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Short Communication

Evaluation of *Bifidobacterium sp.* and *Guazuma ulmifolia* Leaf Extract on Quail (*Coturnix coturnix-japonica*): Influences on Feed Intake, Feed Conversion Ratio, and Quail Day Production

Aprinda Ratna Lovela[1], Widya Paramita Lokapirnasari[2], Mohammad Anam Al Arif[2], Soeharsono[3],Sri Hidanah[2], Sunaryo Hadi Warsito[2], Redilla Prasinta[4], Tiara Hapsari[4], Asafarid Andriani[4]*

[1]Department of Veterinary Agribusiness, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, 60115, East Java, Indonesia
[2]Division of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, 60115, East Java, Indonesia
[3]Division of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, 60115, East Java, Indonesia
[4]Department of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, 60115, East Java, Indonesia

*Correspondence: widya-p-l@fkh.unair.ac.id (Aprinda Ratna Lovela)

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Abstract

Background: Quail farms have a great opportunity as poultry farming businesses in Indonesia due to its short biological cycle and high metabolic rate. The success of a poultry business is closely linked to factors such as feed quality and health management. This study aims to determine the effect of adding *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract into drinking water on quail production performance include feed intake, feed conversion ratio (FCR), and quail day production (QDP). **Methodology:** The research employed an experimental design using a Completely Randomized Design with 96 female quails (*Coturnix coturnix-japonica*), randomly assigned to four treatments with six replications, each consisting of four quails. Different ratios of *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract were administered in each treatment. Data collection occurred during the final week of the research period. **Results:** Each treatment was given different ratio between *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract. The data collected in the last week of the research period were analyzed to evaluate the outcomes. **Conclusion:** The addition of 2 ml/liter *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract to drinking water had a significant effect ($p<0.05$) on feed intake, feed conversion ratio (FCR), and quail day production (QDP).

Keywords
Bifidobacterium sp., health, feed conversion ratio, *Guazuma ulmifolia*, quail day production

1. Introduction

The Japanese quail (*Coturnix coturnix-japonica*) is a broadly cultivated poultry because of its unnecessary egg production. Quail egg production in a year levels from 200-300 eggs for every quail [1]. Although the quail population in Indonesia has experienced growth between 2018 and 2020, there has been a persistent decline in egg production. This decline cannot be solely attributed to the number of quails, but rather it is influenced by factors such as feed intake and the nutritional composition of the feed provided to the quails [2].

Feed is one of the main factors of poultry to decide production results. around 60-70% of necessary costs (total production costs) for quail farming are spent on feed costs, until now it is nevertheless a main problem of poultry within the global [3].

The high cost spent on feed makes farmers use Antibiotic Growth Promoters (AGP) that may optimize productivity. AGP is one of the additives to increase production efficiency and improve feed quality. But currently prohibited considering the negative impacts that AGP can purpose, including that can inflicting residues in animal products, as well as causing inflicting bacterial resistance to antibiotics [4].

Efforts to reduce production costs and use AGP are very critical to support most desirable poultry performance. Alternative substances that can be used to increase production performance are the addition of probiotics and phytobiotic. Probiotics are non-pathogenic microorganisms that assist to maximize the absorption of feed nutrients to increase the efficiency of nutrient utilization in feed [5]. *Bifidobacterium sp.* is a commonly utilized probiotic that possesses the ability to suppress the growth of harmful microorganisms within the gastrointestinal tract. In line with [6], giving probiotic isolates in drinking water had better results than ingesting probiotics in feed.

One of the herb as phytobiotic which are generally utilized in Indonesia is *Guazuma ulmifolia*. The compounds contained in *Guazuma ulmifolia* leaves include bioactive compounds, especially proanthocyanidins, flavonoids and phenolic acids, and among the identified antioxidant compounds are chlorogenic acid, caffeic acid, rutin, quercitrin, quercetin and luteolin [7]. According to a study by [8], flavonoid bioactive compounds have demonstrated positive effects on digestion, intestinal microflora balance, and overall digestive tract function. However, there is a lack of existing research focusing on the combined effects of probiotic *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract. Hence, conducting studies to investigate the potential advantages of using probiotic *Bifidobacterium sp.*, *Guazuma ulmifolia* leaf extract, and their combination in enhancing the production performance of Japanese quail is necessary.

