

Review

A Review of The Potential Applications of Propolis in The Malaysian Poultry Industry

Louisiana Lulu Lukas¹, Nurfaizila Latif¹, Mohammad Nasir Hassan¹, Kamil Latif¹, Lirong Yu Abit¹, Suhaili Mustafa¹, Muhammad Hakim Mohammad Ali Hanafiah¹, Paul Bura Thlama¹, Herinda Pertiwi², Sarah Al-Twain³ and Juriah Kamaludeen^{1,4*}

1. Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus, Bintulu 97008, Sarawak, Malaysia
 2. Faculty of Vocational Studies Airlangga University, Jalan Dharmawangsa Dalam 28-30, Surabaya 60286 Indonesia
 3. Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia
 4. Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
- *Corresponding author: juriahk@upm.edu.my

ABSTRACT

Antibiotics are used in the poultry industry as feed additives to improve growth, prevent disease, treat sick animals, stabilize intestinal microflora, and improve growth performance. Due to the risks regarding long-term antibiotic resistance development of pathogenic bacteria in humans, the utilization of synthetic antimicrobials in livestock feed has been banned in most animal production practices. However, antibiotic prohibition in livestock can result in poor performance of the animals in terms of yield of production, quality, and health status. Therefore, it is important to find natural alternatives such as propolis to substitute for antibiotic usage in livestock feed. Propolis is a natural resin produced by honeybees. There are over 300 beneficial compounds found in propolis. It contains various bioactive compounds such as flavonoids, phenolic acids, and others which contribute to its antimicrobial, antifungal, and antioxidant properties. Various studies have been carried out to determine the biological and chemical activities of propolis as well as its function as an alternative natural feed additive. Thus, this review focuses on propolis composition, the potential of stingless bee propolis in Borneo, its potential as an antioxidant with antimicrobial properties, and the potential of propolis as a natural feed additive for poultry.

Key words: Propolis, feed additive, poultry, performance

Article History

Accepted: 25 October 2023
First version online: 30 December 2023

Cite This Article:

Lukas, L.L., Hassan, M.N., Latif, K., Abit, L.Y., Mustafa, S., Mohammad Ali Hanafiah, M.H., Thlama, P.B., Pertiwi, H., Al-Twain, S. & Kamaludeen, J. 2023. A review of the potential applications of propolis in the Malaysian poultry industry. *Malaysian Applied Biology*, 52(6): 1-9. <https://doi.org/10.55230/mabjournal.v52i6.2666>

Copyright

© 2023 Malaysian Society of Applied Biology

INTRODUCTION

In the past, the application of antibiotics played a major role in the industrialization of animal husbandry. Antibiotics are applied in poultry production to improve average weight gain, stabilize intestinal microorganisms, and prevent diseases caused by pathogenic microorganisms (Tayeb & Sulaiman, 2014). However, increasing knowledge of the negative side effects of antibiotic use especially in terms of strong antibiotic resistance buildup, has led to their prohibition across the poultry industry in many nations (Tayeb & Sulaiman, 2014). Banning antibiotic use as a growth promoter in the poultry industry has led to a reduction in animal performance in yield and product quality as well as higher disease and mortality incidences in this industry. Disease outbreaks have also increased, leading to higher mortality rates and lower quality hence heavy economic losses for the livestock industry (Brian & Delia, 2009).

Furthermore, without antibiotics, higher pathogenic loads in the gut may disrupt the gut microflora's balance leading to an increased feed conversion ratio (FCR) and ultimately increasing the feed cost (Dragana *et al.*, 2012). Many recent studies have focused on the search for antibiotic replacements such as prebiotics, probiotics, and other natural products to improve feed utilization, growth

promotion, and maintenance of intestinal health (Miroslav *et al.*, 2011). These include bee products such as propolis, bee pollen, and bee venom (Rabie *et al.*, 2018). Flavonoids are a key element of propolis produced by plants that can potentially increase the overall performance of birds by improving intestinal health, nutrient digestion, and absorption (Miroslav *et al.*, 2011).

Propolis or bee glue

The word propolis is composed of two syllables from the Greek language, “pro” translates to in defense whereas “polis” means defense as in defense of the hive – to protect the colony and larvae from pathogenic microorganisms and the entrance against intruders. Propolis is a natural resinous bee product that forages on various tree buds, flowers, and plant sources (Ivana *et al.*, 2018). Propolis is a major component of nest construction which also aids in reducing bacterial and fungal levels in their hive (Tayeb & Sulaiman, 2014). Over 300 ingredients have been identified in propolis (Rasha *et al.*, 2018). The chemical composition of propolis can vary depending on the various types of pollens, and vegetation available within a colony’s geographical location, which will be discussed further in the next section.

For centuries, bee products have been extensively used in traditional folk medicine. Natural bee products, including propolis, contain various bioactive compounds, such as flavonoids, and phenolic acids, contributing to their antimicrobial, antifungal, and antioxidant properties (Rasha *et al.*, 2018). Propolis supplementation in poultry diets is one of the best substitutes for antibiotic use (Tayeb & Sulaiman, 2014; Hosseini *et al.*, 2016; Shaddel-Tili *et al.*, 2017). Additionally, propolis contains digestive enzymes that improve digestibility and increase bird egg production (Ramadan *et al.*, 2018). It was found that propolis supplementation in poultry diet positively affected metabolism and maintaining good health (Hosseini *et al.*, 2016). Thus, the antimicrobial properties of propolis give rise to its potential use in both human and veterinary medicines.

