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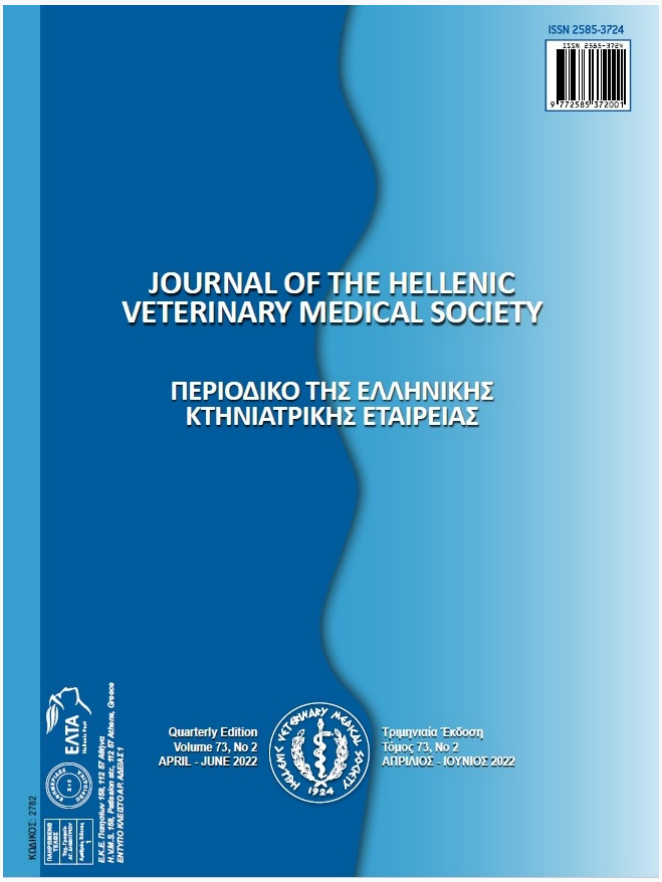

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Review article
Ανασκόπηση

General Properties of Propolis and its Usage in Ruminants

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ABSTRACT: Propolis is a product with many benefits, honey bees produce propolis by mixing the resin substances they collect from the bark, leaves, and plant secretions of trees with honey, wax, pollen, and their salivary enzymes. They use this product for several purposes including protecting bee larvae, honey stores, and honeycombs from infections, repairing damaged areas, covering holes and cracks in the hive. It was also used in folk medicine with its antimicrobial and healing effects since ancient times. Beside these properties, with the advancements in science, various other properties of propolis like immunomodulatory, anti-inflammatory, antitumor, antioxidant, anticarcinogenic, antiprotozoal, antiviral,antiulcer, local anesthetic, cytotoxic, and many more were also discovered.After growing of the concerns about residue problems in food, arbitrary usage of antibiotics in animal farming was restricted or banned by many countries.This led to an increase in the demand for organic products in the public and drove scientists to search for new natural alternative feed additives that can show similar effects with antibiotics in order to prevent economic losses caused by the restriction. The aim of this study was to discuss the effects of propolis in ruminants as a feed additive and its usability as a natural alternative to antibiotics by analyzing studies on the field.

Keywords: Antibiotics, feed additive, immunomodulatory, propolis, ruminants

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INTRODUCTION

Honey bees (*Apis mellifera*) produce a variety of products that are important in human health and nutrition, such as honey, bees wax, bee venom, and propolis. Propolis is one of the most important among these products. The word Propolis is derived from Greek, the word 'pro' means 'at the entrance to' or 'in front', and the word 'polis' means 'city' or 'community' (Wagh, 2013; Yumnam et al., 2017) which means propolis is a substance used in the defense of the hive (Sabir, 2017). Honey bees prepare propolis by mixing the resin substances they collect from the bark, leaves, and plant secretions of trees with honey, wax, pollen, and salivary enzymes they produce (Yücel et al., 2014). Propolis, also called bee glue, is used by bees to protect bee larvae, honey stores, and honeycombs from infections, repair damaged areas, cover holes and cracks in the hive (Wagh, 2013; Sung et al., 2017). They use it for various other purposes, such as embalming invaders that are killed after entering the hive and cannot be transported out (Wagh, 2013). Propolis is thought to be a powerful chemical weapon against pathogenic microorganisms (Siheri et al., 2017). Propolis is rich in phenolic compounds with a wide variety of biological characteristics, as it is made from parts of different plants.