2. Materials and Methods

This study employed a completely randomized design as its experimental design. The research was carried out at the Experimental Animal Cage, located within the Faculty of Veterinary Medicine at Airlangga University in Surabaya. The research took place during the period of March to April 2022. The sample consisted of 96 eight-week-old female quails (*Coturnix coturnix-japonica*). The probiotic *Bifidobacterium sp.* isolate in liquid form, with a concentration of 1.2×10^9 CFU/ ml, was obtained from the collection of Prof. Widya Paramita Lokapirnasari. The *Guazuma ulmifolia* leaf extract used in the study had a concentration of 25%. The drinking water used was free of chlorine, and the quails were fed with commercial feed containing ingredients such as corn, bran, soybean meal, wheat fraction, and leaf meal (proximate analysis can be seen in table 1). This study ensured that variables such as temperature, humidity, lighting, and feed quality were controlled and kept consistent throughout the research.

Tabel 1. The proximate analysis of commercial feed

Dry Matter (%)	Ash (%)	Crude Protein (%)	Crude Lipid (%)	Crude Fiber (%)	Calcium (%)	NFE (%)	ME (kcal/kg)
91.0316	11.7724	18.3716	7.9755	3.7854	3.9557	49.1267	3002.4692

NFE : Nitrogen-Free Extracts ;
ME : Metabolizable Energy

2.1 Extract Making

Guazuma ulmifolia leaf extract was macerated utilizing 70% alcohol with a proportion of 1:2, 1 kg of *Guazuma ulmifolia* leaf powder was macerated in 2 liters of 70% alcohol. The maceration cycle was done for 2 × 24 hours and rehashed 2 × 24 hours. The macerate was allowed to evaporate at a temperature of 40 with a speed of 55 rpm. The extract that we used had a concentration of 25% which was prepared by diluting 25 grams of macerate powder with 100 ml of water.

2.2 Experimental Animal Treatment

The treatment in this study was carried out for 44 days starting when the quail were 8 weeks old, involving four treatments and six replications, with each replication consisting of four quails. The treatments administered were as follows: T0 (control), T1 (*Bifidobacterium sp.* added at a concentration of 2 ml/liter in drinking water), T2 (*Guazuma ulmifolia* leaf extract added at a concentration of 2 ml/liter in drinking water), and T3 (combination of *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract added at a concentration of 2 ml/liter in drinking water).

2.3 Data Collection

Data collection for this study took place during the last week of the treatment period. The process involved calculating the feed intake by taking the difference between the initial amount of feed provided and the remaining feed. The feed conversion ratio was determined by dividing the total amount of feed consumed over a week by the mass of eggs produced during the same period. Egg mass data was obtained by dividing the cumulative weight of all the eggs produced by the population of quails at that specific time. Quail day production (QDP) was calculated by comparing the number of eggs produced on a particular day, measured in grains, with the total population of female quails on that same day, and then multiplying the result by 100%.

2.4 Data analysis

The data collected in this study underwent statistical analysis using analysis of variance (ANOVA). If the analysis revealed a significant effect ($p<0.05$), further comparisons were conducted using Duncan's test. The data analysis was performed using the SPSS 26.0 software program for Windows (IBM Corp., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

The results obtained from the addition of *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract on production performance can be seen in table 2.

