Journal of Advanced Veterinary Research Volume 11, Issue 4 (2021) 254-265

Journal of Advanced Veterinary Research

https://advetresearch.com

Could Phytobiotics replace Antibiotics as Feed Additives to Stimulate Production Performance and Health Status in Poultry? An Overview

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ARTICLE INFO

Review Article

Received:
23 August 2021

Accepted:
27 September 2021

Keywords:

Antibiotics resistance; Antimicrobial properties; Growth promoter; Immunomodulatory; Plant products; Poultry industry

ABSTRACT

In the poultry industry, using antibiotics as growth promoters has been found to significantly increase feed conversion efficiency and growth performance. Nevertheless, excessive use of antibiotics in the poultry production cycle may also lead to antimicrobial resistance in both poultry and humans. With regard to food safety reasons, most developed countries have banned the use of antibiotics in all animal feeds. Consequently, it may be necessary to explore other preventive alternatives for disease prevention and to stimulate fast growth rate in poultry. The interest in using phytobiotics as an alternative feed additive in poultry diets has increased following its natural, residue-free, and less toxic properties in contrast to synthetic antibiotics. Therefore, this review shed the light on the influences of using phytobiotics as a feed additive in commercial poultry diets and the results on the production performances and health status. Phytobiotics like cinnamon, cumin, oregano, clove, thyme, rosemary, sage, green tea, garlic, fenugreek, pepper, ginger, and other plant mixtures were found to consist of growth-promoting properties that enhance digestibility, stimulate feed intake, and improve growth in poultry. The carry-over effect leads to improved carcass characteristics and meat quality as value-added products. Additionally, various studies have also reported that some plant extracts from thyme, turmeric, lemon, green tea, cinnamon, cumin, wild mushroom, and garlic have antimicrobial effects as well as immunomodulatory function when they are complemented in poultry diets. In summary, phytobiotics can be used effectively to replace antibiotics as feed additives in enhancing production and health performances of poultry for food security while preventing antibiotic resistance.

J. Adv. Vet. Res. (2021), 11 (4), 254-265

Introduction

The poultry industry

The poultry industry is a rapidly growing industry, particularly in developing countries (Bahri *et al*., 2019). Based on statistics, there is over 23 billion poultry around the world (Mottet and Tempio, 2017). Thus, there is approximately three poultry for each person, which are five times more than five decades ago (FAOSTAT, 2016). This growth has led to an increase in meat and egg production (Barbut, 2016). In 2016, the global poultry meat production was 116 million metric tons and increased to 120.5 million metric tons in 2017 and 122.5 million tons in 2018, representing a 1.6% increase from 2017 to 2018 (Bahri *et al*., 2019). Comparatively, the human population is now growing at a rate of 1.4% yearly, and it is expected to reach 9.7 billion by 2050 (Barbut, 2016; Jenkins *et al*., 2020). As a result, poultry meat consumption has also increased significantly compared to the last few decades (Mottet and Tempio, 2017). Factors such as decreased in production costs compared to other red meat sources, availability of sophisticated technology to ease primary and secondary processing, inexpensive protein sources with high value of nutrition, as well as having a healthier composition due to its low-fat content have led to the growth of poultry meat production and consumption (Barbut, 2016). Moreover, urbanisation also leads to the rise in people's income, hence surging the demand for meat. Another important factor is the genetic and rigorous breeding selecting of broiler hybrids (Barbut, 2016). In the 1900s, a broiler chicken took around four months to reach a kilogram of body weight, with a feed conversion ratio (FCR) of 5.0, whereas currently it only takes less than 50 days to reach the bodyweight of 2.6 kg with a FCR of 1.9 (Boyd, 2001). This advancement has also contributed by the improvement of the husbandry practices and the use of pharmaceutical drugs such as antibiotics, which reduce the mortality rate to 4% as compared to 20% observed in the 1900s. As a result, all these have assisted the poultry industry to be more cost-effective and inexpensive compared to other meat industries. Although antibiotics have tremendously improved the poultry industry, it also causes certain issues to the birds and ultimately affecting public health (Barbut, 2016).