Characteristic, chemical composition, and functional properties of propolis

Propolis composition varies according to hives, locale/ geographic origins, and seasons (Natalia, 2011; Mahmoud *et al.*, 2015). The color of propolis also depends on the resource availability surrounding their hive (Tayeb & Sulaiman, 2014). Normally, propolis is dark brown, but sometimes different colors such as green, red, black, and white hues (Shaddel-Tili *et al.*, 2017) and light yellow can occur (Tayeb & Sulaiman, 2014). A previous study by Diah *et al.*, (2018) mentioned that different species of bees produced propolis with different compositions. Although its composition slightly varies due to various factors, the basic composition of propolis comprises bee-wax (7%), bee-pollen (5%), resin-polyphenolic fraction (55%), aromatic essential oils (30%), and other minor components (3%) like vitamins (A, C, D, E & B1, B2 & B6), niacin and folate, and some micro and macro minerals like iron, calcium, copper, nickel, zinc, magnesium, manganese, vanadium, strontium, and cobalt (Natalia, 2011; Aygun, 2016; Rasha *et al.*, 2018; Ivana *et al.*, 2018). In addition, flavonoids and phenolic acids are the main chemical components in propolis (Hosseini *et al.*, 2015). The total phenolic content of propolis is also affected by location, harvesting season, water availability, climate, and the solvents used in extraction (Mahmoud *et al.*, 2015). Flavonoids, phenolic acids, and their derivatives are the bioactive compounds in propolis mainly responsible for their strong bactericidal, antiviral, antifungal, and antioxidant effects (Miroslav *et al.*, 2012). Propolis also has strong cytostatic and anti-tumor activity, immunostimulatory, anti-obesity, hepatoprotective effects, and anti-inflammatory properties (Yılmaz *et al.*, 2017). According to Nurul *et al.*, (2020), the aromatic acids and their functional groups such as amine, ester, carbonyl, alkyl, and hydroxyl may be responsible for determining the phenolic and flavonoid contents of propolis. Due to the presence of bioactive components and the high content of vitamins and minerals, it is suggested that these compounds within propolis all contribute to its biological and pharmacological potential (Miroslav *et al.*, 2012).

Propolis production by stingless bee of Borneo

There are 45 verified species of stingless bees occurring from 14 genera found in Malaysia (Rasmussen, 2008). In general, the different species of Malaysian stingless bees produce two general types of propolis, namely, sticky propolis produced by species such as *Heterotrigona itama* (Figure 1) and *Geniotrigona thoracica* as well as hard propolis which is produced by species such as *Tetrigona* sp. (Norafiza *et al.*, 2018). A recent study by Nurul *et al.*, (2020) found that stingless bee propolis collected from Brunei Darussalam had low protein and carbohydrate content but was high in lipid composition. Abdullah *et al.*, (2019), postulated that raw propolis produced by any stingless bee species, on a dry matter basis, comprises <1% of total crude fiber, carbohydrate, and crude protein. This was refuted by Khamsah and Nur Basyirah, (2020), who, in their study, recorded over 50% carbohydrate content in stingless bee propolis samples, and suggested; that the higher carbohydrate content could have been from surplus honey in the analyzed samples. Another independent study by Devequi-Nunes *et al.*, (2018) found that the lipid composition of stingless bee nests was relatively high; between 3 to 5 times higher than those found in the common honeybee, *Apis mellifera* L. The higher lipid content in the

propolis may have been sourced from plant lipids collected by the bees, such as resin, balsam, or wax (Aroujo *et al.*, 2016). Due to the high lipid content of propolis, stingless bee nests are generally more water resistant than a typical honeybee hive (Nurul *et al.*, 2020).

In a study by Nurul *et al.* (2020) the propolis of different species of stingless bees collected from a common area in Tutong District, Brunei Darussalam were found to vary subtly with regards to nutritional composition. They explained that the crude protein content in propolis of *G. thoracica* propolis was approximately five times above those found in *H. itama* and *Tetrigona binghami*. However, in terms of the total carbohydrate content of propolis, those of *H. itama* were found to be more than double that of *G. thoracica* and *T. binghami*. Ibrahim *et al.* (2015) opined that the type of stingless bee species was a major factor in determining the chemical content and biological activity of stingless bee propolis. It was found that the propolis produced by *H. itama* was superior in both flavonoid and phenolic content compared to the propolis of *G. thoracica* collected from the same area. Norafiza *et al.* (2018) found that from 10 types of propolis produced by different stingless bees, *H. itama* had the highest flavonoid and phenolic content. *Heterotrigona itama* belongs to the sub-family Apinae and is among the bee species with corbiculae which are a modification of their hind tibia for storage and transport of large amounts of pollen and resin (pollen basket) relative to their body size (David, 2006).

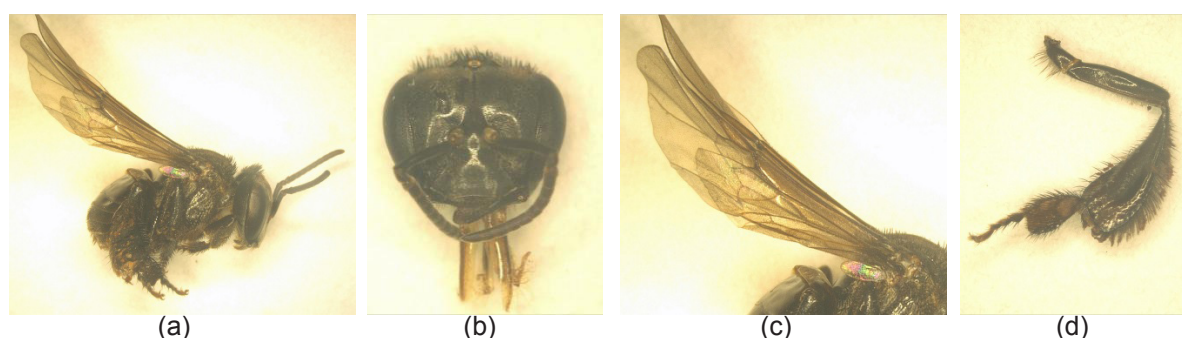


Fig. 1. Principal body parts of *Heterotrigona itama*; a Habitus, Lateral view; b, Frontal view of head; c, Forewings; d, Hind tibia and basitarsus (Source: Parasitology Laboratory, UPM Bintulu Sarawak Campus).