Tabel 2. The effect of *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract on production performance of *Coturnix coturnix-japonica*

Variables	T_0	T_1	T_2	T_3
Feed Intake (g/quail/day)	$22.09^{d} \pm 0.87$	$20.69^{c} \pm 0.90$	$18.73^{b} \pm 0.72$	$17.05^{a} \pm 0.41$
Feed Conversion Ratio	$2.91^{d} \pm 0.13$	$2.48^{c} \pm 0.17$	$2.26^{b} \pm 0.11$	$1.92^{a} \pm 0.09$
Quail Day Production (%)	$66.67^{a} \pm 2.44$	$70.24^{b} \pm 1.46$	$69.64^{b} \pm 2.52$	$72.92^{c} \pm 2.01$

^{a,b,c,d} Different superscripts in the same row showed significant difference ($p<0,05$).

3.1 Feed Intake

The results of the one-way ANOVA analysis on feed intake showed a significant difference ($p<0.05$). Primarily based on table 1, the order of feed intake from the lowest is T3 of 17.05 g/quail/day; T2 of 18.73 g/quail/day; T1 of 20.69 g/quail/day; and T0 of 22.09 g/quail/day. Factors that may influence the level of feed intake are poultry conditions, cage situations, weather, exposure time, and feed quality [9]. Feed intake for 42 days old quail is 22.77 g and the quantity is stable until the rejected quail [10].

The T1 group given probiotics showed lower feed intake compared to the control group. This is due to the fact probiotics have a role in increasing the absorption of feed inside the gastrointestinal tract with the contribution of beneficial micro-organism [11]. When the nutritional and energy requirements in the feed consumed by livestock have been fulfilled, the nutrition needed by livestock has been met [4]. The result of this study is in line with research [18] which reported that probiotic supplementation by drinking water in quail can lower feed intake significantly from 23.0087.

g/quail/day to 21.6528 g/quail/day. The higher concentration of probiotic given the higher bacteria contained in it, and then the quail will be more efficient in consuming feed. The addition of beneficial microbes for animal, preventing the growth of harmful microbes in the digestive tract so can improve the digestion of feed and can minimize feed intake [18].

The T2 group showed lower feed intake values compared to the control treatment. This is hypothesized to be a result of the presence of flavonoids, a chemical compound identified in the leaves of *Guazuma ulmifolia* [12]. The flavonoid is an antibacterial phytochemical with bacterial inhibition mechanisms. The mechanism of action is with antibacterial compounds, including inhibiting cell wall synthesis, inhibiting the integrity of bacterial cell wall permeability, inhibiting enzyme action, and inhibiting the synthesis of nucleic acids and proteins. So, it can kill pathogenic microorganism in the digestive tract and make the utilization of nutrients is maximized [13]. According to the [34] study reported that dietary supplementation of a herbal extract did not significantly effect on feed intake of quails except in the 4th week, but it can helped buffer the negative effects of heat stress of quail that impact to feed intake.

The combination of *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract in the T3 treatment resulted in a reduction in feed intake compared to the T0, T1, and T2 treatments. In this treatment, the feed intake was the lowest was T3. This can be attributed to the synergistic effects of the probiotics and *Guazuma ulmifolia* leaf extract, which enhanced the absorption of nutrients, leading to decreased feed intake. Insufficient nutrients within the digestive tract are responsible for the low feed intake [14]. Utilization of *Guazuma ulmifolia* leaf extract provides a potential alternative because of the antibacterial properties contained in the form of proanthocyanidins. Proanthocyanidins are tannins that have undergone condensation with flavonoids. [15]. Proanthocyanidin compounds protect the walls of the small intestine against toxins excreted by means of pathogenic micro-organism [16]. Desirable and healthy digestive tract conditions have an optimal impact on the absorption of nutrients inside the body of livestock [17]. The addition of beneficial microorganisms can improve food digestion in livestock so that it may streamline feed intake [14].