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ISSN: 2090-6277/2090-6269/

Antibiotics usage in poultry feed

Antibiotics were discovered during the 1920s and have been used successfully in the poultry industry (Gadde *et al.*, 2017). Antibiotics are used in veterinary medicine for treating and preventing diseases. On the other hand, they are also widely used in small amounts in poultry diets for growth promotion to enhance feed efficiency and disease prevention (Giamarellos-Bourboulis and Scaglione, 2010). In the poultry industry, using antibiotics as growth promoters has significantly increase feed conversion efficiency and growth performance by 3 to 5% respectively (Dahiya *et al.*, 2006). However, excessive usage of the substance as a growth promoter may cause drug toxicity as well as the development of antibiotic-resistant pathogenic bacteria (Gheisar and Kim, 2018). Numerous studies have approved that there is a relation in the use of antibiotics at sub dose concentration leading to the development of antimicrobial resistance amongst the gut microflora in poultry (Medeiros *et al.*, 2011; Cosby *et al.*, 2015). The prevalence of these superbugs had been rising due to the misuse of antibiotics which have affected the human consumers (Giamarellos-Bourboulis and Scaglione, 2010; World Health Organization, 2012). Additionally, mixed opinions had suggested that antibiotic resistance genes could transfer from animals to humans (M'ikanatha *et al.*, 2010).

For that reason, the use of in-feed antibiotics has been banned in developed countries due to their harmful effects on human health (Gheisar and Kim, 2018). For example, in 2006, the European Union banned the use of antibiotics as growth promoters on precautionary grounds, especially in egg and meat-producing animals (Windisch *et al.*, 2008). In addition, the United States (FDA) halted the manufacturing of animal drugs to stop the use of in-feed antibiotics for growth promotion. Instead, it is solely used for treatment (FAO, 2014). In 2015, the state of California strictly banned the use of medically significant antimicrobials in animal diets either for growth enhancement or preventing diseases (Gheisar and Kim, 2018). Similarly, the Malaysia Animal Feed Act 2009 has stated that antibiotics (group A) and maximum residue limits for the allowed antibiotics (group B) are banned. In 2014, the Department of Veterinary Services in Malaysia has published a guideline on organic chicken production, which specified that no antibiotics or medicinal substances are allowed to be used in the diets to stimulate growth or production. Supporting this, the Malaysian government has banned the practice of colistin in animal feed in 2019. Colistin is widely used in the poultry sector as a growth promoter. This particular antibiotic is the last line of defence for severe infection and is a common life-saving last resort medicine especially when patients are infected by superbugs (Kempf *et al.*, 2016).

Despite the value of antibiotics in growth promoters and disease prevention, there have been several attempts to replace with better alternatives namely prebiotics, probiotics, toxin binders, phytobiotics, enzymes, oligosaccharides, synbiotics, organic minerals, organic acids, and other feed additives (Hashemi and Davoodi, 2011). These alternatives can enhance performance effectively and have little therapeutic use in veterinary medicine. They also do not lead to deleterious disturbances of the gut normal flora, not absorbed from the gut into tissue that may be edible, and do not cause drug resistance at actual use level (Alghirani *et al.*, 2021). Furthermore, they do not cause a rise in environmental pollution, and they are not toxic to the birds or human beings (Yadav *et al.*, 2016).

Phytobiotics

Approximately 50,000 out of 422,000 types of flowering plants are used for medicinal purposes around the world (El Aziz *et al.*, 2019). For centuries, humans have used plant products to naturally treat ailments with the use of spices and herbs. Additionally, plant products are also used as additives in animals feed in the early cultures (Hashemi and Davoodi, 2011). In the last few decades, the interest of using phytobiotics or plant extract products in veterinary medicine as an alternative to synthetic antibiotics in poultry diets has increased (Vidanarachchi *et al.*, 2005). These biological products have been proven to be natural, residue-free, less toxic, and are supposed to be ideal growth promoters in animal diets compared to synthetic antibiotics (Hashemi and Davoodi, 2011). The therapeutic activity of a medicinal plant is stringently depending on its chemical compounds like secondary metabolites, which are a particularly large group of compounds (Wink, 2012; Stefanović and Comic, 2012).

Phytobiotics are divided into four subgroups namely herbs which include flowering, non-persistent and non-woody plants; botanicals such as bark, root, and leaves; oleoresins based on non-aqueous solvents, and essential oils which produced from hydro purified from plant combinations (Windisch and Kroismayr, 2007). Even though there are various categories of plant families, the chemical compounds can be characterised into several main groups such as saponins, essential alkaloids, tannins, acids and oils, which have their own effective method of extraction (Chung *et al.*, 2018; Muniandy *et al.*, 2020). These chemical compounds have been used widely in many African and Asian countries. Following their promising results, the usage of herbs and spices as feed additives has been practiced in other parts of the world such as Germany, UK, Italy, France, Spain, Belgium, and Netherlands (Polasa and Nirmala, 2003).