Figure 1 depicts the *Heterotrigona itama* specimen collected from Bario, Sarawak (N 03.74° / E115.46°). This particular species possesses a black body and head accompanied by grey forewings. Their wings are monotone coupled with dark brown wing venation. Both the fore and hind legs are covered in coarse, black setae. Body length is between 5–7 mm. The hind legs of this bee are enlarged. *H. itama* usually collects nest-building material from large resin-secreting trees of the Dipterocarpaceae family (Eltz *et al.*, 2003). These bees are fierce and aggressive in behavior (Kwapong *et al.*, 2010); *H. itama* are widely distributed and the most domesticated stingless bee species in Malaysia (Kelly *et al.*, 2014).

Propolis as natural growth promoter

Propolis has been added to poultry feeds as a natural growth promoter mainly due to its strong antioxidant action (Tayeb & Sulaiman, 2014; Hosseini *et al.*, 2016). The micronutrient content in propolis also contributes to its positive effects on bird metabolism (Hosseini *et al.*, 2015). Generally, propolis is also rich with essential amino acids including aspartic acid, glutamic acid, serine, glycine, arginine, histidine, threonine, lysine, alanine, proline, leucine, isoleucine, tyrosine, phenylalanine, valine, methionine, and tryptophan which are essential in enzyme production, animal growth, reproduction, and somatic maintenance (Nazife *et al.*, 2016). According to Klaric *et al.* (2018), the addition of 0.25–1.0 g/kg feed propolis and 20 g/kg feed bee pollen of the common Western honey bee (*Apis Mellifera*) as additives in broiler diets resulted in the production of healthier animals and significantly improved the fattening of chickens. Sakine *et al.* (2016) also suggested that the incorporation of propolis in diet may replace or reduce the use of antibiotics and other synthetic supplements in livestock and poultry diets because, propolis increases the quality of diets due to its high content of flavonoids, phenolic acid, and terpenoids, vitamins (A, B1, B2, B3, & biotin), minerals, protein, and enzymes, which are important to increase growth performance. The addition of between 1.0 – 5.0 g/kg of feed of common Western honey bee propolis may also increase palatability, and lead to increased feed intake (Sakine *et al.*, 2016). The bioactive components of propolis have high antimicrobial potential; thus, they promote intestinal health with increased digestion and absorption, helping to improve the growth performance of poultry (Shaddel-Tili *et al.*, 2017). Broilers supplemented with feeds containing 1.0 – 3.0 g/kg feed of Chinese bee propolis returned better growth performance due to increased digestive iron utilization as well as hemoglobin production efficiency, especially during recovery from anemic syndrome (Rabie *et al.*, 2018). Therefore, the potential of natural additives such as propolis to replace antibiotics as a

growth promoter may positively affect the overall economic use of feed mixtures for poultry and, thus, the overall economics of the poultry industry (Peter *et al.*, 2015; Sakine *et al.*, 2016).

The effect of propolis on the immune system of poultry

The immune status of poultry can be measured through the lymphoid organs' weight. For example, propolis supplementation in broilers could increase the relative weight of the spleen and bursa, which indicates that propolis may have an immune-booster effect (Hosseini *et al.*, 2016). This effect is attributable to the content of propolis, which accelerates the proliferation and differentiation process of the cell, in the immune system in avian birds. Other studies have also indicated that propolis can positively affect immunity through increased macrophage activity, intestinal microbial changes, and lymphatic tissue stimulation (Tayeb & Sulaiman, 2014). These positive effects could be related to bioflavonoids, phenolic acids, vitamins, and phytosterols- that are rich in propolis which encourages high speed of cell regeneration and differentiation in the immune system (Shaban *et al.*, 2018). Propolis supplementation could also enhance natural IgM antibodies in laying hens (Freitas *et al.*, 2011). Propolis can alter the acquired immune system due to its stimulatory potential on different natural immune system cells, such as the macrophages and T and B cells (Sforzin, 2007).

Additionally, flavonoid contents in propolis can suppress lymphoproliferative activity (Hegazi *et al.*, 2013). A study by Sakine *et al.*, (2016) further suggested that the micronutrient components of the natural additive of ethanolic extract from propolis added to the diet could improve the performance and immunity of Japanese quails. However, according to Freitas *et al.* (2011), the biological properties of propolis that enhance the immune response are affected by the concentration, intake period, and route of administration of the propolis.

The effect of propolis on physiological status in poultry

Propolis supplementation has also been reported to impact the physiological status of birds positively. Rabie *et al.* (2018) reported that, the levels of triacylglycerides, cholesterol, LDL (low-density lipoproteins), and LDL: HDL (high-density lipoprotein) ratios were significantly decreased in broilers supplemented with propolis in their diet. The heterophil: lymphocyte (H:L) ratio is one of the important stress indicators in birds, and, this ratio will increase when the birds are exposed to heat stress in the long term (Hosseini *et al.*, 2016). This is supported by Freitas *et al.* (2011), who independently demonstrated a significant decline in heterophils and H:L ratio in birds with propolis supplementation in their diet. The biological properties of propolis, such as lowering cholesterol levels through propolis supplementation, may be associated with the effect of flavonoids which have been shown to lower blood cholesterol levels (Matsui *et al.*, 2004; Krolczewska *et al.*, 2004). Flavonoids affect blood circulation, and metabolites in the blood by stimulating the use of triglycerides from the blood for energy generation. This is due to the ability of both flavonoids and phenolics to promote free radicals scavenging and, at the same time, protect lipids and other components from being destroyed through oxidation during heat stress conditions (Seven *et al.*, 2009).

