efficacy on giardia cyst count regression compared to the previous study. Also, in the present study, the lowest dose of curcumin exhibited the best efficacy.

Not surprisingly, but interestingly, giardia deranges homeostasis of the whole intestinal tract through competition with the commensal gut microbiota (for attachment sites and nutritional conditions). During the latter condition, giardia i) deranges commensal gut microbiota biofilms, ii) exhaust mucus, and iii) diminish the activity of the immune system. Furthermore, at upper-end growing conditions, parasites elevated intestinal epithelial permeability, epithelial apoptosis induction, bacterial translocation promotion, and pro-inflammatory cytokine production (Allain et al. 2017). Even if this was the condition in the present study (not a far relationship), curcumin, primarily used as a rectal enema, could have helped hasten clinical and parasitological recovery. A brief explanation, thus, should include 1) regulation of intestinal barrier functioning (Wang et al. 2017; Ghosh et al. 2018; Liu & Zhu 2022), 2) beneficial effects via gut microbiota [neutralizing diminished butyric acid-producing bacteria along with elevated richness and diversity] (Zhai et al. 2020), 3)anti-apoptotic activity on intestinal epithelial tissue (Loganes et al. 2017), 4) reduction of bacterial translocation and cytokine levels *(*Sözen et al. 2015) all by curcumin. It should not be unwise to draw a preliminary suggestion that curcumin interacts with giardia via the aforementioned probable mechanisms, which could also explain the efficacy of this study.

In a prior investigation (to those in which selected researchers of this manuscript were also involved), the anti-giardial therapeutical efficacy of citrus extract was analyzed. Goat kids, at the age of 12 to 68 days, with giardiasis were classified as the control group (n=8) [without any receipt], and the other relevant group (n=10) received oral bitter orange extract at a dosage of 10 ml/goat kids for seven days. Antigiardial combatting of bitter orange extract was detected by oocyst shedding on days 0 and 10, with 99.9% efficacy on day 10. Interestingly, it is a natural compound against giardiasis with significantly diminished oocyst shedding supporting the anti-giardial activity of bitter orange extract (Ural et al. 2021). In the present study, another natural compound was the choice. The efficacy of both rectal enema and oral curcumin was shown in this study among calves with giardiasis. Group I to III of calves

Table 2. *G. duodenalis* cyst excretion among curcumin-treated and control groups of calves enrolled at days 0 and 10

	Group I (-2 g/kg in rectal enema)	Group II (-80 mg/kg in rectal enema)	Group III (-10 mg/kg in rectal enema)	Group IV (-2 g/calf p.o.)	Control (saline p.o.)	P-value
Day 0	79709±70145	54923±63340	49014±65423	34798±68076	69133±79519	0.792
Day 10	129.9±566^{a}	175±1253^{a}	69.96±253.5^{a}	75.58±563^{a}	23846±16054^{b}	0.005
Cysts Reduction (%)	% 99.84	% 99.68	%99.86	% 99.78	% 65.51	

^{a,b}: Different letters in the same line are statistically different.

Figure 3. Randomized classification to rectal enema curcumin groups involved a) 2 g/kg, b) 10 mg/kg.

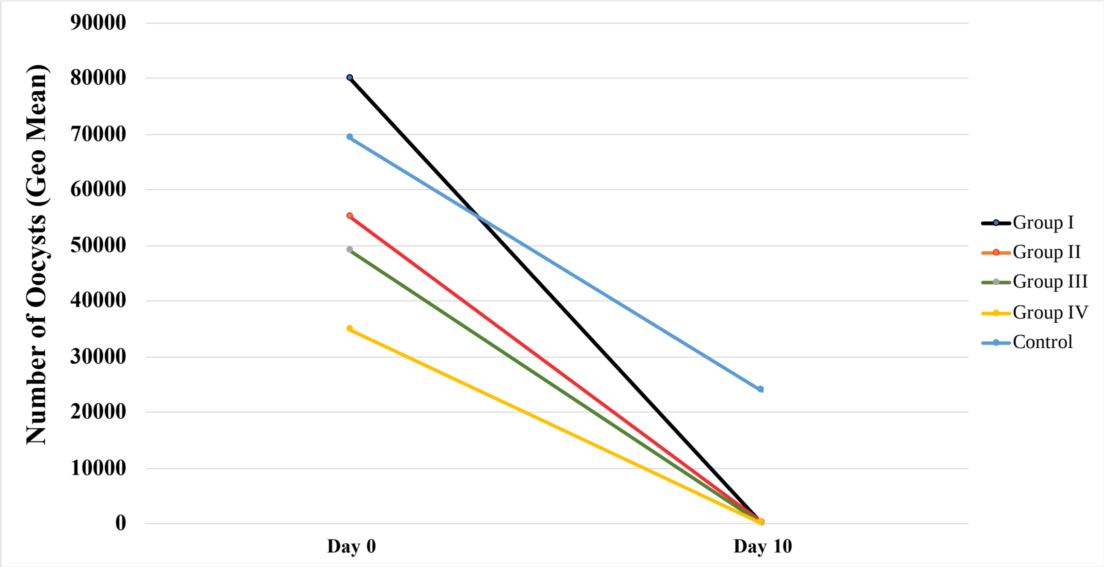

Figure 4. Geometric mean of cyst counts among curcumin-treated and control groups of calves enrolled at days 0 and 10

received rectal enema curcumin at different dosages (apart from the purpose of the present study), resulting in %99.68 to 99.86 efficacy, with the highest reflection in group III. Those calves in group III administered curcumin at a dosage of 10 mg/kg (the lowest among all treated calves) showed the best efficacy. The concept of low-dose curcumin may be beneficial for health promotion (DiSilvestro et al. 2012; Fança-Berthon et al. 2021), lower dose in comparison to higher doses (15 mg. versus 500 mg) showed delivery of higher concentrations, in which our efficacy with the lowest dose at the present study could be briefly explained.

Several frequently observed side effects of oral curcumin might include gastrointestinal complaints (Goulart et al. 2021), whereas, as was also the selected route for the present study with 3 different enema dose regimes, administering curcumin through rectal route (enema), were reported in humans with ulcerative colitis. This methodology could bypass first-pass metabolism within the liver, which is elevated for oral consumption of curcumin (Singla et al. 2014). Our interest was competitively growing in this subject and era of rectal enema curcuminoids, in which our subsequent study would meet these criteria.

CONCLUSION

Our study reveals that curcumin, when administered either orally or via rectal enema, effectively resolved diarrhea within 2 to 4 days in treated groups. Notably, the lowest dosage of curcumin delivered as an enema showed the highest efficacy. By day 10 of treatment, a significant reduction ($p<0.005$) in cyst excretion was observed across all treated groups compared to control calves. Given its cost-effectiveness and favorable tolerance profile, curcumin is a promising, natural therapeutic option, potentially surpassing conventional drugs. This research underscores the potential of curcumin as a therapeutic agent in managing giardiasis in calves, providing invaluable insights for future studies and field applications. The observed benefits of curcumin address immediate clinical outcomes and present broader implications for the long-term health and well-being of treated animals.

REFERENCES

Allain T, Amat CB, Motta JP, Manko A, Buret AG. 2017. Interactions of *Giardia sp*. with the intestinal barrier: Epithelium, mucus, and microbiota. Tissue Barriers. 5:e1274354. DOI:10.1080%2F21688370.2016.1274354.

Benchimol M, Gadelha AP, de Souza W. 2023. Ultrastructural alterations of the human pathogen *Giardia intestinalis* after drug treatment. Pathogens. 12:810. DOI:10.3390/pathogens12060810.

Biswas S, Maji C, Sarkar PK, Sarkar S, Chattopadhyay A, Mandal TK. 2017. Ameliorative effect of two Ayurvedic herbs on experimentally induced arsenic toxicity in calves. J Ethnopharmacol. 197:266-273. DOI:10.1016/j.jep.2016.07.079.

Darlan DM, Hartanto SJ, Rozi MF. 2020. The comparison of efficacy and side effect between albendazole and metronidazole in the management of giardiasis: A systematic review and meta-analysis. EuroMediterr Biomed J. 15:191-196. DOI:10.3269/1970-5492.2020.15.45.

Das R, Roy A, Dutta N, Majumder HK. 2008. Reactive oxygen species and imbalance of calcium homeostasis contribute to curcumin-induced programmed cell death in Leishmania donovani. Apoptosis. 13:867-882. DOI:10.1007/s10495-008-0224-7.

DiSilvestro RA, Joseph E, Bomser J. 2012. Diverse effects of a low dose supplement of lipidated curcumin in healthy middle-aged people. Nutr J. 11:79.

Dyab AK, Yones DA, Ibraheim ZZ, Hassan TM. 2016. Anti-giardial therapeutic potential of dichloromethane extracts of *Zingiber officinale* and *Curcuma longa in vitro* and *in vivo*. Parasitol Res. 115:2637-2645. DOI:10.1007/s00436-016-5010-9.

Escobedo AA, Cañete R, Gonzalez ME, Pareja A, Cimerman S, Almirall P. 2003. A randomized trial comparing mebendazole and secnidazole for the treatment of giardiasis. Annals Trop Med Parasitol. 97:499-504. DOI:10.1179/000349803235002380.

Fança-Berthon P, Tenon M, Bouter-Banon S Le, Manfré A, Maudet C, Dion A, Chevallier H, Laval J, Van Breemen RB. 2021. Pharmacokinetics of a single dose of *Turmeric curcuminoids* depends on formulation: results of a human crossover study. J Nut. 151:1802-1816. DOI:10.1093/jn/nxab087.

Geurden T, Goossens E, Levecke B, Vercammen F, Vercruysse J CE. 2009. Occurrence and molecular characterization of Cryptosporidium and Giardia in captive wild ruminants in Belgium. J Zoo Wildl Med. 40:126-130.

Geurden T, Pohle H, Sarre C, Dreesen L, Vercruysse J, Claerebout E. 2011. The efficacy of a treatment with fenbendazole against an experimental Giardia duodenalis infection in lambs. Small Rumin Res. 96:211-215. DOI:10.1016/j.smallrumres.2010.12.004.

Ghosh SS, He H, Wang J, Gehr TW, Ghosh S. 2018. Curcumin-mediated regulation of intestinal barrier function: The mechanism underlying its beneficial effects. Tissue Barriers. 6:e1425085. DOI:10.1080%2F21688370.2018.1425085.

Goulart RDA, Barbalho SM, Lima VM, Souza GA De, Matias JN, Araújo AC, Rubira CJ, Buchaim RL, Buchaim DV, Carvalho ACA De, et al. 2021. Effects of the use of curcumin on ulcerative colitis and crohn's disease: A systematic review. J Med Food. 24:675-685. DOI:10.1089/jmf.2020.0129.

Gutiérrez-Gutiérrez F, Palomo-Ligas L, Hernández-Hernández JM, Pérez-Rangel A, Aguayo-Ortiz R, Hernández-Campos A, Castillo R, González-Pozos S, Cortés-Zárate R, Ramírez-Herrera MA, et al. 2017. Curcumin alters the cytoskeleton and microtubule organization on trophozoites of *Giardia lamblia*. Acta Trop. 172:113-121. DOI:10.1016/j.actatropica.2017.04.027.

Harder A, Greif G, Haberkorn A. 2001. Chemotherapeutic approaches to protozoa: Giardia, Trichomonas, and Entamoeba - Current level of knowledge and outlook. Parasitol Res. 87:785–786. DOI:10.1007/s00436-001-0589-9.

Hashemzadeh-Cigari F, Khorvash M, Ghorbani GR, Kadivar M, Riasi A, Zebeli Q. 2014. Effects of supplementation with a phytobiotics-rich herbal mixture on performance, udder health, and metabolic status of Holstein cows with various levels of milk somatic cell counts. J Dairy Sci. 97:7487-7497. DOI:10.3168/jds.2014-7989.

Hausen MA, Freitas JCM, Monteiro-Leal LH. 2006. The effects of metronidazole and furazolidone during Giardia differentiation into cysts. Exp Parasitol. 113:135-141. DOI:10.1016/j.exppara.2005.12.009.

Henderson CF, Tilton EW. 1955. Tests with acaricides against the brown wheat mite. J Econ Entomol. 48:157–161. DOI:10.1093/jee/48.2.157.

Holt PR, Katz S, Kirshoff R. 2005. Curcumin therapy in inflammatory bowel disease: A pilot study. Dig Dis Sci. 50:2191-2193. DOI:10.1007/s10620-005-3032-8.

Hoyne GF, Boreham PFL, Parsons PG, Ward C, Biggs B. 1989. The effect of drugs on the cell cycle of Giardia intestinalis. Parasitol. 99:333–339. DOI:10.1017/s 0031182000059047.

Kumar A, Prakash A, Dogra S. 2011. Protective effect of curcumin (*Curcuma longa*) against d-galactose-induced senescence in mice. J Asian Nat Prod Res. 13:42-55. DOI:10.1080/10286020.2010.544253.

Liu X, Zhu H. 2022. Curcumin improved intestinal epithelial barrier integrity by up-regulating ZO-1/Occludin/Claudin-1 in septic rats. 2022: 2884522. Evid Based Complement Alternat Med. DOI:10.1155/2022 /2884522.

Loganes C, Lega S, Bramuzzo M, Brumatti LV, Piscianz E, Valencic E, Tommasini A, Marcuzzi A. 2017. Curcumin anti-apoptotic action in a model of intestinal epithelial inflammatory damage. Nut. 9:578. DOI:10.3390%2Fnu 9060578.

Mehmood, A., ul Haq, Z., Ali, S., Liaquat, I., Hussain M. 2022. To compare the efficacy of 3 days treatment with Nitazoxanide vs 5 days treatment with metronidazole in treating children with diarrhea caused by Giardia-lamblia. Prof Med J. 29:676-680. DOI:10.29309/TPMJ /2022.29.05.6898.

Oh J, Hristov AN, Lee C, Cassidy T, Heyler K, Varga GA WJ. 2013. Immune and production responses of dairy cows to post ruminal supplementation with phytonutrients. J Dairy Sci. 96:7830–7843. DOI:10.3168/jds.2013-7089.

Pérez-Arriaga L, Mendoza-Magaña ML, Cortés-Zárate R, Corona-Rivera A, Bobadilla-Morales L, Troyo-Sanromán R, Ramírez-Herrera MA. 2006. Cytotoxic effect of curcumin on *Giardia lamblia* trophozoites. Acta Trop. 98:152-161. DOI:10.1016/j.actatropica.2006.03. 005

Pinlaor S, Prakobwong S, Hiraku Y, Pinlaor P, Laothong U, Yongvanit P. 2010. Reduction of periductal fibrosis in liver fluke-infected hamsters after long-term curcumin treatment. Eur J Pharmacol. 638:134-141. DOI:10.1016 /j.actatropica.2006.03.005.

Ponce-Macotela M, Gómez-Garduño J, González-Maciel A, Reynoso-Robles R, Anislado-Tolentino V, Martínez-Gordillo MN. 2001. Determinación *in vitro* de la susceptibilidad a la nitazoxanida de cuatro aislados de Giardia duodenalis obtenidos de diferentes huéspedes. Rev Invest Clin. 53:41–45.

Presidente PJA. 1985. Resistance in nematodes to anthelmintic drugs. Australia: CSIRO Division of Animal Health Australian Wool Corporation.

Shahiduzzaman M, Daugschies A. 2011. Curcumin: A natural herb extract with antiparasitic properties. In: Mehlhorn H, Editor. Nature Helps.. Parasitol Res Monogr. Springer (Berlin): Heidelberg. DOI:10.1007/978-3-642-19382-8_6.

Shahiduzzaman M, Dyachenko V, Khalafalla RE, Desouky AY, Daugschies A. 2009. Effects of curcumin on Cryptosporidium parvum *in vitro*. Parasitol Res. 105:1155-1161. DOI:10.1007/s00436-009-1535-5.

Singla V, Pratap Mouli V, Garg SK, Rai T, Choudhury BN, Verma P, Deb R, Tiwari V, Rohatgi S, Dhingra R, et al. 2014. Induction with NCB-02 (curcumin) enema for mild-to-moderate distal ulcerative colitis- A randomized, placebo-controlled, pilot study. J Crohn's Colitis. 8:208-214. DOI:10.1016/j.crohns.2013.08.006.

Sözen S, Aziret M, Bali I, Emir S, Ülgen Y, Binnetoğlu K ... & Çetinkünar S. 2015. The effect of curcumin on an animal intestinal ischemia/reperfusion model for bacterial translocation and inflammatory response. *Int Surg* 100:1352–1359. DOI:10.9738/INTSURG-D-15-0004.1.

Ural DA, Erdoğan S, Balıkçı C, Ayan A, Erdoğan H, Ural K. 2021. Turunçgil ekstraktı anti-giardial terapötik destek sağlayabilir mi?. Atatürk Üniversitesi Vet. Bil. Derg. 16:149-154. DOI:10.17094/ataunivbd.798450.

Ural DA, Ural K, Erdoğan S, Erdoğan H. 2022. Social network analysis of giardiasis positive calves to those of healthy one. IJVAR. 5:113–116.

Ural K, Aysul N, Voyvoda H, Ulutas B, Aldemir OS, Eren H. 2014. Single dose of secnidazole treatment against naturally occurring Giardia duodenalis infection in Sakiz lambs. Rev MVZ Cordoba. 19:4023-4032. DOI:10. 21897/rmvz.122.

Ural K, Erdoğan H, Alıç Ural D, Paşa S, Erdoğan S, Ayan A, Gültekin M. 2022. Enema treatment with the ayuvedic herb curcumin in calves infected with *Giardia duodenalis*: Cohort study. Turk Klin J Vet Sci. 13:35-45. DOI:10.5336/vetsci.2022-92128.

Wang J, Ghosh SS, Ghosh S. 2017. Curcumin improves intestinal barrier function: Modulation of intracellular signaling and organization of tight junctions. Am J Physiol-Cell Physiol. 312:C438-C445. DOI:10.1152/a jpcell.00235.2016.

Wilson JM, Hankenson FC. 2010. Evaluation of an in-house rapid ELISA test for detection of giardia in domestic sheep (*Ovis aries*). JAALAS. 49:809-813.

Zhai SS, Ruan D, Zhu YW, Li MC, Ye H, Wang WC, Yang L. 2020. Protective effect of curcumin on ochratoxin A–induced liver oxidative injury in duck is mediated by modulating lipid metabolism and the intestinal microbiota. Poult Sci. 99:1124-1134. DOI:10.1016/j. psj.2019.10.041.

Characterization in the Egg Quality of Three Species of Phasianidae Birds

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ABSTRAK

Putra WPB, Kırıkçı K, Arslan E. 2023. Karakterisasi kualitas telur pada tiga spesies unggas Phasianidae. JITV 28(4):244-250. DOI: http://dx.doi.org/10.14334/jitv.v28i4.3081.

Ayam Partridge Batu (*Alectoris graeca*), ayam Pegar (*Phasianus colchicus*) dan puyuh Jepang (*Coturnix japonica*) merupakan tiga jenis spesies unggas dari famili Phasianidae yang dipelihara untuk produksi daging dan telur di negara Republik Turki. Penelitian ini bertujuan untuk melakukan karakterisasi kualitas telur yang berasal dari ayam Partridge Batu (RP), ayam Pegar (CP) dan puyuh Jepang (JQ). Parameter yang diukur pada telur yaitu berat telur (EW), indeks bentuk (SI), indeks yolk (YI), indeks albumen (AI), berat kerabang (SW), haugh unit (HU),berat yolk (YW), berat albumen (AW), rasio yolk (YR), rasio albumen (AR) dan rasio kerabang (SR). Sebanyak 372 butir telur unggas terdiri dari 100 butir telur partridge, 200 butir telur pheasant dan 72 butir telur puyuh digunakan pada peneitian ini. Analisis Diskriminan Kanonik (ADK) dan Analisis Klaster Hirarki (AKH) digunakan pada penelitian untuk karakterisasi tiga jenis telur berdeda menggunakan program SPSS 16.0. Hasil penelitian menunjukkan bahwa 9 parameter telur yaitu EW, AR, YI, SI, YW, AW, YR, AI, HU dan SR merupakan variabel pembeda pada ketiga jenis telur yang diteliti. Hasil analisis menunjukkan bahwa nilai korelasi kanonik (R) yang diperoleh termasuk tinggi yaitu 0.98 (Fungsi 1) dan 0.79 (Fungsi 2). Jarak Mahalonobis (D^2) tertinggi dan terendah masing-masing sebesar15.28 (RP - CP) dan 27.94 (CP - JQ). Disimpulkan bahwa unggas Phasianidae pada penelitian ini memiliki sifat kualitas telur yang berbeda dan dapat digunakan untuk penciri spesies.

Kata Kunci: ADK, AKH, Kualitas Telur, Mahalonobis, Unggas Phasianidae

ABSTRACT

Putra WPB, Kırıkçı K, Arslan E. 2023. Characterization in the egg quality of three species of Phasianidae birds. JITV 28(4):244-250. DOI: http://dx.doi.org/10.14334/jitv.v28i4.3081.

Rock partridge (*Alectoris graeca*), Common pheasant (*Phasianus colchicus*), and Japanese quail (*Coturnix japonica*) are three poultry species from the Phasianidae family birds that are kept for meat and egg production in the Republic of Turkey. This research was carried out to characterize the egg quality of Rock partridge (RP), Common pheasant (CP), and Japanese quail (JQ). The eggs parameter of egg weight (EW), shape index (SI), yolk index (YI), albumen index (AI), shell weight (SW), haugh unit (HU), yolk weight (YW), albumen weight (AW), yolk ratio (YR), albumen ratio (AR) and shell ratio (SR) were measured for data analysis. A total of 372 eggs, including 100 eggs of partridges, 200 eggs of pheasant, and 72 eggs of quail were used in this study. A Canonical Discriminant Analysis (CDA) and Hierarchical Cluster Analysis (HCA) were used in this study to characterize three different types of eggs using SPSS. 16.0 package. The results showed that nine egg parameters of AR, YI, SI, YW, AW, YR, AI, HU, and SR are identified as discriminant variables for three egg types. The results showed that the canonical correlation (R) value in this study was high, *i.e.*, 0.98 (Function 1) and 0.79 (Function 2). The Mahalonobis distance (D^2) in this study ranged from 15.28 (RP - CP) to 27.94 (CP - JQ). In conclusion, the Phasianidae poultries in this study have different egg quality traits and can be used as the species marker.

Key Words: CDA, HCA, Egg Quality, Mahalonobis, Phasianidae birds

INTRODUCTION

Egg production is an essential trait in poultry because of its high economic value. Generally, two poultry species of chicken (*Gallus domesticus*) and Japanese quail (*Coturnix japonica*) were kept for meat and egg production in most countries. Despite chicken and quail, two other poultry species, Rock partridge (*Alectoris graeca*) and Common pheasant (*Phasianus colchicus*), were kept for meat and egg production in the Republic of Turkey. According to the IUCN Red List, Common pheasants have the conservation status of Least Concern (Braasch et al. 2011). Meanwhile, the Rock partridge has the conservation status of Near Threatened globally and Vulnerable in Italy (Fontaneto et al. 2022). Despite habitat loss, the decrease in the Rock partridge population can be caused by parasitic helminths of *Cheilospirura hamulosa* (Fanelli et al. 2020). Hence, the breeding program for both bird species for economic purposes is vital to protect them in the wild habitat from extinction.

Rock partridge and Common pheasants are classified as Phasianidae family birds (Shen et al. 2014). The average body weight in mixed-sex Rock partridge at 12 weeks of age was 421.57±4.88 g (Putra & Kırıkçı 2021). Meanwhile, the average body weight in Common pheasant at 13 weeks of age was 1053.00±58.97 g in males and 825.00±74.25 g in females (Kokoszynski et al. 2012). Compared to Rock partridge and Common pheasant, the average body weight of Japanese quail was shown to be lowest, *i.e.,* 203.48±25.21 g in males and 228.21±26.44 g in females (Khalil et al. 2006). Therefore, Rock partridge and Common pheasant can be kept for meat production (Hofbauer et al. 2010; Franco & Lorenzo 2013; Wen et al. 2020).

Interestingly, the quality of milk and eggs can be used to characterize the livestock breed (Haygert-Velho 2018; Putra et al. 2021). A Canonical Discriminant Analysis (CDA) and Hierarchical Cluster Analysis (HCA) are two statistical methods that are used to evaluate livestock animals (Ali et al. 2013). Both statistical analyses represent a valid method to simplify the considerable amount of information available regarding the poultry Farms' routines. An example is cluster analysis, which allows grouping and discriminating between groups, where the Euclidean distances, a measure of similarity, define that the means of nearer observations are in the same group, while the most distant are in separate groups (Todde et al. 2016). In addition, both statistical methods have been used to characterize eggs in poultry (Michalczyk & Kurczab 2018; Putra et al. 2021; Ariza et al. 2021). Presently, a study to discriminate many Phasianidae birds based on egg quality traits has yet to be reported. The egg characteristics can be used for avian species identification (Buss & Keiss 2009) and for determining the Euclidean genetic distance (Putra et al. 2021). Unfortunately, there are few studies on egg characterization in Phasianidae birds. Alaşahan & Günlü (2012) have worked to observe the egg quality traits in partridge, pheasant, quail, and chicken without characterization analysis. Hence, this study aimed to characterize the egg quality of three Phasianidae birds (Rock partridge, Common pheasant, and Japanese quail) and determine the Euclidean genetic distance with their egg quality. The results of this study can be used as early information to observe the similarity of egg characteristics in three species of Phasianidae birds.

MATERIALS AND METHODS

Egg sample and research site

Three species of Phasianidae birds, *i.e.,* Rock partridge (*Alectoris graeca*), Common pheasant (*Phasianus colchicus*), and Japanese quail (*Coturnix japonica*), were involved in this study, as shown in Figure 1. A total of 372 eggs consisting of 100 eggs of Rock partridge (RP), 200 eggs of Common pheasant (CP), and 72 eggs of Japanese quail (JQ) were used in this study for the egg quality analysis. All Phasianidae birds were kept at the Selçuk University Faculty of Veterinary Science Hümeyra Özgen Research and Application Center Farm, Republic of Turkey. The egg collection was performed during the laying period of RP (40-44 weeks of age), CP (44-48 weeks of age), and JQ (10-14 weeks of age).

Management of bird

Typical pheasant eggs were used from matings of 90 females and 15 male pheasants of 44 wk of age. The pheasants were mated inside four semi-open shelters (a 4 × 5 m open section and a 4 × 4 m closed section) as one male: 6 female ratio. Artificial lighting was used 16 hours light in a day. Birds were fed with ration ad libitum, including 18% HP and 2,850 MJ/kg of ME, and water was provided from automatic nipples. Rock partridge eggs were used from mating 72 rock partridges (24 male and 48 female) of 44 wk of age. The partridges were mated as one male and two females in open cages (1.2 m x 6.0 m x 1.2 m). Artificial lighting was used 16 hours a day. An ad libitum ration, including 24% CP and 2,850 MJ/kg of ME was provided, and water was supplied via automatic nipples. Quail eggs were used from matings of 120 quails (40 male and 80 female) at 15 weeks of age. Quails were housed in battery cages with 130 cm^2 space per bird as one male and two females and exposed to a 16 h light daily. Commercial quail layer rations containing 17% crude protein (CP) and 2800 kcal/kg metabolizable energy (ME) were provided for quails. The ration and water were available ad libitum.

Egg quality

Eleven egg quality traits of egg weight (EW), shape index (SI), yolk index (YI), albumen index (AI), shell weight (SW), haugh unit (HU), yolk weight (YW), albumen weight (AW), yolk ratio (YR), albumen ratio (AR) and shell ratio (SR) were measured in this study using animal weighing scale (g) and digital caliper (mm). Therefore, SI, YI, AI, HU, YR, AR, and SR measurements were calculated using equations according to Alkan et al. (2015) and Wijedasa et al. (2020) as follows:

$$SI(\%) = \left(\frac{widht\ of\ egg}{length\ of\ egg}\right) x\ 100$$

$$YI(\%) = \left(\frac{height\ of\ yolk}{diameter\ of\ yolk}\right) x\ 100$$

$$AI(\%) = \left(\frac{albumen\ height}{albumen\ diameter}\right)\ x\ 100$$

$$HU = 100\ x\ Log[(AW + 7.57 - 1.70)x\ EW\ x\ 0.37]$$

$$YR(\%) = \left(\frac{YW}{EW}\right)x\ 100$$

$$AR(\%) = \left(\frac{AW}{EW}\right)x\ 100$$

$$SR(\%) = \left(\frac{SW}{EW}\right)x\ 100$$

Data analysis

The descriptive statistics of egg quality traits were computed with General Linear Model (GLM) using a mathematical model as follows:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where Y_{ij} is the response variable of the jth observation in the group, μj is the overall mean, σ_i is the effect of the ith group, and e_{ij} is random for the jth error. Thus, a Canonical Discriminant Analysis (CDA) was performed to calculate Mahalanobis distance (D^2), tolerance (T), Wilk's lambda (λ) values, and the discriminator variables.

After that, the CDA was applied with the backward-stepping automatic elimination method for the variables, with F value entry = 3.84 and F value removal = 2.71 (Depison et al. 2021). The T value (0 to 1) was computed to detect the level of correlation among variables in the discriminant function. Suppose a variable is highly correlated with one or more of the others. In that case, the T value is minimal, and the resulting estimates of the discriminant function coefficients may be unstable (Asamoah-Boaheng & Sam 2016). The Hierarchical Cluster Analysis (HCA) was used for clustering in each breed with the squared Mahalonobis (D^2) distance (Oliveira et al. 2018). The HCA in this study was performed using the morphometric measurements with the nearest-neighbor method and computed using the SPSS 16.0 computer program.