Phytobiotics have a complex combination of bioactive components due to the presence of their organic chemical compounds along with some anonymous influences of other bio-active composites (Fallah *et al.*, 2013). Owing to this feature, phytobiotics may be used for several purposes in animals including improving the digestibility and feed intake, thus leading to improve growth performance (Windisch and Kroismayr, 2007). Natural plant extracts also play a substantial role in animals' health in terms of its antimicrobial activity, immune stimulant, antioxidant, gut microflora manipulation, nutrigenomics effect, anti-stress properties, digestibility enhancer, cholesterol-lowering effect, as well as they are considered as environmentally friendly pesticide and insecticide (Fallah *et al.*, 2013; Gopi *et al.*, 2014; Dhama *et al.*, 2014; Karangiya *et al.*, 2016). Therefore, this review will comprehensively discuss the application of phytobiotics as a feed supplementation in commercial poultry diets and the effects on the growth performance, carcass traits, meat quality, antimicrobials activities, and immune enhancement.

The effect of phytobiotics on feed intake and growth performance of poultry

Growth-promoting effect

Plant extracts were found to comprise growth-promoting properties that helps enhance the secretion of digestive enzymes, develops palatability and flavour of feed, stimulates feed intake, and improves antimicrobial activity, which can improve the gut function in poultry (Jang *et al.*, 2004; Czech *et al.*, 2009). A variation of spices and herbs such as marjoram, oregano, garlic, rosemary, yarrow, black cumin ginger, green tea, and coriander are used in poultry diets due to their potential as an alternative poultry feed additive to replace antibiotics (Gadde *et al.*, 2017). Additionally, herbs like nishyinda, black pepper, and cinnamon have been reported to have similar growth–stimulating properties. It has been demonstrated that a mixture of polyherbal extracts consisting of nishyinda (*Vitex negundo*) leaves, black pepper (*Piper nigrum*) and cinnamon (*Cinnamomum verum*) at 1% or 1 ml/L in drinking water was found to promote the growth performance in broilers without exhibiting any negative impacts (Molla et. al., 2012). A similar trend was reported by Sang-Oh *et al*. (2013), who asserted that adding 3, 5, and 7% of black cinnamon powder improved growth performance as compared to broilers without any supplementation.

Supporting this, Singh *et al*. (2014) recommended that 0.5% of cinnamon powder can be added to broilers' dietary feed, thus acting as a natural alternative instead of using antibiotic growth promoters. Simultaneously, the addition of 4 g/kg dried ground *Scrophularia striata* and *Ferulago angulate* leaves, 1% of black cumin seeds, 0.35 to 0.7% of fermented *Ginkgo biloba* leaves, and 7.5 g/kg *Euphorbia hirta* leave in broiler diets were found to have similar effects (Hashemi *et al*., 2009; Khalaji *et al*., 2011; Cao *et al*., 2012; Rostami *et al*., 2015). Moreover, Madhupriya *et al*. (2018) stated that essential oils from oregano (50 to 300 mg/kg), clove (100 to 600 mg/kg), thyme (100 mg/kg), rosemary (500 mg/kg), and sage (500 mg/kg) were also used as growth promoters owing to their odour and flavour. All these were attributed by the presence of active ingredients which contained antibacterial and antiviral activity against a wide range of pathogens, improving gut microbiota and nutrient digestibility, antioxidant property contributing to reducing oxidative stress, as well as immunomodulation effect which will ultimately stimulate the growth performance (Singh *et al*., 2014; Madhupriya *et al*., 2018).

Feed intake

Generally, there is an improvement observed in the feed intake of poultry supplemented with natural additives like spices and herbs (Windisch *et al*., 2008). As studied by Kirkpınar *et al*. (2011), an enhancement on the feed intake was observed in broilers fed with 100 and 200 mg/kg of thyme essential oil as well as 300, 500, and 700 mg/kg of oregano essential oil mixed into the diet. Essential oils are found to promote feed intake and performance of poultry by increasing the flavour and palatability of the diets (Windisch *et al*., 2008; Grashorn, 2010). Similar results were obtained by Ulfah (2006), who observed an improvement in the daily feed intake of broilers through the supplementation of 18 mg/kg of cinnamon, thyme, eucalyptus, and oregano essential oils. These results were in agreement with Ertas *et al*. (2005), who also reported that the broilers feed intake improved tremendously after three weeks of supplementation with anise, clove, and oregano essential oils at the rate of 200 and 400 mg/kg in the diets. The active components such as thymol, carvacrol, anetole and eugenol may lead to the appetizing effect towards the respective essential oil mixture, thus leading to the increase in feed intake (Yitbarek, 2015). For example, carvacrol at 0.2 g/kg have shown to increase the feed intake in broiler chickens (Lee *et al*., 2003).