3.2 Feed Conversion Ratio (FCR)

Based the result of one-way ANOVA analysis on the feed conversion ratio showed a significant difference ($p<0.05$). Based on table 1, the order of feed conversion ratio from the lowest is T3 of 1.92 g/quail; T2 of 2.26 g/quail; T1 of 2.48 g/quail; and T0 of 2.91 g/quail. According to Lokapirnasari *et al*. [18], it was indicated that an elevated Feed Conversion Ratio (FCR) implies a lower degree of economic efficiency in feed utilization. Conversely, a situation where the feed intake is minimized while still achieving desirable outcomes such as egg production and weight gain signifies a higher level of feed efficiency. Factors that affect feed conversion include the physical form of feed, animal body weight, maintenance environment, stress, and gender [19]. In *Coturnix coturnix-japonica* the feed conversion ratio varies between 3.3-4.9 g/ quail [20].

The addition of *Bifidobacterium sp.* in the T1 group resulted in a decrease in the FCR compared to the control group. In line with the research [18] that the use of probiotics can decrease feed convertion ratio significantly from 2.1139 to 1.19984. These results also consistent with [33] that supplementation of multi-species probiotic can show the highly significant on FCR from 2.92 to 2.69. This can be attributed to the capacity of probiotics to augment both egg production and weight, resulting in a notable rise in egg mass. The improvement in egg mass efficiency may be attributed to the optimal absorption of nutrients (amino acids, calcium, protein and phosphorus) content in the feed by the probiotics, as well as their positive impact on the gastrointestinal system's function [21].

In the T2 group, the addition of *Guazuma ulmifolia* leaf extract resulted in the lowest FCR compared to the control group. This is likely due to the presence of flavonoids in the extract. According to [22], phytobiotics containing antibacterial and antioxidant substances can reduce feed conversion ratios, improve gastrointestinal function, digestion, and enhance the immune system. This finding is supported by [23], who found that the inclusion of Moringa leaves containing flavonoids can lead to a reduction in the feed conversion ratio. The

result in this study in line with [35] reported an improvement in FCR due to the dietary supplementation of herbal additives. Plants have a wide range of secondary metabolites, especially flavonoids with antioxidant characteristics, which have positive effect on gastrointestinal tract function [36]. Oteiza [37] explain that the systemic effects of flavonoids on the gastrointestinal tract include the ability to protect the intestinal epithelium, modulate nutrient absorption, maintain the integrity of the intestinal barrier, and maintain the intestinal microbiota.

T3 group combination of *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract showed the lowest FCR compared to other treatments, this was presumably due to the synergism between the two as feed additives which showed an increase in digestibility and increased productivity, thus causing a lower feed conversion ratio. This is in line with [24] who argue that the provision of feed additives such as probiotics and phytobiotics singly or in combination can create good synergies and can increase feed efficiency to provide maximum benefits.

3.3 Quail Day Production (QDP)

The one-way ANOVA analysis conducted on quail day production (QDP) revealed a significant difference ($p<0.05$). Subsequent Duncan's test results indicated that T0 significantly differed from T1, T2, and T3. However, there was no significant difference between T1 and T2. Primarily based on table 1 the order of QDP from the highest is T3 of 72.92%; T1 of 70.24%; T2 of 69.64%; and T0 of 66.67%. The results of this study are not much different from the research conducted by where the daily egg production is 60.35%-61.07% [25]. The main factor affecting egg production is the quantity of feed intake and the nutrients contained inside the feed [26].

The T1 group increased in the QDP value as compared to the control, this turned into due to the fact the provision of probiotics to young birds (towards the peak) was able to maximize reproductive performance and absorption of nutrients, so that it could impact the optimization of ova production [4]. At the time of this study, the 9-week-old quail become on its way to peak production, so the combination of probiotics on this study had a positive impact on egg production (QDP). Most effective absorption of nutrients will affect the increase in production due to the fact the protein within the feed may be absorbed optimally, the higher the protein consumption, the higher the egg production [27]. These results consistent with past study [18] that probiotic can improve the quail day production from 55.7837% to 69.6704%.

The T2 group has a higher QDP value compared to the control, this occurred due to the presence of phytochemical substances such as flavonoids and alkaloids within the leaves of *Guazuma ulmifolia*. These compounds enhance antibacterial activity, thereby reducing the population of pathogenic microorganisms. Consistent with [28] [29] the reduction of pathogenic micro-organism in the digestive tract can optimize the absorption of nutrients and increase non-pathogenic micro-organism which can be beneficial to the host. Phytochemical concentrates on optional metabolites, for example, proanthocyanidins showed their antimicrobial and cell reinforcement impacts [30][31][32].