RESULTS AND DISCUSSION

Egg quality

The egg qualities of three Phasianidae poultries in the present study are presented in Table 1. According to Table 1, RP and CP birds have similar AI and SR measurements. Meanwhile, RP and JQbirds have similar SI measurements. Wijedasa et al. (2020) obtained a lower value of HU (81.46±1.43) and EW (9.47±5.64 g) in JQ birds than in the present study. In addition, Alaşahan & Günlü (2012) obtained a lower value of HU in RP (80.34±0.62), CP (80.55±0.56) and JQ (82.88±0.39) birds rather than the present study. Ashraf et al. (2016) obtained lower EW (26.94±5.37 g), YI (32.27±0.86%), and AR (49.30±3.97) values in CP birds rather than in the present study. Kırıkçı et al. (2005) reported that the HU and EW values in different egg colors of CP birds were 83.96±1.41 and 28.10±0.63 g (white); 79.91±2.68 and 26.71±0.62 g (blue); 82.12±0.86 and 31.89±0.34 g (brown); 81.41±0.57 and 31.16±0.23 g

Table 1. Means (±SD) of egg quality in three species of Phasianidae birds

Parameter	Phasianidae birds		
	Rock partridge (N = 100)	Common pheasant (N = 200)	Japanese quail (N = 72)
Egg weight (g)	22.43±1.69^a	31.03±2.26^b	12.49±1.06^c
Shape index	77.04±4.28^a	80.69±3.14^b	77.43±3.61^a
Yolk index	47.88±5.39^a	43.19±4.77^b	38.52±3.51^c
Albumen index	1.56±0.29^a	1.47±0.30^a	2.34±0.63^b
Shell weight (g)	2.34±0.28^a	3.22±0.38^b	1.24±0.25^c
Haugh unit	85.14±4.13^a	82.52±4.48^b	84.39±8.29^a
Yolk weight (g)	8.41±0.97^a	10.20±1.00^b	3.91±0.45^c
Albumen weight (g)	11.68±1.43^a	17.58±1.86^b	7.35±0.71^c
Yolk ratio	37.55±4.07^a	32.96±3.37^b	31.36±2.76^c
Albumen ratio	52.01±4.11^a	56.73±5.14^b	58.85±2.86^c
Shell ratio	10.44±1.03^a	10.40±1.09^a	9.94±2.00^c

^{a,b,c} = superscript of differ significantly (P<0.05); N= Number of bird; SD= Standard deviation

Table 2. Factor selected by stepwise discriminant analysis to discriminate three species of Phasianidae birds

Variable	Tolerance	F to Remove	Min. D^2	Wilk's λ
Egg weight	0.02	13.30	25.38	0.02
Albumen ratio	0.03	7.69	25.67	0.02
Yolk index	0.91	38.10	26.03	0.02
Shape index	0.95	7.69	25.69	0.02
Yolk weight	0.04	5.91	27.00	0.02
Albumen weight	0.02	4.02	26.30	0.02
Yolk ratio	0.04	17.11	26.92	0.02
Albumen index	0.50	59.49	26.98	0.02
Haugh unit	0.48	39.80	26.96	0.02
Shell ratio	0.80	21.19	25.64	0.02

Table 3. Percentage (%) of individual classification per bird species based on discriminant analysis

Birds species	Predicted group membership (N)			Total (N)
	Rock partridge	Common pheasant	Japanese quail	
Rock partridge	100.0 (100)	0.0 (0)	0.0 (0)	100.0 (100)
Common pheasant	2.0 (4)	98.0 (196)	0.0 (0)	100.0 (200)
Japanese quail	0.0 (0)	0.0 (0)	100.0 (72)	100.0 (72)

N= Number of bird

Table 4. The squared Mahalonobis distance (D^2) among three species of Phasianidae birds

Birds species	Rock partridge	Common pheasant	Japanese quail
Rock partridge	1.00	15.28	22.79
Common pheasant		1.00	27.94
Japanese quail			1.00

Alectoris graeca *Phasianus cochiucus* *Coturnix japonica*

Figure 1. Phenotypic characteristics of Rock partridge (A), common pheasant (B), and Japanese quail (C)

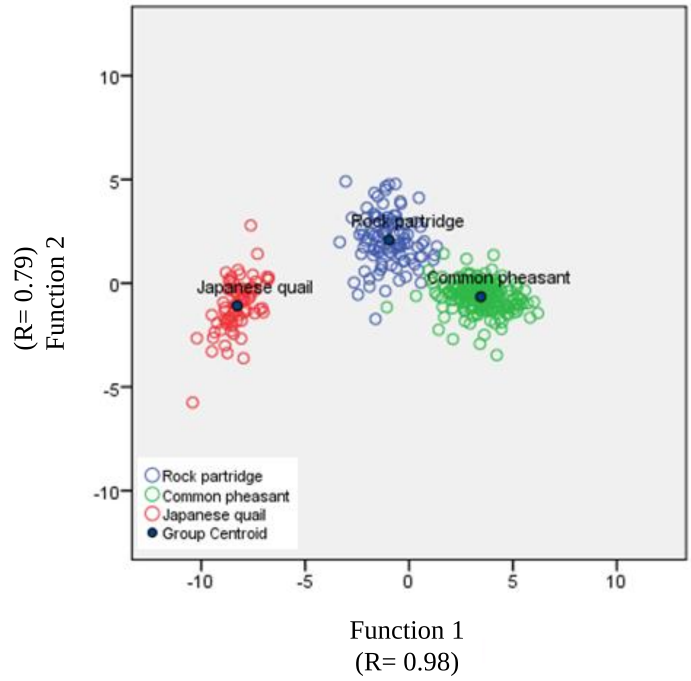

Figure 2. The canonical discriminant plot in the egg quality traits to discriminate three species of Phasianidae birds

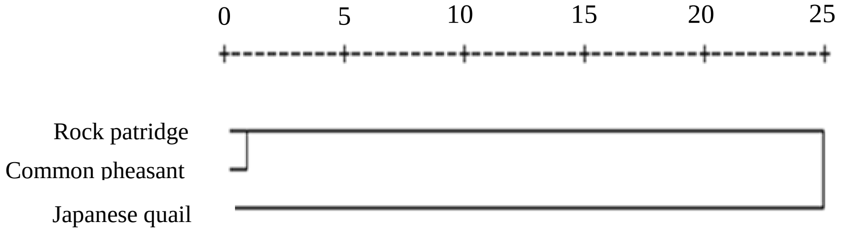

Figure 3. Dendogram distance among three species of Phasianidae birds based on egg quality traits

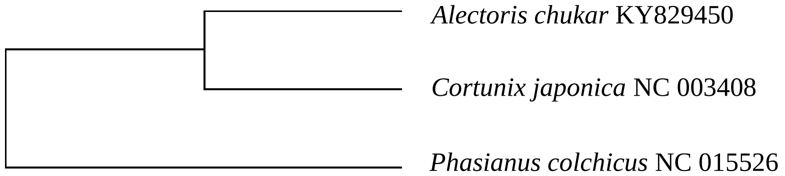

Figure 4. Dendogram among three species of Phasianidae birds based on mitogenome DNA from GenBank database (https://www.ncbi.nlm.nih.gov)

(olive green), respectively. Hence, the HU value of CP birds was higher than in a previous study reported by Kırıkçı et al. (2005). Despite this, the EW of CP birds in the present study was close to the eggs of CP with brown and olive green colors. The egg quality in birds can be affected by nutrient, environmental, and genetic factors.

Canonical discriminant analysis

In this study, the egg quality traits were described as the discriminant variable (Table 2). In addition, ten discriminant variables were able to discriminate three Phasianidae poultries with the canonical correlation (R) of 0.98 (function 1) and 0.79 (function 2), as shown in Figure 2. Hence, ten discriminant variables were able to classify RP (100%), CP (98%), and JQ (100%) birds into their original Phasianidae poultry group (Table 3). The D^2 value among Phasianidae poultries was 15.28 (RP and CP birds), 22.79 (RP and JQ birds), and 27.94 (CP and JQ birds), as shown in Table 4. Hence, RP and CP birds were grouped into a similar cluster based on HCA with egg quality, as shown in Figure 3. Meanwhile, the JQ bird was grouped into a separate cluster. Putra et al. (2021) reported that three egg qualities of yolk diameter, volume, and surface area were the discriminant variables to differentiate White Leghorn, Lohmann Brown, and Ataks chicken breeds. Ariza et al. (2021) reported that partridge has close relatedness with chicken based on egg quality traits. Hence, partridges are a potential bird for egg production in Turkey. In this study, a phylogenetic analysis with the mitogenome DNA sequence of *Alectoris chukar* (GenBank: KY829450), *Phasianus colchicus* (GenBank: NC015526), and *Coturnix japonica* (GenBank: NC003408) was performed to evaluate the findings in the present study. According to the mitogenome DNA sequence, partridge and quail have a close genetic relationship and are grouped into a similar cluster, as shown in Figure 4. Meanwhile, the pheasant was grouped into a separate cluster. Kimball et al. (1999) obtained a similar finding that *Coturnix coturnix* and *Alectoris sp.* have a close genetic relationship based on the mitochondrial Cytochrome-b (Cyt-b) gene.

CONCLUSION

The egg quality traits can be used to differentiate three species of Phasianidae birds, *i.e.*, Rock partridge (*Alectoris chukar*), Common pheasant (*Phasianus colchicus*) and Japanese quail (*Coturnix japonica*). The egg quality traits of Rock Partridge and Common Pheasant were closed. However, a study with the mitogenome DNA revealed that Japanese quail and partridge have a similar maternal lineage. Hence, genetically, Japanese Quails are the closest kinship to Rock partridge. Nonetheless, the egg quality of Rock partridges was close to that of Common pheasant. In the future, Rock partridge and Common pheasant can be managed in poultry farming for meat and egg production.

REFERENCES

Ali AA, Ullah MA, Ejaz MA. 2013. Evaluation of livestock data using multivariate analysis techniques. Pak J Life Soc Sci. 11:208-211.

Alkan S, Galiç A, Karsli T, Karabağ K. 2015. Effects of egg weight on egg quality traits in partridge (*Alectoris chukar*). J Appl Anim Res. 43:450-456. DOI:10.1080/09712119.2014.980419.

Alaşahan S, Günlü A. 2012. Determination of egg quality characteristics of different poultry species with digital image analysis. Kafkas Univ Vet Fak Derg. 18:976-986.

Ariza AG, Arbulu AA, Gonzalez FJN, Bermejo JVD, Vallejo MEC. 2021. Discriminant canonical analysis as a validation tool for multivariety native breed egg commercial quality classification. Foods. 10:632. DOI:10.3390/foods10030632.

Asamoah-Boaheng M, Sam EK. 2016. Morphological characterization of breeds of sheep: a discriminant analysis approach. Springer Plus 5:1-12. DOI:10.1186/s40064-016-1669-8.

Ashraf S, Javid A, Ashraf M, Akram M, Malik S, Irfan, Altaf M. 2016. Influence of egg weight on egg quality parameters and growth traits in Ring Necked pheasant (*Phasianus colchicus*) in captivity. J Anim Plant Sci. 26:331-338.

Braasch T, Pes T, Michel S, Jacken H. 2011. The subspecies of the common pheasant *Phasianus colchicus* in the wild and captivity. Int J Galliformes Conserv. 2:6-13.

Buss A, Keiss O. 2009. Method for identification of avian species by eggshell microstructure. Act Univ Latviensis. 753:89-98.

Depison, Putra WPB, Gushairiyanto, Alwi Y, Suryani H. 2021. Morphometrics characterization of Thin-tail sheep in lowland and highland areas. Trop Anim Sci J. 44:386-398. DOI:10.5398/tasj.2021.44.4.386.

Fanelli A, Tizzani P, Ferroglio E, Belleau E. 2020. *Cheilospirura hamulosa* in the Rock partridge (*Alectoris graeca saxatilis*): epidemiological patterns and prediction of parasite distribution in France. Diversity. 12:484. DOI:10.3390/d12120484.

Fontaneto D, Viola P, Pizzirani C, Chiesa S, Rossetti A, Amici A, Lucentini L. 2022. Mismatches between morphology and DNA in Italian Partridges may not be explained only by recent artificial release of farm-reared birds. Anim. 12:541. DOI:10.3390/ani12050541.

Franco D, Lorenzo JM. 2013. Meat quality and nutritional composition of Pheasants (*Phasianus colchicus*) reared in an extensive system. Br Poult Sci. 54:594-602. DOI:10.1080/00071668.2013.828195.

Haygert-Velho IMP, Conceicao MD, Cosmam LC, Alessio DRM, Busanello M, Sippert MR, Damiani C, Almeida AP, Velho JP. 2018. Multivariate analysis relating milk production, milk composition, and seasons of the year. An Acad Bras Ciênc. 90:3839-3852. DOI:10.1590/0001-3765201820180345.

Hofbauer P, Smulders FJM, Vodnansky M, Paulsen P, El-Ghareeb WR. 2010. A note on meat quality traits of pheasants (*Phasianus colchicus*). Eur J Wildl Res. 56:809-813.

Huang Z, Ke D. 2014. Structure and evolution of the Phasianidae mitochondrial DNA control region. Mitochondrial DNA. 27:350-354. DOI:10.3109/19401736.2014.895987.

Khalil HA, Hassanein AM, Mady ME, Gerken M. 2006. Effect of housing conditions on performance of Japanese quail (*Coturnix coturnix japonica*) under cold stress in winter. Egyptian J Anim Prod. 43:71-82. DOI:10.21608/ejap.2006.93094.

Kimball RT, Braun EL, Zwartjes PW, Crowe TM, Ligon JD. 1999. A molecular phylogeny of the pheasants and partridges suggests that these lineages are not monophyletic. Mol Phylogenet Evol. 11: 38-54. DOI:10.1006/mpev.1998.0562.

Kırıkçı K, Günlü A, Garip M. 2005. Some quality characteristics of Pheasant (*Phasianus colchicus*) eggs with different shell colors. Turk J Vet Anim Sci. 29: 315-318.

Kokoszynski D, Bernacki Z, Duszynski L. 2012. Body conformation, carcass composition and physicochemical and sensory properties of meat from pheasants of different origin. Czech J Anim Sci. 57:115-124. DOI:10.17221/5564-CJAS.

Michalczyk E, Kurczab R. 2018. Assessment of poultry eggs freshness using FTIR spectroscopy combined with HCA and PCA methods. Sci Tech Inov. 2:7-12.

Oliveira EJ, Savegnano RP, de Freitas LA, Freitas AP, Maia SR, Simili FF, Faro LE, da Costa RLD, Junior MLS, C. de Paz CP. 2018. Estimates of genetic parameters and cluster analysis for worm resistance and resilience in Santa Ines meat sheep. Pesq Agropec Braz. 53:1338-1345. DOI:10.1590/S0100-204X2018001200006.

Putra WPB, Kırıkçı K. 2021. The growth curve of body weight in mixed-sex Rock partridge (*Alectoris graeca*) kept in captivity. J Poult Res. 18:19-23. DOI:10.34233/jpr. 901581.

Putra WPB, Ridho M, Nugraha I. 2021. Breeds characterization in three Turkish laying chicken breeds based on egg characteristics. Indonesi J Agric Res. 4:130-141. DOI:10.32734/injar.v4i2.6210.

Shen YY, Dai K, Cao X, Murphy RW, Shen XJ, Zhang YP. 2014. The updated phylogenies Phasianidae based on combined data of nuclear and mitochondrial DNA. PLOS ONE. 9:e95786. DOI:10.1371/journal.pone.0095786.

Todde G, Murgia L, Caria M, Pazzona A. 2016. A multivariate statistical analysis approach to characterize mechanization, structural and energy profile in Italian dairy farms. Energy Reports. 2:129-134. DOI:10.1016/j.egyr.2016.05.006.

Wen Y, Liu H, Liu K, Cao H, Mao H, Dong X, Yin Z. 2020. Analysis of the physical meat quality in partridge (*Alectoris chukar*) and its relationship with intramuscular fat. Poult Sci. 99:1225-1231. DOI:10.1016/j.psj.2019.09.009.

Wijedasa WMRM, Wickramasinghe YHST, Vidanarachchi JK, Himali SMC. 2020. Comparison of egg quality characteristics of different poultry species. J. Agric. Sci.12:331-342. DOI:10.5539/jas.v12n11p331.

Influence of Moringa Leaf Meal As a Feed Additive on Antibody and Bursa Histomorphometry of Hybrid Ducklings

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ABSTRAK

Febriana W, Suprihatin T, Kasiyati. 2023. Pengaruh tepung daun kelor sebagai pakan aditif terhadap antibodi dan histomorfometri bursa anak itik hibrida. JITV 28(4):251-258. DOI: http://dx.doi.org/jitv.v28i4.3153.

Tepung daun kelor mengandung berbagai fitonutrien yang bermanfaat bagi kesehatan hewan dan manusia. Penelitian ini dirancang untuk mengevaluasi tepung daun kelor sebagai pakan aditif terhadap respons antibodi dan histomorfometri bursa Fabricius itik hibrida yang diberi vaksin ND. Penelitian menggunakan rancangan acak lengkap, dengan total tiga puluh dua itik jantan hibrida berumur tiga hari yang dibagi menjadi empat kelompok perlakuan, dan masing-masing perlakuan terdiri atas delapan ulangan. Kelompok pertama diberi pakan standar komersial tanpa penambahan tepung daun kelor (0%), sedangkan kelompok kedua sampai keempat diberi pakan komersial standar dengan penambahan tepung daun kelor 2,5%, 5%, dan 7,5%. Semua kelompok mendapatkan vaksin ND1 dan ND2, masing-masing pada hari ke-tujuh dan ke-empat belas. Hasil penelitian menunjukkan bahwa pakan aditif tepung daun kelor berpengaruh tidak nyata pada titer antibodi, jumlah leukosit, limfosit (L), heterofil (H), rasio H:L, berat bursa, korteks, dan ketebalan medula bursa. Konsentrasi MDA serum, diameter plika, dan folikel bursa dipengaruhi ($P<0,05$) oleh aditif tepung daun kelor. Kesimpulan, pakan aditif tepung daun kelor dapat merangsang proliferasi folikel bursa dan tidak memiliki dampak buruk pada respons imun itik hibrida pascavaksin ND.

Kata Kunci: Tepung Daun Kelor, Itik Jantan, Titer Antibodi, Bursa Fabricius

ABSTRACT

Febriana W, Suprihatin T, Kasiyati. 2023. Influence of moringa leaf meal as feed additive on antibody and bursa histomorphometry of hybrid ducklings. JITV 28(4):251-258. DOI: http://dx.doi.org/jitv.v28i4.3153.

Moringa leaf meal contains various phytonutrients that benefit animal and human health. This research is designed to evaluate Moringa leaf meal as a feed additive on antibody response and histomorphometry of the bursa Fabricius of hybrid duck administrated by ND vaccine. The experimental design used is a Completely Randomized Design (CRD) using thirty-two male hybrid ducks of three days old. They were divided into four treatment groups and eight replicates of each. The first group was standard commercial fed without adding of Moringa leaf meal (0%), while the second until the fourth group was a standard commercial fed with 2.5%, 5%, and 7.5% adding of Moringa leaf meal. All groups were vaccinated by ND1 and ND2, respectively, at the age of seventh and fourteenth days. The results showed that Moringa leaf meal as a feed additive had no significant effect on antibody titer, leukocyte count, lymphocytes (L), heterophils (H), H:L ratio, bursa weight, cortex, and medulla thickness of bursa. The concentration of serum MDA, the diameter of plica, and follicles bursa were significantly influenced ($P<0.05$) by adding Moringa leaf meal. In conclusion, the Moringa leaf meal additive could stimulate the proliferation of bursa follicles and have no adverse effect on the immune response of hybrid ducks post-ND vaccine.

Key Words: Moringa Leaf Meal, Male Ducklings, Antibody Titer, Bursa Fabricius

INTRODUCTION

Hybrid ducks result from crossing two superior breeds that inherit a combination of dominant traits from both parents. Furthermore, hybrid ducks were developed to satisfy the need for breed ducks to produce high eggs or fast growth (Agriflo 2012). According to some research, ducks have a relatively good immune system, but it does not mean they cannot exposed to bacteria, viruses, and fungi, which cause infection or disease. Health management is crucial to avoid spreading disease in the environment (Hofmann et al. 2020). The immune system comprises several components, including leukocytes, detoxification organs, antibody-forming (liver and spleen), and immunomodulatory organs (thymus and Fabricius bursa). The Fabricius bursa is a place to produce B lymphocytes that benefit the humoral response, generating antibodies and differentiating immunoglobulin genes (Sulistiyanto et al. 2019).

Newcastle disease (ND) is well-known as Tetelo in Indonesia. It is an endemic disease in Indonesia, attacking respiratory and systemic, acute, and easily contagious. It is caused by the ND virus, which belongs to the Avian Paramyxovirus genus (Tabbu 2000; Widyaningsih et al. 2020). The pathogenicity of the ND virus varies depending on the host, and birds are susceptible to the ND virus (Tabbu 2000). However, Rozi et al. (2020) stated that cases of ND in chicken or duck farms have a morbidity and mortality rate close to 100% in unvaccinated poultry. Furthermore, Miller et al. (2010) showed that infected ducks do not show clinical or only mild clinical symptoms, even though the same ND virus strain was fatal to chickens. Meanwhile, there is currently no drug to cure it. Prevention is essential through vaccination and improved management of poultry rearing.

Vaccination is an effective way to protect and prevent poultry at various age levels from ND disease (Pratama et al. 2016; Yuliani & Sakan 2018). Unfortunately, the fact shows that the disease still happens in routinely vaccinated poultries (Widyaningsih et al., 2020). It raises the suspicion of the difference between the ND virus circulating in their life and the ND virus in the vaccine (Wibowo et al. 2013). An analysis showed genetic differences between the two ND viruses, giving rise to differences in antigenic responses (Xiao et al. 2012). In addition, low antibody titers cannot protect birds from the virus. Therefore, efforts have been made to increase antibody titers in poultry, which are expected to escalate their immune system. Natural ingredients such as phytonutrient components can help to modulate the immune system by repairing imbalances in the body's defense system, increasing antibody production, cellular immune responses, and the phagocytic ability of macrophages (Amir et al. 2014).

The leaves of the *Moringa oleifera* contain complete nutrients and various phytochemical components for health benefits. It has the main polyphenolic constituents, namely flavonoids in the form of kaempferol, myricetin, quercetin, and phenolic acids such as gallic acid. The flavonoids have a role in hypoglycemic, antibacterial, antifungal, antioxidant, and anti-inflammatory (Fidrianny et al. 2021). Phytochemicals in Moringa leaf, especially polysaccharides, also have potential as immunostimulants and immunomodulators (Li et al. 2020). Dillasamola et al. (2018) also stated that the leaf extract could increase the number of leukocytes. Meanwhile, Soha et al. (2020) found that supplementing the Moringa in broiler food could reduce degenerative changes by regulating mRNA presentation. It could modulate immune responses.

The use of Moringa leaf meal as a feed additive is still being explored today. Feed additives are bioactive compounds intentionally added to the ration in small amounts (Righi et al. 2021). There are many studies about Moringa leaf meal as a feed additive to increase animal production. However, the research on it as the immune response of ND-vaccinated is still much limited. This research is designed to evaluate the effect of Moringa leaf meal as a feed additive on antibody response and histomorphometry of the bursa Fabricius of male hybrid ducklings.

MATERIALS AND METHODS

Experimental animal, raising management, and research design

All experimental animals used in this study were raised following the protocol determined by the Department of Biology, and it has been approved by the Health Research Ethics Commission, Faculty of Medicine Diponegoro University, No. 04/EC/H/FK/-UNDIP/I/2022. The male hybrid ducklings used in this study were a crossbreed of Peking and Magelang ducks. There were 32 ducks, three days old, obtained from hybrid duck breeders in Karangjati, Semarang Central Java, Indonesia. Hybrid ducks had been acclimatized for one week in a box cage and moved into a battery cage sizing of 45×35×65 cm^3. Food and drink were accessible *ad libitum.* The feeding time was two times a day in the morning (07:00 am) and afternoon (03:00 pm). Temperature and humidity are monitored every day.

This study used a completely randomized design with four different concentrations of Moringa leaf meal, *i.e.*, 0% or standard commercial feed without Moringa leaf meal and standard commercial feed with the addition of 2.5%, 5%, and 7.5% Moringa leaf meal. Each treatment consisted of eight replications. The addition of dietary Moringa leaf meal was administered for six weeks.

Feed preparation

The food was a common feed used by breeders, standard commercial feed BR1 for ducks aged 1-3 weeks and standard commercial feed BR2 for those aged 4-8 weeks. The feedstock was made once a week. Meanwhile, the Moringa leaf meal was collected from the local market produced by Flozindo Purbalingga, Central Java Indonesia. BR1 or BR2 was mixed with the Moringa meal homogeneously. The composition of the feed ingredients is presented in Table 1.

Analysis of antioxidant, vitamin D, and vitamin E of Moringa leaf meal

Analysis of antioxidants used the DPPH method. The absorbance value was then measured using a UV-

Table 1. Feed composition and nutrient content of addition of Moringa leaf meal treatments of male hybrid ducklings

Feed Ingredient	Moringa leaf meal concentration in feed (%)							
	Starter Period (1-3 weeks)				Finisher Period (4-8 weeks)			
	0	2.5	5	7.5	0	2.5	5	7.5
Standard commercial feed, (%)[1)]	100	97.5	95	92.5	100	97.5	95	92.5
Moringa leaf meal, (%)	0	2.5	5	7.5	0	2.5	5	7.5
Total, (%)	100	100	100	100	100	100	100	100
Nutrient content								
Metabolic energy (kcal/kg)	2970.20	2947.14	2924.08	2901.01	3040	3016.94	3000.88	2949.81
Crude protein, (%)	21.94	22.29	22.64	22.99	18.63	18.98	19.31	19.74
Crude fat, (%)	3.55	3.49	3.42	3.35	7.26	7.19	7.11	7.07
Crude fiber, (%)	3.56	3.52	3.47	3.43	4.26	4.22	4.12	4.00
Calcium, (%)	0.78	0.79	0.79	0.80	0.87	0.87	0.88	0.88
Phosphor, (%)	0.51	0.50	0.48	0.47	0.50	0.49	0.47	0.47

The standard commercial feed in this study contains yellow corn, rice bran, meat bone meal (MBM), crude palm oil (CPO), pollard, corn gluten meal (CGM), vitamins, and minerals.

Vis spectrophotometer at a wavelength of 517 nm to determine the IC50 (Susanty et al. 2019). Furthermore, analysis of the content of vitamins E and D used the HPLC method with a normal phase. The detector was a UV detector (Widada 2013).

ND (Newcastle Disease) vaccine and antibody titer measurement

This study used the Medivac ND La Sota vaccine for ducks aged 7 and 14 days. Both types of vaccines were administered to ducks through the eye (ocular route) according to the protocol listed by the vaccine manufacturer. Moreover, blood samples for antibody titer measurements were taken at week 0 (pre-vaccine), 1, 2, 3, and 4 post-vaccine.

Blood samples were taken from the cranial tibial vein using a 1 ml syringe. Then, blood was transferred into a non-EDTA tube to produce serum. The collected serum was stored in an Eppendorf tube at -20ºC until the antibody titer was measured using the Haemagglutination Inhibition (HI) test (Novitasari et al. 2018).

Measures of leukocytes, blood MDA, and histomorphometry of Fabricius bursa

Blood sampling for calculating the leukocyte number and its differential was carried out at week 8. It was taken through the brachial vein using a 3 ml syringe. Half of the blood was collected in the EDTA tube, and the rest was in non-EDTA. All blood samples in the EDTA tube were subjected to calculating the leukocyte and its differential through a hematology analyzer in the laboratory. Meanwhile, blood in a non-EDTA tube was prepared for counting the MDA (malondialdehyde) level. The TBA method was then used to measure the level of MDA (Maggi-Capeyron et al. 2002).

The organ for histomorphometry incision was the Fabricius bursa. It was obtained after the ducks were sacrificed by the halal method, by cutting the jugular, oesophageal, and tracheal veins and arteries. The bursa of Fabricius was isolated and then weighed to calculate the ratio of bursa weight to body weight (bursa weight/body weight x 1000). Furthermore, the bursa was fixed into 10% BNF (neutral formalin buffer), and histological preparations were made by paraffin method and hematoxylin-eosin (HE) staining. The histological preparations were then observed using a photomicrograph to obtain a histomorphology of the Fabricius bursa. Furthermore, measurements of plica diameter, number of follicles per plica, follicle diameter, medullary thickness, and cortex thickness of bursa were measured.

Data analysis

The data was analyzed using one-way ANOVA at a significance level of 5%. A further test was using the

Least Significant Difference (LSD). All data analyzed were calculated by SPSS 24 software.

RESULTS AND DISCUSSION

Antibody response

The results of antibody titer analysis in hybrid ducks after the administration of ND1 and ND2 vaccines, followed by the addition of Moringa leaf meal in feed, did not show a significant effect in the first week until the fourth-week post-vaccine (Table 2). At week 0 (pre-vaccine), antibody titers also showed no significant difference. The antibody titer measurement using the HI test was positive if the antibody titer was 2^4 (OIE 2021).

Examining the pre-vaccine antibody titers (week 0 or ducks aged six days) aimed to measure the maternal antibodies. The results (Table 2) showed that hybrid ducks' circulation systems still had maternal antibodies. They derived from transferred parental blood deposited into the yolk to protect the ducklings on the first day post-hatching. Not only that, but they also affect the health of the ducklings because of the gamma globulins inherited from parents through passive immune transfer to protect ducklings from viruses and microorganisms. The average antibody titer of hybrid ducks in this study is equal to the results of the Bali duck antibody titer examination conducted by Novitasari et al. (2018). Windi et al. (2016) stated that maternal antibodies will decrease along with the duckling age.

The antibody titers in the first-week post-ND1 vaccine of all treatment groups showed almost the same mean and had no significant changes. According to criteria from OIE (2021), the mean antibody titer of all measured samples was seronegative because it was less than 2^4. Pratama et al. (2016) stated that passively obtained maternal antibodies could inhibit the formation of immunoglobulins, thus affecting the vaccine. If they are still in the circulating system, the first vaccination should not be too early because these antibodies will neutralize the vaccine antigens.

Measurements at 2 to 4 weeks after booster vaccine (ND2) also showed no significant increase in antibody titer. Generally, antibodies would increase after three weeks or more post-vaccination using an active vaccine. Vaccination in hybrid ducks aged 7 and 14 days in this study was not effective because there was still the influence of maternal antibodies. Pratama et al. (2016) stated maternal antibodies would run out in 10-20 days post-hatching. They effectively prevented the success of vaccination. Kurnianto et al. (2016) and Novitasari et al. (2018) said that the active vaccine, which contains a live attenuated virus, could change the protective antibodies of chickens within three weeks of a booster vaccination. Furthermore, Bhakty et al. (2018) suggested that differences in the immune response level of hybrid ducks could come from several factors, including differences in individual responses and the antigenic ability of the vaccine antigens.

On the other hand, the low total antibody titers post-vaccine ND1 and ND2 in this research might be related to the phytonutrient content in the Moringa leaf meal. The phytonutrients used as feed additives can also affect the vaccine's success. Protein, vitamin D, vitamin E, and carotenoids are phytonutrients in Moringa leaf meal that tend to be immunomodulators. Administration of both vaccines and exposure to pathogens will enhance the acute phase response. The need for nutrients in the liver increases due to the acute-phase protein synthesis. Then, these proteins are distributed throughout the body to protect the epithelial surface and provide epithelial stability. Vitamin D, vitamin E, and carotenoids also play roles as antioxidants. These antioxidants could protect cells that synthesize acute-phase proteins. Moringa leaf meal in this study contained 15.161 mg/L of vitamin E and 7.160 mg/L of vitamin D. In addition, its antioxidant activity has an IC_{50} value of 1.289 mg/mL or equivalent to 1.289 ppm. The value of antioxidant activity in Moringa leaf meal was shallow but still has the potential as an antioxidant. Phongpaichit et al. (2007)

Table 2. Average antibody titers of hybrid ducklings pre-vaccine (week 0) and post-vaccine ND1 and ND2 (week 1 to 4) followed by feeding the addition of Moringa leaf meal

Sampling week	Moringa leaf meal concentration (%)			
	0	2.5	5	7.5
0	$2^{0.75}$±0.18	$2^{0.88}$±0.12	$2^{1.00}$±0.10	$2^{0.88}$±0.20
1*	$2^{1.31}$±0.24	$2^{1.29}$±0.13	$2^{1.23}$±0.26	$2^{1.25}$±0.17
2*	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

*Time of vaccines: There was no significant difference between all treatments. The data are shown as average ±SD stated that the smaller the IC50 value, the higher the antioxidant activity was. Furthermore, Saputra et al. (2020) showed that all antioxidant phytochemical compounds in Moringa leaf meal had functions to neutralize free radicals, protect against oxidative damage, significantly inhibit cell aging, and protect cells from oxidative stress. Okoroafor et al. (2021) proved that vitamin E could enhance immune function against viral pathogens and modulate the function of T cells and cytokines.

Amount of leukocyte, blood MDA, and histomorphometry of bursa

The results of leukocytes, heterophile (H) and lymphocyte (L) differentials, H:L ratio, and blood MDA in hybrid ducklings fed with Moringa leaf meal are presented in Table 3. The number of leukocytes, heterophils, lymphocytes, and H:L ratio (Table 3) showed no significant difference. The administration of vaccines and adding Moringa leaf meal fed to hybrid ducklings did not cause changes in the number of leukocytes and the differential number of leukocytes. The formation and performance of leukocytes were not affected by phytonutrient components in the Moringa leaf meal. However, the leukocyte count in this study was higher than the normal range. According to Saputro et al. (2016), the high number cannot assume the poultry is sick. It could be because the unbalanced nutrient content affected antibodies and leukocytes. Purnomo (2015) stated that the normal leukocyte in poultry ranges from 6–40 × 10^3/ml. Differences in the number of leukocytes, heterophils, lymphocytes, and the ratio of H:L between this study and previous studies may be related to the type of poultry, age, nutrition, treatment, and health status.

The leukocyte differentials (heterophile and lymphocyte) showed the same values for all groups (Table 3), as well as the H:L ratio. It means the hybrid ducklings in this study were at the same level of health, which is in line with Kasiyati et al. (2021), who explained that adding Moringa leaf meal did not cause many lymphocytes, heterophils, and eosinophils of Pengging ducks. The similarity in the ducks' health was because of the bioactive content in the Moringa leaf meal, which had a good impact on the ducks' health. Rahmayanti and Isroli (2019) stated that the antibacterial content of Moringa leaves could reduce parasites. Heterophiles are the front line against bacterial infections, and the number of heterophils will not be affected if there is no infection.

Blood MDA (*Malondyaldehide*) levels (Table 3) showed significantly different (P<0.05). MDA levels decreased along with the increase in the concentration of Moringa leaf meal. The Moringa leaf meal in this study contains natural antioxidants such as beta-carotene, vitamin D, and vitamin E. Natural antioxidants work rapidly to protect cell membranes from oxidative damage or prevent lipid peroxidation due to free radicals or reactive oxygen species (ROS). It could come from the normal processes of metabolism energy or exposure to pathogens. In general, the elimination of pathogens by macrophages or other components of the immune system can release toxic metabolites as weapons to kill pathogens or microorganisms. Side effects of the microbicidal activity of the immune system are host cell killing, cell and tissue injury, oxidative degradation, and production of ROS or free radicals. Overproduction of ROS can result in damage to various macromolecules, so it stimulates pathological conditions and disease. Antioxidants can protect cells from the harmful effects of cytotoxic free radicals or ROS. Free radical concentration could be approached by measuring the MDA. Sun et al. (2022) stated that adding vitamin E supplementation in poultry could significantly increase the activity of antioxidant enzymes and reduce the MDA content in the serum. Similarly, Sahin et al. (2003) reported that vitamin E supplementation also decreased serum and liver MDA concentrations of Japanese quail.

There was no change in the morphology of the bursa of Fabricius in hybrid ducklings that had been vaccinated and fed with Moringa leaf meal additive. Bursal weight, bursa: body weight ratio, follicle diameter, medullary thickness, and bursa cortex thickness were not significantly different (Table 3) in all treatment groups. However, the diameter of the plica and the number of follicles per plica in the bursa were significantly different (P<0.05; Table 3). Raji et al. (2017) stated that the size of the bursa was affected by stress conditions (due to temperature and disease), vaccines, mycotoxins, and rearing systems. The size of the bursa, which was relatively the same in all treatment groups, indicated that ND1 and ND2 vaccines and the addition of Moringa leaf meal in the diet did not increase the number of antibodies (Table 2), did not interfere with the development of the bursa, and did not cause hyperplasia or atrophy of the Fabricius bursa. The bursa weight and ratio of the bursa to body weight are indicators to assess the immune system. Apriliyani et al. (2013) suggested that bursa that often form antibodies can cause follicle depletion and shrinkage. It will affect the relative weight of the bursa.