Propolis has been used for treatment in folk medicine and various other purposes since ancient times. The Egyptians realized the antimicrobial effects of propolis and used it as an embalming material to prevent decay in their dead (Eroglu and Yuksel, 2020; Anjum et al., 2019). Greek and Roman doctors used this substance for the treatment of wounds and as an oral disinfectant. Propolis was used by the Incas in Central America to reduce fever, and in the 11th century, Ibn Sina recommended propolis to the soldiers to treat their wounds due to its antiseptic properties (Yonar, 2017). Between the 17th and 20th centuries propolis became very famous in Europe due to its antibacterial activity (Toreti et al., 2013). It was used in various Soviet clinics for the treatment of tuberculosis, as it was determined to be effective in the treatment of appetite problems and the decrease in lung problems during the Second World War (Wagh, 2013). In the Balkan countries, propolis was used to treat burns, wounds, stomach ulcers, and sore throat (Pandya and Roy, 2016).

COMPOSITION AND FUNCTIONAL PROPERTIES OF PROPOLIS

The composition of propolis can vary depending on the season, region, illumination, flora, and collector type (Toreti et al., 2013). Although the melting point of propolis is between 60-70 °C, some types melt at 100 °C, propolis is extracted commercially with solvents such as methanol, ethanol,ether, chloroform, and acetone, but ethanol is reported to be the best solvent (Martinotti and Ranzato, 2015; Anjum et al., 2019). Propolis typically consists of resins (50%), waxes (30%), pollen (5%), essential oils (10%), and other organic components (5%) including vitamins (B1, B2, B3, and B6), steroids, benzoic acid, cetons, fatty acids, quinones, esters, lactones, and sugars (Abdulkhani et al., 2017). As a result of studies conducted in different samples, more than 300 components were identified in the content of propolis (Altun and Aydemir, 2020). Flavonoids, diterpenic acids, aromatic acids, and phenolic compoundsare the main actors in the biological activities of propolis (Silici and Kutluca, 2005). Biological and pharmacological effects of some components contained in propolis are presented in Table 1.

Propolis was widely used for its antimicrobial effects in the early ages, along with the development of technology, besides antibacterial (Przybyłek and Karpiński, 2019), antifungal (Mendonça et al., 2015), antiviral (Alp, 2018), antiprotozoal (Anjum et al., 2019) properties. It has also been determined to have a large number of other useful biological activities such as antioxidant (Torres et al., 2018), anti-inflammatory (Machado et al., 2016), antitumor (Doğanet al., 2020), immunomodulatory (Pandya and Roy, 2016), antiulcer (da Silva et al., 2018), local anesthetic, cytotoxic (Bonamigoet al., 2017) properties. Due to these properties, the use of propolis in fields of classical medicine, alternative types of therapy (apitherapy) and biocosmetics is becoming more popular nowadays. It is known that propolis is effective in the regulation of blood lipid level, in the treatment of various allergies and infections, asthma, fever, migraine headache, hypertension, acne, ulcer, and arthritis, as well as digestive, blood circulation, and respiratory system disorders. Besides, it is stated that it strengthens the immune system, increases the body's resistance against diseases, and accelerates the recovery and regeneration of tissues in diseases such as burns, cuts, and wounds in the skin (Yılmaz et al., 2004).

USE OF PROPOLIS ON RUMINANT DIETS

With all these above-mentioned properties, propolis, which has been used in folk medicine for centuries, has recently also been used in farm animals.

Table 1. Biological and pharmacological activities of some compounds contained in propolis

