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 cytoplastic and anti-tumor activity, immunostimulatory, anti-obesity, hepatoprotective effects, and anti-inflammatory properties (Yilmaz 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).

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 systems, such as the macrophages and T and B cells (Sforcin, 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 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; Kroliczewska 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 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; Erkmen & 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...
Propolis, a natural product from the African honey bee, has been explored as a potential substitute for antibiotics in poultry diets. The ability of propolis to provide a natural defense against bacterial infections has been well-documented. Several studies have shown that the inclusion of propolis in the diet of broiler chickens can reduce the antibiotic resistance genes in the gut, which is a critical factor in the antibiotic resistance crisis. Propolis has been shown to promote the growth of beneficial bacteria and inhibit the growth of pathogenic bacteria, thereby improving the health and productivity of poultry.

In a study by Mahmoud et al. (2017), the inclusion of propolis in the diet of broiler chickens significantly increased the average daily gain (ADG) in broilers (Hosseini et al., 2015). This is due to the presence of flavonoids and phenolic compounds in propolis, which have antioxidant and anti-inflammatory properties. These compounds help to reduce the oxidative stress caused by heat stress, improving the health and productivity of the birds.

Some studies have shown that propolis supplementation can improve the gut health and nutrient absorption ability of the mucosal membrane of poultry digestive systems. This can be attributed to the presence of bioactive compounds in propolis, such as flavonoids and phenolic acids, which can interact with the gut microbiome and improve nutrient absorption. (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 hemorhaging 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 (Yılmaz 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 may 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 (Ian 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 (HavlòÂk 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.

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ETHICAL STATEMENT

Not applicable.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

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