The diameter of the plica and the number of follicles per plica in the bursa increased (P<0.05) in the 5% Moringa leaf meal group. The diameter of plica in a 5% group was the largest, with the highest number of follicles compared to the control group (0% Moringa leaf meal). The diameter and number of follicles increase in line with the age of the birds. Figure 1 showed that the hybrid ducklings group feeding Moringa leaf meal of 2.5% and 5% had many follicles

Table 3. Average leukogram, blood MDA, morphometry of the Fabricius bursa of hybrid ducklings post-vaccine ND1 and ND2 and followed by feeding the addition of Moringa leaf meal

Parameter	Moringa leaf meal concentration (%)			
	0	2.5	5	7.5
Leukocytes, ($\times 10^3$/ml)	157.4±22.29	161.7±25.99	149.3±31.39	135.2±22.37
Heterophile, ($\times 10^3$/ml)	3.01±0.24	2.49±1.01	2.83±0.78	2.91±0.35
Lymphocytes, ($\times 10^3$/ml)	148.8±21.45	153.8±25.81	141.3±28.28	127.1±21.15
H:L ratio	0.06±0.01	0.04±0.03	0.05±0.03	0.06±0.01
Blood MDA (nmol/ml)	2.35^{a}±0.18	1.99^{b}±0.09	1.75^{c}±0.09	1.39^{d}±0.11
Bursal Weight, (%)	0.76±0.04	0.75±0.02	0.78±0.04	0.81±0.05
Bursa: Body weight ratio	1.11±0.27	1.07±0.12	1.27±0.21	1.43±0.29
Diameter of plica, (mm)	2.13±0.06^{b}	2.28±0.12^{b}	2.75±0.23^{a}	2.48±0.36^{ab}
Number of follicles per plica	11.42±1.75^{b}	20.49±8.19^{ab}	31.87±10.84^{a}	19.83±0.56^{ab}
Follicle diameter (mm)	0.72±0.09	0.66±0.05	0.73±0.09	0.74±0.13
Medullary thickness (mm)	0.20±0.05	0.18±0.02	0.20±0.03	0.22±0.05
Cortex thickness (mm)	0.16±0.01	0.15±0.01	0.20±0.04	0.19±0.04

$^{a\text{-}d}$= Numbers followed by different superscripts in the same row indicate significantly different ($P<0.05$); The data are shown as average ± SD; MDA=Malondyaldehide; H=Heterophile; L= Leukocytes

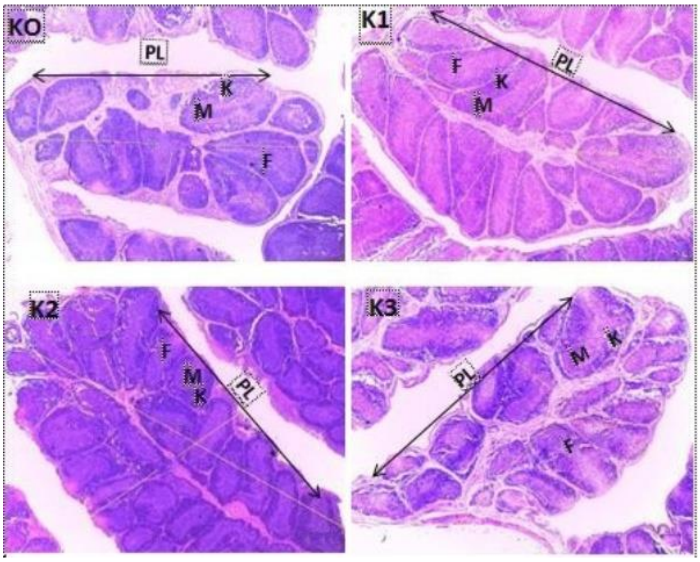

Figure 1. Bursa Fabricius hybrid ducklings with ND1-2 vaccine and Moringa leaf meal. KO (control), K1 (2.5% added Moringa leaf meal), K2 (5% Moringa leaf meal added), and K3 (7.5% Moringa leaf meal added). PL: Plica, F: Follicle, M: Medulla, K: Cortex. HE staining, 40× magnification

with a tight arrangement, regular interfollicular septa, no space between follicles, and no signs of system damage. The increasing number of bursa follicles indicated that the research ducks could recognize the virus administration in the form of the ND vaccine. It successively stimulated bursa follicles to proliferate to compensate for the production of lymphocytes. The escalating number of follicles per plica contributes to raising the size of the plica's diameter.

Vaccination in poultry can affect the histomorphometry of the bursa. In general, there are three stages of vaccine exposure to the pathophysiology and histology of the bursa, namely 1) the acute inflammatory stage, characterized by the rising size of the bursa, occurring about four days post-virus infection (vaccine exposure), 2) the sub-acute stage, characterized by spontaneous regression of the bursa. Quickly, the bursa can return to its original size, and 3) the final stage, characterized by atrophy of the bursa, an increase in the stock exchange index, and the ratio of stock exchange weight to body weight (Raji et al. 2017). Phytonutrient components of Moringa leaf meal were also indicated to be active in the proliferation of bursa follicles and maintaining follicle size. Phytonutrients, such as polyphenols, flavonoids, flavonols, and non-phenolic components (vitamins, proteins, oligosaccharides), are antioxidants to boost the immune system and poultry health.

CONCLUSION

Adding 5% Moringa leaf meal in feed could optimally stimulate the proliferation of bursa follicles and improve the immune status of hybrid ducklings. This study can be applied, but it is necessary to evaluate the administration of the ND vaccine to increase the efficacy of the ND vaccine in ducks.

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REFERENCES

Agriflo. 2012. Itik Potensi Bisnis dan Kisah Sukses Praktisi. Jakarta (Indones): Penebar Swadaya.

Ahmad S, Khalique A, Pasha TN, Mehmoof S, Sohail Ahmad S, Khan AM, Hussain K. 2018. Influence of *Moringa oleifera* leaf meal used as phytogenic feed additive on the serum metabolites and egg bioactive compounds in commercial layers. Brazilian J Poultry Sci. 20:325-332. DOI:10.1590/1806-9061-2017-0606.

Amir S, Ahmad T, Umer M, Rehman A, Hussain Z. 2014. Prevention and control of Newcastle disease. IJAIR. 3:454-460.

Apriliyani F, Suthama N, Wahyuni HI. 2013. Rasio Heterofil Limfosit dan Bobot Relatif Bursa Fabricius Akibat Kombinasi Lama Pencahayaan dan Pemberian Porsi Ransum Berbeda Pada Ayam Broiler. Anim Agric J. 2:393-399.

Bhakty ZW, Kencana GAY, Suartha IN. 2018. Titer antibodi ayam petelur pascavaksinasi *Avian Influenza* pada peternakan komersial di Desa Denbantas, Kecamatan Tabanan. Indones Med Vet. 7:123-131. DOI:10.19087 /imv.2018.7.2.123.

Dillasamola D, Aldi Y, Fakhri M, Diliarosta S, Biomechy OP. 2018. Immunomodulatory effect test from Moringa leaf extract (*Moringa oleifera* L.) with carbon clearance method in male White Mice. Asian J Pharm Clin Res. 11:241-245. DOI:10.22159/ajpcr.2018.v11i9.26703.

Fidrianny I, Kenapa I, Singgih M. 2021. Phytochemistry and pharmacology of Moringa tree: an overview. Biointerface Res Appl Chem. 11:10776-10789. DOI:10.33263/BRIAC113.1077610789.

Hofmann T, Schmucker SS, Bessei W, Grashorn M, Stefanski V. 2020. Impact of housing environment on the immune system in chickens: a review. Anim. 10:1-26 DOI:10.3390/ani10071138.

Kasiyati K, Djaelani MA, Sunarno S. 2021. Hematological Responses of pengging ducks fed Moringa (*Moringa oleifera*) leaf meal as feed additives. J Vet. 22:8–15. DOI:10.19087/jveteriner.2021.22.1.8.

Kurnianto AB, Kencana GAY, Astawa INM. 2016. Respons antibodi sekunder terhadap penyakit tetelo pada ayam petelur pascavaksinasi ulangan dengan vaksin tetelo aktif. J Vet. 17:331-336. DOI:10.19087/jveteriner. 2016.17.3.331.

Li C, Dong Z, Zhang B, Huang Q, Liu G, Fu X. 2020. Structural characterization and immune enhancement activity of a novel polysaccharide from *Moringa oleifera* leaves. Carbohydr Polym. 234:115897.

Lokapirnasari WP, Agustono B, Arif MAA, Maslachah L, Chandra EH, Yulianto AB. 2022. Effect of probiotic and *Moringa oleifera* extract on performance, carcass yield, and mortality of Peking duck. Vet World. 15:694-700. DOI:10.14202/vetworld.2022.694-700.

Maggi-Capeyron MF, Cases J, Badia E, Cristol JP, Rauanet JM. 2002. A diet high in cholesterol and deficient in vitamin E indices lipid peroxidation but does not enhance antioxidant enzyme expression in rat liver. J Nutr Biochem. 13:296-301.

Meel MS, Sharma T. 2021. Effect of feeding *Moringa oleifera* leaf meal as feed additive on the performance and carcass characteristics of broiler chicks. Int J Curr Microbiol App Sci. 10:40-46 DOI:10.20546/ijcmas. 2021.1001.006.

Miller PJ, Decanini EL, Afonso CL. 2010. Newcastle Disease: evolution of genotypes and the related diagnostic challenges, infection, genetics, and evolution. Infect Gen. 10:26-35.

Novitasari A, Suardana IBK, Sampurna IP. 2018. Respon imun itik Bali pascavaksinasi Newcastle Disease. Bul Vet Udayana. 10:127. DOI:10.24843/bulvet.2018.v10.i02.p04.

[OIE] Office International des Epizooties. 2021. Newcastle Disease (infection with Newcastle Disease virus). Manual of diagnostic test and vaccines for terrestrial animals 2021. p:10.

Okoroafor ON, Ogunniran TM, Ikenna-ezeh NH. 2021. Effects of dietary supplementation of vitamins E and C on oxidative stress induced by a Nigerian velogenic strain of the Newcastle Disease virus (KUDU 113) in the brain and bursa of fabricius of broiler chickens. 14:2452–2461.

Phongpaichit S, Nikom J, Rungjindamai N, Sakayaroj J, Towatana NH, Rukachaisirikul V, Kirtikara K. 2007.

Biological activities of extracts from endophytic fungi isolated from garcinia plants. FEMS Immunol Med Microbiol. 51:517-525. DOI:10.1111/j.1574-695X. 2007.00331.x.

Pratama L, Santosa PE, Siswanto. 2016. Pengaruh dosis vaksin Newcastle Disease inaktif pada itik betina terhadap jumlah sel darah putih dan titer antibodi. J Ilmiah Peternak Terpadu.4:211-216.

Rahmayanti RP, Isroli. 2019. Pengaruh penambahan tepung daun kelor (*Moringa oleifera*) dan tepung bawang putih (*Allium sativum*) dalam pakan terhadap total leukosit dan diferensial leukosit ayam broiler. Proceeding Seminar Nasional Fakultas Pertanian UNS. p:138-144.

Raji AA, Mohammed B, Oladele SB, Saidu L, Jibril AH, Cazaban G. 2017. Bursa body index as a visual indicator for the assessment of bursa of fabricius. J Vet Med Anim Health. 9:32-38. DOI:10.5897/JVMAH 2016.0456.

Righi F, Pitino R, Manuelian CL, Simoni M, Quarantelli A, De Marchi M, Tsiplakou E. 2021. Plant feed additives as natural alternatives to the use of synthetic antioxidant vitamins on poultry performances, health, and oxidative status: a review of the literature in the last 20 years. Antioxid. 10. DOI:10.3390/antiox10050659.

Rozi F, Rahmahani J, Purnama MTE, Hamid IS, Yudhana A, Praja RN. 2020. Seroprevalensi antibodi Newcastle Disease (ND) pada Itik di Desa Temuasri, Sempu, Banyuwangi. J Med Vet. 3:108-113.

Sahin K, Sahin N, Onderci M, Gursu MF, Issi M. 2003. Vitamin C and E can alleviate negative effects of head stress in Japanese quails. Food Agric Environ. 1:244-249.

Saputra A, Arfi F, Yulian M. 2020. Literature review: analisis fitokimia dan manfaat ekstrak daun kelor (*Moringa oleifera*). Amina. 2:114–119.

Saputro BE, Sutrisna R, Santosa PE, Fathul F. 2016. Pengaruh ransum yang berbeda pada itik jantan terhadap jumlah leukosit dan diferensial leukosit. J Ilmiah Peternak Terpadu. 4:176–181.

Soha A, Frejus O, Ohouko H. 2020. Immunomodulatory properties of *Moringa oleifera* in animal and human nutrition. Pharmacogn Phytother. 12:1-9. DOI:10.5897 /JPP2019.0551.

Purnomo D, Sugiharto, Isroli. 2015. Total leukosit dan diferensial leukosit darah ayam broiler akibat penggunaan tepung onggok fermentasi *Rhizopus oryzae* pada ransum. J Ilmu-Ilmu Peternak. 25:59–68. DOI:10.21776/ub.jiip.2015.025.03.08.

Sulistiyanto B, Kismiati S, Utama CS. 2019. Tampilan produksi dan efek imunomodulasi ayam broiler yang diberi ransum berbasis wheat pollard terolah. J Vet. 20:352. DOI:10.19087/jveteriner.2019.20.3.352.

Sun Q, Yang H, Yu J, Liang J, Xu X, Wang Z. 2022. Effect of dietary vitamin E on growth performance, immunity and antioxidant capacity in male Jiangnan White Goslings from 1 to 28 d of age. Agric. 12:1–9. DOI:10.3390 /agriculture12010083.

Susanty RNA, Chaerrudin A, Yudistirani SA. 2019. Antioksidan daun kelor. J UMJ. 21:1–7.

Tabbu CR. 2000. Penyakit ayam dan penanggulangannya. Yogyakarta (Indones): Kanisius.

Wbowo SE, Asmara W, Wibowo MH, Sutrisno B. 2013. Perbandingan tingkat proteksi program vaksinasi Newcastle Disease pada broiler. JSV. 31.

Widada H. 2013. analisis kandungan vitamin E pada Buah *Borassus flabellifer* Linn. menggunakan high performance liquid chromatography (HPLC). J. Kedokt Kesehat Mutiara Medika. 13:143–150.

Widyaningsih PO, Sudira IW, Suardana IBK. 2020. Titer antibodi ayam kampung yang diberikan jamu daun ashitaba (*Angelica Keiskei)* menurun pascavaksinasi penyakit tetelo. Indones Med Vet. 9:446-455. DOI:10.19087/imv.2020.9.3.446.

Winddi A, Purnama ES, Sri S. 2016. Pengaruh pemberian dosis vaksin AI (*Avian Influenza)* inaktif pada itik betina terhadap titer antibodi yang dihasilkan. J Ilm Peternak Terpadu. 4:140-142.

Xiao S. 2012. Complete genom sequences of Newcastle Disease virus strain circulating in chickens populations of Indonesia. J Virol. 86:5969-5970.

Yang SL, Yang RC, Zhou X, Yang SH, Luo LL, Zhu YC, Boonanuntan S. 2020. Effects of feeding diets with processed Moringa oleifera stem meal on growth and laying performance, and immunological and antioxidant activities in laying ducks. Poult Sci. DOI:10.1016%2Fj.psj.2020.04.002.

Yuliani NS, Sakan GYI. 2018. Respon titer antibodi pada ayam broiler yang divaksinasi ND dan diberi herbal rempah. Pertner. 23:696-704.

Fermentation of Cocoa Pods Husk Using Turmeric Powder and *Aspergillus niger*: Effects on Fiber Composition and Antinutrients

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ABSTRAK

Atmaja IGM, Ismartoyo, Natsir A, Syahrir S. 2023. Fermentasi kulit buah kakao menggunakan kunyit bubuk dan *Aspergillus niger*: pengaruh terhadap komposisi serat dan anti-nutrisi. JITV 28(4):259-267. DOI:http://dx.doi.org/10.14334/jitv.v28i4.3180.

Pengolahan kulit buah kakao (KBK) secara fermentasi dengan *Aspergillus niger* serta tambahan herbal seperti kunyit, diyakini mampu meningkatkan kualitas bahan pakan asal limbah perkebunan. Tujuan penelitian ini adalah untuk menurunkan kandungan fraksi serat dan antinutrisi KBK melalui proses fermentasi menggunakan *A.niger* dan penambahan kunyit dengan level yang berbeda. Percobaan dilaksanakan berdasarkan rancangan acak lengkap yang terdiri dari delapan perlakuan dan tiga ulangan. Perlakuan terdiri dari: P0: kontrol, P1: fermentasi KBK dengan *A. niger*, P2, P3 dan P4: fermentasi (KBK + 0,5; 1,0 dan 1,5% kunyit bubuk) dengan *A. niger* P5, P6 dan P7: fermentasi KBK dengan *A. niger* + 0.5; 1,0 dan 1,5% kunyit bubuk. Variabel yang diamati adalah komposisi serat dari KBK serta antinutrisi. Hasil penelitian menunjukkan bahwa fermentasi KBK menggunakan *A. nige*r dengan kadar bubuk kunyit yang berbeda nyata menurunkan kandungan fraksi serat yaitu; NDF, ADF, dan hemiselulosa dibandingkan dengan KBK yang tidak difermentasi. Kandungan anti-nutrisi, fermentasi menggunakan *A. niger* dengan kadar bubuk kunyit yang berbeda nyata menurunkan tanin dan cenderung menurunkan kandungan lignin KBK dibandingkan dengan KBK yang tidak difermentasi. Kesimpulannya, fermentasi KBK menggunakan *A. niger* dengan penambahan 1,5% kunyit sebelum fermentasi merupakan perlakuan yang paling efektif dalam menurunkan kandungan serat dan komponen antiutrisi pada KBK.

Kata Kunci; Antinutrisi, *Aspergillus niger*, Fraksi Serat, Kulit Buah Kakao, Kunyit

ABSTRACT

Atmaja IGM, Ismartoyo, Natsir A, Syahrir S. 2023. Fermentation of cocoa pods husk using turmeric powder and *Aspergillus niger*: effects on fiber composition and antinutrients. JITV 28(4):259-267. DOI:http://dx.doi.org/10.14334/jitv.v28i4.3180.

Processing of cocoa pods husk (CPH) by fermentation with *Aspergillus niger (A. niger)* and additional herbs such as turmeric is believed to be able to improve the quality of feed ingredients from plantation byproducts. The purpose of this study was to evaluate the effectiveness of the fermentation process using *A. niger* and the addition of different levels of turmeric in reducing the fiber fraction and antinutrient contents of CPH. The experiment used a completely randomized design of eight treatments and three replications. The treatments consisted of P0: control; P1: CPH fermented with *A. niger*; P2, P3 and P4: fermented (CPH + 0.5; 1.0 and 1.5% turmeric powder) with *A. niger*; P5, P6 and P7: fermented CPH with *A. niger* + 0.5; 1.0 and 1.5 % turmeric powder. Variables observed were the fiber composition of CPH as well as antinutrients. The results showed that CPH fermentation using *A. niger* with different levels of turmeric powder significantly reduced the content of fiber fractions, i.e., NDF, ADF, and hemicellulose, compared to that of unfermented CPH. For antinutritional content, fermentation using *A. niger* with different levels of turmeric powder significantly decreased tannin. It tended to decrease the lignin content of CPH compared to unfermented CPHs. In conclusion, fermentation of CPH using *A. niger* with an addition of 1.5% of turmeric before the fermentation is the most effective treatment in decreasing the fiber and antinutritional Components In CPH.

Keywords: Antinutritional, *Aspergillus niger*, Cocoa Pods Husk, Fiber Fraction, Turmeric

INTRODUCTION

Production of fresh cocoa pods in Indonesia in 2020 was 767 thousand tonnes (Syafira, 2022). High cocoa pod production is in line with waste production. Fresh cocoa pods produce the main products in the form of seeds, fruit pods, and placenta with a ratio of 21.74:75.67:2.59% (Wasmun et al. 2016). Cocoa pod husk (CPH) still contains sufficient nutrients that can be used as feed for livestock, which is cheap and available continuously. The chemical composition of CPH is relatively comparable to forage (mainly grass), so CPH biomass has the potential as an alternative feed to replace forage (Suryanto et al. 2014). Some of the nutritional components of CPH are crude protein (6.3%), crude fiber (24%), fat (0.5%), non-nitrogen extract (32.1%) (Yahya et al. (2015); Adamafio, 2013; Haruna and Rasbawati (2020).

However, CPH has some disadvantages, such as high fiber fraction content, including 68.70% acid detergent fiber (ADF), 75.36% neutral detergent fiber (NDF), 30.25% cellulose, and 6.66% hemicellulose (Agus and Budisatria 2012), which limited its digestibility. These antinutritional compounds in the form of lignin, tannins, and theobromine affect the availability of nutrients. Theobromine is a heterocyclic compound that can inhibit digestion (Adamafio 2013). Efforts to overcome these deficiencies are generally carried out through processing either mechanically, chemically, or biologically to break the bonds between lignocellulose and hemicellulose with lignin and reduce theobromine and tannin content.

The fermentation process is one method that can be used to reduce the antinutrient contents in CPH. Fermentation with *A. niger* can increase the nutritional content of CPH. *A. niger* is one of the fast-growing and produces several enzymes such as amylase, pectinase, amyloglucosidase, and cellulase. *A. niger* produces tannase enzymes that can degrade tannins into soluble gallic acid and glucose (Chávez-González, Rodríguez-Duran, et al. 2018). The fermentation of CPH using *A. niger* and *A. oryzae* can reduce the fiber fraction (crude fiber, NDF, and ADF) and increase protein content (Rakhmani and Purwadaria 2018). The lignin content in CPH can also be reduced by using the laccase enzyme oxidizing phenol to phenoxyl. In addition, the laccase enzyme is also able to degrade theobromine which is helpful as a carbon source, nitrogen source, and energy source for *A. niger* (Zhang et al. 2014).

In the fermentation process, adding herbal plants can also improve the quality of agricultural waste. Using herbal plants, especially turmeric, could improve the quality of feed ingredients from agricultural and plantation waste. According to a study by Khanifah et al. (2021) that combined commercial cocoa products and turmeric in a ratio of 50%:50% showed the presence of flavonoids and tannins, but it did not contain alkaloids. Turmeric rhizome fermented with *Aspergillus oryzae* significantly increases the antioxidant content compared to those without fermentation (Sulasiyah et al. 2018). Fermentation of turmeric rhizome with EM-4 produced higher levels of curcumin than unfermented turmeric extract, which was analyzed using the TLC-densitometry method (Yasa et al. 2015). These results align with research done by Lim et al. (2021), which showed that fermented turmeric with *Rhizopus oligosporus* for seven days can increase curcumin phenolics' and antioxidant content. Turmeric contains amylase, lipase, and protease enzymes, which help improve the digestion of feed ingredients such as carbohydrates, fats, and proteins. In addition, turmeric also contains secondary metabolites of flavonoids, alkaloids, and tannins (Sidiq and Wardani, 2014). Based on the description above, it is necessary to study a new approach to reducing the fiber fraction and antinutritional content of CPH by combining *A.niger* and turmeric powder in the fermentation of CPH. The purpose of this study was to evaluate the effectiveness of the fermentation process using *A.niger* with the addition of turmeric powder at different levels in reducing fiber fraction and antinutritional contents of CPH.

MATERIALS AND METHODS

Materials preparation

This study was conducted in the Animal Feed Chemistry Laboratory, Department of Animal Nutrition and Animal Feed, Faculty of Animal Science, Hasanuddin University, Makassar, and the Integrated Research and Testing Laboratory at Gajah Mada University from February through April 2022. The materials used in the study were fresh CPH obtained from South Sulawesi Province, Indonesia. The CPH was collected from local types of cocoa trees that were superior in terms of fruit production, fruit quality, and health. The CPH was then immersed in a 0.5% sodium metabisulfite solution for 15 minutes to maintain the phenolic and flavonoid levels in the CPH (Sartini et al. 2017). Furthermore, the CPH was melted down with a grinding process to increase the surface area, and then the CPH was dried under sunshine until dry (moisture content ±15%). Turmeric powder and fermenter in the form of *A. niger* were commercial products that were available in the market. Apart from these three ingredients, water, granulated sugar, urea, and NPK were also used during the incubation period. The equipment to activate the fermenter were buckets with a minimum capacity of 10 liters, aerators, and wooden sticks as stirrers. The activation process of *A. niger* followed the method of (Fransistika et al. 2013).

Experimental design

The study was carried out according to a completely randomized design (CRD), which consisted of eight treatments and three replications for each treatment, giving a total number of experimental units of 24. The treatments were P0: CPH without fermentation (Control), P1: Fermented CPH with 5×10^{-3} mL/L *A. Niger*, P2: Fermented (CPH + 0.5% Turmeric powder) with 5×10^{-3} mL/L *A. Nige*, P3: Fermented (CPH + 1.0% Turmeric powder) with 5×10^{-3} mL/L *A. Nige*, P4: Fermented (CPH + 1.5% Turmeric powder) 5×10^{-3} mL/L with *A. Niger*, P5: Fermented CPH with 5×10^{-3} mL/L *A. niger* + 0.5% Turmeric powder, P6: Fermented CPH with5×10^{-3} mL/L *A. niger* + 1.0% Turmeric powder, P7: Fermented CPH with 5×10^{-3} mL/L *A. niger* + 1.5% Turmeric powder.

Procedures

The CPH fermentation process for P2 to P4 treatments was carried out by spreading CPH and adding turmeric powder according to the treatment with a thickness of 2 cm covering an area of 100 cm^2 and spraying with 5 x 10^{-3} mL/L A. *niger* activation solution until the water content was 40%, then mixed until homogeneous. The CPH fermentation process for treatments P1, P5 to P7 was carried out by spreading CPH with a thickness of 2 cm covering an area of 100 cm^2 and spraying A. *niger* activation solution until the water content was 40%, then mixed until homogeneous. After completion, labeling was conducted to make it easier to control. The fermentation process lasted for seven days with the anaerobic principle. After seven days, the storage area for CPH and turmeric was opened and dried under the sun for 1-2 days to stop the fermentation process. After the next, treatments P5 to P7 were added with turmeric powder according to the treatment. Furthermore, the sieving process was carried out to make a uniform size and put it into a temporary container before analyzing the content of fiber fractions and antinutrients.

Parameters measured

The variables observed were fiber fraction and antinutrients. The fiber fractions, including NDF, ADF, cellulose, hemicellulose, and lignin, were analyzed using the procedure of Van Soest (1994). The tannin content was determined according to the redox titration analysis method using $KMnO_4$ reagent Aliwarga & Victoria (2019) in the Animal Feed Chemistry Laboratory, Faculty of Animal Science, Hasanuddin University. The theobromine variable was determined using the HPLC method at the Integrated Research and Testing Laboratory at Gadjah Mada University.

Data analysis

The data obtained were analyzed using analysis of variance (ANOVA) based on a completely randomized design (Noto 2015). The significant differences among the treatments were further analyzed using contrast orthogonal. All data were analyzed using the statistical package of SPSS version 22.

RESULTS AND DISCUSSION

Fiber fraction analysis of fermented CPH and turmeric powder by *A. niger*

The data in Table 1 shows that the treatment of CPH fermentation with *A. niger* resulted in significantly lower ADF, NDF, and cellulose values compared to CPH without fermentation. The reduced content of fiber components due to fermentation using *A. niger* on CPH was due to the degradation of complex cell walls into simpler components, as well as the dissolution of some proteins and hemicelluloses in the cell walls in organic acid solutions. An organic acid solution can be produced from the fermentation process; this follows Setiawan's (2014) and Peng et al. (2012) opinion that acid solutions hydrolyze hemicellulose. The cellulose-breaking enzymes produced by *A. niger*, such as the enzymes protease, glucoamylase, lipase, cellulase, amylase, hemicellulase, pectinase, oxidase, and catalase (Yusriani and Puastuti 2020) will make it easier to break down the fiber contained in the fermented substrate, and this follows the opinion of Maulana et al. (2021) that the cellulase enzymes produced by microbes can overhaul the structure of cellulose into more straightforward sugar products that will be useful as an energy source. This phenomenon is followed by the research of Rahmat (2020), which stated that cellulase enzymes produced by microbes can break down the structure of cellulose into more straightforward sugar products, which will be useful as an energy source.

The average results of this study Fermentation treatment compared to fermentation treatment of *A. niger* added with turmeric powder significantly reduced the content of ADF and hemicellulose compared to CPH, which was fermented without the addition of turmeric powder. The reduced content of ADF and hemicellulose is an indication of the effectiveness of adding turmeric powder to reduce the fiber component of CPH fermented with *A. niger*. The main nutritional content, flavonoids, and various enzymes in turmeric can streamline the fermentation process so that it has an impact on reducing the fiber component of CPH, which is fermented and added to turmeric powder, that is in line with Berlian et al. (2017); Ergina, Nuryanti, and Pursitasari (2014); Cobra, Amini, and Putri (2019); and Udayani, Ratnasari, and Nida (2022) who explained that the main ingredients in turmeric rhizome include essential oil, curcumin, resin, oleoresin, *desmetoxicurcumin*, *bidesmetoxiccurcumin*, fat, protein, calcium, phosphorus, iron, alkaloid, flavonoid and tannin. These compounds are included in the content of secondary metabolites, which can be utilized as antioxidants, anti-inflammatory, anti-blood coagulants, anti-cancer, anti-biotics, and can inhibit carcinogenic effects (Ergina et al. 2014).

The low NDF content in fermented CPH is caused by the breakdown of crude fiber content by *A. niger* mold into organic acids during the fermentation process. As a result, the breakdown of cellulose and the weakening of the crude fiber complex bonds decrease the crude fiber content. Research by (Nurdin et al. 2021) showed that the NDF content decreases due to the production of the cellulase enzyme from *A. niger* during the incubation process. Therefore, the amount of *A. niger* added will

Table 1. The mean of fiber fraction of fermented CPH and turmeric powder by *A. niger*

Treatment	Variable			
	ADF	NDF	Cellulose	Hemicellulose
Control (P0)	65.41	70.13	26.16	4.73
Fermented CPH (P1)	61.87	68.80	25.00	6.93
Fermented (CPH+Turmeric)				
0,5% (P2)	64.03	68.94	25.95	4.91
1,0% (P3)	63.95	66.9	25.65	2.97
1,5% (P4)	62.87	66.65	24.38	4.08
Fermented CPH+Turmeric				
0,5% (P5)	63.57	68.82	25.71	5.24
1,0% (P6)	62.89	71.13	25.34	8.24
1,5% (P7)	62.59	68.21	24.74	5.61
Contrast Test				
P0 Vs. P1, P2, P3, P4, P5, P6, P7	65.42 Vs. 63.11*	70.13 Vs. 68.54*	26.16 Vs. 25.25*	4.73 Vs. 5.42*
P1 Vs. P2, P3, P4, P5, P6, P7	61.88 Vs. 63.32*	68.80 Vs. 68.50^{Ns}	25.00 Vs. 25.29^{Ns}	6.93 Vs. 5.17*
P2, P3, P4, Vs. P5, P6, P7	63.62 Vs. 63.02^{Ns}	67.61 Vs.69.39*	25.33 Vs.25.27^{Ns}	3.99 Vs. 6.37*
P2 Vs. P3	64.03 Vs. 63.95^{Ns}	68.94 Vs. 66.90*	25.95 Vs. 25.65^{Ns}	4.91 Vs. 2.97*
P2 Vs. P4	64.03 Vs. 62.87^{Ns}	68.94 Vs. 66.65*	25.95 Vs. 24.38*	4.91 Vs. 4.08^{Ns}
P3 Vs. P4	63.95 Vs. 62.87^{Ns}	66.90 Vs. 66.65^{Ns}	25.65 Vs. 24.38*	2.97 Vs. 4.08*
P5 Vs. P6	63.57 Vs. 62.89^{Ns}	68.82 Vs. 71.13*	25.71 Vs. 25.34^{Ns}	5.24 Vs. 8.24*
P5 Vs. P7	63.57 Vs. 62.89^{Ns}	68.82 Vs. 68.21^{Ns}	25.71 Vs. 24.74^{Ns}	5.24 Vs. 5.61^{Ns}
P6 Vs. P7	62.89 Vs. 62.89^{Ns}	71.13 Vs. 68.21*	25.34 Vs. 24.74^{Ns}	8.24 Vs. 5.61*

*= Significantly different (P<0.05), Ns= No significant (P>0.05), ADF= Acid detergent fiber, NDF= Neutral detergent fiber, P0= CPH without fermentation (Control), P1= Fermented CPH with 5 x 10^{-3} mL/L *A. niger*, P2= Fermented (CPH + 0.5% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P3= Fermented (CPH + 1.0% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P5= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 0.5% Turmeric powder, P6= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.0% Turmeric powder, P7= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.5% Turmeric powder

affect the production of cellulase enzymes, which play a role in breaking cell wall bonds, so that the crude fiber contained in the material will be degraded. *A. niger* mold can produce cellulase enzymes which play a role in breaking down the crude fiber content. The fermentation process also breaks lignin bonds to reduce the NDF content (Ananda 2021).