Compounds	Biological and Pharmacological Effects
- Flavonoids	- Reducing capillary permeability, antimicrobial, anti-inflammatory, antioxidant
- Chrysine	- Anticarcinogenic
- Apigenin	- Healing gastric ulcer
- Acacetin	- Preventing inflammation
- Quercetin	- Antiviral, strengthening of capillaries, anticarcinogenic, spasmolytic
- Kaempferol	- Spasmolytic
- Galangin	- Bacteriostatic, antimicrobial, antimycotic, *Helicobacter pylori* growthinhibitor
- Pinocembrin	- Bacteriostatic, antimicrobial, antimycotic, local anesthetic, *Helicobacter pylori* growthinhibitor
- Pinobanksin	- Antimicrobial, antimycotic
- Luteolin	- Antiviral, healing gastric ulcer
- Artepillin C	- Anticarcinogenic, antileukemic
- Ferulic acid	- Antibacterial, agglutinant, collagenic
- Isoferulic acid and Cinnamic acid	- *Staphylococcus aureus* growth inhibitor
- Benzoic acid	- Bacteriostatic and bactericidal
- Caffeic acid	- Antiviral, antibacterial, anti-inflammatory
- Caffeic acid phenethyl ester (CAPE)	- Anticarcinogenic
- Etheric oils	- Antimicrobial, anti-inflammatory

(Yılmaz et al., 2004)

In livestock enterprises, running a profitable business largely depends on the health of the animals in the herd. As a result of diseases that are an important source of stress for animals, high yield losses occur in enterprises. For decades, antibiotics were widely used in farm animals in the treatment of various diseases and as a growth promoter. It is estimated that total use of antibiotic in animal husbandry range roughly from 63,000 to 240,000 tons per year (Manav et al., 2020), and between 2010 and 2030 this amount is expected to increase by 67% (Kuppusamy et al., 2018). However, in recent years, consumers' demand for organic products in plant and animal production has been increasing. Moreover, antibiotic resistance is identified as one of the three major threats to human health by the World Health Organization (WHO) (Anonymous, 2014). Since antibiotics create residues in foods and cause resistance in some microorganisms, the use of antibiotics in animals has been restricted in many countries (Şen Y., 2020). The European Union has banned the use of antibiotics as a growth promoter in animal feeds in 2006 (Commission, 2005). As a result of the restrain on the use of antibiotics, scientists have focused on discovering alternative feed additives in order to prevent economic losses caused by restriction (Erhan, 2015; Soltan et al., 2016). Considering all the pharmacological properties, propolis stands out as a safe natural alternative (Prado et al., 2010). Many researchers reported that the use of propolis, its extracts, and flavonoids extracted from propolis show positive effects on ruminants and it may be a promising alternative to antibiotics.

Effects of Propolis on Growth and Development of Young Ruminants

Many researchers reported the positive effects of propolis administration on growth performance and feed conversion ratio in ruminants. In a study examining the effects of propolis supplementation in milk fed to lambs, a control group and three different propolis dose groups were formed. Results of the study indicated that lambs receiving propolis at the dose of 150 µL/day had 2.94kg greater weight gainin the trial which lasted 42 days compared to the control group. (Cécere et al., 2021). Abd-Allah and Daghash (2019) stated that the use of propolis with or without flavomycin as a feed additive in Egyptian Buffalo calves had a positive effect on the growth and live weight gain of calves. The weaning weights of the calves fed control ration plus 50 mg propolis/head/daywas 7.7 kg greater than the control group. In a similar study carried out on Simmentalcalves and lasted 21 days

after birth, it was reported that 4 ml of EEP (Propolis ethanol extract) group had 213,9 gr greater average daily live weight gain compared to the control group (Kupczyński et al., 2012). Zawadzki et al., (2011) examined the effects of sodium monensin and propolis administration on finishing performance and carcass traits of Nellore bulls and stated that the supplementation of propolis extract on bull's diets significantly increased daily live weight gain (1.17 kg) compared to control group (0.87 kg).The daily live weight gains were reported as 458.3 and 470.5 g/day for the male and 312.8 and 392.8 g/day for the female Holstein Friesian calves for control and propolis groups by Yücel et al. (2015).These results illustrate that propolis can efficiently be used in ruminant feeding to promote and accelerate growth and development as a safe and natural alternative to antibiotics.