Flavonoids have been shown to inhibit lipid peroxidation. Lipid peroxidation can interfere with platelet accumulation, capillary permeability, and vulnerability functions (Havsteen *et al.*, 2002). Klaric *et al.*, (2018) also suggested that compounds contained in propolis could enhance lipid metabolism and positively affect liver and kidney functions, which is indicated by lower triglycerides and cholesterol levels in the blood. Propolis supplementation in broilers can also reduce plasma creatinine and uric acid levels, which is a common biomarker for tissue damage in poultry, due to the inhibition of xanthine oxidase (XOD) activity (Mahmoud *et al.*, 2017; Rabie *et al.*, 2018). The bioactive components found in propolis such as chrysin, galangin, caffeic acid phenethyl ester, and p-coumaric acid have protection protective effect through the suppression of the expression of XOD and lipid oxidation in the cell membrane which has a positive effect on the liver and kidney thus, promoting a reduction in plasma creatinine and uric acid levels. It has been reported by Freitas *et al.* (2011) that there was a significant decline in heterophils (numbers/levels) and H:L ratio in birds with propolis supplementation (50 mg/kg feed) in their diet. The heterophil: lymphocyte (H:L) ratio is also an important stress indicator in birds, and, this ratio increases when birds are exposed to heat stress for the long term (Hosseini *et al.*, 2016).

The effect of propolis on the intestinal microbial population of poultry

The bioactive compounds of propolis, such as flavonoids, phenolic acids, and their derivatives, could be attributable to the bactericidal, antiviral, antifungal, and antioxidant effects (Tosi *et al.*, 2007; Erkmén & Özcan, 2008). The digestive system of broilers, particularly their cecum, and ileum, contains different species of bacteria (Miroslava *et al.*, 2011). Miroslava *et al.* (2012) and Lan *et al.* (2005) mentioned that the microflora population in the gut has a significant role in the gut system, and it could improve or reduce the host nutrition quality, health, and growth performance. Furthermore, a high load of gut microflora in fast-growing chickens could cause a nutritional burden due to the increased energy requirement for gut microflora maintenance, resulting in reduced nutrient utilization efficiency (Dibner & Richards, 2005). According to Miroslava *et al.* (2011), propolis helps control *E. coli* and

Clostridium rates in the gastrointestinal tract (GIT) of birds. The authors also suggested propolis may be a natural alternative to control the microbial loads in the GIT of broilers instead of probiotics or antibiotics. Propolis supplementation could increase the number of beneficial lactic acid bacteria and reduce Enterobacteriaceae family counts in crops, which may be associated with the antibacterial activity of propolis (Miroslava *et al.*, 2012). The inclusion of propolis in the diet of broiler chicken could promote pathogenic bacterial proliferation in the gut and thus improve digestion and nutrient absorption capacity increasing the average daily gain (ADG) in broilers (Hosseini *et al.*, 2015).

The effect of propolis in intestinal histomorphology in poultry

The health and nutrient absorption ability of the mucous membrane of poultry digestive system is indicated by the villus height and crypt depth; higher villus-to-crypt (VCR) ratios indicate better nutrition digestion and absorption (Shaban *et al.*, 2018). According to Hosseini *et al.* (2015), propolis supplementation in broilers chicken significantly increased the VCR ratios and ADG because, the bioactive components of propolis promoted pathogenic bacteria proliferation, which protects the intestinal mucosa from possible harm and also improved the digestion and absorption capacity. Other studies have shown significant improvements in villus height in the jejunum, crypt depth in the duodenum, jejunum, and ileum, the VCR ratio in the jejunum, and the number of goblet cells in both duodenum and ileum in broiler chicken after being supplemented with propolis in their diet indicating efficient digestion in the broiler (Torki *et al.*, 2015).

The role of propolis in alleviating heat stress in poultry

Several studies have shown that supplementation of natural antioxidants such as propolis is a potential method to minimize the detrimental effects of heat stress in birds (Mahmoud *et al.*, 2017; Gamal *et al.*, 2017; Ramadan *et al.*, 2018; Shaban *et al.*, 2018). Dietary natural antioxidants such as propolis are one of the best methods to resolve the adverse effects of heat stress in broiler meat. Creatine kinase (CK) and lactate dehydrogenase (LDH) are biomarkers of heat stress. Antioxidants from propolis supplementation may reduce both CK and LDH activity in breast muscle and, thus, promote myopathy reduction in broilers (Hosseini *et al.*, 2015). Deep pectoral myopathy causes changes in meat color to deep red due to the hemorrhaging of blood from ruptured vessels thus, reducing meat quality. In the study of Mahmoud *et al.* (2015), it was reported that broiler chickens fed with 250 – 750 mg/kg feed of Chinese honey bee propolis and exposed to heat stress showed better growth performance than broilers fed with 250 mg/kg vitamin C due to the better ability of propolis to reduce harmful effects of oxidative stress. The bioactive compounds of Egyptian honey bee propolis comprise 10.2% flavonoids and 5.6% polyphenols amounts in the propolis samples could reduce protein denaturation and then, decrease protein breakdown (Gamal *et al.*, 2017). This is also reflected in the high production performance of quail birds even when they are subjected to heat stress conditions (Sahin *et al.*, 2006). These studies have confirmed that propolis supplementation in quail diets would likely increase the feed conversion ratio and nutrient digestibility (Gamal *et al.*, 2017).

During heat stress events, the shell thickness and weight of layer eggs could be increased with propolis supplementation. This may be due to increased calcium digestibility and absorption aided by acid derivatives such as benzoic, and 4-hydroxy-benzoic of propolis (Yilmaz *et al.*, 2017). According to Ramadan *et al.* (2018), pigeons fed with 0.5 g/kg feed of Egyptian honey bee propolis exhibited the highest egg production rate during heat stress. Moreover, the antioxidant components of propolis, such as flavonoids, act similarly to anabolic agents with estrogenic effects that may alleviate the detrimental effect of heat stress on the reproductivity of pigeons (Vidda *et al.*, 2008). The bioactive compounds of propolis are responsible for their antibacterial, antiviral, and antifungal potentials, which play a significant role, especially during heat stress events, by reducing the detrimental oxidative effect, improving bird immunity and health status (Ramadan *et al.*, 2018).