The addition of turmeric to the CPH fermentation process with *A. niger* markedly reduced the fiber components in the form of NDF and hemicellulose compared to turmeric, which was added to CPH without being involved in the fermentation process. These results reinforce the statement that turmeric streamlines the CPH fermentation process. That is also supported by the data shown in Figure 1.

Figure 1 shows that increasing turmeric powder to the CPH fermentation process with increasing levels decreases the CPH fiber components, especially the NDF, cellulose, and hemicellulose components. This decrease was achieved by using up to 1.5% of turmeric powder, compared to CPH, which was fermented and then added to turmeric. However, the turmeric did not participate in the fermentation process.

Antinutritional content of fermented CPH and turmeric using *A. niger*

The data in Table 2 shows that the treatment of CPH fermentation with *A. niger* resulted in significantly lower tannin content and tended to reduce the lignin content compared to CPH without fermentation. The reduced content of antinutritional components due to fermentation using *A . niger* on CPH is due to the

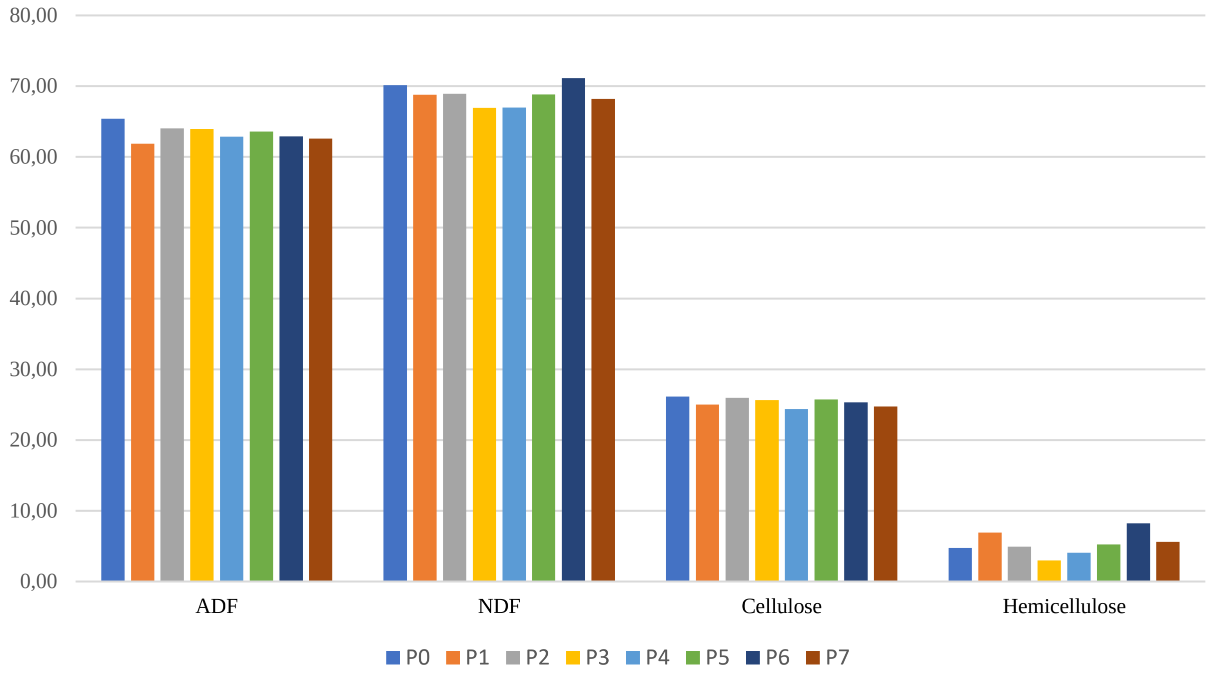

Figure 1. Average Results of Van Soest of Fermented CPH + Turmeric (%). Means of fiber fractions of CPH according to the treatment. ADF= Acid detergent fiber, NDF= Neutral detergent fiber, P0= CPH without fermentation (Control), P1= Fermented CPH with 5 x 10^{-3} mL/L *A. niger*, P2= Fermented (CPH + 0.5% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P3= Fermented (CPH + 1.0% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P5= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 0.5% Turmeric powder, P6= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.0% Turmeric powder, P7= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.5% Turmeric powder

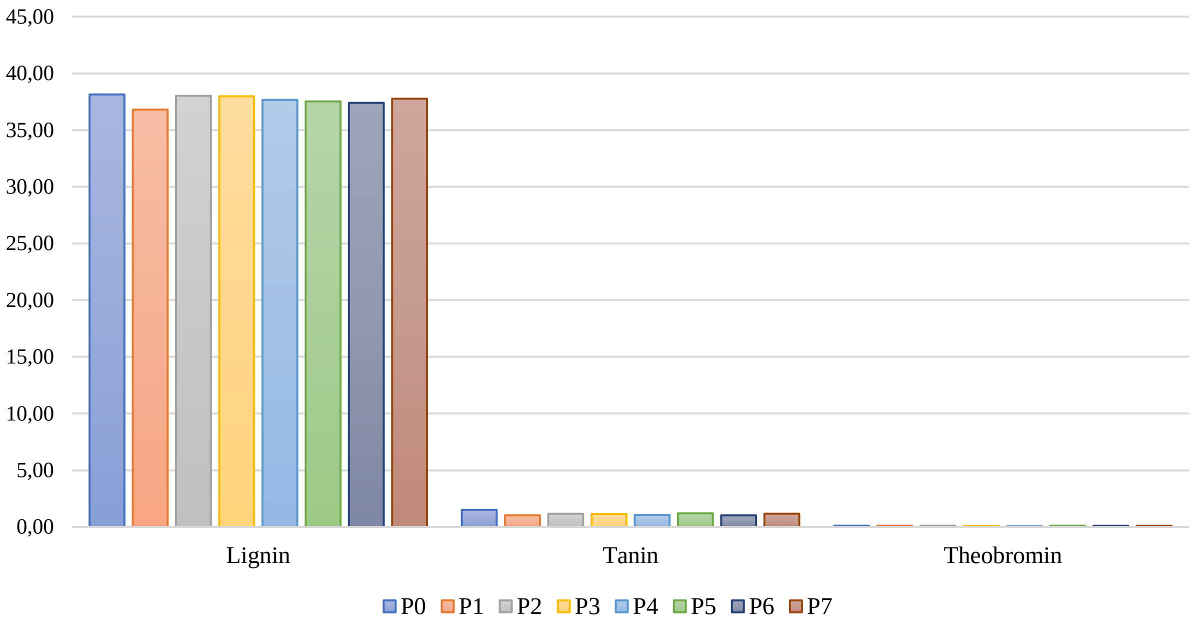

Figure 2. The mean of antinutritional components of fermented CPH and Turmeric by *A. Niger*. P0= CPH without fermentation (Control), P1= Fermented CPH with 5 x 10^{-3} mL/L *A. niger*, P2= Fermented (CPH + 0.5% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P3= Fermented (CPH + 1.0% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P5= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 0.5% Turmeric powder, P6= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.0% Turmeric powder, P7= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.5% Turmeric powder

Table 2. Analysis results of antinutritional content of fermented CPH and turmeric using *A. niger*

Treatment	Variable		
	Lignin	Tannin	Theobromine
Control (P0)	38.14	1.50	0.11
Fermented CPH (P1)	37.66	1.05	0.11
Fermented (CPH+Turmeric)			
0,5% (P2)	38.02	1.16	0.11
1,0% (P3)	37.98	1.14	0.09
1,5% (P4)	36.80	1.03	0.07
Fermented CPH+Turmeric			
0,5% (P5)	37.52	1.20	0.11
1,0% (P6)	37.40	1.03	0.10
1,5% (P7)	37.76	1.16	0.10
Contrast Test			
P0 VS P1, P2, P3, P4, P5, P6, P7	38.14 Vs. 37.59^{Ns}	1.51 Vs.1.11*	0.11 Vs. 0.09^{Ns}
P1 VS P2, P3, P4, P5, P6, P7	36.80 Vs. 37.72^{Ns}	1.03 Vs. 1.12^{Ns}	0.11 Vs. 0.09^{Ns}
P2, P3, P4 VS P5, P6, P7	37.89 Vs. 37.56^{Ns}	1.12 Vs. 1.13^{Ns}	0.09 Vs. 0.10^{Ns}
P2 Vs. P3	38.02 Vs. 37.98^{Ns}	1.16 Vs. 1.14^{Ns}	0.11 Vs. 0.09^{Ns}
P2 Vs. P4	38.02 Vs. 36.80*	1.16 Vs. 1.03^{Ns}	0.11 Vs. 0.07*
P3 Vs. P4	37.98 Vs. 36.80*	1.14 Vs. 1.03^{Ns}	0.09 Vs. 0.07^{Ns}
P5 Vs. P6	37.52 Vs. 37.40^{Ns}	1.20 Vs. 1.03*	0.11 Vs. 0.10^{Ns}
P5 Vs. P7	37.52 Vs. 37.76^{Ns}	1.20Vs. 1.16^{Ns}	0.11 Vs. 0.10^{Ns}
P6 Vs. P7	37.40 Vs. 37.76^{Ns}	1.03 Vs. 1.16^{Ns}	0.10 Vs. 0.10^{Ns}

*= Significantly different (P<0.05), Ns= No significant (P>0.05), P0= CPH without fermentation (Control), P1= Fermented CPH with 5 x 10^{-3} mL/L *A. niger*, P2= Fermented (CPH + 0.5% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P3= Fermented (CPH + 1.0% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P4= Fermented (CPH + 1.5% Turmeric powder) with 5 x 10^{-3} mL/L *A. niger*, P5= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 0.5% Turmeric powder, P6= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.0% Turmeric powder, P7= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.5% Turmeric powder, P7= Fermented CPH with 5 x 10^{-3} mL/L *A. niger* + 1.5% Turmeric powder

degradation of complex antinutrients into simpler components, as well as the hydrolysis of tannins by the tannase enzyme produced from the fermentation process. Tannase enzymes can be produced from a solid phase fermentation process with *A. niger* 1.401 U/mL (Chávez-González, Guyot, et al. 2018). Tannase is an extracellular enzyme produced by several microorganisms, such as bacteria, yeast, and fungi. Research by Lekshmi et al. (2021) suggests that the fungus genera *Aspergillus* and *Penicillium* are the best producers of the tanase enzyme. Rakhmani and Purwadaria (2018) fermentation with *A. niger* produces more mannanase and cellulase enzymes than *Aspergillus oryzae*. These enzymes help break down and loosen lignocellulosic bonds; high cellulose can reduce the lignin content. The trend of decreasing lignin content was due to the breakdown of lignocellulose bonds by cellulase enzymes produced by *A. niger* during the fermentation process. Lignocellulosic and hemicellulose bonds in the cell walls of cocoa pod shells will be stretched in the presence of lignocellulose and cellulase enzymes produced by *A. niger*, that aligns with the statement (Dalimunthe et al. 2021), which states that fermentation aims to break down lignocellulosic complex bonds and produce cellulose content to be broken down by cellulase enzymes produced by microbes. Fiber treatment in akaa leaves heated in turmeric solution can increase cellulose levels and reduce lignin levels, while hemicellulose levels are almost constant (Renreng et al. 2017). Lignin is a phenylpropanoid polymer that is challenging to break down because of its heterogeneous and very complex structure. The composition of plant material is 30% lignin. Therefore, lignin can provide strength to wood against attacks by microorganisms (Carniel et al. 2021).

Fermentation treatment compared to *A. niger* fermentation treatment added with turmeric powder had no significant effect on the antinutritional components of CPH. The absence of anti-nutrition content indicates that the addition of turmeric powder in CPH fermentation with *A. niger* has not been effective in reducing tannins, lignin, and theobromine in CPH. The addition of turmeric to the CPH fermentation process with *A. niger* and turmeric added to CPH without being involved in the fermentation process did not significantly affect the decrease in the antinutritional content of CPH.

Figure 2 shows that increasing turmeric powder to the CPH fermentation process impacts reducing the antinutritional components of CPH in the tannin 11.20%, lignin 2.54%, and theobromine 29.00% components. This decrease was achieved by using up to 1.5% of turmeric powder, compared to CPH, which was fermented and then added to turmeric. However, the turmeric did not participate in the fermentation process. The trend of decreasing theobromine content at treatment levels using turmeric powder up to 1.5%, which A. niger ferments, indicates that theobromine degradation by *A. niger* occurs via the demethylase route and involves the expression of enzymes such as theobromine *demethylase, theobromine oxidase, xanthine dehydrogenase, xanthine oxidase, urease* and *uricase* Yamaoka-Yano and Mazzafera (1999); Summers et al. (2015); and Cornelis et al. (2016). Some of these statements indicate that *A. niger* microbes can degrade theobromine (Bentil et al. 2015).

CONCLUSION

Compared to unfermented CPH, the addition of turmeric powder up to 1.5% to the CPH fermentation using *A. niger* significantly impacts decreasing the CPH fiber components, especially the NDF, ADF, and cellulose. The impact is more profound in reducing NDF fraction when the turmeric is added to CPH before the fermentation process than adding turmeric after the fermentation of CPH using *A. niger*. Concerning the antinutrient components, the addition of turmeric powder up to 1.5% to the CPH fermentation using *A. niger* only has a profound effect on reducing the tannin content of CPH and tends to decrease the lignin component. Adding turmeric either before or after the fermentation of CPH using *A. niger* has similar effects.

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REFERENCES

Adamafio NA. 2013. Theobromine toxicity and remediation of cocoa byproducts: An overview. J Biol Sci. 13:570–576. DOI:10.3923/jbs.2013.570.576.

Agus A, Budisatria IGS. 2012. Performa domba yang diberi complete feed kulit buah kakao terfermentasi. Bul Peternak. 36:162–168. DOI:10.21059/buletinpeternak.v36i3.1624.

Aliwarga L, Victoria A V. 2019. Pengendapan titanium pada larutan pasis besi dalam asam sulfat. Acid Solut. 15:109–118. DOI:10.30556/jtmb.Vol15.No2.2019.989.

Ananda S. 2021. Pengaruh lama inokulasi ampas sagu (Metroxylon sagu) dengan *Aspergillus niger* terhadap kandungan ADF Dan NDF ampas sagu. J Peternak Sriwij. 10:1–7. DOI:10.33230/JPS.10.1.2021.12388.

Bentil JA, Dzogbefia VP, Alemawor F. 2015. Enhancement of the nutritive value of cocoa (*Theobroma cacao*) bean shells for use as feed for animals through a two-stage solid state fermentation with Pleurotus ostreatus and *Aspergillus niger.* Int J Appl Microbiol Biotechnol Res. 3:20–30.

Berlian Z, Pane ER, Hartati S. 2017. Efektivitas kunyit (*Curcuma domestica*) Sebagai pereduksi formalin pada tahu. J SainHealth. 1:1–14.

Carniel FC, Fortuna L, Zanelli D, Garrido M, Vázquez E, González VJ, Prato M, Tretiach M. 2021. Graphene environmental biodegradation: Wood degrading and saprotrophic fungi oxidize few-layer graphene. J Hazard Mater. 414:125553. DOI:10.1016/j.jhazmat.2021.125553.

Chávez-González ML, Guyot S, Rodríguez-Herrera R, Prado-Barragán A, Aguilar CN. 2018. Exploring the degradation of gallotannins catalyzed by tannase produced by *Aspergillus niger* GH1 for ellagic acid production in submerged and solid-state fermentation. Appl Biochem Biotechnol. 185:476–483. DOI:10.1007/s12010-017-2663-5.

Chávez-González ML, Rodríguez-Duran L V, Buenrostro-Figueroa JJ, Sepúlveda-Torre L, Ascacio-Valdés JA, Rodríguez-Herrera R, Aguilar CN. 2018. Tannin degrading enzymes: catalytic properties and technological perspectives. J Enzym Food Technol. 7:125–141. DOI:10.1007/978-981-13-1933-4_7.

Cobra LS, Amini HW, Putri AE. 2019. Skirining fitokimia ekstrak sokhletasi rimpang kunyit (*Curcuma longa*) dengan pelarut etanol 96 %. J Ilm Kesehat Karya Putra Bangsa. 1:12–17.

Cornelis MC, Kacprowski T, Menni C, Gustafsson S, Pivin E, Adamski J, Artati A, Eap CB, Ehret G, Friedrich N. 2016. Genome-wide association study of caffeine metabolites provides new insights into caffeine metabolism and dietary caffeine-consumption behavior. Hum Mol Genet. 25:5472–5482. DOI:1093/hmg/ddw334.

Dalimunthe M, Purnama D, Jasmidi J, Amdayani S, Annazilli H, Sihombing JL. 2021. Teknologi pakan ternak silase dari limbah pelepah daun kelapa sawit di Desa Perkebunan Amal Tani. JPKM TABIKPUN. 2:47–54. DOI:10.23960/jpkmt.v2i1.22.

Ergina E, Nuryanti S, Pursitasari ID. 2014. Uji kualitatif senyawa metabolit sekunder pada daun palado (*Agave angustifolia*) yang diekstraksi dengan pelarut air dan etanol. J Akad Kim. 3:165–172.

Fransistika R, Idiawati N, Dest L. 2013. Pengaruh waktu fermentasi campuran trichoderma reesei dan *Aspergillus niger* terhadap kandungan protein dan serat kasar ampas sagu. JKK. 1:35-39.

Haruna H, Rasbawati R. 2020. Optimalisasi hara in situ melalui intergrasi tanaman dan ternak:. J Din Pengabdi. 6:62–70. DOI:10.20956/jdp.v6i1.9416.

Khanifah F, Puspitasari E, Awwaludin S. 2021. Uji kualitatif flavonoid, alkaloid, tanin pada kombinasi kunyit (*Curcuma longa*) cokat (*Theobroma cacao* L). J Sains dan Terap Kim. 15:91–99.

Lekshmi R, Nisha SA, Vasan PT, Kaleeswaran B. 2021. A comprehensive review on tannase: Microbes associated production of tannase exploiting tannin-rich agro-industrial wastes with special reference to its potential environmental and industrial applications. Environ Res. 201:111625. DOI:10.1016/j.envres.2021.111625.

Lim J, Nguyen TTH, Pal K, Park C, Kim SW, Kim D. 2021. Phytochemical properties and functional characteristics of wild turmeric (*Curcuma aromatica*) fermented with Rhizopus oligosporus. Food Chem X. 13:100198. DOI:10.1016/j.fochx.2021.100198.

Maulana F, Nuraini N, Mirzah M. 2021. Kandungan dan kualitas nutrisi limbah sawit fermentasi dengan *Lentinus edodes*. JPI. 23:174–182.

Noto MS. 2015. Efektivitas pendekatan metakognisi terhadap penalaran matematis pada matakuliah geometri transformasi. Infin. 4:22–31. DOI:10.22460/infinity. v4i1.p22-31.

Nurdin AJ, Muwakhid B, Wadjdi MF. 2021. Pengaruh tingkat penambahan *Aspergillus niger* pada haylase complate feed berbasis bagas tebu dan kotoran ayam kering terhadap kandungan neutral detergent fiber (NDF) dan selulosa. Din Rekasatwa. 1:90-93.

Peng F, Peng P, Xu F, Sun R-C. 2012. Fractional purification and bioconversion of hemicelluloses. Biotechnol Adv. 30(4):879–903.

Rahmat G. 2020. Pemamfaatan Campuran Limbah Sawit dan Deda Padi Yang Difermentasi Dengan Pleurotus ostreatus Dalam Ransum Terhadap Performa Dan Kualitas Telur Puyuh.

Rakhmani SI, Purwadaria T. 2018. Improvement of nutritional value of cocoa pod husk fermented with Aspergillus spp. and two levels of urea and ammonium sulphate. J Ilmu Ternak dan Vet. 22(3):101. https://doi.org/10.14334/jitv.v22i3.1670

Renreng I, Soenoko R, Pratikto, Irawan YS. 2017. Effect of turmeric (Curcumae longae) treatment on morphology and chemical properties of Akaa (Corypha) single fiber. J Eng Sci Technol. 12(8):2229–2237.

Sartini S, Asri RM, Ismail I. 2017. Pengaruh pra perlakuan sebelum pengeringan sinar matahari dari kulit buah kakao terhadap kadar komponen fenolik dalam ekstrak. Bioma J Biol Makassar. 2(1):15–20.

Setiawan G. 2014. Pengaruh Penambahan Mikroba Lokal (MOL) Terhadap Kadar Neutral Detergent Fiber Dan Acid Detergent Fiber Pada Ransum Lengkap Terfermentasi. Students e-Journal. 3(2).

Sidiq F, Wardani WW. 2014. Aktivitas anti-oksidan dari curcumin dalam mengurangi dampak stres oksidatif pada unggas yang terpapar cekaman panas. Trouw Add Sci. 3:1–3.

Sulasiyah S, Sarjono PR, Aminin ALN. 2018. Antioxidant from Turmeric Fermentation Products (Curcuma longa) by Aspergillus Oryzae. J Kim Sains dan Apl. 21(1):13–18.

Summers RM, Mohanty SK, Gopishetty S, Subramanian M. 2015. Genetic characterization of caffeine degradation by bacteria and its potential applications. Microb Biotechnol. 8(3):369–378.

Suryanto E, Bulkaini B, Ashari A, Karda IW. 2014. Carcass quality, marbling and cholesterol content of male Bali cattle fed fermented cocoa shell. J Indones Trop Anim Agric. 39(4):249–255.

Syafira L. 2022. Penerapan autoregressive distribusi lag (ARDL) pada prediksi produksi kakao indonesia. J Math UNP. 7(3):74–82.

Udayani NNW, Ratnasari NLAM, Nida IDAAY. 2022. Penetapan Kadar Senyawa Fitokimia (Alkaloid, Flavonoid dan Tanin) pada Ekstrak Etanol Rimpang Kunyit Hitam (Curcuma caesia Roxb.). J Pendidik Tambusai. 6(1):2088–2093.

Wasmun H, Rahim A, Hutomo GS. 2016. Pembuatan minuman instan fungsional dari bioaktif pod husk kakao. AGROTEKBIS E-JURNAL ILMU Pertan. 4(6):650–658.

Yahya MA, Al-Qodah Z, Ngah CWZ. 2015. Agricultural bio-waste materials as potential sustainable precursors used for activated carbon production: A review. Renew Sustain Energy Rev. 46:218–235.

Yamaoka-Yano DM, Mazzafera P. 1999. Catabolism of caffeine and purification of a xanthine oxidase responsible for methyluric acids production in Pseudomonas putida L. Rev Microbiol. 30:62–70.

Yasa PI, Winata I, Oktavianawati I. 2015. Studi Kadar Kurkumin Hasil Fermentasi Kunyit (Curcuma longa)

Dengan EM4 Menggunakan Metode KLT-Densitometri. :67–69.

Yusriani Y, Puastuti W. 2020. Implementasi Silase Kulit Buah Kakao dan Zinc-Metionin pada Ransum Kambing Boerka di Kabupaten Pidie Jaya. In: Pros Semin Nas Teknol Peternak dan Vet. [place unknown]; p. 428–437.

Zhang W, Xue B, Li M, Mu Y, Chen Z, Li J, Shan A. 2014. Screening a strain of *Aspergillus niger* and optimization of fermentation conditions for degradation of aflatoxin B1. Toxins (Basel). 6(11):3157–3172.

Quality of Bebek Ungkep as Affected by Natural Feed Additives

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ABSTRAK

Mardiana NA, Kurniawan D, Widigdyo A, Kurniawan H. 2023. Pengaruh pakan tambahan alami terhadap kualitas bebek ungkep. JITV 28(4):268-273. DOI: http://dx.doi.org10.14334//jitv.v28i4.3178.

Penelitian ini bertujuan untuk mengetahui pengaruh pemberian natural feed additive terhadap kualitas bebek ungkep berdasarkan parameter kimia, mikrobiologi dan organoleptik. Rancangan percobaan yang digunakan dalam penelitian adalah rancangan RAL (Rancangan Acak Lengkap) terdiri dari 2 perlakuan yaitu P0 (bebek yang diberi pakan komersial selama proses budidaya) dan P1 (bebek yang diberi pakan natural feed additives selama proses budidaya) dengan masing-masing perlakuan diulang sebanyak 5 ulangan. Variabel yang diamati karakteristik kimia (kadar air, kadar protein, kadar abu, kadar lemak, kadar kolesterol, dan kadar asam lemak jenuh), karakteristik mikrobiologi (TPC, number of *Enterobacteriaceae, Salmonella sp., Staphylococcus aureus,* dan *Listeria monocytogenes* colonies) dan kualitas organoleptik (warna, aroma, tekstur, rasa dan keseluruhan). Data yang didapat di analisa dengan one-way ANOVA. Hasil penelitian menunjukkan bahwa terjadi penurunan kadar kolesterol pada bebek ungkep yang diberi feed additives. Selain itu, tidak ada perbedaan yang nyata pada parameter mikroorganisme. Untuk organoleptik tidak ada perbedaan nyata terhadap semua parameter (rasa, warna, tekstur, secara keseluruhan), kecuali aroma.

Kata Kunci: Bebek Ungkep, Kimia, Mikrobiologi, *Natural Feed Additives*, Organoleptic

ABSTRACT

Mardiana NA, Kurniawan D, Widigdyo A, Kurniawan H. 2023. Effect of a natural feed additive on quality of bebek ungkep. JITV 28(4):268-273. DOI: http://dx.doi.org/jitv.v28i4.3178.

This study aimed to determine the effect of natural feed additives on the quality of ducks based on chemical, microbiological, and organoleptic parameters. The experimental design used in this study was the Completely Randomized Design, which consisted of 2 treatments, namely P0 (ducks were fed commercial feed during rearing) and P1 (ducks were given additional natural feed during rearing), with each treatment pentaplicates. The observed variables were chemical properties (moisture content, protein content, ash content, fat content, cholesterol content, and saturated fatty acid content), microbiological properties (TPC, number of *Enterobacteriaceae* colonies, *Salmonella sp., Staphylococcus aureus,* and *Listeria monocytogenes*) and organoleptic quality (color, aroma, texture, taste and overall). The data collected in this study were analyzed by one-way ANOVA. The results showed an alteration of cholesterol content in Bebek Ungkep with natural additives during rearing. Besides that, there was no significant difference in the parameters of the microorganisms. For organoleptic, there is no significant difference for all parameters (taste, color, texture, overall) except aroma.

Key Words: Bebek Ungkep, Chemical, Microbiological, Natural Feed Additives, Organoleptic

INTRODUCTION

Bebek ungkep is a famous Indonesian dish that involves cooking duck meat in a mixture of aromatic spices and herbs. The preparation consists of marinating the duck in a blend of garlic, shallots, turmeric, and other spices, then cooking slowly (92-96°C) over low heat until the duck is tender and delicious. Ducks are the second most produced poultry after chickens in Indonesia. Not only that, the number of duck populations has grown quite rapidly. Based on (Badan Pusat Statistik 2022) data, the population of ducks in Indonesia has increased by 2 million compared to 2020. The high population and production of ducks are influenced by efforts to prevent and control disease in animals carried out by breeders. Generally, breeders use Antibiotic Growth Promoters (AGP) to prevent or treat sick animals.

The antibiotic Growth Promoter (AGP) mechanism suppresses the population of microorganisms that cause infection, thus increasing the body weight of livestock, and the feed conversion ratio can be optimized (Maria Cardinal et al. 2019). However, the Antibiotic Growth Promoter (AGP) can also be absorbed with nutrients and accumulate in meat carcasses; thus, consumers also indirectly receive low-dimension antibiotics. This antibiotic can interfere with human health because it can cause bacterial antibiotic resistance (Untari et al. 2021). Therefore, the government has issued regulations to regulate the use of AGP.

The prohibition and restriction on the use of AGP raises a new problem, namely a decrease in duck production; thus, natural supplements are needed to replace the role of AGP. One of the natural supplements that can be used is derived from herbs and spices. Herbs and spices contain numerous active ingredients that can exert various effects (bactericidal, immunomodulatory, and antioxidant) on animals. Thus, they can affect animal health status, productivity, and the quality of animal products (Krzysztof et al. 2018). However, only a few publications are related to post-harvest, especially the characteristics of duck carcasses processed into Bebek Ungkep products.

Based on this description, quality testing of Bebek Ungkep products was carried out. The parameters tested were chemical, microbiological, and organoleptic. This study aimed to test the chemical, microbiological, and organoleptic quality of Bebek Ungkep products.

MATERIALS AND METHODS

Sample

The sample used in this study was a hundred 42-day-old hybrid ducks given additional natural feed additives and commercial feed during rearing. Ducks reared for 42 days, then slaughtered at Dimoro Slaughterhouse, Blitar City. The research sample used was duck carcasses, which were processed into Bebek Ungkep.

Preparation of bebek ungkep

The duck carcass is cut into four parts. The duck carcass is cooked with spices such as 21.3% galangal, 17.9% shallots, 9% garlic, 2.1% coriander, 5.9% candlenut; 1.3% turmeric; 5.3% ginger; 0.4% nutmeg; 0.8% pepper; 6.3% lemongrass; 1.3% lime leaves; 1.3% bay leaves; 5.2% salt; and 20.8% sugar for 1 hour. Next, drain the steamed duck and wait for it to cool. The roasted duck was vacuum packed in PE plastic packaging and stored in a freezer at -18^{o}C.

Chemical evaluation

The chemical evaluation was carried out to determine the nutrition in food. In this study, we measured the ash content (AOAC 2016), carbohydrate content (by difference method), fat content (Monakhova et al. 2013), moisture content (AOAC 2016), protein content (AOAC 2016), cholesterol content (Dinh et al. 2012), and saturated fatty acid content (Dahimi et al. 2014).

Microbiology analysis

The samples were tested for several microorganism groups: Total Plate Counts (ISO 4833-1), *Enterobacteriaceae* (ISO 21528-2), *Salmonella* (ISO 6579), *Staphylococcus aureus* (SNI ISO 6888-1), dan *Listeria monocytogenes* (SNI ISO 11290-1).

Organoleptic analysis

Organoleptic analysis was carried out using the hedonic test. Organoleptic attributes are commonly used to describe the sensory properties of food, such as color, appearance, shape, taste, and texture. Product appearance is the most essential attribute of a product. When choosing a product, consumers consider product appearance first, ignoring other sensory attributes. Good product appearance tends to be seen as high quality and tasty (Tarwendah 2017). Therefore, in this study, we use the sensory parameters observed by the panelists: aroma, taste, color, texture, and overall parameters.

The hedonic test followed Mardiana's et al. (2021) method. The hedonic test measured panelists' preference for Bebek Ungkep products. The hedonic test was carried out with 39 trained panelists. Panelists were asked to give a score from 1-5; score 1 had the lowest preference level, while score 5 indicated the highest.

Statistical analysis

The experiments were performed in 5 replications. The significant differences between the means of parameters were analyzed by the t-test (paired comparison) using the Minitab program (version 17.0).

RESULTS AND DISCUSSION

Chemical analysis

Ash, carbohydrate, moisture, protein, fat, cholesterol, and saturated fat content of Bebek Ungkep are depicted in Table 1. The ash and carbohydrate content of Bebek Ungkep with different treatments showed no significant difference (P>0.05). Bebek ungkep without natural feed additives was 2.30±0.14%, while Bebek Ungkep with natural feed additives was 2.00±0.24%. Our results are in agreement with (Farghly et al. 2019), who found that the moisture content in Muller duck meat, ranging from 1.78% to 2.26%. Different feed diets in broilers do not affect broiler meat's chemical composition, such as ash content. Meanwhile, the carbohydrate content of Bebek Ungkep without natural feed additives was 1.35±0.14%, while for Bebek Ungkep with natural feed additives was 1.17±0.16%. According to (Kementerian Kesehatan Republik Indonesia 2018) data, the carbohydrate content of raw

Table 1. Proximate composition of bebek ungkep

Parameter	Samples		T-test value
	Bebek Ungkep Without Natural Feed Additives	Bebek Ungkep With Natural Feed Additives	
Ash Content (%)	2.30±0.14	2.00±0.24	0.123
Carbohydrate content (%)	1.35±0.14	1.17±0.16	0.483
Moisture content (%)	65.24±1.00	61.16±2.48	0.040*
Protein content (%)	16.63±1.04	16.55±1.35	0.916
Fat content (%)	14.24±0.76	19.27±1.31	0.000*
Cholesterol (mg/100 g)	202.55±2.96	124.18±3.19	0.000*
Saturated Fatty Acid Content (%)	7.90±0.76	6.16±0.51	0.134

*****significantly difference

duck meat was zero. However, during the cooking process, with the addition of spices and herbs, the carbohydrate content of fried duck is 4.5%.

The study results showed that a difference in feed affects moisture content on Bebek Ungkep ($P<0.05$). The moisture of Bebek Ungkep without natural feed additives during rearing was 65.24±1.00%, while Bebek Ungkep with natural feed additives was 61.16±2.48%. The results are consistent with those obtained by Džinić (2011), confirming that feed alters carcasses' or processed meat's moisture content. However, cooking methods such as boiling affect the moisture content in products. Water as a heat transfer medium enters the duck meat tissue; thus, the moisture content will increase (Nurmala et al. 2014).