Effects of Propolis on Diarrhoea and General Health Status

Calf diarrhoea is among the most serious problems of cattle enterprises in the world and causes significant economic losses especially in the first 2-3 weeks after birth (Şahal et al., 2017) and some infectious diseases contribute to these losses as well. In various studies, it has been stated that propolis can be an alternative to antibiotics in the prevention of lamb and calf diarrhoea and in tackling infectious diseases. Infectious diarrhoea in calves is usually associated with enterotoxigenic *Escherichia coli, Salmonella* (Muktar et al., 2015), *Cryptosporidium parvum, rotavirus, coronavirus*, or some combinations of these pathogens (Foster and Smith, 2009). Veiga et al. (2017) reported antibacterial effects of poplar propolis against gram-negative, gram-positive microorganisms and multidrug-resistant bacteria. Propolis and its extracts have been found to have improving effects on productivity, and immunity against intestinal parasites when used in critical periods such as pregnancy, flushing, and lactation (Aguiar et al., 2014; Soltan et al., 2016). Also, in many studies, propolis has been reported to be effective against many types of microorganisms, such asbacteria, yeasts, parasites, and viruses (Al-Ani et al., 2018). İnhibiting effect of propolis on the activity of microorganisms that cause infections of the upper respiratory tract and otitis media has been reported by Yilmaz *et al*. (2004). It has also been demonstrated in various studies that propolis is efficient on *Escherichia Coli* and *Salmonella* (Przybyłek and Karpiński, 2019) which are two main type of bacteria cause diarrhoea. Furthermore, propolis has been reported to show antiviral activity by preventing virus entry into cells and creating disruption in viral replication that leads to the destruction of RNA (Sforcin, 2016; Anjum et al., 2019). In various studies, the effects of propolis on diarrhoea in ruminants have been studied. The effects of propolis administration in Lacaune lambs were examined and it was reported that there was a decrease in the amount of *Escherichia coli* and total coliform in samples taken from faeces at all doses (Cécere et al., 2021). In similar studies conducted on Holstein Friesian calves, it was reported that diarrhoea status in calves has decreased significantly with propolis administration on diets (Yücel et al., 2015; Slanzon et al., 2019). These findings indicate that propolis could be a promising alternative feed additive to antibiotics to raise healthy animals with all aforementioned effects.

Effects of Propolis on Immunity and Certain Blood Parameters

Active immunity in calves is gradually acquired after birth. Calves are therefore extremely vulnerable to external disease factors when they are born. Colostrum protects calves by providing passive immunity with a high content of antibodies (immunoglobulins), but the content of colostrum varies due to various factors, and in some cases, colostrum may not be enough, or calves may not consume sufficient levels of colostrum. It has been stated that the mortality rate is high and the risk of pneumonia is twice as high in newborn calves when Immunoglobulin (Ig) G level is lower than 10 g / L (Kozat, 2019). In various studies, propolis has been reported to affect the Immunoglobulin levels of animals. Shedeed et al. (2019) reported that with the supplementation of propolis, a significant increase was observed in blood Immunoglobulin (Ig) A levels in sheep compared to the control group, but there was no change in Ig M and G levels. In a similar study, propolis administration was reported to significantly increase blood Ig A levels in lambs (Cécere et al., 2021). In a study conducted on Hanwoo calves for 90 days beginning from birth, the effects of different feed additives (propolis, illite, neomycin (antibiotic)) on some blood parameters were examined and the highest Immunoglobulin (IgA, IgM, IgG) levels were reported in the propolis applied group (Sarker and Yang, 2010).

Effects of propolis administration on blood parameters in ruminants were examined in various studies. Morsy et al. (2021) reported that supplementation of brasilian red propolis raised serum erythrocyte, total

leukocyte, hemoglobin, total protein, globülin and glucose levels in late pregnant Santa Ines ewes. Kupczyński et al. (2012) stated that propolis extract had a positive effect on blood erythropoiesis and iron content in calves. Morsy et al. (2016) reported that the administration of propolis extract in Santa Ines sheep increased the blood concentrations of total leukocytes, protein, globulin, and glucose levels. Another study was conducted on Holstein Friesian calves for 70-days beginning from birth and the results showed that there was no significant difference between the groups in the concentrations of blood metabolites (glucose, cholesterol, triglyceride, albumin, urea, and globulin) among the control, 500 and 1000 ppm propolis extract supplemented groups (Seif et al., 2017). Abd-Allah and Daghash (2019) investigated the effects of using flavomycin and propolis powders as feed additives on buffalos and reported that serum triglyceride and total cholesterol levels decreased significantly in the propolis group compared to the control group. Slanzon et al. (2019) found that the application of propolis extract caused a decrease in the total number of red blood cells in calves. İncrease in the immunogloblin and total leukocytes levels may explain the immune-stimulating effects of propolis.