Conclusion current prospect of propolis potential as a natural additive in poultry feed

It is crucial to find natural products such as propolis as a viable substitute for antibiotics in animal production. Antibiotic substitutes must have similar or enhanced abilities to improve the immune system and fight against pathogens for both humans and animals. Without functioning antibiotics in livestock production, the poultry industry may be exposed to the risk of disease and, without the ability to prevent the spread of infection; it will also result in the animals' poor performance yield and product quality (Ilan *et al.*, 2004).

Propolis propolis-supplemented diet may reduce the use of antibiotics and other synthetic supplements in livestock and poultry diets (Shaddel-Tili *et al.*, 2017). In contrast to antibiotics, propolis is a natural product with no known negative side effects and is useful in the food chain (Ihsan *et al.*, 2013). Recently, there has been a profound interest in finding natural antioxidant with no known negative side effects, and is useful in the food chain (Ihsan *et al.*, 2013) due to the carcinogenic potential of synthetic antioxidant (Mahmoud *et al.*, 2015). However, an intensive investigation is necessary to study the mechanism of propolis towards all species of birds' productivity performance, immune response,

and meat quality. Several studies have shown that propolis had a positive effect on poultry performance (Miroslav *et al.*, 2012; Hosseini *et al.*, 2015; Klaric *et al.*, 2018), whereas other studies could not confirm this (Abbasali *et al.*, 2017). The inconsistent results in regards to the effectiveness of propolis in poultry may be related to the dose and dosage administered, study conditions such as stressful or non-stressful conditions (Shaban *et al.*, 2018), the extraction methods (Fokt *et al.*, 2010), stingless bee species (Diah *et al.*, 2018) and source of propolis (Natalia, 2011; Konstantia *et al.*, 2016) even to different species of the birds. Therefore, further studies are required to find the right extraction method and dose and/ or dosage of propolis supplementation for optimum production in animals.

The propolis concentration supplemented in the feed may have different effects on the growth performance parameters of birds. For example; quails fed with higher concentrations of honey bee propolis (300 – 500 mg/kg) showed significant improvement in growth performance whereas, quails fed with lower honey bee propolis concentrations (100 – 200 mg/kg) showed less improvement in growth performance (Tayeb & Sulaiman, 2014). Studies are also needed to investigate the propolis compositions according to species and origin, as the same species of stingless bees may produce different compositions of propolis. 2391.0 mg/mL (GAE/g) and 275.20 mg/mL (QE/g) of total phenolic and flavonoids, were extracted from *Heterotrigona itama* stingless bee's samples collected in Brunei Darussalam by Nurul *et al.* (2020). Meanwhile, *H. itama* propolis collected from Kelantan, Malaysia contained 28.09 mg/mL (GAE/ g) and 7.85 mg/mL (QE/g) of total phenolic and flavonoids (Siti *et al.*, 2019). Different compositions for both total phenolic and flavonoid may also affected by methods of extraction where; Nurul *et al.* (2020) used maceration in 96% ethanol as a method of extraction, whereas, Siti *et al.* (2019) used 70% ethanol coupled with freeze drying- as their method of extraction. *Heterotrigona itama* propolis showed a higher total phenolic and flavonoid contents (56.9 µg/mL and 163.9 µg/mL) than *Geniotrigona thoracica* (29.1 µg/mL and 61.5 µg/mL) stingless bee propolis collected from the same area in Terengganu, Malaysia (Ibrahim *et al.*, 2016). Therefore, extensive studies and knowledge on propolis compositions, extraction methods, and levels of incorporation in animal feed are extremely valuable to the problem of propolis standardization.

Future research should focus on propolis's mode of action or mechanisms to prevent pathogenic bacteria proliferation and indigenous bacteria adjustment to promote poultry's health, immune status, and performance. Other than that, determining the effects and mechanism of natural products such as propolis on human and animal health may be of importance at present due to the increasing practice of organic agriculture and the increasing importance attached to safe food production (Torki *et al.*, 2015). Studies on propolis potential to alleviate heat stress in poultry are also crucial because global warming is a major challenge for the poultry industry (Gamal *et al.*, 2017). Additionally, heat stress exposure during poultry production could substantially decrease poultry production and meat quality which will have a huge impact worldwide especially in terms of poultry prices (HavlöÅk *et al.*, 2014). Furthermore, propolis has an immunostimulatory effect in poultry species by increasing the production of antibodies; thus, studies on using propolis to increase antigen-specific antibody responses to vaccines could be important (Freitas *et al.*, 2011). Further studies are necessary to investigate whether propolis enhances the production of antibodies and other antigens to protect birds against disease.

To our knowledge, no previous studies have been conducted on the effects of stingless bee propolis as a natural additive for quail. The increase in stingless bee rearing or meliponiculture activities in Malaysia is a golden opportunity to study stingless bee propolis as a natural animal feed additive due to the abundant availability of stingless bee propolis available at cheaper prices.

CONCLUSION

It could be emphasized that propolis is one of the most valuable candidates to replace antibiotics in livestock production. The bioactive compositions of propolis, such as phenolic acids and flavonoid contents are responsible for its antimicrobial, antifungal, and antioxidant properties which is comparable to antibiotic. Additionally, its bioactive compound is shown to affect heat stress alleviation positively which is important in Malaysian poultry production which has a tropical climate that is hot and humid throughout the year. Therefore, propolis supplementation in poultry diets may improve their growth performance, gut health, physiology, and immunity. However, extensive study will be required to standardize the phenolic acids and flavonoid compositions and their levels of inclusion in the feed to be used as an effective feed additive.