The study results showed that a difference in feed does not affect moisture content on Bebek Ungkep ($P>0.05$). The protein content of Bebek Ungkep without natural additives was 16.63±1.04%, while the protein content of Bebek Ungkep with natural feed additives was 16.55±1.35%. This study's result is lower than that of Nurmala et al. (2014), in which the protein content of duck meat was between 17.78% and 23.63%. Differences might happen due to different types of ducks, feed, and cooking methods. Different types of birds will have a metabolism system to utilize feed for poultry to grow and other vital processes. Meanwhile, in making Bebek Ungkep, we cooked duck meat with water and spices in low heat for a long time. It is suspected that there is a process of leaching nitrogenous compounds into the boiling water because the protein dissolves easily in water. (Olagunju & Nwachukwu 2020). Also, boiling and grilling may reduce protein content in meat due to protein denaturation. Thus, it lowers the protein content in duck.

Table 1 presents the fat and cholesterol content between the two samples. The study results showed that a difference in feed does not affect fat and cholesterol content on Bebek Ungkep ($P<0.05$). The fat content of Bebek Ungkep with natural feed additives is higher (19.27±1.31%) than Bebek Ungkep without natural feed additives (14.24±0.76%). This study disagreed with the observation of Elis (2020) that the addition of phytogenic feed additives in feed reduces the fat content in duck meat and improves the duck flavor. In contrast, the cholesterol content of Bebek Ungkep with natural feed additives is lower (124.18±3.19 mg/100 g) than without natural feed additives (202.55±2.96 mg/100 g). According to (Lestari et al. 2021), different types of feed will influence cholesterol metabolism. High consumption caused a high intake of ducks. Carbohydrates and fatty acids are converted into triglycerides deposited in the liver and muscle. Excess amounts of triglycerides cause more cholesterol deposits in muscle; thus, the cholesterol content in duck meat will increase, leading to health problems for humans who consume it. In humans, cholesterol has a function to produce hormones, as a building block for human tissues, and assists in bile production in the liver. However, daily high cholesterol and saturated fatty acid food intake will increase heart disease risk (Zampelas & Micha 2015). Thus, lower cholesterol in Bebek Ungkep will minimize the health risk.

The study results showed that a difference in feed does not saturated fatty acid content on Bebek Ungkep ($P>0.05$). The saturated fatty acid content in Bebek Ungkep without natural feed additives is higher (7.90±0.76) than natural feed additives (6.16±0.51%). Natural feed additives can lower saturated fatty acid in duck meat. It is supported by Jakubczyk's et al. (2020) research, which is similar to ours. Natural feed additives such as herbs and spices have antioxidant properties that may affect the fatty acid profile in tissue lipids and the oxidative stability of meat, as well as limit the deterioration of meat quality in storage. Besides that, herbs in feed rations reduced the percentage of SFA of

Table 2. Bacteria counts of bebek ungkep samples

Parameter	Samples		Acceptable microbial limit*
	Bebek Ungkep Without Natural Feed Additives	Bebek Ungkep With Natural Feed Additives	
Total Plate Count (colony/gram)	<10	<10	10^4
Enterobacteriaceae (colony/ gram)	<10	<10	10
Salmonella sp. (/25 gram)	Negative	Negative	Negative
Staphylococcus aureus (colony/gram)	<10	<10	10^2
Listeria monocytogenes (/25 gram)	Negative	Negative	Negative

*based on Peraturan badan pengawas obat dan makanan nomor 13 tahun 2019

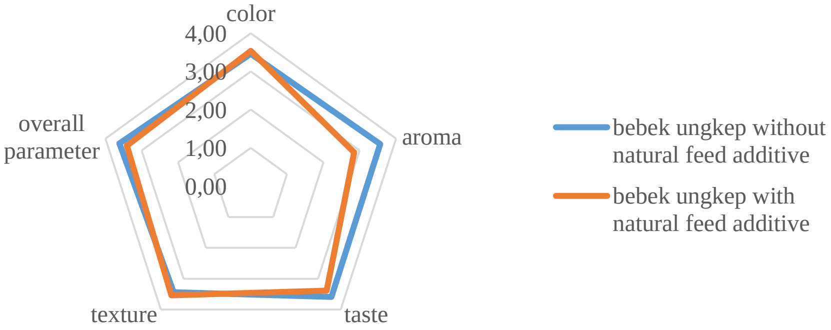

Figure 1. Organoleptic test of Bebek Ungkep

abdominal fat (Xing et al. 2020). Saturated fatty acids can harm consumer health, while monounsaturated fatty acids (MUFA) and PUFAs affect health positively. Thus, lowering the saturated fat content in Bebek Ungkep impacts human health.

Microbiology analysis

Microbiology analysis was aimed at detecting the presence of microbial contaminants in Bebek Ungkep products. Total Plate Count is a method to determine whether pathogenic or non-pathogenic microbes exist in food and the hygienic status of food produced (Arifan et al. 2019). Besides that, we also count four pathogenic bacteria isolated from Bebek Ungkep samples: *Enterobacteriaceae, Salmonella sp., Staphylococcus aureus, and Listeria monocytogenes*. *Enterobacteriaceae* is coliform, and their presence in any food sample reflects fecal contamination; hence, they should not be isolated in food samples (Genome et al. 2018). These bacteria could be attributed to poor hygienic standards and could be suspected to be the poultry contamination source.

Meanwhile, *Salmonella sp*. is among the most foodborne outbreaks worldwide. Salmonella occurs naturally in the intestines of animals such as poultry. It can contaminate and survive for extended periods in food products (Eng et al. 2015). *Listeria monocytogenes* can grow in refrigerated temperatures and tolerate salty environments, unlike many other foodborne bacteria (Ariyanti 2010). Under the National Agency of Drug and Food Control Law, the absence of *Salmonella sp*. and *Listeria monocytogenes* is required in frozen meat products. *Staphylococcus aureus* is usually a contaminant in food and causes clinical infection. This disease is associated with cross-contamination of ready-to-eat food with either raw or cooked food and poor hygienic practices of food handlers (Provincial Health Services Authority 2022). Based on Table 2, the number of colonies *of Salmonella sp*. and *Listeria monocytogenes* were zero. Meanwhile, TPC and the number of colonies *of Enterobacteriaceae* and *Staphylococcus aureus* were less than ten colonies/grams. There was no significant difference between the two samples, which means the addition of natural feed additives will not influence microorganisms in Bebek Ungkep. However, microorganisms loaded in the sample depend on the cooking process. According to

Rai et al. (2016), enough heat in the cooking process can inactivate pathogenic microorganisms and ensure the safety of products.

Table 2 presents the bacterial load in all the samples, generally within the acceptable microbial limit decided by the National Food Drug Agency (2019). This value proved that food safety practices were correctly done during the cooking and post-cooking of Bebek Ungkep. Proper cooking time and temperature are essential to kill microorganisms (James et al. 2021). Proper food handling, food storage at the correct temperature, and proper hygiene practices also need to prevent contamination of microorganisms after cooking (Chekol et al. 2019)

Organoleptic analysis

The organoleptic test was carried out using the hedonic test method. The hedonic test is a method that aims to identify and assess a product based on the preference level of each attribute in the product. The organoleptic test of samples can be seen in Figure 1. Based on Graph 1, all the samples were well-accepted by the respondents. There was no significant difference in color, taste, texture, and overall parameters between the two groups. This carcass quality agrees with the findings of Babalola (2016), who reported that dried lettuce in a fed diet did not affect the quality of carcass snails. In contrast, there was a significant difference in aroma between the two groups. Replenishment of natural feed additives during the rearing process may cause a change in the aroma of the carcass due to the components of the active compounds contained in natural feed additives entering the muscle tissue (Sugiharto 2022). In addition, there is a change in the fatty acid profile where fatty acids have a significant contribution to the aroma of a product (Song et al. 2022). Besides that, during the cooking process, volatile components such as pyrans, alcohols, ketones, and other components that contribute to aroma production were formed (Shahidi & Hossain 2022).

CONCLUSION

Feed influenced the nutrient content of duck carcass. The use of natural feed additives affects fat and cholesterol content; thus, it predisposes the acceptability of panelists to the aroma of Bebek Ungkep. In contrast, other parameters, such as color, taste, and texture, have no alteration by applying natural feed additives during rearing.

ACKNOWLEDGEMENT

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REFERENCES

AOAC. (2016). Official methods of analysis of AOAC International, 20th ed. Maryland (USA): AOAC International.

Arifan F, Winarni S, Wahyuningsih W, Pudjihastuti I, Broto R W. 2019. Total Plate Count (TPC) Analysis of processed ginger on Tlogowungu Village. ICoMA. 167:377–379. DOI:10.2991/icoma-18.2019.80.

Babalola, O. O. (2016). Performance, nutrient digestibility, carcass analysis, and organoleptic assessment of snailets of African giant land snail (*Archachatina marginata*) fed diets containing graded levels of dried lettuce. Anim Feed Sci Technol. 216:169–175. DOI:10.1016/j.anifeedsci.2016.03.013.

Badan Pusat Statistik. 2022. Produksi daging itik/itik Manila menurut provinsi. [Accessed December 1st 2022]. Badan Pusat Statistik. https://www.bps.go.id/indicator/24/489/1/produksi-daging-itik-itik-manila-menurut-provinsi.html.

Chekol FA, Melak MF, Belew AK, Zeleke EG. 2019. Food handling practice and associated factors among food handlers in public food establishments, Northwest Ethiopia. BMC Res Notes. 12. DOI:10.1186/S13104-019-4047-0.

Dahimi O, Hassan MS, Rahim AA, Abdulkarim SM, Masitoh AS. 2014. Differentiation of lard from other edible fats by gas chromatography- flame ionisation detector (GC-FID) and chemometrics. J Food Pharma Sci. 2:27–31. DOI:10.14499/jfps.

Dinh TTN, Thompson LD, Galyean ML, Chance Brooks J, Boylan ML. 2012. Determination of total cholesterol in meat and poultry by gas chromatography: single-laboratory validation. J AoaC Int. 95. DOI:10.5740/jaoacint.11-224.

Eng S, Pusparajah P, Mutalib NA, Ser H, Lee L. 2015. *Salmonella*: A review on pathogenesis, epidemiology, and antibiotic resistance. Front Life Sci. 8:284-293. DOI:10.1080/21553769.2015.1051243.

Farghly MFA, Elsagheer MA, Jghef MM, Taha AE, El-hack MEA, Jaremko M., El-tarabily KA, Shabaan M. 2019. Consequences of supplementing duck's diet with charcoal on carcass criteria, meat quality, nutritional composition, and bacterial load. Poult Sci. 102:102275. DOI:10.1016/j.psj.2022.102275.

Genome M, Kang E, Crouse A, Chevallier L, Pontier SM, Alzahrani A, Silué N, Campbell-Valois, François-Xavier, Montagutelli, · Xavier, Gruenheid S, Malo D. 2018. Enterobacteria and host resistance to infection. Mammal Gen. 1:1–13. DOI:10.1007/s00335-018-9749-4.

Jakubczyk K, Kałduńska J, Kochman J, Janda K. 2020. Chemical profile and antioxidant activity of the kombucha beverage derived from white, green, black, and red tea. Antioxidants. 9:447. DOI:10.3390/antiox9050447.

James C, Dixon R, Talbot L, James SJ, Williams N, Onarinde BA. 2021. Assessing the impact of heat treatment of food on antimicrobial resistance genes and their potential uptake by other bacteria—A critical review. Antibiot. 10:1–16. DOI:10.3390/antibiotics10121440.

Kementerian Kesehatan Republik Indonesia. 2018. Tabel komposisi pangan indonesia 2017. In Izwardy D, Editor. Jakarta (Indones): Direktorat Jenderal Kesehatan Masyarakat Direktorat Gizi Masyarakat.

Lestari RD, Lokapirnasari WP, Al Arif MA, Hidanah S, Soeharsono S, Lamid M. 2021. The effect of additional feed fermentation of *Moringa oleifera* leaves on the cholesterol level of Mojosari laying ducks. J Medik Vet. 4:221. DOI:10.20473/jmv.vol4.iss2.2021.221-225.

Mardiana NA, Galih DP, Prayitno SA, Chotimah C. 2021. Physicochemical properties and sensory evaluation of fermented mustard with difference ratio of rice water and tal palm sap. Kontribusia. 5:15. DOI:10.30587/kontribusia.v5i1.2930.

Cardinal KM, Kipper M, Andretta I, Ribeiro AML. 2019. Withdrawal of antibiotic growth promoters from broiler diets: Performance indexes and economic impact. Poult Sci. 98 6659–6667. DOI:10.3382/ps/pez536.

Monakhova YB, Godelmann R, Andlauer C, Kuballa T, Lachenmeier DW. 2013. Identification of imitation cheese and imitation ice cream based on vegetable fat using NMR spectroscopy and chemometrics. Int J Food Sci. 2013:1–9. DOI:10.1155/2013/367841.

National Food Drug Agency. 2019. Peraturan Badan Pengawas Obat dan Makanan Nomor 13 Tahun 2019 tentang Batas maksimal cemaran mikroba dalam pangan olahan. Jakarta (Indones): Indonesian Drug and Food Control. p.1–48.

Nurmala I, Rachmawan O, Suryaningsih L. 2014. Pengaruh metode pemasakan terhadap komposisi kimia daging itik jantan hasil budidaya secara intensif. Students E-J. 3:1–10.

Olagunju A, Nwachukwu D. 2020. The differential effects of cooking methods on the nutritional properties and quality attributes of meat from various animal sources. Croatian J Food Sc Technol. 12:37–47. DOI:10.17508/CJFST.2020.12.1.06.

Provincial Health Services Authority. 2022. *Staphylococcus aureus* (food poisoning). [Accessed November 28th, 2022). BC Centre for Disease Control. http://www.bccdc.ca/health-info/diseases-conditions/staphylocococcus-aureus

Rai R, Bhattacharyya D, Praveen PK, Ganguly S, Dalai N, Shekhar S. 2016. Effect of different cooking procedures on the microbiological quality of Chevon meatballs. Asian J Anim Sci. 11:30–32. DOI:10.15740/HAS/TAJAS/11.1/30-32.

Shahidi F, Hossain A. 2022. Role of lipids in food flavor generation. Mol. 27. DOI:10.3390/molecules27155014.

Song S, Zheng F, Tian X, Feng T, Yao L, Sun M, Shi L. 2022. Evolution Analysis of free fatty acids and aroma-active compounds during tallow oxidation. Mol. 27. DOI:10.3390/molecules27020352.

Sugiharto S. 2022. Potential benefits of plant-derived products on broiler meat characteristics—A short review. J Sain Peternak Indones. 17:29–43. DOI:10.31186/jspi.id.17.1.29-43.

Tarwendah IP. 2017. Studi komparasi atribut sensoris dan kesadaran merek produk pangan. J Pangan Agroindust. 5:66–73.

Untari T, Herawati O, Anggita M, Asmara W, Endang Tri Wahyuni AETH, Wibowo MH. 2021. The effect of antibiotic growth promoters (AGP) on antibiotic resistance and the digestive system of broiler chicken in Sleman, Yogyakarta. BIO Web Conf. 33:04005. DOI:10.1051/bioconf/20213304005.

Xing Y, Wu X, Xie C, Xiao D, Zhang B. 2020. Meat quality and fatty acid profiles of Chinese Ningxiang pigs following supplementation with N-carbamylglutamate. Anim. 10:1–10. DOI:10.3390/ani10010088.

Zampelas A, Micha R. 2015. Antioxidants in health and disease. Antioxid Health Dis. 1–302. DOI:10.1201/b18539.

Molecular Characteristics and Evolutionary Relationships of Toll-Like Receptor (TLR21) of Indonesian KUB-1 Chicken

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ABSTRAK

Suryohastari RRB, Sumarsono SH, Rachman EAG. 2023. Karakteristik molekuler *toll-like receptor* (TLR21) Ayam KUB-1 dan hubungan kekerabatanya secara evolusionaris. JITV 28(4):274-286. DOI:http://dx.doi.org/10.14334/jitv.v28.i4.3266.

Pada hewan vertebrata, sistem imun memiliki *Toll-like receptor* (TLR) untuk mengenali pola molekul terkait patogen tertentu. Pada aves, TLR21 dikenal sebagai homolog fungsional dari TLR9 pada mamalia. Penelitian ini bertujuan untuk mengetahui karakteristik molekuler TLR21 pada ayam Kampung Unggul Balitbangtan (KUB-1) dan hubungan kekerabatannya secara evolusinaris. Gen *TLR21* diperoleh dari ekstraksi RNA ovarium dan disintesis menjadi cDNA melalui transkripsi terbalik. Kami menemukan bahwa cDNA *TLR21* ayam KUB-1 memiliki nukleotida sepanjang 3504 pasang basa (pb), yang termasuk *open reading frame* (ORF) 2823 pb, yang mengkodekan protein putatif dari 940 asam amino (aa). Protein TLR21 ayam KUB-1 hasil deduksi, dibangun dari 720 aa pada domain ekstrasel yang mengandung 20 LRR, 23 aa pada domain transmembran, dan 141 aa pada domain intrasel, serta memiliki berat molekul 107 kDa. TLR21 ayam KUB-1 masing-masing memiliki homologi sekitar 99%, 76%, dan 43% terhadap TLR21 ayam galur lain, unggas non-ayam, dan ikan. Sepanjang evolusi pada TLR21 ayam, karakter molekulernya masih dilestarikan sehingga TLR21 ayam KUB-1 tidak jauh berbeda dengan Broiler. Evolusi TLR21 telah terjadi pada itik dan angsa, meskipun fungsinya tetap sama. Secara evolusioner, TLR21 *Gallus gallus*_KUB-1 yang termasuk dalam Famili TLR11, telah terdiferensiasi dari TLR21 ikan dan membentuk hubungan parafiletik dengan TLR21 pada *Anser cygnoides* dan *Anas platyrhynchos*.

Kata Kunci: *Gallus gallus*, KUB-1, Ovarium, Transkripsi Terbalik, TLR21

ABSTRACT

Suryohastari RRB, Sumarsono SH, Rachman EAG. 2023. Molecular characteristics and evolutionary relationships of toll-like receptor (TLR21) of Indonesian KUB-1 chicken. JITV 28(4):274-286. DOI:http://dx.doi.org/10.14334/jitv.v28.i4.3266.

In vertebrates, the immune system has several Toll-like receptors (TLRs) to recognize specific pathogen-associated molecular patterns (PAMPs). In aves, TLR21 is known to be a functional homolog of TLR9 in mammals. This study aimed to determine the molecular characteristics of TLR21 in KUB-1 chicken, a superior breed of Indonesian local chicken, and their evolutionary relationship. The TLR21 gene was obtained from the ovarian RNA extraction and synthesized into cDNA by reverse transcription. We found that the cDNA sequence of the TLR21 of KUB-1 chicken has a length of 3504 bp, including a 2823 bp open reading frame (ORF), which encodes a putative protein of 940 amino acids (aa). The deduced KUB-1 chicken TLR21 protein consisted of 720 aa in the extracellular domain containing 20 LRRs, 23 aa in the transmembrane region, and 141 aa of Toll-IL-1 receptor in the intracellular domain, and had a molecular weight of 107 kDa. The KUB-1 chicken TLR21 had homology of about 99%, 76%, and 43% with other TLR21 from other breeds of chicken, non-chicken poultry, and fish, respectively. Its molecular character was conserved throughout the evolution of TLR21 in chicken, so the KUB-1 chicken was not much different from the broiler. However, there has been an evolution in duck and goose, although the function is still the same. Evolutionarily, *Gallus gallus*_KUB-1 TLR21, which belongs to the TLR11 Family, has differentiated from fish TLR21 and formed a paraphyletic relationship with TLR21 in *Anser cygnoides* and *Anas platyrhynchos*.

Key Words: *Gallus gallus*, KUB-1, Ovary, Reverse Transcription, TLR21

INTRODUCTION

The sensory receptors capable of connecting the environment with the immune system have been studied since the discovery of the Toll-like Receptors (TLRs) family. After the discovery of human TLRs, it was immediately followed by the characterization of mammalian TLRs, and since then, members of that family have also been found in birds (Keestra et al. 2013; Juul-Madsen HR. 2013). TLRs play a role in innate immunity with their function as pattern recognition receptors (Song et al. 2015) and are type I transmembrane glycoproteins (Zhang et al. 2016). Functional studies of TLRs detected their ability to recognize various Pathogen-associated molecular patterns (Ruan et al. 2015). The high level of evolutionarily conserved TLRs in vertebrates has allowed their phylogenetic classification into groups of receptors that respond to similar ligands. They can be divided into cell surface TLRs (TLR1, 2, 4, 5, and 15) and intracellular TLRs (TLR3, 7, and 21), which are localized in the endosome that recognizes nucleic acids from bacteria and viruses (Keestra et al. 2013; Nawab et al. 2019).

In chicken, TLR3, 4, 5, and 7 are close orthologs of TLRs in other vertebrates (Świderská et al. 2018), and TLR1, 6, and 10 in mammals are replaced by TLR1La/b and TLR 2a/2b from chicken are both orthologs of a single TLR2 in mammals (St. Paul et al. 2013). Avian TLR21 is an ortholog of the TLR21 of teleost (Lai et al. 2019). Cells transfected with the chicken TLR21 gene can recognize CpG-ODN, which plays the same role as TLR9 in mice (Yeh et al. 2013). Furthermore, TLR21 protein generally consists of 965-986 amino acids (aa), which includes a series of leucine-rich repeats (LRRs) in the extracellular domain (ECD), a transmembrane (TM) region, and a Toll-IL-1 receptor (TIR) on the intracellular domain (ICD) in the cytosol (Wu et al. 2018). The individual LRR consists of 20-30 aa (Matsushima et al. 2007). Based on ECD architecture, vertebrate TLRs are classified into eight families (Family 1/3/4/5/7/11/13/15), and TLR21 is a member of Family 11 (Wang et al. 2016).

Each TLR family is characterized by its LRR motifs that can be divided into highly conserved (LRRh) and variable (LLRv). The LRRh consists of LxxLxLxxNxL or LxxLxLxxCxxL motifs, where L residues at positions 1, 4, 6, and 11 participate in the hydrophobic core (Matsushima et al. 2015). The chicken TLR21 has a "typical type" (LRR-Typ) at LRR2/3/14/15/15/17 (Li et al. 2018). The LRR domain adopts an arc shape that plays a role in pathogen recognition and binding. Most LRR structures have a cap consisting of two or four cysteines to protect the hydrophobic core (Mokhtari et al. 2021). The TIR of chicken TLR21 contains 125-200 residues required for protein-protein interactions and the conserved sequence at residue 778-943 (Wu et al. 2018). The TIR transmits signals to the cytosol by recruiting the adapter protein responsible for producing pro-inflammatory cytokines (Wicherska-pawłowska et al. 2021). The genetics of chicken breeds significantly influence the production of pro-inflammatory cytokines in response to the challenge of *Salmonella* (Kaiser et al. 2022).

Salmonella can contaminate chicken eggs (Popa & Popa 2021). Transovarial transmission can be through *Salmonella* colonization in the ovaries, a site of innate immune activation (Wigley 2014). The primary understanding for studying innate immunity and its relationship to host defense is regarding TLRs as ancient and evolved pattern recognition receptors (Fitzgerald & Kagan 2020). The reproductive tract contains an innate immune system with various TLRs, and TLR21 in chicken is known to be a functional homolog of TLR9 in mammals (Rehman et al. 2021). The genetics of the chicken breed strongly influence the immune response of chicken to Salmonella challenges (Kaiser et al. 2022). Indonesian local chicken have 32 breeds (Winaya et al. 2023) that are the result of the domestication of *Red junglefowl* and an important asset for the formation of superior breeds because of their ability to adapt well (Tamzil & Indarsih 2022). This study used KUB-1 chicken, which has been designated as a superior strain through the Decree of the Minister of Agriculture of the Republic of Indonesia No. 274/kpts/SR.120/2/2014, with superior characteristics in disease resistance (Sartika & Iskandar 2019). Therefore, this study aimed to characterize the molecular of TLR21 in KUB-1 chicken and their evolutionary relationships.

MATERIALS AND METHODS

Ethics statement

The protocol for tissue sampling of the reproductive tract in KUB-1 chicken in this study was approved by the Ethics Commission of the Institut Teknologi Bandung, Indonesia, through the issuance of an Ethics Decree Number 02/KEPHP-ITB/10-2019.

Sample collection

RNA samples were taken from the ovaries of reproductive adult female KUB-1 chicken that had laid eggs at least five times—collection samples from necropsied chicken with dissecting equipment. Hen ovary samples were typically taken from a tiny yellow follicle, which was then collected in cryotubes and stored at -80 °C.

RNA extraction

KUB-1 chicken ovaries stored at -80 C were removed from the freezer and waited until defrosted. Each ovary was sliced and weighed to obtain 500 mg of tissue for RNA extraction. Total RNA was extracted using the Quick-RNA Miniprep Plus Kit (R1058, Zymo Research) according to the manufacturer's protocol. NanoPhotometer® N60/N50 Microvolume Spectroscopy (Implen) was used to measure the quality and quantity of total RNA. The product from the RNA extraction was stored at -80 °C.

Reverse transcription

Total RNA was synthesized into cDNA by reverse transcription using the SensiFAST™ cDNA Synthesis

Table 1. PCR primer sequences for three fragments of the TLR21 gene in KUB-1 chicken

Primer	Sequence	TA (°C)	Size (bp)
1st Fragment	F1: TCC CAC TGC TGT CCA CTC R1: CCA GGC AAG ACG GCA GTT	58	1136
2nd Fragment	F2: GAG CTG GAC CTG TCC TGG AA R2: CAA AGC TGT CAT AGA GGT AGC A	61	1417
3rd Fragment	F3: AGT GCT GCT GCT GCT GGT R3: GAA ACT CTG TTC TGG GGA AGA	58	1343

Kit (Bioline®) according to the manufacturer's protocol. PCR was carried out with a Thermal Cycler T100™ machine (Bio-Rad). Reverse transcription conditions were set at 25 °C for 10 minutes for primer annealing, 42 °C for 15 minutes for reverse transcription, 85 °C for 5 minutes for inactivation, and 4 °C for hold. The cDNA was stored at -20 °C.

PCR amplification *TLR21* gene and sequencing

The PCR method used three pairs of primers designed based on the *Gallus gallus TLR21* sequence (NM_001030558.3). The primary design used Primer3 Plus (https://www.bioinformatics.nl/cgi-bin/primer 3plus/primer3plus.cgi) and Oligocalculator (http://biotools.nubic.northwestern.edu/OligoCalc.html). Primers were synthesized by Macrogen (Korea) with specific sequences and annealing temperatures in PCR (Table 1). PCR was run for 35 cycles with setting conditions of 95 °C for 15 seconds, 58 °C/ 61 °C for 15 seconds, and 72 °C for 10 seconds. The PCR reaction mixture used a MyTaq HS Red Mix master mix (Bioline®) with a protocol according to the manufacturer's instructions. Sequencing in this study was carried out by sending 30 µl amplicons for each KUB-1 chicken TLR21 gene fragment to Macrogen (Korea), along with 10 µl of F-primers and 10 µl of R-primers for each fragment, until Macrogen sent nucleic acid data from each fragment.

Visualization of *TLR21* fragments of KUB-1 chicken

Amplicons of the TLR21 gene were electrophoresed on 2% agarose gel at 100 volts for 35 minutes and documented by Syngene InGenius³ (Synoptic Ltd). The electropherogram of the TLR21 gene of KUB-1 chicken was read based on a 1 Kb DNA Ladder (Geneaid®).

Bioinformatics analysis

After obtaining three fragments of the TLR21 gene, the nucleic acid sequence data were processed by a bioinformatics device, trimming the nucleic acid sequence of each TLR21 fragment by Chromas version 2.6.6 (https://technelysium.com.au/wp/). BioEdit made nucleic acid sequence consensus for each TLR21 fragment—assembly for nucleic acid sequences from three KUB-1 chicken TLR21 gene fragments by Geneious Prime 2020. Furthermore, the homology of the KUB-1 chicken TLR21 nucleic acid sequence was analyzed for identity by BLAST (https://blast.ncbi.nlm.nih.gov/Blast .cgi). The orf-finder obtained the ORF area (https://www.ncbi.nlm.nih.gov/orffinder). Then, the TLR21 amino acid sequences were obtained using the ExPASy Translate tool (https://www.expasy.org/). Prediction of KUB-1 chicken TLR21 protein domain structure was made by ScanProsite (https://prosite.expasy.org/scanprosite). TLR21 amino acid sequences of chicken KUB-1 were reassigned through Multiple sequences alignment (MSA) with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). The characterization of the KUB-1 chicken TLR21 included analysis to determine the amino acid sequence and the characteristics of LRRs, a TM region, TIR, hydrophobicity, solubility, molecular weight (MW), and isoelectric point (pI) of the deduced TLR21 protein. *Neighbor-Joining* constructed the phylogenetic tree with 1000x bootstrap at MEGA7.

RESULTS AND DISCUSSION

In this study, the molecular characterization of the KUB-1 chicken TLR21 gene was obtained based on the analysis of the nucleic acid sequence of cDNA TLR21 in the ovary of KUB-1 chicken and based on the amino acid sequence of the deduced protein. The evolutionary relationship of KUB-1 chicken TLR21 protein was obtained from phylogenetic analysis.

Molecular characteristic of TLR21 of KUB-1 chicken

The KUB-1 chicken used in this study was female and reproductively mature, with the character determined by the Indonesian Ministry of Agriculture based on the Decree of the Minister of Agriculture of the Republic of Indonesia No. 274/kpts/SR.120/2/2014

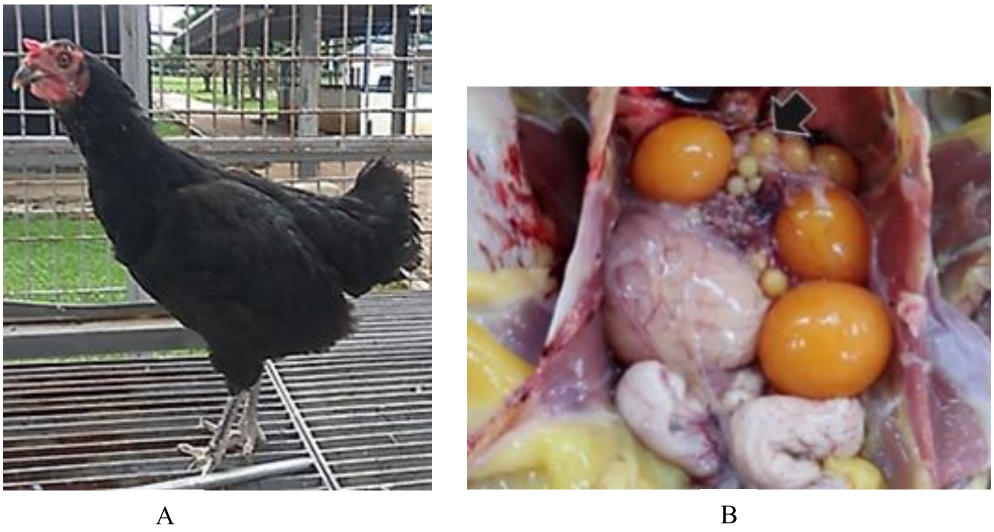


Figure 1. Female KUB-1 Indonesian local chicken has primarily black feathers, a yellow to black beak, gray to black legs, an oval head shape, a single comb, and pea-shaped (A), the ovaries in the visceral abdomen of KUB-1 chicken, the SYF (marked with a black arrow) for RNA extraction (B)

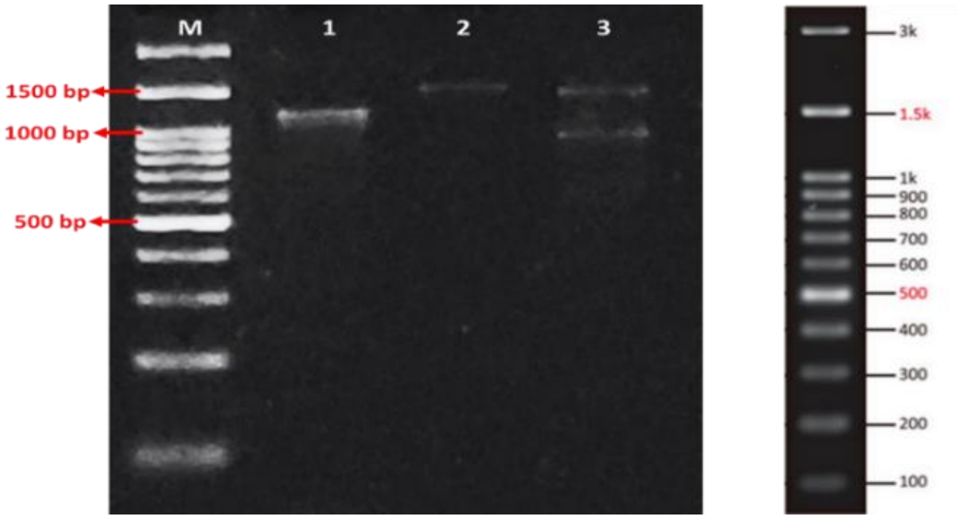


Figure 2. Electropherogram of three fragments in *TLR21* gene of KUB-1 chicken. Electrophoresis was carried out in 2% agarose gel. M= Marker (DNA Ladder 100 bp), 1= 1st fragment (1136 bp), 2= 2nd fragment (1417 bp), and 3= 3rd fragment (1343 bp)

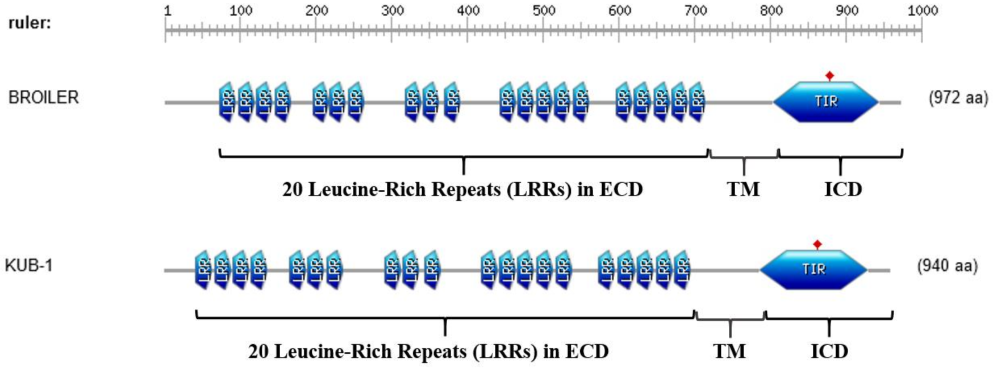


Figure 3. Characteristics of the chicken TLR21 protein profile**.** The deduced KUB-1 chicken TLR21 protein is composed of 940 amino acids. It has an extracellular domain profile containing 20 LRRs and one TIR with an active site (red diamond) in the intracellular domain, as well as broiler chicken TLR21, which is composed of 970 amino acids

(Figure 1A). The total RNA was obtained from the ovaries of KUB-1 chicken with the criteria for small and yellow ovaries (Figure 1B). Selection of ovaries with small yellow follicles (SYF) used for RNA extraction, as reported by Chen et al. (2021) that the total number of differentially expressed genes from SYF was much greater than large white follicles (LWF) and large yellow follicles (LYF). Nie et al. (2022) reported that in the ovary, there are several pre-hierarchical follicles and one hierarchical follicle at each developmental stage, and the color of the follicles gradually changes from white to yellow.