On the contrary, a diet supplemented with 100 mg/kg of CRINA® Poultry containing thymol does not show any impact on feed intake (Lee *et al*., 2003). Similarly, Jang *et al*. (2007) obtained the same results when CRINA® Poultry was fed at the rate of 25 or 50 mg/kg. Confirming this, Cross *et al*. (2007) observed that feed intake of broilers decreases by 10% when supplemented with thyme essential oil at the level of 1 g/kg. According to Yitbarek (2015), thymol has the ability to enhance digestion and balance the gut microbial ecosystem which can improve growth performance, but this was not observed in few studies mentioned earlier. For that reason, it is clear that feed intake of poultry can be improved by supplementing certain phytobiotics in accurate levels but should not be a sole indicator to determine the growth performance. Although these active compounds are mostly accepted in the European Union to be used in livestock feed, it is important to identify the composites that may have side effects and also to understand the suitable dosage to be used effectively (Anadón *et al*., 2019).

Body weight gain

The body weight gain of poultry can be improved tremendously through the application of phytobiotics which are similar to those of antibiotic growth promoters (El-Ghany and Ismail, 2014). Gheisar *et al*. (2015a) found that, a diet containing 0.075% of a phytobiotics blend increases the body weight gain of broiler chickens about 3.9% and also increased the feed consumption ratio about 3.4%. This was consistent to a current study by Rashid *et al*. (2019), who described that adding a mixture blend of 3% w/v peppermint, green tea, black cumin, cinnamon, and garlic in chicken diets did not show any significance towards feed intake ($P > 0.05$) but showed significant increase in the body weight ($P < 0.05$) in contrast to the antibiotic treatment chickens. Besides, Jamroz and Kamel (2002), also observed an improvement in daily weight gain of 8.1% and FCR of 7.7% after feeding broilers with diets complemented with 300 mg/kg of a plant extract comprising carvacrol, capsaicin, and cinnamaldehyde. In addition, the treatment of broiler birds with dry fruit powder of *Emblica officinalis* and dry leaves of *Ocimum sanctum* mixture at 3 g/kg feed for 2 weeks also showed an increase in the broilers' body weight (Mode *et al*., 2009). When applied individually in poultry diets, both Lewis *et al*. (2003) and El-Latif *et al*. (2013) reported that the body weight gain of broilers increased significantly when supplemented with 1.64 g/kg of garlic oil and 100 mg/kg of garlic essential oil, respectively. In a different study, garlic essential oil was shown to increase the body weight and feed intake of ducks by 2.6% and 3.5%, respectively (Gheisar *et al*., 2015b). Furthermore, the use of rosemary powder at 500 mg/kg in poultry feed has shown to increase the body weight gain of chickens (Spernakova *et al*., 2007). Additionally, Biavatti *et al*. (2003) stated that *Alternanthera brasiliana* extracts supplemented at 180 ml / 200 kg in feed could help enhance broiler chickens' weight and performance in between the rearing age of 14 to 21 days. Okitoi *et al*. (2007) posited that hydrophyllic extracts from fresh green tea liquid at 0.1 or 0.2 g/kg in broilers' diet improved the body weight gain, feed efficiency, plus carcass traits. Likewise, using thyme essential oil in Japanese quail diets promoted cumulative feed intake, body weight, and feed conversion (Awuah, 1995). The active thyme oil acts to maintain the significance of microbial flora content in gut, and as a digestibility enhancer that stimulates the secretion of endogenous digestive enzymes, then consequently improves the growth performance in poultry. On top of that, the addition of thyme essential oil at 0.15 ml/L in broilers' diet has shown a higher weight gain (Roller *et al*., 2004).

Nonetheless, there were no significant effects on the body weight gain and FCR of Cobb broilers fed with 50 and 100 mg/kg of oregano essential oil added as an antibiotic replacement to a wheat-corn-soybean diet (Lee *et al*., 2004). In accordance to previous studies the inclusion of black cumin (*Nigella sativa* L.) at 0.7, 1.4, 2.1, and 2.8%, or oregano powder at 0.15 and 0.3 g/kg as antibiotic alternative in broiler diets also did not yield any positive result on the growth performance of those broilers (Windisch *et al*., 2008; Karimi *et al*., 2010; Al-Mufarrej, 2014).