ACKNOWLEDGEMENTS

The authors would like to thank the Universiti Putra Malaysia Bintulu Sarawak Campus for supporting resources and facilities. This study is funded by RECODA (Regional Corridor Development Authority of Sarawak, Malaysia) for an integrated rural community development project.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Abbasali, G., Shekofa, S. & Nasir, L. 2017. Effect of ethanolic extract of propolis as an alternative to antibiotics as a growth promoter on broiler performance, serum biochemistry and immune responses. *Veterinary World*, 10(2): 249-254. <https://doi.org/10.14202/vetworld.2017.249-254>
- Abdullah, N.A., Ja'afar, F., Yasin, H., Taha, H., Petalcorin, M., Mamit, M., Kusriani E. & Usman. A. 2019. Physicochemical analyses, antioxidant, antibacterial, and toxicity of propolis particles produced by stingless bee *Heterotrigona itama* found in Brunei Darussalam. *Heliyon*, 5(9): 1-8. <https://doi.org/10.1016/j.heliyon.2019.e02476>
- Araujo, K.S.D.S., Joaquim, D.S.J., Marcello, O.S., Fernanda, D.B., Isamar, M.S., Robson, B., Tarso, D. S.A., Sergio, D.A. & Sandra, M.B.M. 2016. Physicochemical properties and antioxidant capacity of propolis of stingless bees (meliponinae) and Apis from two regions of Tocantins, Brazil. *Acta Amazonica*, 46(1): 61-68.
- Aygun, A. 2016. The effects of in-Ovo injection of propolis on egg hatchability and starter live performance of Japanese quails. *Brazilian Journal of Poultry Science*, 2: 087- 094. <https://doi.org/10.1590/1806-9061-2015-0198>
- Brian, P. & Delia, G. 2009. The impacts of livestock diseases and their control on growth and development processes that are pro-poor. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 364: 2643-2655. <https://doi.org/10.1098/rstb.2009.0097>
- David, W.R. 2006. Stingless bee nesting biology. *Apidologie*, 37(2): 124-143. <https://doi.org/10.1051/apido:2006026>
- Devequi-Nunes, D., Machado, B.A.S., de Abreu Barreto, G., Silva, J.R., da Silva, D.F., da Rocha, J.L.C., Brandão, H.N., Borges, V.M. & Umsza-Guez, M.A. 2018. Chemical characterization and biological activity of six different extracts of propolis. *PLoS ONE*, 13(12): e0207676. <https://doi.org/10.1371/journal.pone.0207676>
- Diah, K.P., Abdul, M., Andini, S. & Muhamad, S. 2018. Phytochemical profile and antioxidant activity of propolis ethanolic extract from Tetragonula bee. *Pharmacognosy Journal*, 10(1): 128-135. <https://doi.org/10.5530/pj.2018.1.23>
- Dibner, J.J. & Richards, J.D. 2005. Antibiotic growth promoters in agriculture: History and mode of action. *Poultry Science*, 84: 634-643. <https://doi.org/10.1093/ps/84.4.634>
- Dragana, S., Stuart, E.D., Robert, J.H., Mark, S.G., Tamsyn, M.C., Honglei, C., Volker, R.H. & Robert, J.M. 2012. Intestinal microbiota associated with differential feed conversion efficiency in chickens. *Applied Microbiology and Biotechnology*, 96: 1361-1369. <https://doi.org/10.1007/s00253-011-3847-5>
- Eltz, T., Bruhl, C.A., Imiyabir, Z. & Linsenmair, K.E. 2003. Nesting and nest trees of stingless bees (Apidae: Meliponini) in lowland Dipterocarp forests in Sabah, Malaysia, with implications for forest management. *Forest Ecology and Management*, 172, 301-313. [https://doi.org/10.1016/S0378-1127\(01\)00792-7](https://doi.org/10.1016/S0378-1127(01)00792-7)
- Erkmen, O. & Özcan, M.M. 2008. Antimicrobial effect of Turkish propolis, pollen, and Laurel on spoilage and pathogenic food-related microorganisms. *Journal of Medicinal Food*, 11: 587-592. <https://doi.org/10.1089/jmf.2007.0038>
- Freitas, J.A., Vanat, N., Pinheiro, J.W., Balarin, M.R.S., Sforzin, J.M. & Venancio, E.J. 2011. The effects of propolis on antibody production by laying hens. *Poultry Science*, 90: 1227-1233. <https://doi.org/10.3382/ps.2010-01315>
- Fokt, H., Pereira, A., Ferreira, A. M., Cunha, A. & Aguiar, C. 2010. How do bees prevent hive infections? The antimicrobial properties of propolis. In: *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. A. Méndez-Vilas (Ed.). Formatex. pp. 481-493.
- Gamal, M.K.M., Rania, M.I., Adel, A.D., Hosam, M.S., Osama, A.E. & Ahmed, O.A. 2017. The importance of propolis in alleviating the negative physiological effects of heat stress in quail chicks. *PLoS ONE*, 12(10): e0186907. <https://doi.org/10.1371/journal.pone.0186907>
- Havlíček, P., Valin, H., Herrero, M., Obersteiner, M., Schmid, E. & Rufino, M.C. 2014. Climate change mitigation through livestock system transitions. *Proceedings of the National Academy of Sciences*, 111: 3709-3714. <https://doi.org/10.1073/pnas.1308044111>
- Havsteen, B. 2002. The biochemistry and medical significance of the flavonoids. *Pharmacology and Therapeutics*, 96: 67-202. [https://doi.org/10.1016/S0163-7258\(02\)00298-X](https://doi.org/10.1016/S0163-7258(02)00298-X)
- Hegazi, A., Abdou, M. & Allah, F.A. 2013. Influence of honey on immune response against Newcastle disease vaccine. *International Journal of Basic and Applied Virology*, 2: 1-5.
- Hosseini, S.M., Afshar, M., Ahani, S. & Vakili Azghandi, M. 2015. Heat shock protein 70 mRNA expression and immune response of heat-stressed finishing broilers fed propolis (bee glue) supplementation. *Archives Animal Breeding*, 58: 407-413. <https://doi.org/10.5194/aab-58-407-2015>
- Hosseini, S.M., Vakili, A.M., Ahani, S. & Nourmohammadi, R. 2016. Effect of bee pollen and propolis (bee glue) on growth performance and biomarkers of heat stress in broiler chickens reared under high