Furthermore, the measurement of RNA concentration with a nanodrop spectrophotometer obtained total RNA quality that complies with the requirements for cDNA synthesis through reverse transcription. The PCR amplification obtained three amplicons from the first, second, and third fragments in the KUB-1 chicken TLR21 gene. The electropherogram (Figure 2) showed that the length of the first fragment was 1136 bp, the second was 1417 bp, and the third was 1343 bp. A single band was obtained at the first and second fragments of the KUB-1 chicken TLR21 gene, while a double band was in the third fragment. However, the amplicons in the upper position matched the third primer target (1343 bp). In primer design, sequences with active binding sites can lead to the appearance of double bands; this is consistent with the results of ScanProsite from KUB-1 chicken TLR21, that in the third fragment, there is a TIR domain containing the active site at the residue of 847. The upper band in lane 3 will continue the sequencing process. Assembling three fragments of the KUB-1 chicken TLR21 gene, a nucleic acid sequence of 3504 bp was obtained.

Subsequently, analysis for the open reading frame (ORF) region (https://www.ncbi.nlm.nih.gov/orffinder) showed that the 3504 bp nucleic acid sequence in KUB-1 chicken TLR21 gene contained 2823 bp ORF encoded 940 aa, while in broiler chicken TLR21 (NM_001030558.3) contained 2919 bp ORF that encoding 972 aa. The prediction of protein domain structure using ScanProsite Expasy (https://prosite.expasy.org/scanprosite/) showed that in the KUB-1 chicken TLR21 and broiler chicken TLR21 (NP_001025729.3), both had extracellular domain containing 20 LRRs—illustration for the domain profile of chicken TLR21 protein as shown in Figure 3.

From the ScanProsite, the location and sequence of amino acids in each LRR and TIR can be known, but the information on the TM region cannot be obtained. The results of the analysis of the TM region of KUB-1 chicken TLR21 protein based on HMMTOP (http:// www.enzyme.hu/hmmtop/html/submit.html) found that the number of TM was only one, the length was 23 aa (LGLYLFAGTAPAVLLLLVVPVVY), and the location of the transmembrane helix sequence was in residue 721-743. The transmembrane Prediction server (https://tmdas.bioinfo.se/DAS/index.html) for the Dense Alignment Surface (DAS) curve of KUB-1 chicken TLR21 (Figure 4) illustrates the location of the TM region of this protein. Based on the results of ScanProsite on 940 aa of KUB-1 chicken TLR21 and the result of the analysis of the TM region, the amino acid sequences at LRR1–LRR20 in the ECD, TM, and TIR with the binding site in the ICD can be seen in Table 2.

It is known from Table 2 that each LRR of KUB-1 chicken TLR21 consists of 22-24 aa, where LRR17 contains 24 aa, while the other LRR consists of 22 aa. This result is consistent with the previous study (Wang et al. 2016) that each LRR module has 12-25 aa. The LRR structure with a conserved xLxxLxxLxLxxNxLxxLPxxxFx motif has a hydrophobic core, asparagine network, and variable region as reported by Manavalan et al. (2011) and illustrated as shown in Figure 5.

Each LRR of KUB-1 chicken TLR21 (Table 2) was observed to have an N (asparagine) residue, as reported by Wang et al. (2016) that among the LRR modules, there is a conserved asparagine which plays a vital role in maintaining the shape of the ECD by forming a continuous hydrogen bond network and forming the asparagine ladder. Asparagine can be replaced by other amino acids capable of donating hydrogens, such as threonine, serine, and cysteine. Furthermore, asparagine contained in each LRR of KUB-1 chicken TLR21 has an intact asparagine ladder on the concave surface of the adjacent LRR module to stabilize the overall shape of the ECD responsible for ligand recognition. The ECD of single-domain TLRs with intact asparagine ladders allows for binding to nucleic acids or hydrophilic proteins.

Furthermore, Matsushima et al. (2007) and Batkhishig et al. (2020) reviewed that LRR has a highly conserved segment (LRRh) and a variable segment (LLRv), where the LLRh motif is LxxLxLxxNxL or LxxLxLxxNxxL or LxxLxLxxCxxL. The LRRh motif at KUB-1 chicken TLR21 was found in LRR2 (QLHTLDLTYNLLETLSPGAFNG). In LRR3, 14-17 KUB-1 chicken TLR21 also has the LRRh motif. These results are similar to the previous studies (Li et al. 2018) that found that LRR2, LRR3, and LRRs14-17 have an LRR Typ. Meanwhile, Wu et al. (2018) reported that LRRs 14-18 had relatively higher conserved scores than other LRR motifs, implying that they have a role in protein-protein interfaces for dimerization or binding to other members of the TLR family. Matsushima et al. (2007) reported that other hydrophobic amino acids can replace the conserved L residue. Meanwhile, LRR4 (NLSSLQVQHNPLSTVSPSALLP) and LRR10 (KLEVLTVEHNLLKKLPSCLGAQ) in the L residue are replaced by V (Val), in which the "L" residue is Leu, Ile, Val, or Phe. Four L residues at amino acids at position.

Table 2. Amino acid positions and sequences in the domain of KUB-1 chicken TLR21

Domain	LRR	Aa position	Aa Sequences
ECD	1	42 – 63	HAIALNLSYNKMRCLQPSAFAH
	2	66 – 87	QLHTLDLTYNLLETLSPGAFNG
	3	90 – 111	VLVVLDLSHNKLTTLAEGVFNS
	4	114 – 135	NLSSLQVQHNPLSTVSPSALLP
	5	164 – 185	QLELLDLCENNLTTLGPGPPLP
	6	187 – 208	SLLTLQLCNNSLRELAGGSPEM
	7	211 – 232	HVKILDLSYNSISQAEVFTQLH
	8	286 – 307	ALRRLQLQRNGLKVLHCNALQL
	9	310 – 331	VLRELDLSWNRLQHVGCAGRLL
	10	338 – 359	KLEVLTVEHNLLKKLPSCLGAQ
	11	411 – 432	NLTELRLDNNLLTDLYHNSFID
	12	435 – 456	RLRTLNLRNNRVSVLFSGVFQG
	13	459 – 480	ELQTLDLGGNNLRHLTAQSLQG
	14	483 – 504	KLRRLYLDRNRLLEVSSTVFAP
	15	508 – 529	TLGVLDLRANNLQYISQWLRKP
	16	564 – 585	RLQQLSLSQNMLRSIPPDVFED
	17	588 – 611	QLRSLALadSSNGLHDLPDGIFRN
	18	614 – 635	NLRFLDLENAGLHSLTLEVFGN
	19	638 – 659	RLQVLHLARNELKTFNDSVASR
	20	662 – 683	SLRYLDLRKCPLSCTCDNMWLQ
TM		721 – 743	LGLYLFAGTAPAVLLLLVVPVVY
ICD	TIR	772 – 912	YLYDSFVSYNSADESWVLQKLVP ELEHGAFRLCLHHRDFQPGRSIIDN IVDAVYNSRKTVCVXSRSYLRSEW CSLEVQLAsYRLLDERRDILVLVLL EDVGDAELSAYHRMRRVLLRRTY LRWPLDPAAQPLFWARLKRAL

Conserved sequences in the TIR domain are marked in the box. ECD= extracellular domain, LRR= leucine-rich repeat, TM= transmembrane, ICD= intracellular domain, TIR= Toll-IL-1 receptor

positions 1, 4, 6, and 11 participate in the hydrophobic core in the LRR arc. The LRRv motif in KUB-1 chicken TLR21 was thought to be present in LRR1 (HAIALNLSYNKMRCLQPSAFAH) and LRR20 (SLRYLDLRKCPLSCTCDNMWLQ) consistent with Matsushima et al. (2007) that the first LRR in the N-terminus (LRR1) is often irregular, for example, (L/x)xx(L/A)xCxx(L/R)xLxxVPxxIPxx, and most LRR structures have closures containing two or four cysteine residues to protect the hydrophobic core from the first LRR at the N-terminus (LRRNT) or the last LRR at the C-terminus (LRRCT).

The TIR of KUB-1 chicken TLR21 is located at residues 772-912, with the active site at the amino acid position 847 (marked by E in bold in the TIR sequence). The number of amino acids that comprise the TIR in KUB-1 chicken TLR21 consists of 141 aa. This result is similar to the TIR domain structure in chicken TLR21 predicted by Wu et al. (2018) that there is about 125-200 aa in the TIR domain, which is required for protein-protein interactions and the position of the 778-943 residue forms a highly conserved structure in the cytoplasmic region of chicken TLR21. The results of this study, in the TIR domain of KUB-1 chicken TLR21, obtained three conserved sequences: YDSFVSYN, LCLHHRDFQPGR, and LFWAR, whose sequences were marked in boxes. A previous Li et al. (2018) study reported that the conserved sequences in boxes 1 and 2 mediate receptor molecules in signal transduction pathways. In contrast, the function of the sequences in box 3 is to control the subcellular location of these receptors. Meanwhile, Chuang et al. (2020) also reported three conserved sequences in the TIR domain of the TLR, in which TIR-conserved sequences for mammalian TLR signaling were also conserved in the TLR21.

Based on previous data that KUB-1 chicken TLR21 protein had a hydrophobic core region in each LRR, the hydrophobicity analysis (https://fasta.bioch.virginia.edu/fasta_www2/fasta_www.cgi?rm=misc1) and solubility analysis (https://protein-sol.manchester.ac.uk/) were carried out by the Kyle-Dolittle plot on the hydropathy curve of KUB-1 chicken TLR21 protein (Figure 6A). The characteristics of the ECD and the TM region of KUB-1 chicken TLR21 tend to be hydrophobic, while the TIR domain tends to be hydrophilic. The results of the solubility scale for KUB-1 chicken TLR21 protein was 0.208 with a solubility value <0.45 (Figure 6B) that was predicted to be insoluble in E. coli (assessed to have a low probability of being expressed in E. coli). Hebditch et al. (2017) described this result as the solubility value scale (QuerySol), the predicted solubility. The population means for the experimental dataset (PopAvrSol) was 0.45. Therefore, a scaled solubility value greater than 0.45 was predicted to have a higher solubility than the mean from the experimental solubility dataset of *E.coli*-soluble protein, and any protein with a scale solubility lower than 0.45 was expected to be less soluble. In addition, the analysis of molecular weight (MW) and isoelectric point (pI) (https://web.expasy.org/ compute_pi/) showed that KUB-1 chicken TLR21 protein has 9.09 pI and 106,947.22 Da of MW.

Furthermore, the multiple sequence alignments (MSA) for amino acids sequences using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) that retrieved the TLR sequences from the database in the Protein Database (PDB) obtained the homology of the tertiary structure of KUB-1 chicken TLR21 protein vs. other vertebrate TLRs, and the top three results are shown at

Table 3. BLAST results of KUB-1 chicken TLR21 protein sequences aligned with sequences in the Protein Database (PDB)

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
TLR7 [*Homo sapiens*]	*Homo sapiens*	162	162	67%	4e-40	28.02%	1049	7CYN_A
TLR13-ssRNA13 complex [*Mus musculus*]	*Mus musculus*	155	155	75%	1e-38	29.61%	709	4Z0C_A
TLR5 fitted into an electron microscopy [*Homo sapiens*]	*Homo sapiens*	137	191	64%	1e-32	26.99%	844	3J0A_A

Table 4. BLAST result of KUB-1 chicken TLR21 protein sequence

Description	Query Cover	E value	Per. Ident	Acc. Len	Accession
TLR21 *Gallus gallus* breed Beijing White 939	97%	0.0	99.89%	972	AFD61602.1
TLR21 *Gallus gallus* (broiler >< layer, bGalGal1)	97%	0.0	99.89%	967	XP_040562958.1
TLR21 *Gallus gallus* (broiler.GRCg7b)	97%	0.0	99.89%	972	NP_001025729.3
TLR21 *Gallus gallus* breed White Leghorn	97%	0.0	99.78%	972	AFD61603.1
TLR21 *Gallus gallus* breed White-Feather Silky	97%	0.0	99.45%	972	AFD61606.1
TLR21 *Gallus gallus* breed Nongda No.3	97%	0.0	99.56%	972	AFD61605.1
TLR21 *Anas platyrhynchos*	97%	0.0	75.90%	976	AUO17544.1
TLR21 *Anser cygnoides*	97%	0.0	76.89%	976	AMB20882.1
TLR21 *Danio rerio*	97%	0.0	43.66%	989	CAQ13807.1
TLR21 *Cyprinus carpio*	98%	0.0	43.96%	964	AVX48323.1

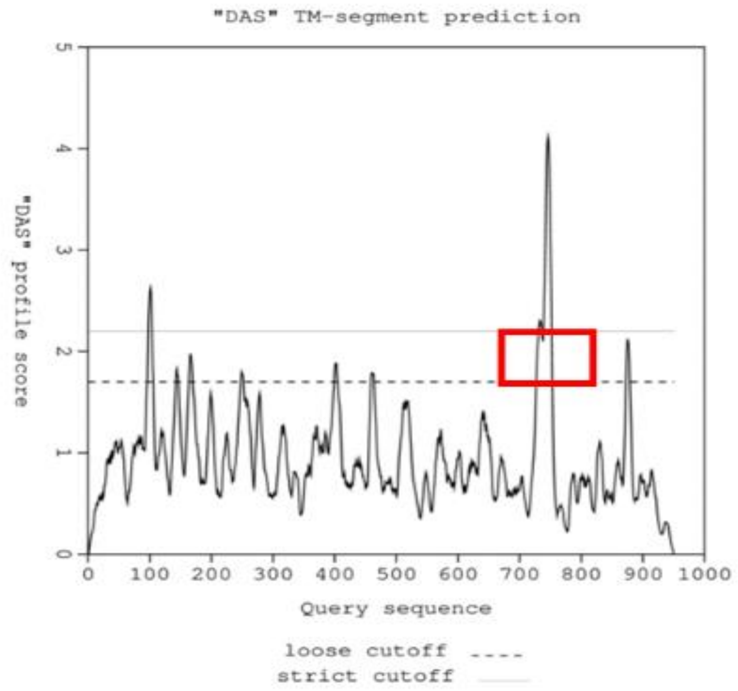


Figure 4. DAS curve of KUB-1 chicken TLR21 protein with a red box in the curve indicates the TM region. This curve is obtained by pairwise comparison of the proteins in the test set in an "each against the rest" fashion. Two cutoffs are on the plots: a "strict" one at 2.2 DAS score and a "loose" one at 1.7. The hit at 2.2 is informative in terms of the number of matching segments, while a hit at 1.7 gives the actual location of the TM segment

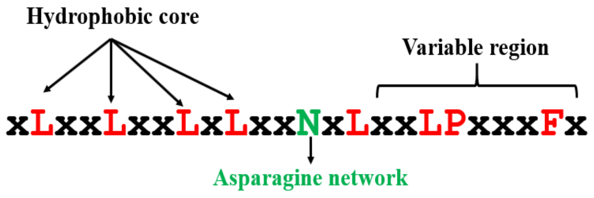


Figure 5. Consensus for LRR. Amino acid residues form a hydrophobic core, asparagine ladder, and variable regions. L, P, and F for Leucine, Proline, and Phenylalanine in red = hydrophobic, N for Asparagine in green = hydroxyl/amine/base, and x for any amino acid

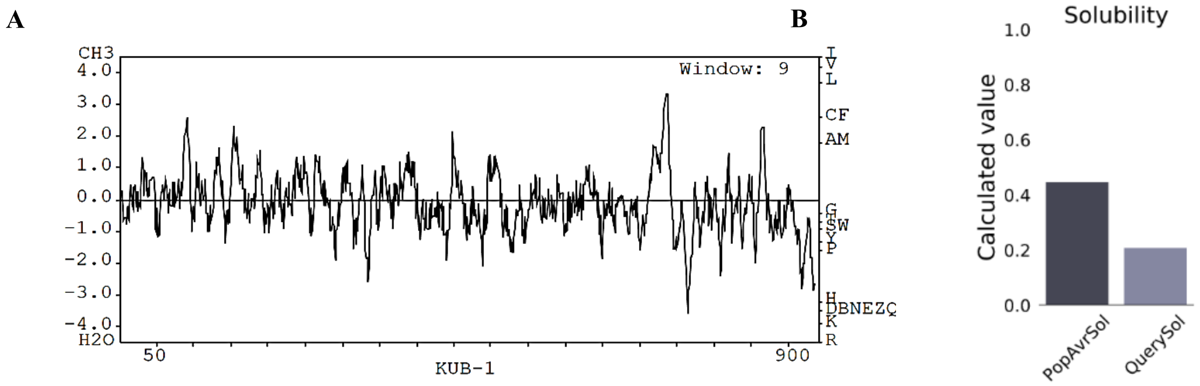
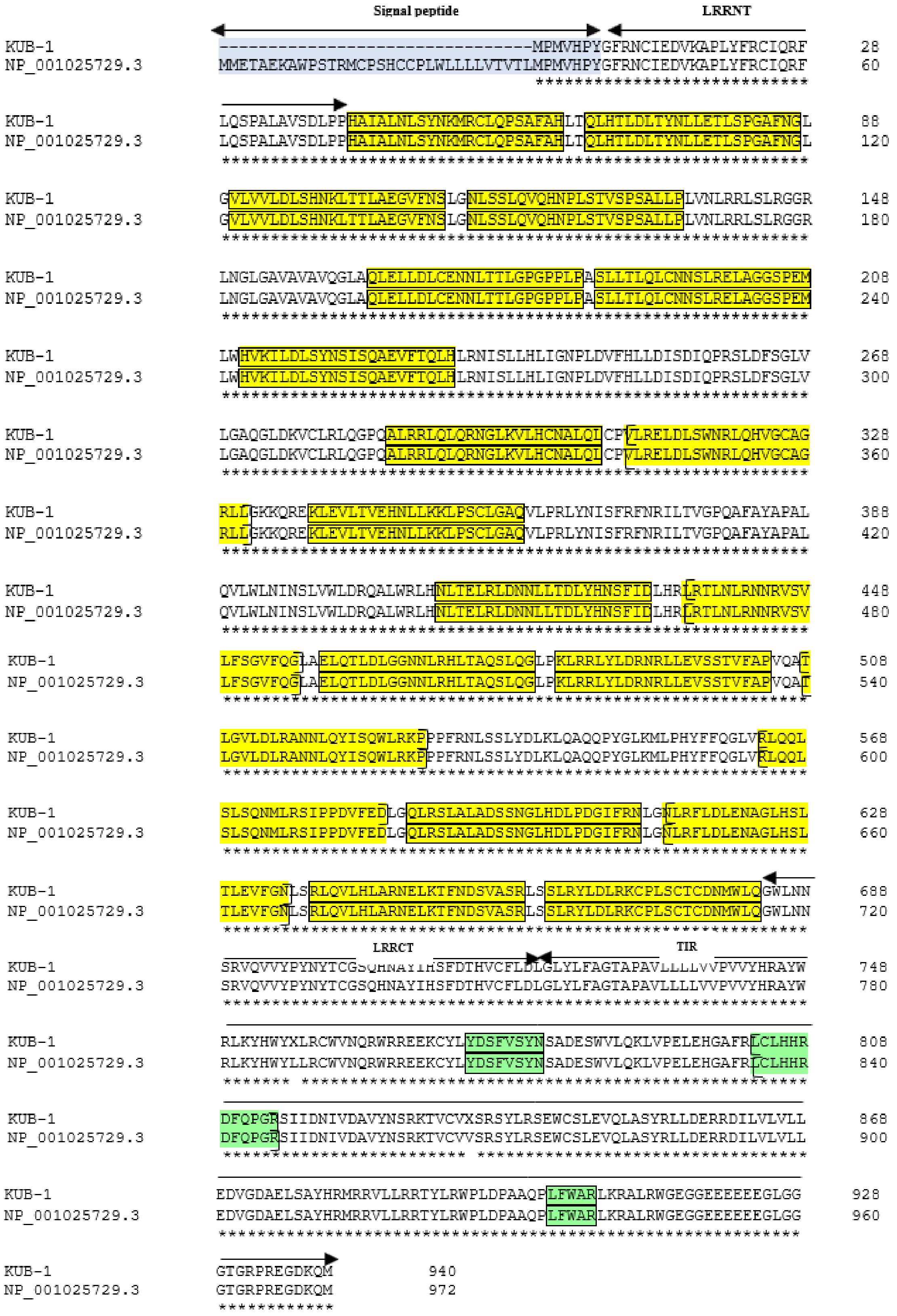


Figure 6. Predicted hydrophobicity and solubility of KUB-1 chicken TLR21 protein. KUB-1 chicken TLR21 protein tends to be hydrophobic (A); the predicted solubility scale for KUB-1 chicken TLR21 protein was 0.208, with a solubility value <0.45 (B)



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KUB-1             --------------------------------MPMVHPYGFRNCIEDVKAPLYFRCIQRF    28
NP_001025729.3    MMETAEKAWPSTRMCPSHCCPLWLLLLVTVTILMPMVHPYGFRNCIEDVKAPLYFRCIQRF    60
                                                  ****************************

KUB-1             LQSPALAVSDLPPHAIALNLSYNKMRCLQPSAFAHLTQLHTLDLTYNLLETLSPGAFNGL    88
NP_001025729.3    LQSPALAVSDLPPHAIALNLSYNKMRCLQPSAFAHLTQLHTLDLTYNLLETLSPGAFNGL    120
                  ************************************************************

KUB-1             GVLVVLDLSHNKLTTLAEGVFNSLGNLSSLQVQHNPLSTVSPSALLPLVNLRRLSLRGGR    148
NP_001025729.3    GVLVVLDLSHNKLTTLAEGVFNSLGNLSSLQVQHNPLSTVSPSALLPLVNLRRLSLRGGR    180
                  ************************************************************

KUB-1             LNGLGAVAVAVQGLAQLELLDLCENNLTTLGPGPPLPASLLTLQLCNNSLRELAGGSPEM    208
NP_001025729.3    LNGLGAVAVAVQGLAQLELLDLCENNLTTLGPGPPLPASLLTLQLCNNSLRELAGGSPEM    240
                  ************************************************************

KUB-1             LWHVKILDLSYNSISQAEVFTQLHLRNISLLHLIGNPLDVFHLLDISDIQPRSLDFSGLV    268
NP_001025729.3    LWHVKILDLSYNSISQAEVFTQLHLRNISLLHLIGNPLDVFHLLDISDIQPRSLDFSGLV    300
                  ************************************************************

KUB-1             LGAQGLDKVCLRLQGPQALRRLQLQRNGLKVLHCNALQLCPVLRELDLSWNRLQHVGCAG    328
NP_001025729.3    LGAQGLDKVCLRLQGPQALRRLQLQRNGLKVLHCNALQLCPVLRELDLSWNRLQHVGCAG    360
                  ************************************************************

KUB-1             RLLGKKQREKLEVLTVEHNLLKKLPSCLGAQVLPRLYNISFRFNRILTVGPQAFAYAPAL    388
NP_001025729.3    RLLGKKQREKLEVLTVEHNLLKKLPSCLGAQVLPRLYNISFRFNRILTVGPQAFAYAPAL    420
                  ************************************************************

KUB-1             QVLWLNINSLVWLDRQALWRLHNLTELRLDNNLLTDLYHNSFIDLHRLRTLNLRNNRVSV    448
NP_001025729.3    QVLWLNINSLVWLDRQALWRLHNLTELRLDNNLLTDLYHNSFIDLHRLRTLNLRNNRVSV    480
                  ************************************************************

KUB-1             LFSGVFQGLAELQTLDLGGNNLRHLTAQSLQGLPKLRRLYLDRNRLLEVSSTVFAPVQAT    508
NP_001025729.3    LFSGVFQGLAELQTLDLGGNNLRHLTAQSLQGLPKLRRLYLDRNRLLEVSSTVFAPVQAT    540
                  ************************************************************

KUB-1             LGVLDLRANNLQYISQWLRKPPPFRNLSSLYDLKLQAQQPYGLKMLPHYFFQGLVRLQQL    568
NP_001025729.3    LGVLDLRANNLQYISQWLRKPPPFRNLSSLYDLKLQAQQPYGLKMLPHYFFQGLVRLQQL    600
                  ************************************************************

KUB-1             SLSQNMLRSIPPDVFEDLGQLRSLALADSSNGLHDLPDGIFRNLGNLRFLDLENAGLHSL    628
NP_001025729.3    SLSQNMLRSIPPDVFEDLGQLRSLALADSSNGLHDLPDGIFRNLGNLRFLDLENAGLHSL    660
                  ************************************************************

KUB-1             TLEVFGNLSRLQVLHLARNELKTFNDSVASRLSSLRYLDLRKCPLSCTCDNMWLQGWLNN    688
NP_001025729.3    TLEVFGNLSRLQVLHLARNELKTFNDSVASRLSSLRYLDLRKCPLSCTCDNMWLQGWLNN    720
                  ************************************************************

KUB-1             SRVQVVYPYNYTCGSQHNAYIHSFDTHVCFLDLGLYLFAGTAPAVLLLLVVPVVYHRAYW    748
NP_001025729.3    SRVQVVYPYNYTCGSQHNAYIHSFDTHVCFLDLGLYLFAGTAPAVLLLLVVPVVYHRAYW    780
                  ************************************************************

KUB-1             RLKYHWYXLRCWVNQRWRREEKCYLYDSFVSYNSADESWVLQKLVPELEHGAFRLCLHHR    808
NP_001025729.3    RLKYHWYLLRCWVNQRWRREEKCYLYDSFVSYNSADESWVLQKLVPELEHGAFRLCLHHR    840
                  ******* ****************************************************

KUB-1             DFQPGRSIIDNIVDAVYNSRKTVCVXSRSYLRSEWCSLEVQLASYRLLDERRDILVLVLL    868
NP_001025729.3    DFQPGRSIIDNIVDAVYNSRKTVCVVSRSYLRSEWCSLEVQLASYRLLDERRDILVLVLL    900
                  ************************* **********************************

KUB-1             EDVGDAELSAYHRMRRVLLRRTYLRWPLDPAAQPLFWARLKRALRWGEGGEEEEEEGLGG    928
NP_001025729.3    EDVGDAELSAYHRMRRVLLRRTYLRWPLDPAAQPLFWARLKRALRWGEGGEEEEEEGLGG    960
                  ************************************************************

KUB-1             GTGRPREGDKQM        940
NP_001025729.3    GTGRPREGDKQM        972
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Figure 7. MSA Clustal Omega of KUB-1 chicken TLR21 to broiler TLR21 (NP_001025729.3). The LRR1–LRR20 is highlighted in yellow, and three conserved sequences in the TIR domain are highlighted in green

Table 3. The result showed that the deduced protein of KUB-1 chicken TLR21 had a homology of ~30% against *M. Musculus* TLR13, *H. sapiens* TLR7, and *H. sapiens* TLR5. Wang et al. (2016) reported that the chicken TLR21 has a single-domain architecture which is also shared by TLR5 (Family 5), TLR7 (Family 7), and TLR13 (Family 11). In addition, the results of MSA Clustal Omega (Figure 7) showed that in the middle of the KUB-1 chicken LRR sequence, there was a region containing 51 residues between LRR10 and LRR11. Matsushima et al. (2007) reported that the TLR in Family 7 includes a region containing 58-73 residues in the middle part of the LRR domain, where the middle has a vital role in the structure and function of the TLR. Regarding the homology to TLR13, Wu et al. (2018) reported that the number of LRR motifs in *G. gallus* TLR21 had similarities with *M. musculus* TLR13 and *M. musculus* TLR9, where the individual LRR consisted of about 22-33 residues, except for the LRRCT and LRRNT sequences in the ECD of chicken TLR21. Then, structural comparison of the ECD and the TIR domain in the TLR was reported by Wu et al. (2018) that in the chicken TLR21, there is no Z-loop between the LRR14 and LRR15 modules in the center of the chicken TLR21 ECD, which is very different from the crystal structure of the human TLR9 ECD (PDB code: 3wpc), but similar to the TLR13 ECD (PDB code: 4z0c). Therefore, although chicken TLR21 is a mammalian TLR9 homology, the structural biology of the chicken TLR21 ECD structure does not have a Z-loop as found in mice TLR9. Subsequently, the homology of the amino acids sequence of KUB-1 chicken TLR21 against other TLR21 based on the BLAST result showed that the deduced protein of KUB-1 chicken TLR21 had homology of about 43%, 76%, and 99% against fish TLR21, non-chicken poultry TLR21, and other breed chicken TLR21 respectively, as shown in Table 4.

Phylogenetic analysis of TLR21 protein of KUB-1 chicken

To determine the relationship between KUB-1 chicken TLR21 protein and other vertebrate TLRs, the amino acid sequence data from Table 3 and Table 4 were used in the phylogenetic analysis. A phylogram (Figure 8) was constructed for the KUB-1 chicken TLR21 amino acid sequence for evolutionary kinship with the vertebrate TLRs. The result shows that *Gallus galus*_KUB-1 TLR21 and *Mus musculus* TLR13 are in one cluster. This result is consistent with the phylogenetic analysis in a previous study that TLR21 and TLR13 were classified in Family 11 (Wang et al. 2016) due to both of them having an intact asparagine ladder in single domain architecture at the ECD of their LRRs.

Subsequently, to determine the evolutionary kinship of KUB-1 chicken TLR21 against TLR21 from duck, goose, fish, and other breeds of chicken, the data in Table 4 were used in the phylogenetic analysis, and the phylogram (Figure 9) shows that the KUB-1 chicken TLR21 is an ortholog of the TLR21 fish (*Danio rerio* and *Cyprinus carpio*). As reported in a previous study by Lai et al. (2019), this result shows that chicken TLR21 is the ortholog of TLR21 in fish and amphibians. Meanwhile, Rehman et al. (2021) reported that the paralogue in the chicken TLR comes from gene duplication, and the chicken TLR only has two paralogs. Due to TLR21 being a non-mammal TLR type, this phylogenetic analysis was not compared to the mammalian TLR21 reported by Priyathilaka et al. (2014). The phylogenetic analysis showed that TLR21 in this study still conserved evolutionarily, consistent with Rehman et al. (2021), who found that TLRs in chicken have exhibited conserved traits during evolution.