Digestibility

Phytobiotics have been shown to benefit chicken's gastrointestinal enzymatic activity which improve nutrient digestibility (Jang *et al*., 2004). Supplementation of plant extracts also have promising biological effects on the digestibility by decreasing pathogenic bacteria in diverse fragments of digestive tract as well as increasing villus height in different parts of the small intestine; essentially in the duodenum (Ganguly, 2013). Henceforth, using phytobiotics in feed may alter the morphological changes in chicken's intestinal tissues, which may be beneficial to the digestive tract by increasing the villi length and reducing crypt depth in the jejunum and colon (Joshi, 2001; Murali *et al*., 2012). This is supported by Hernandez *et al*. (2004), who reported that essential oils from oregano, cinnamon, and pepper compounds can improve the digestibility of nutrients in chicken. Madhupriya *et al*. (2018) also reported that the addition of 125 mg/kg essential oil extracted from oregano leaves, anise seeds, and citrus peel could improve the apparent ileal fat digestibility of broiler Cobb. In addition, there are minimum influences of phytogenic feed extracts on the ecosystem and also towards the activity of caecal microbial flora of poultry which is related to a reduction in microbial activity in the gastrointestinal gut (Platel and Srinivasan, 2004; Windisch and Kroismayr, 2007). Commercial poultry product like CRINA® Poultry containing 29% active ingredients (thymol) could enhance the secretion of endogenous digestive enzymes and balance the gut microbial ecosystem, which in turn, improve the growth performance (Yitbarek, 2015). Besides, Narimani-Rad *et al*. (2011) indicated that adding a mixture of 5 g/kg peppermint, 5 g/kg Ziziphora, and 10 g/kg oregano in diets of broilers improve FCR, which is attributable to the higher crude protein digestibility.

In brief, the efficacy of phytobiotics as feed supplements and their effect on the growth performance may also be influenced by other biological factors (plant species, harvest conditions, and growing location), manufacturing (stabilization and extraction/distillation), and storage environments (temperature, humidity, light, time, and oxygen tension) (Huyghebaert *et al*., 2011). Based on previous studies (Table 1 and Table 2), it can be posited that plant extracts can be used as a natural alternative to increase poultry growth performance, digestibility, feed intake, and body weight gain provided they are used in the correct concentration. If they are not used in an optimum quantity, no benefits will be yielded, but it may affect the final poultry performance as well as incurring a higher cost of production (Chung *et al*., 2019). Moreover, few works have concluded that better results were obtained by using a mixture of phytobiotics in poultry diets, which have different mechanisms of action that might complement each other.

The effect of phytobiotics on carcass characteristics and meat quality of poultry

Carcass characteristics

Phytobiotics could be utilised to enhance the value of poultry product by enhancing carcass microbial hygiene and carcass traits due to their antimicrobial and antioxidant characteristics (Aksit *et al*., 2006). Using phytogenic compounds like essential oils from herbs and spices in food animals could contribute positively to the microbiological quality and food safety during storage either in a raw or cooked form (Soltan *et al*., 2008). Stanaćev and Puvača (2011) posited that the dietary intake of phytogenic feed additives plays a significant role in food safety by decreasing pathogens in the gut, thus promoting a healthy gut environment, and also reducing carcass contamination during slaughter. For instance, complementing essential oil extracted from rosemary plant at 10 g/kg has demonstrated a progressive improvement towards carcass hygiene of turkey breast during refrigerated storage (Govaris *et al*., 2007). In addition, oregano was also shown to have antimicrobial activity on broiler carcass, whereas addition of 0.1% oregano essential oil at 15 g/kg into the Ross 308 broiler chickens diet can reduce the entire viable bacteria or specific pathogens like *Salmonella* on chicken carcasses (Aksit *et al*., 2006). Besides, supplementing plant extracts to poultry diet was reported to improve the breast muscle percentage of the eviscerated carcass by 1.2% (Stanaćev and Puvača, 2011). Few researchers have stated that both black pepper and fenugreek had different effects on the carcass characteristics of broilers when supplemented at different concentrations in the diets. The group fed with 0.5% of black pepper seeds showed a heavier evisceration percentage due to the enhancement effect of the digestive tract, while the group fed with 10 g/kg of fenugreek seed powder exhibited a lower abdominal fat content in broilers' meat because of the anti-cholesterimic influence (Weerasingha and Atapattu, 2013; Abou-Elkhair *et al*., 2014). Furthermore, the addition of 0.5, 1, and 1.5% of ginger powder into broiler diets help improved the thigh and breast muscle yield. (Prabakar *et al*., 2016). The same study also concluded the abdominal fat of broilers were decreased significantly when supplemented with 1.5% of ginger powder.