- ambient temperature. *Journal of Animal and Feed Sciences*, 25: 45-51. <https://doi.org/10.22358/jafs/65586/2016>
- Ian, P., Mark, C., Tony, C., Brad, D., Christina, F., Ron, J., Charles, N., Rodney, P. & John, W. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy*, 53: 28-52. <https://doi.org/10.1093/jac/dkg483>
- Ibrahim, N., Zakaria, A.J., Ismail, Z. & Mohd, K.S. 2015. Antibacterial and phenolic content of propolis produced by two Malaysian stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica*. *International Journal of Pharmacognosy and Phytochemical Research*, 8(1): 156-161.
- Ihsan, M.S., Balqees, H.A. & Abd-alameer, H.Z. 2013. Effect of propolis in diet supplementation on the histopathological changes in some organs and challenge tests against Newcastle disease in broiler chicks. *Magazin of Al-Kufa University for Biology*, 5(2): 2073-8854.
- Ivana, K., Mirela, P., Ivan, M., Valerija, B., Albina, D. & Maja, M. 2018. Influence of dietary supplementation of propolis and bee pollen on liver pathology in broiler chickens. *Animals*, (8)54: 54. <https://doi.org/10.3390/ani8040054>
- Kelly, N., Farisya, M.S.N., Kumara, T.K. & Marcela, P. 2014. Species diversity and external nest characteristics of stingless bees in meliponiculture. *Pertanika Journal of Tropical Agricultural Science*, 37(3): 293-298.
- Khamsah, S.M. & Nur Basyirah, M.Z. 2020. Chemical and biological investigation of apiculture products from stingless bees *Heterotrigona itama*. *Journal of Agrobiotechnology*. 2020, 11(1): 7-19. <https://doi.org/10.37231/jab.2020.11.1.183>
- Klaric, I., Miskulin, I., Seruc, V., Dumic, A., Jonjic, J. & Miskulin, M. 2018. The effects of propolis and bee pollen supplementation on biochemical blood parameter of broilers. *Acta Veterinaria- Beograd*, 68 (2): 190-200. <https://doi.org/10.2478/acve-2018-0017>
- Konstantia, G., Milena, P., Olga, G., Vassya, B. & Ioanna, C. 2016. Characterization and biological evaluation of selected Mediterranean propolis samples. Is it a new type? *Food Science and Technology*, 65: 261-267. <https://doi.org/10.1016/j.lwt.2015.08.025>
- Kroliczewska, B., Jankowska, P., Zawadzki, W. & Oszmianski, J. 2004. Performance and selected blood parameters of broiler chickens fed diets with skullcap (*Scutellaria baicalensis* Georgi) root. *Journal of Animal and Feed Sciences*, 13: 35-38. <https://doi.org/10.22358/jafs/70289/2004>
- Kwapong, P., Aidoo, K., Combey, R. & Karikari, A. 2010. Stingless bee: Importance, management and utilisation: A training manual for stingless bee keeping. (Carson A. G., Ed). Unimax Macmilland Ltd.
- Lan, Y., Verstegen, M.W., Tamminga, S. & Williams, B.A. 2005. The role of the commensal gut microbial community in broiler chickens. *World's Poultry Science Journal*, 61: 95-104. <https://doi.org/10.1079/WPS200445>
- Mahmoud, M.A.M., Abdel-Mohsein, H.S. & Farghali, M.R.F. 2015. Antioxidant properties of Chinese propolis in Ross broilers exposed to heat stress in Egypt. *Open Journal of Veterinary Medicine*, 5: 197-209. <https://doi.org/10.4236/ojvm.2015.59027>
- Mahmoud, U.T., Amen, O.A., Applegate, T.J. & Cheng, H.W. 2017. Brazilian propolis effects on growth, productivity performance, gut characteristics and physiological changes in broiler chickens. *International Journal of Poultry Science*, 16(15): 169-179. <https://doi.org/10.3923/ijps.2017.169.179>
- Matsui, T., Ebuchi, S., Fujise, T., Abesundara, K.J., Doi, S., Yamada, H. & Matsumoto, K. 2004. Strong antihyperglycemic effects of water-soluble fraction of Brazilian propolis and its bioactive constituent, 3,4,5-tri-*o*-caffeoylquinic acid. *Biological and Pharmaceutical Bulletin*, 27: 1797-1803. <https://doi.org/10.1248/bpb.27.1797>
- Miroslav, K., Margita, Č., Jana, B., Monika, L., Peter, H. & Viera, D. 2012. Effect of nutrition with propolis and bee pollen supplements on bacteria colonization pattern in Gastrointestinal Tract of broiler chickens. *Scientific papers Animal Science and Biotechnologies*, 45(1): 63-67.
- Miroslav, K., Peter, H., Lukáš, H., Jaroslav, P., Martin, M., Vladimíra, K., Jadža, L. & Henrieta, A. 2011. Bee products effect to microbial colonization of chickens gastrointestinal tract. *Potravinárstvo*, 5: 372-376.
- Natalia, O. 2011. The Immunological efficiency of Vaccine Ma5 + Clone 30 administrated by spray method separate and in combination with the Hydroalcoholic solution of propolis. *Bulletin of University of Agricultural Sciences and Veterinary Medicine*, 68(2): 235-239.
- Nazife, E., Senem, A., Mustafa, Y., Baris, A. & Sibel, S. 2016. Amino acid and vitamin content of propolis collected by native Caucasian honeybees. *Journal of Apicultural Science*, 60(2): 101-109. <https://doi.org/10.1515/jas-2016-0021>
- Norafiza, A., Nora'aini, A., Fadzilah, A.A.M., Sofiah, H. & Shamsul, B.A.R. 2018. Total flavonoids and phenolic contents of sticky and hard propolis from 10 species of Indo-Malayan stingless bees. *Malaysian Journal of Analytical Sciences*, 22(5): 877-884. <https://doi.org/10.17576/mjas-2018-2205-15>
- Nurul, A.A., Nadzirah, Z., Siti, N.Z.Z., Hussein, T., Fatimah, H. & Anwar, U. 2020. Phytochemicals, mineral contents, antioxidants, and antimicrobial activities of propolis produced by Brunei stingless bees *Geniotrigona thoracica*, *Heterotrigona itama*, and *Tetrigona binghami*. *Saudi Journal of Biological Sciences*, 27: 2902-2911. <https://doi.org/10.1016/j.sjbs.2020.09.014>