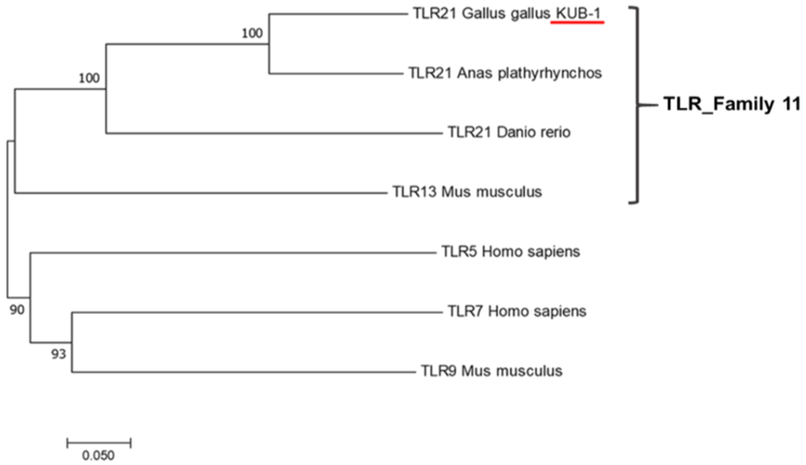

Figure 8. Phylogram of KUB-1 chicken TLR21 against other vertebrate TLRs constructed using the Neighbor-Joining algorithm with 1000x bootstrapping of the TLR21 amino acid sequence that has been aligned with ClustalW on MEGA7 shows that TLR21 in birds and fish are grouped in one cluster with *Mus musculus* TLR13, while *Mus musculus* TLR9, *Homo sapiens* TLR5 and *Homo sapiens* TLR7 were in a different cluster

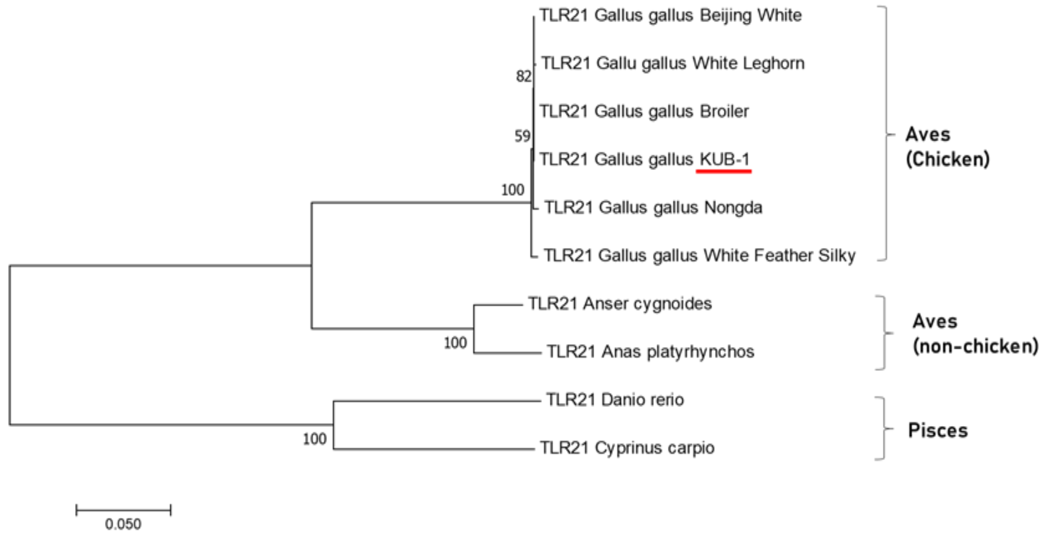

Figure 9. Phylogram of KUB-1 chicken TLR21 against other TLR21. The tree constructed using the Neighbor-Joining algorithm with 1000x bootstrapping on the amino acid sequences of TLR21 that have been aligned with ClustalW on MEGA 7 shows that TLR21 from Aves is grouped in one cluster, which is divided into two sub-clusters: chicken TLR21 and non-chicken (duck and goose) TLR21. In contrast, Pisces TLR21 is in a different cluster from Aves TLR21. The phylogenetic tree showed that *Gallus gallus* TLR21 formed a paraphyletic relationship with TLR21 in *Anser cygnoides* and *Anas platyrhynchos*

CONCLUSION

The molecular characteristic of KUB-1 cDNA TLR21 has nucleotides 3504 bp, which include a 2823 bp ORF, which encodes putative proteins of 940 aa. The deduced protein consisted of 720 aa, 23 aa, and 141 aa in ECD, TM, and ICD, respectively. Evolutionarily, KUB-1 chickens' TLR21 protein, which belongs to the TLR11 family, has differentiated from fish and formed a paraphyletic relationship with ducks and geese.

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REFERENCES

Batkhishig D, Enkhbayar P, Kretsinger RH, Matsushima N. 2020. A strong correlation between consensus sequences and unique super secondary structures in leucine rich repeats. Prot Struct Funct Bioinform. 88:840–852. DOI:10.1002/prot.25876.

Chen X, Sun X, Chimbaka IM, Qin N, Xu X, Liswaniso S, Xu R, Gonzalez JM. 2021. Transcriptome analysis of ovarian follicles reveals potential pivotal genes associated with increased and decreased rates of chicken egg production. Front Gen. 12. DOI:10.3389/fgene.2021.622751.

Chuang YC, Tseng JC, Yang JX, Liu YL, Yeh DW, Lai CY, Yu GY, Hsu LC, Huang CM, Chuang TH. 2020. Toll-like receptor 21 of chicken and duck recognize a broad array of immunostimulatory cpg-oligodeoxynucleotide sequences. Vaccine, 8:1–17. DOI:10.3390/vaccines8040639

Fitzgerald KA, Kagan JC. 2020. Toll-like Receptors and the control of immunity. Cell. 180:1044–1066. DOI:10.1016/j.cell.2020.02.041.

Hebditch M, Carballo-Amador MA, Charonis S, Curtis R, Warwicker J. 2017. Protein-Sol: A web tool for predicting protein solubility from sequence. Bioinform. 33:3098–3100. DOI:10.1093/bioinformatics/btx345.

Juul-Madsen HR, Viertlböeck B, Härtle S, Smith AL, Göbel TW. 2013. Innate immune responses. In avian immunology: 2nd ed. p. 121–147. DOI:10.1016/B978-0-12-396965-1.00007-8.

Kaiser MG, Hsieh J, Kaiser P, Lamont SJ. 2022. Differential immunological response detected in mRNA expression profiles among diverse chicken lines in response to *Salmonella* challenge. Poult Sci. 101:101605. DOI:10.1016/j.psj.2021.101605.

Keestra AM, de Zoete MR, Bouwman LI, Vaezirad MM, Van Putten JPM. 2013. Unique features of chicken Toll-like receptors. Develop Comp Immunol. 41:316-323. DOI:10.1016/j.dci.2013.04.009.

Lai CY, Yu GY, Luo Y, Xiang R, Chuang TH. 2019. Immunostimulatory activities of CpG-oligodeoxynucleotides in teleosts: Toll-like receptors 9 and 21. Front Immunol. 10:179. DOI:10.3389 /fimmu.2019.00179

Li H, Li T, Guo Y, Li Y, Zhang Y, Teng N, Zhang F, Yang G. 2018. Molecular characterization and expression patterns of a non-mammalian toll-like receptor gene (TLR21) in larvae ontogeny of common carp (*Cyprinus carpio* L.) and upon immune stimulation. BMC Vet Res. 14:153. DOI:10.1186/s12917-018-1474-4.

Manavalan B, Basith S, Choi S. 2011. Similar Structures but Different Roles - An updated perspective on TLR structures. Front Physiol. 2:41. DOI:10.3389 /fphys.2011.00041.

Matsushima N, Miyashita H, Enkhbayar P, Kretsinger RH. 2015. Comparative geometrical analysis of leucine-rich repeat structures in the NOD-like and Toll-like receptors in vertebrate innate immunity. Biomol. 5: 1955-1978. DOI:10.3390/biom5031955.

Matsushima N, Tanaka T, Enkhbayar P, Mikami T, Taga M, Yamada K, Kuroki Y. 2007. Comparative sequence analysis of leucine-rich repeats (LRRs) within vertebrate toll-like receptors. BMC Gen. 8:1224. DOI:10.1186/1471-2164-8-124.

Mokhtari Y, Pourbagheri-Sigaroodi A, Zafari P, Bagheri N, Ghaffari SH, Bashash D. 2021. Toll-like receptors (TLRs): An old family of immune receptors with a new face in cancer pathogenesis. J Cell Mol Med. 25:639–651. DOI:10.1111/jcmm.16214.

Nawab A, An L, Wu J, Li G, Liu W, Zhao Y, Wu Q, Xiao M. 2019. Chicken toll-like receptors and their significance in immune response and disease resistance. Int Rev Immunol. 38:284–306. DOI:10.1080/08830185.2019. 1659258.

Nie R, Zheng X, Zhang W, Zhang B, Ling Y, Zhang H, Wu C. 2022. Morphological characteristics and transcriptome landscapes of chicken follicles during selective development. Anim. 12:713. DOI:10.3390/ani12060713.

Popa GL, Popa MI. 2021. *Salmonella* spp. Infection–a continuous threat worldwide. GERMS. 11:88-96. DOI:10.18683/germs.2021.1244.

Priyathilaka TT, Elvitigala DAS, Whang I, Lim BS, Jeong HB, Yeo SY, Choi CY, Lee J. 2014. Molecular characterization and transcriptional analysis of non-mammalian type Toll like receptor (TLR21) from rock bream (Oplegnathus fasciatus). Gene. 553:105–116. DOI:10.1016/j.gene.2014.10.008.

Rehman MS, Rehman S, Yousaf W, Hassan FU, Ahmad W, Liu Q, Pan H. 2021. The potential of Toll-Like receptors to modulate avian immune system: exploring the effects of genetic variants and phytonutrients. Front Genet. 12: 671235. DOI:10.3389/fgene.2021.671235.

Ruan W, An J, Wu Y. 2015. Polymorphisms of chicken TLR3 and 7 in different breeds. PLoS ONE. 10:e0119967. DOI:10.1371/journal.pone.0119967.

Sartika T, Iskandar S. 2019. The productivity of 4th generation KUB-2 chicken. JITV. 24:151-157. DOI:10.14334 /jitv.v24i4.2033.

Song W, Wang J, Han Z, Zhang Y, Zhang H, Wang W, Chang J, Xia B, Fan S, Zhang D, Wang J, Wang HW, Chai J. 2015. Structural basis for specific recognition of single-stranded RNA by Toll-like receptor 13. Nat Struct Mol Biol. 22:782-787. DOI:10.1038/nsmb.3080.

St. Paul M, Brisbin JT, Abdul-Careem MF, Sharif S. 2013. Immunostimulatory properties of Toll-like receptor ligands in chickens. Vet Immunol Immunopathol. 152:191-199. DOI:10.1016/j.vetimm.2012.10.013.

Świderská Z, Šmídová A, Buchtová L, Bryjová,A, Fabiánová A, Munclinger P, Vinkler M. 2018. Avian Toll-like receptor allelic diversity far exceeds human polymorphism: an insight from domestic chicken breeds. Sci Rep. 8:17878. DOI:10.1038/s41598-018-36226-1.

Tamzil MH, Indarsih B. 2022. Revisit the development of native Indonesian chickens from Red Jungle Fowls (*Gallus gallus* bankiva) to commercial chickens. Wartazoa. 32. DOI:10.14334/wartazoa.v32i1.2585.

Wang J, Zhang Z, Liu J, Zhao J, Yin D. 2016. Ectodomain architecture affects sequence and functional evolution of vertebrate Toll-like receptors. Sci Rep. 6. DOI:10.1038/srep26705.

Wicherska-pawłowska K, Wróbel T, Rybka J. 2021. Toll-like receptors (TLRs), nod-like receptors (NLRs) and rig-i-like receptors (RLRs) in innate immunity. TLRs, NLRs, and RLRs ligands as immunotherapeutic agents for hematopoietic diseases. Int J Mol Sci. 22:13397. DOI:10.3390/ijms 222413397.

Wigley P. 2014. *Salmonella enterica* in the chicken: How it has helped our understanding of immunology in a non-biomedical DOI:10.3389/fimmu.2014.00482.

Winaya A, Fahmiady DI, Suyatno S, Malik A, Mahmud A, Jaganathan R. 2023. Morphometric diversity and genetic relationship of “Bangkok” chicken (Thai Game Fowl) in East Java, Indonesia. JJBS. 16:189-197. DOI:10.543 19/jjbs/160203.

Wu H, Wang H, Jiang W, Lian Z. 2018. The evolutionary characteristics and structural biology of *Gallus* Toll-like receptor 21. J Mol Recognit. 31:e2696. DOI:10.1002/jmr.2696.

Yeh DW, Liu YL, Lo YC, Yuh CH, Yu GY, Lo JF, Luo Y, Xiang R, Chuang TH. 2013. Toll-like receptors 9 and 21 have different ligand recognition profiles and cooperatively mediate activity of CpG-oligodeoxynucleotides in zebrafish. Proc Natl Acad Sci USA. 110:20711-20716. DOI:10.1073/pnas.13052 73110.

Zhang Z, Ohto U, Shibata T, Krayukhina E, Taoka M, Yamauchi Y, Tanji H, Isobe T, Uchiyama S, Miyake K, Shimizu T. 2016. Structural analysis reveals that toll-like receptor 7 is a dual receptor for guanosine and single-stranded RNA. Immun. 45:737-748. DOI:10.1016/j.immuni.2016.09.011

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UDC: 581.35

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Pengaruh konsentrasi spermatozoa dan penambahan antioksidan melatonin pada pengencer terhadap kualitas spermatozoa domba post-thawing (Influence of sperm number and antioxidant melatonin addition in extender on the quality of post-thawing sheep spermatozoa)

(Org: Eng)

JITV 28(1): 1-10

This study aimed to examine the effect of spermatozoa concentration and the effectiveness of melatonin supplementation in diluent on the quality of post-thawing semen. Ejaculated semen was collected using the artificial vaginal method (MVB). The study was carried out in two stages, firstly semen was frozen in andromed diluent with different concentrations in one straw (50, 100, 200 million per straw), and the second was frozen semen in diluent supplemented with melatonin with different doses (0, 0.5, 1, 0 and 1.5 mM melatonin). Parameters observed were the movement of spermatozoa using Computer Assisted Sperm Analysis, membrane integrity, and acrosome integrity. Data were analyzed using ANOVA and further tested using Duncan's test. The results showed no significant difference in the quality and movement pattern of sheep semen when frozen at concentrations of 50, 100, or 200 million per straw ($P>0.05$). Furthermore, adding melatonin to the diluent in this study affected spermatozoa's total motility and progressive motility at a concentration of 1.0 mM ($P<0.05$) but did not significantly affect the percentage of spermatozoa motility pattern characteristics. From the results, it can be concluded that the concentration of per straw spermatozoa does not affect the quality of sheep semen, and melatonin supplementation in diluent can reduce the effects of the frozen-thawed process on motility, acrosome cap, and plasma membrane integrity in sheep semen. Melatonin supplementation with a concentration of 1 mM in the extender was the highest quality concentration in this study.

(Author)

Key Words: Cryopreservation, Melatonin, Post-thawing Quality, Sperm Number

UDC: 57.089.3

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Kompetensi pematangan inti dan pembentukan pronukleus oosit domba yang disuplementasi L-ergothioneine pada medium maturasi (Nuclear maturation competence and pronuclear formation in ovine oocytes supplemented with L-ergothioneine in maturation medium)

(Org: Eng)

JITV 28(1):11-21

This study aimed to determine the efficacy of supplementing maturation medium with L-ergothioneine on nuclear maturation competence and pronuclei formation of ovine oocytes *in vitro*. In the first study, oocytes were divided into four groups and matured for 24 hours in a maturation medium supplemented with 0 mM (control), 10 mM, 15 mM, and 20 mM LE. In the second study, oocytes were divided into two groups and matured for 24 hours in a maturation medium supplemented with 0 mM LE (control) and 10 mM LE (optimal dose from the first study), then co-incubated with sperm in a fertilization medium for 12 hours. The results of the first study showed that the maturation rate of oocytes enriched with LE 10 mM (88.74±1.79%) and 15 mM (87.52±2.30%) in maturation medium was significantly different ($P<0.05$) compared to the control (78.55±1.78%) and LE 20 mM (77.37±1.21%). Furthermore, the results of the second study revealed that the fertilization rate of oocytes with 10 mM LE (75.55±3.47%) supplementation in maturation medium had a significantly higher percentage of two pronuclei formation than control (63.78±3.49%). In conclusion, supplementing 10 mM LE in a maturation medium improved oocyte nuclear maturation competence and fertilization rates.

(Author)

Key Words: *In vitro* Maturation, *In vitro* Fertilization, L-ergothioneine, Oocyte, Ovine

UDC: 57.085

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Profil fermentasi rumen pada ransum berbasis indeks sinkronisasi protein-energi: studi *in vitro* (Rumen fermentation profiles of protein-energy synchronization index-based ration: an *in vitro* study)

(Org: Eng)

JITV 28(1): 22-33

The study examined the effect of protein-energy synchronization (PES) index-based rations on the rumen fermentation profile. The material used was the Jawa Randu goat's rumen fluid, collected soon after the goat was slaughtered. The treatment ration consisted of elephant grass, lamtoro (Leucaena leucocephala), coconut meal, tofu waste, bran, cassava waste, and mineral mix arranged based on the PES index of each feed ingredient. The research was conducted in vitro using a completely randomized design (CRD). The treatment consisted of 4 PES indexes, namely 0.55 (R1); 0.6 (R2); 0.65 (R3); 0.7 (R4), and each had 5 replications. Data were analyzed by ANOVA and orthogonal polynomials (OP). The results of the ANOVA showed that the PES index did not affect the production of acetate (C2) and butyrate (C4) but had a highly significant effect on dry matter digestibility (DMD), organic matter digestibility (OMD), pH, a total of volatile fatty acids (VFA), propionate (C3), C2:C3 ratio, methane (CH_4), ammonia (NH_3), and microbial protein synthesis (MPS). The analysis of OP showed a quadratic effect on all variables with the equation $Y= -81.601X^2+375.04X–310.78$ (DMD), $Y= -433.69X^2+522.69X–128.75$ (OMD), $Y=-44X^2+54.04X– .9$ (pH), $Y= -2160X^2+2576.8X–604.2$ (VFA Total), $Y= -481.8X^2+585.01X–143.45$ (C3), $Y = 50.93X^2–60.177X+21.067$ (C2:C3), $Y = 202.45X^2–223.18X+103.41$ (CH_4), $Y= 436X^2 – 552.28X+181.08$ (NH_3), dan $Y= -1012X^2+1260X–311.64$ (MPS). Protein-energy synchronization (PES) index of 0.6 achieved the best rumen fermentability. The formulation of the PES index-based ration of 0.6 had the most effective compared to other indexes based on the high of propionate, the lowest methane, the lowest NH_3, and the best microbial protein synthesis. The ration with an index of 0.6 is composed of 30% elephant grass, 30% Leucaena leucocephala, 10% coconut meal, 10% tofu waste, 10% rice brand, 9% cassava waste, and 1% mineral mix.

(Author)

Key Words: Ration, Protein-Energy Synchronization Index, Rumen Fermentation, *In Vitro*

UDC: 576.32

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Filogeografi maleo senkawor (*Macrocephalon maleo* Sal. Muller 1846) berdasarkan gen Cytochrome B di Sulawesi dan identifikasi jenis kelaminnya menggunakan teknik sexing molekuler (Phylogeography of the Maleo Senkawor (*Macrocephalon maleo* Sal. Muller 1846) based on Cytochrome B Gene in Sulawesi and their sex determination using molecular sexing)

(Org: Eng)

JITV 28(1): 34-44

The Maleo Senkawor bird (*Macrocephalon maleo*) is endemic and widely distributed in Sulawesi. Maleo Senkawor bird is monomorphic so it is difficult to distinguish between male and female individuals. This study aimed to determine the kinship relationship among *M. maleo* in various geographic areas in Sulawesi through the use of the gene marker *Cytochrome-b* (Cyt-b), determine the sex ratio, and examine the reliability of the 2550F/2718R primer. Fifteen (feather and eggshell) samples from North Sulawesi, Southeast Sulawesi, and Central Sulawesi were collected. Total DNA isolation was performed using the *Dneasy® Blood and Tissue kit* (Cat. No. 69504) following a Qiagen protocol with modification. PCR amplification (35 cycles) used a forward primer MMCytb_F (5'- GAAAATCCCACCCCCTACTA-3'), a reverse primer MMCytb_R (5'-GTTGGCTACGAGGAGTCAGA-3') and a primer for sexing used 2550F/2718R. Analysis was performed on the length of 903 bp Cyt-b mtDNA gene sequences of *M. maleo* along with their amino acid sequences (301 AA) and *Chromo Helicase DNA-Binding* (CHD) gene sequences. Based on the genetic distance of the *Kimura 2-Parameter model* and *p-distance*, the interpopulation of *M. maleo* in Sulawesi (the population of Southeast Sulawesi was separated from the population of Central Sulawesi and North Sulawesi) was 0.002 (0.2%) – 0.003 (0.3%). Each population has formed a different haplotype. Primer 2550F/2718R was able to amplify the CHD gene and could distinguish the sex identification. Male individuals are more dominant than female individuals with a sex ratio of 6.5:1.

(Author)

Key Words: *Macrocephalon maleo*, Phylogeography, Cyt-b gene, Sex determination, Sulawesi

UDC: 636.52

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Penampilan ayam persilangan antara ayam kampung dan ayam petelur dan prediksi kurva pertumbuhannya (Performance and growth curve prediction of crossbred chickens from crossing between local roosters and layer hens)

(Org: Eng)

JITV 28(1): 46-56

Demand for crossbred chicken (Indonesian native chicken x laying hens) continues to increase. This study aimed to find the best-crossbred chicken resulting from crossing between local/native roosters and laying hens which produce the highest body weight up to eight weeks of age, and its growth prediction using the Gompertz function. Eighteen (18) native roosters (9 Bangkok roosters, 9 Pelung roosters) and 36 laying hens of strains Isa brown, Lohman brown, and Hyline brown (12 each) were used in this study to produce 360 crossbred chickens. Factorial 2x3 (two male local roosters versus three strains of laying hens) using a completely randomized design with 3 replications was applied in this study. The results showed an interaction between native roosters and layer hens on the body weight of crossbred chickens at eight weeks of age. Crossbred chicken from crossing between Pelung rooster and Isa brown layer hen was the best in body weight at eight weeks of age. This crossbred chicken would reach a mature weight of 3026.89 g with an inflection weight of 1113.53 g and an inflection age of 7.40 weeks. In conclusion, crossbred chicken from crossing between Pelung roosters and layer hens of Isa brown strain was the best crossbred with the highest body weight at eight weeks of age. This crossbred chicken could be an alternative for fulfilling the demand for local chicken in Indonesia because the slaughter age could be shortened from 8 months to 8 weeks.

(Author)

Key Words: Crossbreeding, Local Rooster, Layer Hen, Body Weight, Gompertz Function

UDC: 612.617

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Pengaruh jenis karbohidrat dan fenotipe terhadap kualitas semen beku ayam KUB pasca pencairan (Effect of carbohydrate type and phenotype on the quality of post-thawing frozen semen of KUB chicken)

(Org: Eng)

JITV 28(1): 56-68

The superior Balitbangtan Kampung Chicken (KUB) chickens have different phenotypes. It was reported that the chicken phenotype was related to semen quality. This study aimed to determine the post-thawing characteristics and quality of KUB chicken semen with different phenotypes frozen in Ringer's lactate egg yolk (RLEY) diluent with the addition of fructose or glucose. Semen was collected using the massaging method from 20 KUB chickens with a single comb phenotype and black or dark brown feather color with a red feather neck (SCNR), green-black single comb with white feather neck collar (SCNW), pea comb and black feathers or dark brown fur with a red neck (PCNR), and a green-black pea comb with a white neck (PCNW). Semen from each chicken phenotype was divided into three parts or frozen in three types of diluents: RLEY, RLEY+fructose (RLEYF), and RLEY+glucose (RLEYG). The highest sperm motility was found in the diluent with the addition of glucose in the SCNR and PCNW phenotypes ($P<0.05$). The highest sperm viability was shown in the RLEYG diluent in the PCNW phenotype ($P<0.05$). The highest abnormality was found in the RLEY and RLEYF diluents in the SCNW, PCNR, and PCNW groups, whereas in the RLEYG group, it was only found in the PCNR group. From the results of this study, it can be concluded that the type of glucose and chicken phenotype influences the quality of post-thawing semen. The best is found in diluents with glucose attachments in the SCNR phenotype.

(Author)

Key Words: Chicken Semen, Comb, Extender, Medium Supplementation, Freezin

UDC: 598.618

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Pengaruh pemberian pakan dengan level energi dan protein berbeda terhadap performa puyuh petelur (Effect of dietary with different energy and protein levels on laying quails performance)

(Org: Eng)

JITV 28(1): 69-75

The performance of quail is a function of several production factors, one of which is feed. However, the optimum metabolizable energy and crude protein for the performance of laying quails currently needs to be revised. This study aimed to evaluate the effects of feeding diets with different metabolizable energy and crude protein levels on the performances of laying quails. Five hundred forty (540) laying quails aged eight weeks were randomly allocated into nine dietary treatments. Each treatment consisted of four replicates with 15 laying quails per replicate. Dietary treatments comprised three metabolizable energy levels (2700, 2800, and 2900 kcal/kg) and three crude protein levels (17, 19, and 21%) in a factorial research methodology arrangement. After one week of the adaptation period, the feeding trial was conducted for five weeks (9-13 weeks old). Interaction between metabolizable energy and crude protein levels was recorded on feed intake ($P<0.05$). Feed intake was reduced along with the increase in the metabolizable energy when crude protein levels were 17 and 19%. However, the feed intake tended to be improved when high metabolizable energy was combined with high crude protein contents (2900 kcal/kg and 21%, respectively). No interaction was recorded on the other variables (egg production, egg weight, egg mass, and feed conversion ratio) ($P>0.05$). Feeding diets with different metabolizable energy levels had a significant effect on feed conversion ratio ($P<0.05$) but without any significant impact on egg production, egg weight, and egg mass of laying quails ($P>0.05$). Crude protein as a single factor significantly affects egg production, weight, mass, and feed conversion ratio of laying quails ($P<0.05$). Linear regression analysis indicated that metabolizable energy intake linearly improved egg production, weight, mass, and feed conversion ratio. Moreover, crude protein intake linearly improved egg production, weight, mass, and feed conversion ratio. Therefore, feeding diets containing 2900 kcal/kg metabolizable energy and 21% crude protein are required to provide optimum performance of laying quails.

(Author)

Key Words: Laying Quail, Metabolizable Energy, Crude Protein, Feed Conversion Ratio, Egg Production

UDC: 637.54’658

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Kualitas fisik dan sensori daging dada ayam kampug super yang dimarinasi dengan konsentrasi marinade yang berbeda (The quality of physical and sensory of super native chicken breast marinated with herbs and spices with different levels of marinade concentration)

(Org: Eng)

JITV 28(1): 76-85

Super-native chickens result from crosses between male native chickens and laying hens. The diversification of processed products from super-native chicken can be developed into ready-to-cook products by marinating them with local herbs and spices. This research was conducted on the effect of marination concentrations of different local herbs and spices on the hedonic quality of super-native chicken breast. The research material consisted of super-native chicken breast, chili powder, sugar, salt, pepper, garlic, cinnamon, palm oil, lime juice, monosodium glutamate, and ginger. The treatment levels of marination concentration were 10, 20, and 30% of the weight of the meat. Physical quality parameters tested were color, pH value, moisture, cooking loss, water-holding capacity, yield, and hardness. Each treatment consisted of 3 replications. In addition, sensory quality was tested by a hedonic quality test by 40 untrained panelists. The hedonic quality parameters tested were color, flavor, taste, texture, tenderness, and juiciness. The data from the physical quality test were analyzed by analysis of variance. The hedonic quality test results were analyzed by non-parametric analysis through the Kruskal-Wallis hedonic test. If there was a significant difference ($P<0.05$), the test was continued with Duncan's New Multiple Range Test. The results showed that the concentration level of different local herbs and spices marination could affect the physical quality (redness, pH value, and moisture) and sensory (color and texture) of super-native chicken breast. The marination concentration of local herbs and spices at 20% level was the best treatment based on hedonic quality with a smooth meat texture and a score of 3.98.

(Author)

Key Words: Local Spices, Breast Meat, Phsycal Quality, Marination, Sensory

UDC: 636.52

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Persepsi dan perilaku peternak yang mempengaruhi tingkat adopsi ayam KUB (Effect of supplementing ground leaf of misai (Farmers' perceptions and behavior that affect the adoption rate of KUB chickens)

(Org: Eng)

JITV 28(1): 84-98

As a superior breed produced by Indonesian Agency for Agricultural Research and Development (IAARD), KUB chicken becomes one of the solutions to increase the need for animal protein. KUB chicken breeds have been successfully disseminated to all provinces in Indonesia. Thus, breeders' adoption rate and behavior in maintaining the sustainability of KUB chicken rearing needs to be analyzed because the disseminated breeds may need to be successfully developed in a particular area. This research will collaborate with two theories; the "Theory of Planned Behavior" (TPB) and the "Technology Acceptance Model" (TAM). It was conducted in June 2022 in the City of Salatiga, Semarang, and Magelang, Central Java Province. The determination of the research location was based on the consideration that a KUB chicken breeders group already had a KUB chicken breeder association called AnaKUB (KUB Chicken Breeders Association) and a breeding population that met the sampling requirements. This research aimed to analyze the perception factors and breeders' behavior that affect the adoption rate of KUB Chicken Technology in Central Java Province, Indonesia. The combined results of path analysis (path) and regression analysis on the Structural Equation Modeling (SEM) technique, it can be concluded that perceived benefits (25.3%), ease of raising KUB chickens (23.9%), attitudes (21.2%), subjective norms (16.7%) and perceptions of control behavior (17.3%) have a positive effect on the intention to adopt KUB chickens. The perceived benefits (45.8%) and the perceived ease of raising KUB chickens (50.1%) have a very positive effect on the attitude variable of the farmer. Important people, including family members, have an important role in supporting the maintenance of KUB chickens. In contrast, extension workers have yet to contribute much in supporting breeders to adopt KUB chickens. The collaboration of two theories has a mutually influencing relationship, especially towards attitudes and intentions to adopt KUB chickens, which positively affect the adoption rate. The adoption rate of KUB chicken is 3.32 (66.40%); this shows that the adoption rate of breeders in the Central Java region is classified as high adoption.

(Author)

Key Words: Adoption Rate, Breeder Behaviour, KUB Chicken, TAM, TPB

UDC: 316.647.7

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Polimorfisme indel 84-bp gen *Sterol Regulatory Element-Binding Protein 1* (*SREBP1*) pada beberapa bangsa sapi di Indonesia. (The 84-bp Indel Polymorphism of *Sterol Regulatory Element-Binding Protein 1* (*SREBP1*) gene in several cattle breeds in Indonesia)

(Org: Eng)

JITV 28(2): 102-111

Sterol regulatory element-binding protein 1 (*SREBP1*) gene is a gene that encodes SREBP1, a family of transcription factors that have a key role in lipid homeostasis as well as fatty acid metabolisms. The present study aimed to detect the 84-bp indel polymorphism in the intron 5 of the SREBP1 gene (84-bp indel) in several cattle breeds in Indonesia. A total of 740 cattle of six breeds (Limousin, Simmental, Holstein-Friesian, Bali, Sumbawa, and Pasundan) from two National Artificial Insemination Centers (NAICs) and smallholder farmers were used in this study. The detection of 84-bp indel polymorphism was performed using the polymerase chain reaction (PCR) method and visualized through a gel agarose electrophoresis system. The study showed that the L allele (insertion-type) was common and is fixed in Holstein-Friesian, Bali, and Sumbawa cattle (1.00). Meanwhile, the S allele (deletion-type) was found in Limousin, Simmental, and Pasundan cattle with a frequency of 0.24, 0.16, and 0.01, respectively. The presence of the S allele in Pasundan cattle was only found in the form of the heterozygous LS genotype (0.03). In conclusion, the 84-bp indel of the SREBP1 gene was found to be polymorphic in Limousin, Simmental, and Pasundan cattle, but monomorphic in Holstein-Friesian, Bali, and Sumbawa cattle.

(Author)

Key Words: Fatty Acid, Indel, Indonesian Local Cattle, *SREBP1* Gene

UDC: 613.287.5

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Perubahan komposisi kualitatif susu sapi Holstein selama stress musim panas (Changes in the qualitative composition of the milk of Holstein cows during summer chronic heat stress)

(Org: Eng)

JITV 28(2): 112-121

Seasonal summer heat stress leads to significant economic losses, resulting in a drop in milk yield in dairy cows and a deterioration in milk quality. The purpose of this study was to determine the changes in some qualitative parameters of milk in Holstein cows during chronic heat stress on one of the largest commercial dairy farms Ukraine (50°49′14″ N, 31°49′23″ E). Five multiparous cows of medium lactation with a milk yield of about 30 kg per day were randomly selected into the reference (in the spring, at the end of May) and the experimental group (in the summer, at the end of August). Milk samples were taken from cows during the morning milking. Qualitative analysis of milk included the identification of milk solids-not-fat, density, mass fraction of lactose, fat, protein and minerals, as well as the freezing point, electrical conductivity and active acidity using ultrasonic method. Animals were kept in naturally ventilated barns. The total mixed single-type balanced diet consisting of corn silage and concentrates that the cows received remained unchanged. The sampling of milk from cows of the experimental group was preceded by a continuous 26-day hot period, during which the maximum daily values of the temperature and humidity index did not fall below 72 units. The results showed that in the milk of the animals of the experimental group there was a significant decrease in the content of milk solids-not-fat, the mass fraction of fat and protein ($P<0.05$). The mass fraction of lactose and minerals tended to increase. It should be noted that the average daily milk yield of dairy cows in the herd in spring and summer was almost at the same level. In conclusion, the results of the study showed that high summer temperatures lead to a deterioration in the quality of milk in Holstein cows. Despite the decrease in milk density and freezing point, these figures met the requirements of the standard. Organoleptic indicators of milk, electrical conductivity and active acidity of milk did not change in hot weather, their value indicated the naturalness of milk. The mass fraction of milk fat, which undergoes the greatest change under the influence of seasonal heat stress, is one of the most valuable components of milk, which has a direct effect on nutritional value and purchase price of raw milk. Therefore, a further deeper study of the fatty acid composition of milk using the method of chromato-mass spectrometry will provide valuable data necessary to search for possible herd management strategies to maintain high milk quality under conditions of seasonal heat stress.