The T3 group, which received a combination of probiotic *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract, exhibited the highest quail day production (QDP) value. This can likely be attributed to the synergistic effect of the combined feed additive on the gastrointestinal system. The combination appears to help maintain a balanced microflora in the gastrointestinal tract, leading to increased nutrient absorption and ultimately resulting in higher egg production. Adding feed additives can increase absorption of feed substances is able to maximize egg production [14].

CONCLUSION

According to the findings of this study, it can be inferred that the addition combination of *Bifidobacterium sp.* and *Guazuma ulmifolia* leaf extract at a concentration of 2 ml/liter in drinking water can lead to a reduction in feed intake, a decrease in feed conversion ratio (FCR), and an increase in quail day production (QDP) for *Coturnix coturnix-japonica*.

Author Contributions
Methodology, W.P.L, M.A.A., S., S.H., and S.H.W.; Writing Original – Review & Editing

A.R.L., W.P.L., M.A.A., S., S.H., S.H.W., R.P., T.H., A.A; Project Administration A.R.L., R.P., T.H., A.A.; Supervision W.P.L.; Formal Analysis M.A.A., S.; Conceptualization, W.P.L.

Ethics Approval

It is important to note that this research has undergone ethical evaluation and has been approved by the Research Ethics Commission of Universitas Brawijaya, with the ethical clearance number 021-KEP-UB-2022.

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Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

[1] Narinc, D., Karaman, E., Aksoy, T., & Firat, M.Z. (2013). Investigation of Nonlinear Models to Describe Long-Term Egg Production in Japanese Quail. Poultry Science, 92, 1676–1682. https://doi.org/10.3382/ps.2012-02511

[2] Directorate General of Livestock and Animal Health (Ditjen PKH). (2021). Livestock and Animal Health Statistics 2021. Indonesian Ministry of Agriculture. Retrieved Jan 5, 2022, from https://ditjenpkh.pertanian.go.id

[3] Thirumalaisamy, G., Muralidharan, J., Senthilkumar, S., Sayee, R.H., & Priyadharsini, M. (2016). Cost-Effective Feeding of Poultry. International Journal of Science, Environment and Technology, 5(6), 3997-4005. https://api.semanticscholar.org/CorpusID:189954869

[4] Huda, K., Lokapirnasari, W. P., Soeharsono., Hidanah, S., Harijani, N., & Kurnijasanti, R. (2019). The Effect of Probiotic *Lactobacillus acidophilus* and *Bifidobacterium sp.* on Production Laying of Infected *Escherichia coli*. Indonesian Journal of Animal Science, 14(2), 154-160. https://doi.org/10.31186/jspi.id.14.2.154-160

[5] Karwanti, N. W., Arumdani, D. F., Yulianto, A. B., Marbun, T. D., Sherasiya, A., Al Arif, M. A & Lokapirnasari, W. P. (2023). Efficacy of *Moringa oleifera* Lam. Extracts and *Pediococcus pentosaceus*, *Lactobacillus acidophilus, Lactobacillus plantarum* Probiotic During Starter Period on Growth Performance of Male Broiler Chicken. F1000Research, 12(215), 215. https://doi.org/10.12688/f1000research.130072.1

[6] Lokapirnasari, W. P., Agustono, B., Al Arif, M. A., Maslachah, L., Chandra, E. H., & Yulianto, A. B. (2022). Effect of Probiotic and *Moringa oleifera* Extract on Performance, Carcass Yield, and Mortality of Peking Duck. Veterinary World, 15(3), 694. https://doi.org/10.14202/vetworld.2022.694-700