On the contrary, supplementing some phytobiotics in broilers diet like anise seeds did not show any enhancement towards the carcass dressing percentage (Jamroz *et al*., 2005; Soltan *et al*., 2008). Similarly, Hassan *et al*. (2016), also observed that supplementation of *Moringa oleifera* leaves into

Table 1. The effect of phytobiotics on feed intake and digestibility of poultry

Plants	Plant compounds	Feed intake	References
Thyme	100 and 200 mg/kg of essential oil	Increase feed intake of broilers	Kirkpınar *et al.* (2011)
	1 g/kg of essential oil	Decrease feed intake	Cross *et al.* (2007)
Oregano	300, 500 and 700 mg/kg of essential oil	Increase feed intake of broilers	Kirkpınar *et al.* (2011)
Cinnamon, thyme, eucalyptus, and oregano	18 mg/kg of essential oil for each	Increase daily feed intake of broilers	Ulfah (2006)
Anise, clove, and oregano	200 and 400 mg/kg of essential oil	Improve feed intake of broilers	Ertas *et al.* (2005)
Carvacrol	0.2 g/kg of essential oil	Increase the feed intake	Lee *et al.* (2003)
CRINA® Poultry	25 or 50 mg/kg containing thymol essential oil	No effect on broilers	Jang *et al.* (2007)
Plants	**Plant compounds**	**Digestibility**	**References**
Oregano, anise, and citrus	125 mg/kg of essential oil	Improve apparent ileal fat digestibility of broiler Cobb	Madhupriya *et al.* (2018)
CRINA® Poultry	29% of thymol essential oil	Enhance secretion of digestive enzymes and balance gut microbial ecosystem	Yitbarek (2015)
Peppermint, Ziziphora and oregano	Mixture of 5 g/kg peppermint, 5 g/kg Ziziphora, and 10 g/kg oregano	Increase crude protein digestibility and improve FCR of broilers	Narimani-Rad *et al.* (2011)

Table 2. The effect of phytobiotics on growth performance and body weight gain of poultry

Plants	Plant compounds	Growth Promoter	References
Nishyinda leaves, black pepper, and cinnamon mixture	1% or 1 ml/kg of polyherbal extracts mixture in drinking water	Promote the growth performance (live weight and weight gain) in broilers	Molla *et. al.* (2012)
Black Cinnamon	3, 5, and 7% of powder	Improve growth performance (body weight) in broilers by stimulating the growth of cells of major immune organs and boosting the immunoglobulin IgG content	Sang-Oh *et al.* (2013)
	0.5% of powder	Improve growth performance (body weight and feed intake) in broilers	Singh *et al.* (2014)
Scrophularia striata and Ferulago angulate	4 g/kg of dried ground leaves mixture	Improve growth performance (overall ADG and FG) in Ross 308 male broilers	Rostami *et al.* (2015)
Black cumin	1% of seeds	Improve growth performance (significantly increased body weight and decreased FCR without any adverse effect on feed intake) in male Ross 308 broilers	Khalaji *et al.* (2011)
Ginkgo biloba	0.35 to 0.7% of fermented leaves	Improve growth performance (improved FCR and body weight gain) in broilers	Cao *et al. (*2012)
Euphorbia hirta	7.5 g/kg of leaves	Improve growth performance (improved body weight gain) in broilers	Hashemi *et al.* (2009)
Oregano, clove, thyme, rosemary and sage	50 to 300 mg/kg oregano, 100 to 600 mg/kg clove, 100 mg/kg thymol, 500 mg/kg rosemary and sage of essential oil extracts	Improve growth promoter in broilers (increased feed intake, body weight and stimulate digestion)	Madhupriya *et al.* (2018)
Plants	Plant compounds	Body weight gain	References
Phytobiotics blend	0.07%	Increase the body weight gain of broilers	Gheisar *et al.*, *(*2015a)
Mixture of peppermint, green tea black cumin, cinnamon, and garlic	3% w/v blend	Increase the body weight gain of broilers	Rashid *et al.* (2019)
Plant extracts	300 mg/kg of a plant extract comprising carvacrol, capsaicin, and cinnamaldehyde	Increase the body weight gain of broilers	Jamroz and Kamel (2002)
Emblica officinalis and *Ocimum sanctum*	3 g/kg combination of *Emblica officinalis* dry fruit powder and *Ocimum sanctum* dry leaves	Increase the body weight gain of broilers	Mode *et al.* (2009)
Garlic	1.64 g/kg of garlic oil	Increase the body weight gain of broilers	Lewis *et al.* (2003)
	100 mg/kg of garlic essential oil	Increase the body weight gain of broilers	El-Latif *et al.* (2013)
Rosemary	500 mg/kg of powder	Increase the body weight gain of chickens	Spernakova *et al.* (2007)
Alternanthera brasiliana	180ml/200kg of extract	Enhance broiler chickens' weight at day 14 to 21	Biavatti *et al.* (2003)
Green tea	0.1 to 0.2 g/kg of hydrophyllic extracts	Improve the body weight gain, feed efficiency plus carcass traits	Okitoi *et al*. (2007)
Thyme	0.15 ml/L of essential oil	Increase the body weight gain of broilers	Roller *et al*. (2004)
Oregano	50 and 100 mg/kg of essential oil	No effect on broiler Cobb	Lee *et al. (*2004)
	0.15 and 0.3 g/kg of powder	No effect on broilers	Karimi *et al.* (2010)
Black cumin (*Nigella sativa* L.)	0.7, 1.4, 2.1, and 2.8% of grinded seed	No effect on broilers	Al-Mufarrej (2014)