- Peter, H., Ibrahim, O.E., Miroslav, K., Marek, B., Miroslava, K., Jozef, G., Milan, Š. & Ahmed, A.S. 2015. The influence of propolis as supplement diet on broiler meat growth performance, carcass body weight, chemical composition and lipid oxidation stability. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 2(63): 411-418. <https://doi.org/10.11118/actaun201563020411>
- Rabie, A.H., El-Kaiaty, A.M., Hassan, M.S.H. & Stino, F.K.R. 2018. Influence of some honeybee products and a growth promoter supplementation on productive and physiological performance of broiler chickens. *Egyptian Poultry Science Journal*, 38(2): 513-531.
- Ramadan, D., Ghada, S. & Ahmed, S.O. 2018. Impact of heat stress on reproductive behavior, performance and biochemical parameters of pigeon: A trial to alleviate heat stress by propolis or wheat diets. *Alexandria Journal of Veterinary Sciences*, 56(2): 84-96. <https://doi.org/10.5455/ajvs.292429>
- Rasha I.M.H., Gamal, M.M.M. & Hala Y.A.E. 2018. Effect of feeding propolis on growth performance of broilers. *Journal of Advanced Veterinary and Animal Research*, 8(3): 66-72.
- Rasmussen, C. 2008. Catalog of the Indo-Malayan/Australasian stingless bees (Hymenoptera: Apidae: Meliponini). *Zootaxa*, 1935: 1-8. <https://doi.org/10.11646/zootaxa.1935.1.1>
- Sahin, K., Onderci, M., Sahin, N., Gursu, M.F., Khachik, F. & Kucuk, O. 2006. Effects of lycopene supplementation on antioxidant status, oxidative stress, performance and carcass characteristics in heat-stressed Japanese quail. *Journal of Thermal Biology*, 31: 307-312. <https://doi.org/10.1016/j.jtherbio.2005.12.006>
- Sakine, B., Shaeban, R., Mohammad, A. K. T., Golamhosein, T. & Seyed, N.K.M. 2016. Effects of propolis, royal jelly, honey and bee pollen on growth performance and immune system of Japanese quails. *Veterinary Research Forum*, 7(1): 13-20.
- Seven, T., Yilmaz, S., Seven, I., Cerci, I.H., Azman, M.A. & Yilmaz, M. 2009. Effect of propolis on selected blood indicators and antioxidant enzyme activities in broilers under heat stress. *Acta Veterinaria Brunensis*, 78: 75-83. <https://doi.org/10.2754/avb200978010075>
- Sforcin, J.M. 2007. Propolis and the immune system: A review. *Journal of Ethnopharmacology*, 113: 1-14. <https://doi.org/10.1016/j.jep.2007.05.012>
- Shaban, C., Ali, K. & Hassan, R. 2018. Alleviation of thermal and overcrowding stress in finishing broilers by dietary propolis supplementation. *Italian Journal of Animal Science*, 17(2): 377-385. <https://doi.org/10.1080/1828051X.2017.1360753>
- Shaddel-Tili, A., Eshratkhan B., Kouzehgari H. & Ghasemi-Sadabadi M. 2017. The effect of different levels of propolis in diets on performance, gastrointestinal morphology and some blood parameters in broiler chickens. *Bulgarian Journal of Veterinary Medicine*, 20(3): 215-224. <https://doi.org/10.15547/bjvm.986>
- Siti, U.M., H'ng, S.H., Darlene T.T.L. & Jayaraman, S. 2019. Comparison of total phenolic and flavonoids contents in Malaysian propolis extract with two different extraction solvents. *International Journal of Engineering Technology and Sciences*, 6(2): 1-11. <https://doi.org/10.15282/ijets.v6i2.2577>
- Tayeb, I.T. & Sulaiman, B.F. 2014. Effect of propolis supplementation on productive performance in local quail. *Iranian Journal of Applied Animal Science*, 4: 621-627.
- Torki, M., Soltani, J. & Mohammadi H. 2015. Effects of adding ethanol extract of propolis and cumin essential oil to diet on the performance, blood parameters, immune response and carcass traits of broiler chicks. *Iranian Journal of Applied Animal Science*, 5(4): 911-918.
- Tosi, A.E., Re' E., Ortega, M.E. & Cazzoli, A.F. 2007. Food preservative based on propolis: bacteriostatic activity of propolis polyphenols and flavonoids upon *Escherichia coli*. *Food Chemistry*, 104: 1025-1029. <https://doi.org/10.1016/j.foodchem.2007.01.011>
- Vidda, M., Ruiz-N, Y., Fernández, L. & Perez-Alvarez, J. 2008. Functional properties of honey, propolis, and royal jelly. *Journal of Food Science*, 6: 117-123.
- Yilmaz, S., Tatli Seven, P. & Kaya, E. 2017. Effects of propolis, royal jelly, bee pollen and Ronozyme supplementation in diets of Japanese quails (*Coturnix Coturnix Japonica*) on yolk lipid peroxidation. *International Journal of Veterinary Health Science & Research*, 5(5): 183-189. <https://doi.org/10.19070/2332-2748-1700037>