[7] Lokapirnasari, W. P., Al Arif, M. A., Maslachah, L., Kirana, A. L. P., Suryandari, A., Yulianto, A. B., & Sherasiya, A. (2023). Evaluation of *Lactobacillus acidophillus* and *L. lactis* Probiotics and *Guazuma ulmifolia* Lam. extract as Feed Additives with Different Application Times to Improve Nutrient Intake and Feed Efficiency in *Coturnix coturnix-japonica* Females. Journal of Animal and Feed Sciences, 32(1), 59-67. https://doi.org/10.22358/jafs/156018/2022

[8] Ramiah, S. K., Zulkifli, I., Rahim, N. A. A., Ebrahimi, M., & Meng, G. Y. (2014). Effects of Two Herbal Extracts & Virginiamycin Supplementation on Growth Performance, Intestinal Microflora Population & Fatty Acid Composition in Broiler Chickens. Asian-Australasian Journal of Animal Sciences, 27(3), 375 – 382. https://doi.org/10.5713/ajas.2013.13030

[9] Lordelo, M., Fernandes, E., Bessa, R.J.B., & Alves, S.P. (2016). Quality of Eggs from Different Laying Hen Production Systems, from Indigenous Breeds and Specialty Eggs. Poultry Science, 96(5), 1485–1491. https://doi.org/10.3382/ps/pew409

[10] Lokapirnasari, W. P. (2017). Nutrisi dan Manajemen pakan burung Puyuh. Airlangga University Press. http://118.97.240.83:5758/inlislite3/opac/detail-opac/index?id=82824

[11] Yaqoob, M.U., Abd El-Hack, M.E., Hassan, F., El-Saadony, M.T., Khafaga, A.F., Batiha, G.E., Yehia, N., Elnesr, S.S., Alagawany, M., El-Tarabily, K.A., & Wang, M. (2021). The

Potential Mechanistic Insight and Future Implications for the Effect of Prebiotics on Poultry Performance, Gut Microbiome, and Intestinal Morphology. Poultry Science, 100(7), 101143. https://doi.org/10.1016/j.psj.2021.101143

[12] Rafi, M., Meitary, N., Septaningsih, D.A., & Bintang, M. (2020). Phytochemical Profile And Antioxidant Activity of *Guazuma ulmifolia* Leaves Extracts Using Different Solvent Extraction. Indonesian Journal of Pharmacy, 31(3), 171-180. https://doi.org/10.22146/ijp.598

[13] Riyadi, F.M., Prajitno, A., Fadjar, M., Syaifurrisal, A., & Fauziyyah, A.I. (2021). Potential of Moringa (*Moringa olieifera*) Leaf Extract to Inhibit the Growth of Pathogenic Bacteria *Edwardsiella tarda*. Joutnal of Auaqulture and Fish Health, 10(3), 321-330. https://doi.org/10.20473/jafh.v10i3.25057

[14] Agustono, B., Lokapirnasari, W. P., Yunita, M. N., Kinanti, R. N., Cesa, A. E., & Windria, S. (2022). Efficacy of Dietary Supplementary Probiotics as Substitutes for Antibiotic Growth Promoters During the Starter Period on Growth Performances, Carcass Traits, and Immune Organs of Male Layer Chicken. Veterinary World, 15, 324–330. https://doi.org/10.14202/vetworld.2022.324-330

[15] Heinrich, M., Barnes, J., Prieto-Garcia, J., Simon, G., & Williamson, E.M. (2017). Fundamentals of Pharmacognosy and Phytotherapy 3th Edition. Churchill livingstone. https://doi.org/10.1590/S1516-93322004000300027

[16] Castillejo, R.R., Garcimartín, A., Hernández-Martín, M., López-Oliva, M. E., Bocanegra, A., Macho-González, A., Bastida, S., Benedi, J, & Sánchez-Muniz. (2023). Proanthocyanidins: Impact on Gut Microbiota and Intestinal Action Mechanisms in the Prevention and Treatment of Metabolic Syndrome. International Journal of Molecular Sciences, 24(6), 53-69. https://doi.org/10.3390/ijms24065369