broilers diet up to 0.3% had no significant effect on the carcass relative weight, breast, thigh, gizzard, liver, heart, and abdominal fat. Even though several studies approved the positive impacts of phytobiotics on carcass traits, researchers are still unable to conclude the probable efficiency of phytobiotics feed towards carcass hygiene enhancement (Gheisar and Kim, 2018).

Antioxidant effects

Phytobiotics could be applied during heat stress conditions due to their antioxidant activity (Wei and Shibamoto, 2007; Chung *et al*., 2020a). Spices and herbs namely oregano and thyme contain a great amount of thymol, carvacrol, and monoterpenes that have a positive effect on certain antioxidant enzymes such as superoxide dismutase and glutathione peroxidase that help to control the lipid metabolism in animals whereas, other varieties of plants such as mint, sage, and rosemary have direct antioxidant properties (Filazi and Yurdakok-Dikmen, 2019). Their antioxidant properties may be contributed by the concentration of phenolic substances (hydrolysable tannins, phenolic terpenes proanthocyanidins, flavonoids, and phenolic acids) and the presence of certain vitamins (A, C, and E) (Suganya *et al*., 2016). Plants that are rich in flavonoids like green tea and other Chinese herbs naturally have antioxidant properties, thus they can also be used in poultry feed to protect lipids from rapid oxidation (Wei and Shibamoto, 2007). The same effect was reported by Suganya *et al*. (2016), who discovered that both onion and garlic inhibit the oxidation of low-density lipoproteins. Khan *et al*. (2014) revealed that 2 and 3 g/kg garlic powder complemented in broiler diets could significantly reduce the serum triglycerides, cholesterol, and low-density lipoprotein while increasing the high-density lipoprotein. Supporting this, adding garlic and rosemary essential oils to poultry's diet act as an antioxidant factor which has a positive influence on the glutathione redox system in the liver (Khan, 2014). In addition, Hashemipour *et al*. (2013) stated that supplementing thymol at 0.06, 0.1, and 0.2 g/kg into poultry diets will help slow down the antioxidant effect. According to Botsoglou *et al*. (2002), adding oregano extracts into chicken diets significantly increased the levels of α-tocopherol in blood serum and body tissues. Similarly, the same researcher observed a strong antioxidant effect in the broiler tissues after supplementing 50 to 100 mg/kg of oregano to the broilers' diet. Therefore, these researchers had recommended that various plant products like oregano, thyme, mint, sage, rosemary, green tea, onion, and garlic can be used in poultry diet to optimise lipid profile and ultimately produce healthier broiler meat.

Meat quality

Likewise, phytobiotics may be used to improve the protection value of processed meats and also decreases the muscle drip loss during the process of thawing of cold freeze products (Windisch *et al*., 2008). As a result, several studies have concluded that certain plant-based extracts can develop oxidative stability on the product derived from poultry (Karangiya *et al*., 2016). It can be utilised to improve the stability of chicken egg and meat products (Janz *et al*., 2007). In a study by Kirkpınar *et al*. (2011), supplementing garlic, pepper, and fenugreek powder at 10 g/kg in the broilers' diet could decrease the tyrosine and thiobarbit uric acid reactive substances in broiler meat. This is because those plants possess antioxidant activities that inhibits the synthesis of fatty acid in the liver and lipid oxidation (Kırkpınar *et al*., 2011). In addition, sage extracts, oregano, garlic powder, and rosemary powder have also shown positive effect on meat quality due to their antioxidant properties which defer lipid oxidation (Stanaćev *et al*., 2011a). Supporting this, supplementing rosemary powder at 0.5 g/kg help delay lipid oxidation in broiler chicken meat in comparison to the control diet (Spernakova *et al*., 2007). Al–Hajo (2008) has also summarised that the quality and sensory properties of minced poultry meat patties could be enhanced by using 0.05, 0.10, and 0.15% of rosemary powder owing to the antioxidant properties. The moisture content, pH, and water holding capacity were improved significantly. Apart from the growth promoter, Sang-Oh *et al*. (2013) also reported that 3, 5, and 7% of black cinnamon powder was beneficial to enhance the meat quality of broilers. Moreover, *Achillea millefolium*, *Levisticum officinale*, and *Hypericum peforatum* were used to enhance meat sensory characteristics besides their use as antibiotics (Cuppett and Hall, 1998).