(Author)

Key Words: Dairy Cows, Organoleptic Indicators, Physico-Chemical Properties of Milk, Prolonged Hot Weather

UDC: 316.647.7

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Asosiasi gen DGAT1 terkait flavor, odor, kolesterol, dan mineral pada domba Indonesia (Association of DGAT1 Gene related to flavor, odor, cholesterol, and mineral in Indonesian sheep)

(Org: Eng)

JITV 28(2): 122-128

Diacylglycerol acyltransferase 1 (DGAT1) is a potential candidate gene for improving Indonesian lamb's quality and nutrient value. The study aimed to identify the diversity of the DGAT1 gene with SNP g.8539 C>T and its relation to Indonesian lamb's flavor and odor, cholesterol, and mineral. Total of 254 ten to twelve months old sheep consisted of 20 Javanese fat-tail sheep (JFTS), 107 Javanese thin-tail sheep (JTTS), 10 Garut composite sheep (GCS), 10 Compass agrinak sheep (CAS), 10 Barbados cross sheep (BCS), 20 Garut sheep (GS), 27 Jonggol sheep (JS), and 50 Jambi local sheep (JLS). One hundred sheep were used to analyze the association of the DGAT1 gene with flavor and odor content, cholesterol, and mineral. The diversity of DGAT1|*Alu*I was analyzed with the PCR-RFLP method. The association of the DGAT1 gene with the nutritional value of meat was analyzed using the GLM (General Linear Model) method. The results showed that the DGAT gene was polymorphic in JTTS, GS, and JFTS and monomorphic in CAS, BCS, JLS, and GCS. CC and CT genotypes were found in JTTS, GS, and JFTS. SNP g.8539 C>T of DGAT1 gene had a significant association ($P<0.05$) with flavor and odor, 4-Ethyloctanoic Acid (EOA). However, the DGAT1 gene had no significant association ($P>0.05$) with cholesterol and mineral. The DGAT1 gene might be marker-assisted selection for improving lamb flavor and odor in Indonesian sheep.

(Author)

Key Words: Cholesterol, DGAT1, Flavor, Odor, Minera

UDC: 612.617

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Performa produksi dan karakteristik sperma puyuh jantan (*Coturnix-coturnix japonica*) dengan konsentrasi immunoglobulin yolk berbeda [Production Performance and

Sperm Characteristics of Quail (*Coturnix-coturnix japonica*) with Different Concentrations of Yolk Immunoglobulin]

(Org: Eng)

JITV 28(2): 129-135

Quails are classified according to their immunity to different IgY concentrations. Quail IgY concentrations range from 0 to 1200 µg ml-1, and these IgY concentrations may affect production and reproductive performance. This study aimed to analyze IgY concentrations in male quail and to compare production and reproductive performance at different IgY concentrations. Forty-two male quail at five weeks of age participated in the study, including 29 quail with low IgY concentration (210-393 µg ml^{-1}) and, 13 with intermediate IgY concentration (403-564 µg ml^{-1}). The observed productive performances were feed intake, initial body weight, final body weight, weight gain, feed conversion, morbidity, and mortality. In addition, this study observed testicular weight and macroscopic and microscopic semen quality for reproductive traits. Differences in production and reproductive performance of each group were analyzed using an independent-sample t-test. The result showed that male quail with different IgY concentrations were equal in all productive traits (P>0.05). This means that male quail with low IgY and medium IgY concentrations are equally good. Testicular weight, semen color and pH were identical at different IgY concentrations. Quails with low IgY concentrations have better sperm consistency, which is related to the weight of the testes, which are heavier in quails with low IgY concentrations than in those with moderate IgY concentrations. This consistency is related to the concentration of sperm in the semen. The result concludes IgY concentrations in male quail did not affect production performance. Quails with low IgY concentration had thicker sperm consistency and higher sperm concentration.

(Author)

Key Words: IgY Concentration, Performance, Quail, Semen Characteristic

UDC: 613.2.038

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Pengaruh suplementasi zinc dalam pakan terhadap performa, karkas, organ pencernaan, dan morfologi intestinal bebek Sikumbang Jati betina (Effect of zinc supplementation in the diet on Sikumbang Janti female duck performance, carcass, digestive organs, and intestinal morphology)

(org: Eng)

JITV 28(2): 136-142

The research aimed to evaluate the effect of Zn supplementation in diet of Sikumbang Janti female duck on its performance, carcass, digestive organs, and intestinal morphology. The study used 96 female ducks aged 8 weeks. This research used a completely randomized design with four treatments (0, 30, 60, and 90 mg Zn/kg), each treatment was repeated four times. Performance, carcass, digestive organs, and intestinal morphology were observed. The results showed that Zn supplementation significantly (P<0.05) affected body weight, body weight gain, feed conversion ratio, carcass weight, abdominal fat, and intestinal morphology, but it did not affect (P>0.05) feed consumption, carcass percentage, and digestive organs. In conclusion, supplementation of 60 mg Zn/kg in the diet improved performance, intestinal morphology, and the health of visceral of Sikumbang Janti female ducks aged 8 to 16 weeks.

(Author)

Key Words: Dietary Zn Supplementation, Growth Performance, Intestinal Morphology, Local Duck, Visceral

UDC: 599.735.51

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Karakteristik morfologi Kambing Kacang betina dewasa di daerah lahan kering (Morphological characterization of doe Kacang Goat in the dry land area)

(Org: Eng)

JITV 28(2): 143-151

This study was conducted to select a breed based on the morphological characteristics of Kacang goats in the dry land area, by assessing 31 doe Kacang goats. Semi-intensive raising system is carried out (without giving concentrate), yet water; forage (such as *Gliricidia sepium leaves, Sesbania grandiflora leaves, Leucaena leucocephala leaves*) and local grass are given by applying *ad libitum* method. Description of morphological characteristics, Pearson's correlation between body weight and body size, and body index, were analyzed using SPSS 25.0 program. The result of descriptive analysis for BW, BL, WH, CG, CW, CD, RW, RH, RL, HL, HW, and HH are 20.72±3.26 kg, 44.34±8.17 cm, 55.42±7.84 cm, 62.68±7.12 cm, 10.98±2.07 cm, 22.74±2.12 cm, 8.44±1.38 cm, 54.96±6.44 cm, 12.55±0.85 cm, 14.07±0.55 cm, 10.19±0.36 cm, dan 11.69±0.45 cm respectively. The value of the morphological index for WS, BI, DI, LI, PI, B, HS, FL, and CI are 0.78+0.12, 71.06±12.38, 0.42±0.06, 0.81±0.18, 67.55±11.93, 0.43±0.08, 11.18±5.78, 32.68±7.40, dan 72.48±2.82, respectively. The conclusion is that the body weight and body measurements in the dry land area are still below the quality National standards of Indonesia. The correlation coefficient of body weight with body size is in the positive range of moderate to high except, the correlation of body weight with head height which shows a negative correlation. The correlation coefficient of body weight with the morphological index is in the low positive range except for the correlation coefficient of body weight with width slope, balance, and height slope which has a negative correlation coefficient.

(Author)

Key Words: Doe Kacang Goat, Dry Land, Morphological Index

UDC: 591.151

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Polimorfisme sintase asam lemak domba Awassi dan pengaruhnya pada komposisi asam lemak (Fatty acid synthase polymorphism of Awassi sheep and its impact on fatty acid composition)

(Org: Eng)

JITV 28(3): 152-158

Livestock intramuscular fat content is determined by fatty acid synthase (FASN). The fatty acid profile of the fat is of relevance to human health. Thus, this study explores the relationship between the polymorphism of the *FASN* gene in Awassi sheep and its impact on fatty acid composition. The study used 100 Awassi rams, ranging in age from 1 to 2.5 years. Molecular DNA was isolated from each blood sample; genotyping, sequencing reactions, and *in silico* tools were subsequently used to confirm the variants in amplified fragments. The results revealed two genotypes (GG and GA) of the ovine *FASN* gene (exon 2). The novel missense c.186 G>A was identified in the genotype GA. The GA genotype had significantly (P<0.05) increased intramuscular fat, higher unsaturated fatty acid content, and lower saturated fatty acid content than the GG genotype. Cumulative *in silico* analysis indicated a detrimental effect of the SNP c.186 G>A on FASN activity. The intramuscular GA genotype of Awassi sheep had a low saturated content relative to unsaturated fats. The result confirmed that the c.186 G>A SNP in ovine *FASN* gene variation is potentially helpful in assessing carcass traits, and this might lead to a more efficient selection of animals with healthier fatty acid profiles, resulting in higher-quality meat.

(Author)

Key Words: Awassi Sheep, *FASN* Gene, Fatty Acids Composition

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UDC: 613.2.038

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Imunitas dan kelangsungan hidup anak domba dari induk yang diberi ransum flushing mengandung EPA dan DHA (Immunity and survival rate of Lamb born from ewe fed flushing diet containing EPA and DHA)

(Org: Eng)

JITV 28(3): 159-168

Nutrition can influence lamb survival and the immune status during the neonatal phase until weaning. This study aimed to investigate the effect of flushing of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from Lemuru fish oil in the Ewe's diet on lamb immunity and survival. Twenty-eight neonatal lambs were divided into four treatments according to Ewe's diet. Four treatments: control concentrate (P1), flushing concentrate with 6% palm oil (P2), flushing concentrate with 3% lemuru oil and 3% palm oil (P3), and flushing concentrate with 6% lemuru oil (P4). The Ewe were fed Napier grass and concentrated in a 30:70% ratio based on dry matter. Ewe and their lambs remained together until weaning at about two months of age. The parameter observed in ewes was colostrum Immunoglobulin G (IgG). The parameters in the Lamb are blood Ig G, neonatal behavior of the Lamb, physiological response, and hematology of the Lamb at birth and weaning. The results showed that feeding P4, P3, and P2 to the Ewe resulted in a shorter latency to stand-in lamb (P<0.05). P1 has higher leukocytes (P<0.05) during weaning than P2 and P3. P1 has the same leukocyte as P4. Treatment has a non-significant effect (P>0.05) on Ig G and physiological response. In conclusion, administering 6% Lemuru oil containing EPA and DHA twice can shorten the latency to stand in newborn lambs.

(Author)

Key Words: Flushing, Immunity, Immunoglobulin, Lamb Survival, Lemuru Oil

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UDC: 613.2.038

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Performa pedet Pasundan pascasapih yang diberi ransum ekstra pada sistem penggembalaan ekstensif (Performance of post-weaned Pasundan calves fed extra diets in an extensive grazing system)

(Org: Eng)

JITV 28(3): 169-176

Changing from the pre-weaning to the post-weaning period is a transition from liquid to solid feed accompanied by a dramatic transformation of the GI tract. Supplemental solid feeding of grazing post-weaning calves is expected to improve their productivity. The study aimed to determine the effect of extra diet on the performances of post-weaned Pasundan calves (15 males and 15 females) that were grazed, using a completely randomized design of 2x3 factorials (two sex factors x three diet factors). The diet treatments were that post-weaned Pasundan calves were grazed: without being given extra diets (grazing/control), extra diets without urea-impregnated zeolite (extra diet-1), and extra diets with urea-impregnated zeolite (extra diet-2). The parameters were diet consumption (only for extra diets, unmeasured grazing consumption), body weights, and body condition. The data were analyzed with GLM Univariate. The results showed: The interaction between diet treatment and sex did not affect all the observed parameters. Consumption of extra diet-1 (529.37±3.06 g DM/head/day) was higher (P<0.05) than extra diet-2 consumption (525.17±1.96 g DM/head/day), but after a correction of body weight, the result was no different. Sex factors did not affect all observed parameters. The ADG of post-weaned calves fed extra diet-1 (155.8±27.5 g), or extra-2 diet (124.2±56.3 g) was higher (P<0.05) than the grazing (66.67±35.8 g). The extra diet-1 produced a higher change in body condition (1.8±0.78, P<0.05) than extra diet-2 or grazing (1.2±0.42). In conclusion, feeding extra diet-1 to post-weaned Pasundan calves increased ADG by 234% and improved the body condition by 150% better than calves that were only grazed in an extensive grazing system.

(Author)

Key Words: Body Condition, Body Weight Gain, Extensive Grazing, Post-Weaned Pandan Calves, Urea-Impregnated Zeolite

UDC: 591.151

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Polimorfisme gen *melanocortin-4 receptor* dan asosiasinya terhadap sifat pertumbuhan sapi Bali (Polymorphism of melanocortin-4 receptor gene and its association with growth traits in Bali cattle)

(Org: Eng)

JITV 28(3): 177-189

The melanocortin-4 receptor (MC4R) is a gene that controls growth traits. This gene is embedded in the leptin-melanocortin pathway and regulates body weight. Previous studies have successfully identified the genetic diversity of the MC4R associated with growth traits. However, studies on Bali cattle are limited. This study aimed to identify the SNP markers of the MC4R gene and its effect on birth body weight, weaning body weight, weaning chest girth, weaning withers height, weaning body length, yearling body weight, yearling chest girth, yearling withers height, and yearling body length in Bali cattle (n=43). The MC4R genotype was identified by sequencing and aligned using BioEdit v.7.2.5. The association between genotype and growth traits was analyzed using the GLM procedure and DMRT in RStudio program v.2022.02. Four SNPs were identified in the exon region: g.355G>T, g.394C>T, g.463G>A, and g.682G>A. Based on the Chi-square test, the population was fitted with HWE (p>0.05). Genotype-phenotype association based on SNPs showed no significant result (p>0.05) where the g.682G>A (AG) locus had values of WB (104.33±17.15 kg), WCG (112.83±3.66 kg), WBL (89.50±3.94 kg), YB (124.17±22.44 kg), YCG (120.50±5.50 kg), and YBL (92.83±4.31 kg) higher than the other genotypes. Association based on haplotype type showed significant results (p<0.05) in BB, where haplotype 2 had the highest BB (21.17±0.75 kg). In conclusion, the SNP markers found in this study were polymorphic but did not affect growth traits in Bali cattle.

(Author)

Key Words: Bali Cattle, Genetic Diversity, Growth Traits, MC4R Gene, Single Nucleotide Polymorphism

UDC: 612.621.9

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Produksi dan perkembangan embrio sapi donor double-muscled dan persilangannya yang disuperovulasi (Embryo production and development from superovulated donors in double-muscled cattle and their crosses)

(Org: Eng)

JITV 28(3): 187-196

Belgian Blue was introduced in Indonesia to increase the biodiversity of livestock genetic resources. Belgian Blue was crossed with Ongole grade to increase the productivity of local cattle. Therefore, this study evaluates reproduction traits, especially the response to superovulation, embryonic development, and quality of Belgian Blue, Ongole grade, and their crosses. Estrous was synchronized with intravaginal progesterone Cue-Mate before artificial insemination (AI). Superovulation was performed with Follicle Stimulating Hormone (FSH) intramuscularly with non-surgical embryo flushing. In addition, embryo quality was assessed microscopically according to the International Embryo Transfer Society (IETS) criteria. The study was performed in a quasi-experimental design, and data were analyzed with an analysis of variance. After superovulation, oocytes/embryos were obtained from all donor breeds. Oocyte and embryo production from Ongole grade and Belgian Blue differed at 11.83±1.91 and 4.86±1.33, respectively, P<0.05 (mean ± SEM). In addition, there are differences in recovery rate (89.63% vs. 75.35%) and fertilization rate (77.35% vs. 68.22%) between Ongole grade and Belgian Blue, respectively (P<0.05). There is no difference in embryo development quality and proportion of transferable embryos between Ongole grade, Belgian Blue, and their crosses. This study concluded that the cross-bred Belgian Blue x Ongole donor had identical oocyte and embryo production, recovery rate, fertilization rate, and degenerative embryos compared to its purebred.

(Author)

Key Words: Cattle, Embryo Flushing, *In Vivo*, Pre-implantation, Superovulatio

UDC: 637.54'658

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Karakteristik kualitas daging ayam IPB-D1 dan final stock-nya dari lokasi yang berbeda (Meat quality characteristics of IPB-D1 chicken and the final stock from different locations)

(Org: Eng)

JITV 28(3): 197-207

The increasing demand for animal protein encourages innovations development of new livestock types or breeds. IPB-D1 chicken is an improved local chicken for their productivity. Its rearing was carried out in Sukabumi and Bekasi Regency. Physical, chemical, and microbiological quality testing of IPB-D1 chicken meat was carried out at the Integrated IPTP Laboratory and the Microbiology Laboratory of Livestock Products, and organoleptic testing was carried out in the Organoleptic Room, both of the Faculty of Animal Husbandry, IPB University. The research was carried out from February to June 2022. The scope of this research includes chicken rearing at two locations and sampling that aims to test the physical, chemical, microbiological, and organoleptic quality. The chickens tested were the IPB-D1 Sukabumi chicken, IPB-D1 Bekasi chicken, IPB-D1 final stock Sukabumi chicken, IPB-D1 Bekasi final stock chicken, and Kampong, Sentul, and broiler chickens, which consist of

15 chickens each. At each location, sampling was carried out 10 times as a replication. The sampling technique used is simple random sampling. The results showed that the quality of IPB-D1 chicken meat and its Final Stock did not have a significant difference (P>0.05) in physical quality aspects such as pH and water holding capacity (WHC) but had a significant difference (P<0.05) in cooking loss and tenderness than Kampong, Sentul, and broilers. Meanwhile, the chemical quality of IPB-D1 broilers and their final stock showed significantly different results (P<0.05) in the fat content of the meat. As for the parameters of ash content, protein, minerals, and cholesterol showed no significant difference (P>0.05). Microbiological quality analysis in the form of total bacterial colonies (total plate count) of IPB-D1 broilers and its Final Stock was 105 CFU/g. These results are still within the Maximum Microbial Contamination Limits set by the Indonesian National Standardization Agency in 2009. Based on the results of this study, it is necessary to improve handling methods, hygienic packaging, and sanitation so that the quality of broilers can be improved and microbial contamination can be minimized.

(Author)

Key Words: Characteristics, Chicken Meat, Final Stock, IPB-D1, Microbiology, Organoleptic

UDC: 613.287.5

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Evaluasi empiris pilihan kebijakan peningkatan produksi susu di Indonesia: pendekatan sistem dinamik (An empirical evaluation of policy options for increasing dairy production in Indonesia: a system dynamics approach)

(Org: Eng)

JITV 28(3): 208-219

Domestic dairy production in Indonesia grows slower than consumption, resulting in an excess demand that imports must fulfill. Accelerating dairy production can no longer be solved partially; a holistic system approach is required. This study aims to empirically evaluate the policy options for accelerating dairy production in Indonesia. The data used in this study were secondary data from Badan Pusat Statistik, the Ministry of Agriculture, the Ministry of Trade, the Ministry of Industry, the Coordinating Ministry for Economic Affairs, the Ministry of Cooperatives and SMEs, Bank Indonesia, and FAO. A system dynamics approach was used to construct the model and describe the short-, medium-, and long-term impacts based on policy scenario options. The empirical results showed that the calf rearing program policy, increased female dairy cattle imports, higher conception rates, and import tariffs all positively impacted the share of domestic dairy production to dairy demand. On the other hand, a policy of increasing dairy consumption, if not accompanied by an increase in domestic dairy production, harms the domestic dairy production share. Except for the policy of increasing dairy consumption, all policy scenarios increased the long-term share of domestic dairy production more than the short- and medium-term. It is concluded that an optimistic policy through calf rearing, increased import of female cows, conception rate, and maintaining import tariffs was the best policy with the highest impact on increasing the share of domestic dairy production compared to pessimistic and moderate policies.

(Author)

Key Words: Dairy Demand, Dairy Production, Policy Analysis, Scenarios, System Dynamics

UDC: 581.145

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Pengaruh wilayah terhadap beberapa parameter reproduksi domba Ouled Djellal (Influence of region on some reproductive parameters in Ouled Djellal sheep)

(Org: Eng)

JITV 28(4): 220-226

The present study aims to assess the reproductive performance of Ouled Djellal ewes at four different Biskra locations: El Hadjeb, Sidi Okba, Ouled Djellal, and Chaiba. All flocks were kept under extensive management. For this study, 357 clinically healthy and non-pregnant Ouled Djellal ewes have been used. Mating was practiced in an accessible mode, conducted over two months: May and June 2015 (61 days) for all herds. The total number of lambing ewes and lambs in all flocks was determined during the lambing period. We compared fertility, fecundity, and prolificacy rates using the $\chi 2$ test to verify the relationship between the measured rates and the four sites and multiple comparisons that revealed significant differences. The variables assessed in this study encompassed fecundity, prolificacy, and fertility. The overall mean values obtained were 78% for fecundity, 117% for prolificacy, and 92% for fertility. These averages were significantly lower than those observed in intensively managed herds. However, the lowest rates were recorded at site 4 (Chaiba), with 65% for fertility, 109% for prolificacy, and 71% for fecundity. The results of this study clearly show that the region has a significant effect on fertility (P= 0.001) and fecundity (P= 0.0001) but no significant effect on the prolificacy rate (P= 0.074).

(Author)

Key Words: Ewes, Fecundity, Fertility, Prolificacy

UDC: 637.14

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Perbandingan karakteristik fisikokimia, mikrobiologi dan organoleptik dali, dangke, dan keju segar asal susu kambing (Comparison of physicochemical, microbiological and organoleptic characteristics of dali, dangke and fresh cheese from goat milk)]

(Org: Eng)

JITV 28(4): 227-236

Indonesian people utilize goat milk to be traditionally processed into food products. Goat milk processing into dairy products is expected to be an alternative way to increase the value of goat milk consumption without reducing the benefit. The traditional dairy products from goat milk are Dali from North Sumatra and Dangke from South Sulawesi. This study aimed to examine the physiochemical, microbiological, and organoleptic characteristics of dali with Agave angustifolia leaf extract coagulant (alo-alo leaf), dangke with papaya latex coagulant, and fresh cheese with rennet coagulant. The experimental design used a randomized block design with three different levels of coagulant treatment, namely Rennet, papaya latex, and Agave angustifolia leaves as casein coagulating enzymes for dali, dangke, and fresh cheese. The variance and non-parametric data test used the Kruskal-Wallis test, followed by Tukey's multiple comparison test. The results showed that the values of aw, pH, and fat between dali, dangke, and fresh cheese were not different. Meanwhile, there is a significant difference ($P<0.05$) for yield value, dry matter, ash, protein, and carbohydrate. The results showed that the total plate count, yeast, and mold count of fresh cheese, dali, and dangke were not significantly different. The results of the hedonic test showed that fresh cheese differs significantly from others ($P<0.05$). Fresh cheese had a better organoleptic value than dangke and dali.

(Author)

Key Words: Dali, Dangke, Keju Segar, Susu Kambing

UDC: 613.2.038

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Pengaruh dosis curcumin Enema terhadap pengurangan giardia cyst pada sapi pedet (Effect of curcumin enema dosage regimes on reducing giardia cyst among calves)

(Org: Eng)

JITV 28(4): 237-243

Giardiasis is one of the most significant and neglected parasitic infestations among calves, resulting in intestinal infections. This protozoan agent gained diagnostic and therapeutical importance because of its zoonotic potential, in which novel alternative/substitute therapeutical interventions are important, and this is because commonly used antiparasitic drugs for control and eradication for battling this infection commonly presented side effects. Therefore, the present project evaluated the anti-protozoan activity of curcumin at different dose regimes used via the rectal route, the main constituent of turmeric. Rectal enema groups [Group I: 2 gr/kg, Group II: 80 mg/kg, and Group III: 10 mg/kg] and oral treatment Group IV: 2 gr/calf consisted of curcumin treatment at appropriate dosages showed herein in which the control group V were untreated. All treatment groups showed a statistically significant decrease in cyst counts on day 10, compared to day 10 values of the control group ($p<0.005$). Among the treatment groups, the highest cyst reduction was evident at 99.86% in curcumin 10 mg/kg rectal enema-administered calves (Group III), which received the lowest dose of the study. Curcumin at all dosages, primarily used in the rectal route at the lowest dosage regimen of 10 mg/kg, could have hastened clinical and parasitological efficacy via probable inhibition of Giardia proliferation and adhesion, as described previously. This treatment modality via the rectal route can change the game, highlighting novel treatment protocols.

(Author)

Key Words: Curcumin, Giardia, Oral, Rectal, Treatment

UDC: 613.286

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Karakterisasi kualitas telur pada tiga spesies unggas Phasianidae (Characterization in the egg quality of three species of Phasianidae birds)

(Org: Eng)

JITV 28(4): 244-250

Rock partridge (Alectoris graeca), Common pheasant (Phasianus colchicus), and Japanese quail (Coturnix japonica) are three poultry species from the Phasianidae family birds that are kept for meat and egg production in the Republic of Turkey. This research was carried out to characterize the egg quality of Rock partridge (RP), Common pheasant (CP), and Japanese quail (JQ). The eggs parameter of egg weight (EW), shape index (SI), yolk index (YI), albumen index (AI), shell weight (SW), haugh unit (HU), yolk weight (YW), albumen weight (AW), yolk ratio (YR), albumen ratio (AR) and shell ratio (SR) were measured for data analysis. A total of 372 eggs, including 100 eggs of partridges, 200 eggs of pheasant, and 72 eggs of quail were used in this study. A Canonical Discriminant Analysis (CDA) and Hierarchical Cluster Analysis (HCA) were used in this study to characterize three different types of eggs using SPSS. 16.0 package. The results showed that nine egg parameters of AR, YI, SI, YW, AW, YR, AI, HU, and SR are identified as discriminant variables for three egg types. The results showed that the canonical correlation (R) value in this study was high, i.e., 0.98 (Function 1) and 0.79 (Function 2). The Mahalonobis distance (D^2) in this study ranged from 15.28 (RP - CP) to 27.94 (CP - JQ). In conclusion, the Phasianidae poultries in this study have different egg quality traits and can be used as the species marker

(Author)

Key Words: CDA, HCA, Egg Quality, Mahalonobis, Phasianidae birds

UDC: 636.085.8

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Pengaruh tepung daun kelor sebagai pakan aditif terhadap antibodi dan histomorfometri bursa anak itik hibrida (Influence of moringa leaf meal as feed additive on antibody and bursa histomorphometry of hybrid ducklings)

(Org: Eng)

JITV 28(4): 251-258

Moringa leaf meal contains various phytonutrients that benefit animal and human health. This research is designed to evaluate Moringa leaf meal as a feed additive on antibody response and histomorphometry of the bursa Fabricius of hybrid duck administrated by ND vaccine. The experimental design used is a Completely Randomized Design (CRD) using thirty-two male hybrid ducks of three days old. They were divided into four treatment groups and eight replicates of each. The first group was standard commercial fed without adding of Moringa leaf meal (0%), while the second until the fourth group was a standard commercial fed with 2.5%, 5%, and 7.5% adding of Moringa leaf meal. All groups were vaccinated by ND1 and ND2, respectively, at the age of seventh and fourteenth days. The results showed that Moringa leaf meal as a feed additive had no significant effect on antibody titer, leukocyte count, lymphocytes (L), heterophils (H), H:L ratio, bursa weight, cortex, and medulla thickness of bursa. The concentration of serum MDA, the diameter of plica, and follicles bursa were significantly influenced ($P<0.05$) by adding Moringa leaf meal. In conclusion, the Moringa leaf meal additive could stimulate the proliferation of bursa follicles and have no adverse effect on the immune response of hybrid ducks post-ND vaccine.

(Author)

Key Words: Moringa Leaf Meal, Male Ducklings, Antibody Titer, Bursa Fabricius

UDC: 636.085.8

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Fermentasi kulit buah kakao menggunakan kunyit bubuk dan *Aspergillus niger*: pengaruh terhadap komposisi serat dan anti-nutrisi (Fermentation of cocoa pods husk using turmeric powder and *Aspergillus niger*: effects on fiber composition and antinutrients)

(Org: Eng)

JITV 28(4): 259-267

Processing of cocoa pods husk (CPH) by fermentation with *Aspergillus niger (A. niger)* and additional herbs such as turmeric is believed to be able to improve the quality of feed ingredients from plantation byproducts. The purpose of this study was to evaluate the effectiveness of the fermentation process using *A. niger* and the addition of different levels of turmeric in reducing the fiber fraction and antinutrient contents of CPH. The experiment used a completely randomized design of eight treatments and three replications. The treatments consisted of P0: control; P1: CPH fermented with *A. niger*; P2, P3 and P4: fermented (CPH + 0.5; 1.0 and 1.5% turmeric powder) with *A. niger*; P5, P6 and P7: fermented CPH with *A. niger* + 0.5; 1.0 and 1.5 % turmeric powder. Variables observed were the fiber composition of CPH as well as antinutrients. The results showed that CPH fermentation using *A. niger* with different levels of turmeric powder significantly reduced the content of fiber fractions, i.e., NDF, ADF, and hemicellulose, compared to that of unfermented CPH. For antinutritional content, fermentation using *A. niger* with different levels of turmeric powder significantly decreased tannin. It tended to decrease the lignin content of CPH compared to unfermented CPHs. In conclusion, fermentation of CPH using *A. niger* with an addition of 1.5% of turmeric before the fermentation is the most effective treatment in decreasing the fiber and antinutritional Components In CPH.

(Author)

Key Words: Antinutritional, *Aspergillus niger*, Cocoa Pods Husk, Fiber Fraction, Turmeric

UDC: 636.085.8

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Pengaruh pakan tambahan alami terhadap kualitas bebek ungkep (Effect of a natural feed additive on quality of bebek ungkep)

(Org: Eng)

JITV 28(4): 268-273

This study aimed to determine the effect of natural feed additives on the quality of ducks based on chemical, microbiological, and organoleptic parameters. The experimental design used in this study was the Completely Randomized Design, which consisted of 2 treatments, namely P0 (ducks were fed commercial feed during rearing) and P1 (ducks were given additional natural feed during rearing), with each treatment pentaplicates. The observed variables were chemical properties (moisture content, protein content, ash content, fat content, cholesterol content, and saturated fatty acid content), microbiological properties (TPC, number of *Enterobacteriaceae* colonies, *Salmonella sp.*, *Staphylococcus aureus,* and *Listeria monocytogenes*) and organoleptic quality (color, aroma, texture, taste and overall). The data collected in this study were analyzed by one-way ANOVA. The results showed an alteration of cholesterol content in Bebek Ungkep with natural additives during rearing. Besides that, there was no significant difference in the parameters of the microorganisms. For organoleptic, there is no significant difference for all parameters (taste, color, texture, overall) except aroma.

(Author)

Key Words: Bebek Ungkep, Chemical, Microbiological, Natural Feed Additives, Organoleptic

UDC: 316.647.7

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Karakteristik molekuler *toll-like receptor* (TLR21) Ayam KUB-1 dan hubungan kekerabatanya secara evolusionaris [Molecular characteristics and evolutionary relationships of toll-like receptor (TLR21) of Indonesian KUB-1 chicken]

(Org: Eng)

JITV 28(4): 274-286

In vertebrates, the immune system has several Toll-like receptors (TLRs) to recognize specific pathogen-associated molecular patterns (PAMPs). In aves, TLR21 is known to be a functional homolog of TLR9 in mammals. This study aimed to determine the molecular characteristics of TLR21 in KUB-1 chicken, a superior breed of Indonesian local chicken, and their evolutionary relationship. The TLR21 gene was obtained from the ovarian RNA extraction and synthesized into cDNA by reverse transcription. We found that the cDNA sequence of the *TLR21* of KUB-1 chicken has a length of 3504 bp, including a 2823 bp open reading frame (ORF), which encodes a putative protein of 940 amino acids (aa). The deduced KUB-1 chicken TLR21 protein consisted of 720 aa in the extracellular domain containing 20 LRRs, 23 aa in the transmembrane region, and 141 aa of Toll-IL-1 receptor in the intracellular domain, and had a molecular weight of 107 kDa. The KUB-1 chicken TLR21 had homology of about 99%, 76%, and 43% with other TLR21 from other breeds of chicken, non-chicken poultry, and fish, respectively. Its molecular character was conserved throughout the evolution of TLR21 in chicken, so the KUB-1 chicken was not much different from the broiler. However, there has been an evolution in duck and goose, although the function is still the same. Evolutionarily, *Gallus gallus*_KUB-1 TLR21, which belongs to the TLR11 Family, has differentiated from fish TLR21 and formed a paraphyletic relationship with TLR21 in *Anser cygnoides* and *Anas platyrhynchos*.

(Author)

Key Words: *Gallus gallus*, KUB-1, Ovary, Reverse Transcription, TLR21

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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.
c. Van Soest P. 2018. Nutritional ecology of the ruminant. 2nd ed. New York (USA): Cornell University Press.

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 socioeconomic 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/.

Patent:

Raab RM, Lazar G, Shen B. 2022. AGRIVIDA Inc, assignee. Engineered phytases in animal feed. 2022 Feb 8.

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