[17] Lokapirnasari, W. P., Dewi, A. R., Fathinah, A., Hidanah, S., Harijani, N., Soepranianondo, K & Andriani, A. D. (2018). Effect of Probiotic Containing Enterobacter Spp., Bacillus Spp., Cellulomonas Spp., Actinomyces Spp. Supplementation on The Laying Performance and Egg Cholesterol of Quail. In IOP Conference Series: Earth and Environmental Science, 187(1), 012030. https://doi.org/10.1088/1755-1315/187/1/012030

[18] Lokapirnasari, W. P., Dewi, A. R., Fathinah, A., Hidanah, S., Harijani, N., Soeharsono., Karimah, B., & Andrian, A. D. (2017). Effect of Probiotic Supplementation on Organic Feed to Alternative Antibiotic Growth Promoter on Production Performance and Economics Analysis of Quail. Veterinary World, 10(12), 1508-1514. https://doi.org/10.14202/vetworld.2017.1508-1514

[19] Andriani, A. D., Lokapirnasari, W. P., Karimah, B., Hidanah, S., & Al-Arif, M. A. (2020). Potency of Probiotic on Broiler Growth Performance and Economics Analysis. Indian Journal of Animal Sciences, 90(8), 1140-1145. https://doi.org/10.56093/ijans.v90i8.109294

[20] Khalil, M.M. (2015). Use of Enzymes to Improve Feed Conversion Efficiency in Japanese Quail Feed a Lupin-based Diet. Australia: Thesis The University of Western Australia. https://doi.org/10.21157/ijtvbr.v4i1.13804

[21] Wu, G., Bryant, M.M., Gunawardana, P., & Roland Sr, D.A. (2007). Effect of Nutrient Density on Performance, Egg components, Egg Solids, Egg Quality, and Profits in Eight Commercial Leghorn Strains During Phase One. Poultry Science, 86(4), 691-697. https://doi.org/10.1093/ps/86.4.691

[22] Prabakar, G., Gopi, M., Karthik, K., Shanmugana, S., Kirubakara, A., & Pavulraj, S. (2016). Phytobiotics: Could the Greens Inflate the Poultry Production?. Asian Journal of Animal and Veterinary Advances, 11(7), 383–392. https://doi.org/10.3923/ajava.2016.383.392

[23] Satria, E.W., Sjofjan, O., & Djunaidi, I.H. (2016). Effect of Moringa (*Moringa oleifera*) Leaf Meal Supplementation in Layer Chicken Diet on Production Performance and Egg Quality. Buletin Peternakan, 40(3),197-202. https://doi.org/10.21059/buletinpeternak.v40i3.11203

[24] Wijayanti. A. D., Rosetyadewi, A. W., Fitriana, I., & Pratama, A.M. (2021). Addition of Phytobiotics and Probiotics Increases Feed Conversion Ratio and the Percentage of Broilers Abdominal Fat. Indonesian Veterinary Journal, 22(3), 303-308. https://doi.org/10.19087/jveteriner.2021.22.3.303

[25] Fransela, T., Sarajar, C.L.K., Montong, M.E.R., & Najoan, M. (2017). Performans of Quail (*Coturnix coturnix japonica*) Provided Conch Paddy (*Pila ampullaceal*) Floura as a Substitute for Fish Flour in the Ration. Zootek journal, 37(1), 62-69. https://doi.org/10.35792/zot.37.1.2017.14223

[26] Brand, Z., Brand, T.S. & Brown, C.R. (2003). The Effect of Dietary Energy and Protein Levels on Production in Breeding Female Ostrich. British Poultry Science, 44(4), 598-606. https://doi.org/10.1080/00071660310001618343

[27] Lokapirnasari, W.P., Pribadi, T.B., Arif, A.A., Soeharsono, Hidanah, S., Harijani, N., Najwan, R., Huda, K., Wardhani, H.C.P., Noorrahman, N.F., & Yulianto, A.B. (2019). Potency of Probiotics *Bifidobacterium sp.* and *Lactobacillus casei* to Improve Growth Performance and Business Analysis in Organic Laying Hens. Veterinary World, 12(6), 860-867. https://doi.org/10.14202/vetworld.2019.860-867