Effects of Propolis on Ruminal Parameters

Different antibiotis, such as monensin, hainanmycin and virginiamycin, were used to improve ruminal fermentation and the efficiency of nutrient utilization in ruminants for years (Ren et al., 2018). Several studies were carried out to determine the effects of propolis administration on rumen parameters, methane formation, and its usability as an alternative to antibiotics. Morsy et al., (2015) studied the effects of propolis application on rumen degradation of nutrients and methanogenesis and reported that the use brasilian red and egyptian brown propolis resulted in a decrease in the methane (CH4) formation of the rumen and enhanced the true organic matter degradability. Morsy et al., 2021 reported similar results on late pregnant Santa Ines ewes after the use of brasilian red propolis. Several researchers stated that propolis has an antiprotozoal effects (Soltan et al., 2016; Soltan and Patra, 2020; Morsy et al., 2021), thus reduction in methanogenesis could be attributed to these effects of propolis (Soltan et al., 2016; Morsy et al., 2021). It was also reported that levels of rumen short-chain fatty acids (acetate, butyrate, propionate) were raisedwith the application of brasilian red propolis (Morsy et al., 2021). Similar findings were reported by Stradiotti Júnior et al. (2004). Aguiar et al., (2014) studied the effects of different levels of propolis-based products (Brasilian red propolis) on ruminal parameters in dairy cows fed 591.9 g/kg corn silage and 408.1 g/kg concentrate (Dry matter basis) diet and reported that propolis-based products administration did not affect rumen pH, the efficiency of microbial protein synthesis and blood parameters.However, administration of different phenolic compounds in propolis reduced ruminal ammonia-nitrogen (NH3-N) production and increased intestinal digestibility. It was also reported by Morsy et al. (2021) that supplementation of brasilian red propolis improves the digestability and microbial protein synthesis in late pregnant Samta Ines ewes. In another study, Ozturk et al., (2010) reported that propolis supplementation on 60% forage and 40% concentrate diet decreased total ruminal total bacteria count, decreased Ammonia nitrogen (NH3-N) concentration but had no effect on production of total short-chain fatty acids, dry matter digestibility ruminal pH, and total protozoa count. Due toits higher antimicrobial activity against gram-positive than gram-negative bacteria, propolis use might be a more appropriate and natural way to decrease the energy loss as methane (CH4) by modifying rumen microbial fermentation than antibiotics (Morsy et al., 2015; Soltan et al., 2016).

Antioxidant Effects of Propolis

Free radicals can trigger pathological changes by creating oxidative damage in cell lipids, proteins, and DNA. Antioxidants, on the other hand, protect cells from these damages by neutralizing free radicals. Most of the free radicals in the organism are radicals made up of oxygen. Reactive oxygen species (ROS) are formed from the partial reduction of the oxygen molecule. It has been reported in many studies that propolis has an antioxidant effect (Seven et al., 2007; Torres et al., 2018; Anjum et al., 2019). Several studies on the subject were also conducted in ruminants. Cecere et al. (2021) reported a decrease in serum ROS level with propolis application as a result of their study on lambs. Moreover, Shedeed et al., (2019) stated that propolis administration increased the concentration of enzymes with antioxidant effects, such as Superoxide dismutase (SOD), HP (Horseradish peroxidase), NO (Nitric Oxide), and MDA (Malondialdehyde), in sheep in the periparturient period.

CONCLUSION

In conclusion, with all the above-mentioned prop-

erties propolis which is an extremely beneficial product and having been used by humans for different purposes since ancient times is also advantageous and suitable for use in animal husbandry. For many years, antibiotics were widely used in farm animals as a growth promoter and for the treatment of diseases. But after antibiotics are discovered to create residues in foods and cause resistance in some microorganisms, antibiotics use in animals has been restricted in many states. This increased the consumer demand for organic products in plant and animal production. The studies carried out on ruminants indicate that propolis is useful to promote growth and development, increase feed conversion ratio, prevent diarrhoea and infectious diseases, raise healthy animals, reduce oxidative stress, modify rumen fermentation and decrease the loss of energy as methane (CH4). Because of these effects, propolis could be a natural and promising alternative to antibiotics.

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