[28] Brisbin, J. T., Gong, J., Lusty, C. A., Sabour, P., Sanei, B., Han, Y., & Sharif, S. (2008). Influence of in Feed Virginiamycin on the Systemic & Mucosal Antibody Response of Chickens. Poultry Science, 87(10), 1995–1999. https://doi.org/10.3382/ps.2008-00159

[29] Yulianto, A. B., Suwanti, L. T., Widiyatno, T. V., Suwarno, S., Yunus, M., Tyasningsih, W., & Lokapirnasari, W. P. (2021). Probiotic *Pediococcus pentosaceus* ABY 118 to Modulation of ChIFN-γ and ChIL-10 in Broilers Infected by *Eimeria tenella* Oocyst. Veterinary Medicine International. https://doi.org/10.1155/2021/1473208

[30] Morais, S.M., Calixto-Júnior, J.T., Ribeiro, L.M., Sousa, H.A., Silva, A.A.S., Figueiredo, F.G., Matias, E.F.F., Boligon, A.A., Athayde, M.L., Morais-Braga, M.F.B., & Coutinho, H.D.M. (2017). Phenolic Composition and Antioxidant, Anticholinesterase and Antibiotic-Modulating Antifungal Activities of *Guazuma ulmifolia* Lam. (Malvaceae) Ethanol Extract. South African Journal of Botany, 110, 251–257. https://doi.org/10.1016/j.sajb.2016.08.003

[31] Assis, R.Q., Andrade, K.L., Batista, L.E.G., Rios, A.O., Dias, D.R., Ndiaye E.A., & de Souza, É.C. (2019). Characterization of Mutamba (*Guazuma ulmifolia* LAM.) Fruit Flour and Development of Bread. Biocatalysis and Agricultural Biotechnology, 19, 101120. https://doi.org/10.1016/j.bcab.2019.101120

[32] Pereira, G.A., Araujo, N.M.P., Arruda, H.S., Farias, D.P., Molina, G., & Pastore, G.M. (2019). Phytochemicals and Biological Activities of Mutamba (*Guazuma ulmifolia* Lam.): A Review. Food Research International, 126, 108713. https://doi.org/10.1016/j.foodres.2019.108713

[33] Premavalli, K., Sangilimadan, K., & Omprakash, A. (2018). Effect of Supplementation of Multi-Species Probiotic on Production Performance of Japanese Quail. International Journal of Chemical Studies, 6, 2164-2166. https://www.academia.edu/36051412

[34] Kaplan, C., & Köksal, B.H. (2021). Effect of Dietary Supplementation with a Herbal Extract on Growth Performance and Meat Quality in Quails Raised under Thermal-Neutral and Heat Stress Conditions. Poultry Science Journal, 9(1), 73-84. https://doi.org/10.22069/psj.2021.18502.1649

[35] Hassan, I.I., Askar, A.A., & El-Shourbagy, A.G. (2004). Influence of Some Medicinal Plants on Performance, Physiological and Meat Quality Traits of Broiler Chicks. Egyptian Poultry Science, 24, 247-266. https://api.semanticscholar.org/CorpusID:89342410

[36] Shin, D. H., Lee, J. Y., Hwang, K. Y., Kim, K. K., & Suh, S. W. (1995). High-resolution Crystal Structure of the Non-Specific Lipid-Transfer Protein from Maize Seedlings. Structure London, 3(2), 189–199. https://doi.org/10.1016/s0969-2126(01)00149-6

[37] Oteiza, P.I., Fraga, C.G., Mills, D.A., & Taft, D.H. (2018). Flavonoids and the Gastrointestinal Tract: Local and Systemic Effects. Molecular Aspects of Medicine, 61, 41-49. https://doi.org/10.1016/j.mam.2018.01.001