Referring to previous studies, there are different effects of phytobiotics on the carcass characteristics and meat quality in poultry (Table 3). In general, it can be summarised that these plant-derived compounds could be utilised effectively to im-

Table 3. The effect of phytobiotics on carcass characteristics and meat quality of poultry

Plants	Plant compounds	Carcass traits	References
Rosemary	10 g/kg of essential oil	Improve breast muscle hygiene of turkey	Govaris *et al.* (2007)
Oregano	0.1% of essential oil added into 15 g/kg of feed	Reduce *Salmonella* pathogen on chicken carcass	Aksit et al. (2006)
Black pepper	0.5% of seeds	Produce heavier evisceration percentage	Abou-Elkhair *et al. (*2014)
Fenugreek	10 g/kg of seed powder	Lower abdominal fat content	Weerasingha and Atapattu (2013)
Ginger	0.5, 1, and 1.5% of powder	Improve thigh and breast muscle yield, plus decreased abdominal fat content at 1.5% level	Prabakar *et al.*, (2016)
Plants	Plant compounds	Antioxidant effects	References
Garlic	2 and 3 g/kg of powder	Reduce serum triglycerides, cholesterol, and low-density lipoprotein, while increase high-density lipoprotein	Khan *et al.* (2014)
Thyme	0.06, 0.1, and 0.2 g/kg of thymol	Slow down the antioxidant effect in feed	Hashemipour *et al. (*2013)
Oregano	50 to 100 mg/kg of extract	Increase the levels of α-tocopherol in blood serum and tissues of chickens	Botsoglou *et al.* (2002)
Plants	Plant compounds	Meat quality	References
Garlic, pepper, and fenugreek	10 g/kg of powder	Decrease the tyrosine and thiobarbit uric acid reactive substances in broiler meat	Kırkpınar *et al.*, (2011)
Rosemary	0.5 g/kg of powder	Delay lipid oxidation in broiler chicken meat	Spernakova *et al.* (2007)
	0.05%, 0.10, and 0.15% of powder	Increase moisture content, pH, and WHC of minced poultry meat patties	Al–Hajo (2008)
Black Cinnamon	3, 5, and 7% of powder	Improve meat quality of broiler	Sang-Oh *et al.* (2013)

prove carcass characteristics as well as carcass hygiene to enhance the shelf life of those meat products. Besides having antioxidant properties, plant-based additives were found to improve meat quality by improving the muscle drip loss, pH, water holding capacity, decreasing serum cholesterol, triglycerides, and low-density lipoprotein, as well as increasing high-density lipoprotein. Therefore, the effect of the potential use of plant-derived phytobiotic on the carcass traits, meat quality, and food safety warrant further investigation.

The effect of phytobiotics as antimicrobials in poultry

Numerous studies have reported that some plants have antimicrobial effects when they are supplemented in the poultry diet. According to Anadón *et al.* (2019), active plant extracts include allium, astragalus, carvacrol, extracted soybean oil, curcuma, echinacea, capsicum, origanum, and achy have antimicrobial activities which are considered to be natural and safe due to their plant-derived origin. The antimicrobial activity of phytobiotics does not just kill pathogens, but also influence the key events in the pathogenic development (Liu and Pop, 2009). For example, those plant extracts could prevent the critical pathogenic events of toxin production and colonisation by diarrheal pathogens (Beceiro *et al.*, 2013). These are achieved in the gut via moderating the cellular membrane of pathogens that causes membrane interruption, raising the hydrophobicity of the pathogenic species that might affect the surface features of pathogenic cells by influencing the virulence characters of the microbes, motivating favourable bacterial growth like bifidobacteria and lactobacilli in the gut, as well as acting as an immunostimulatory material or defending the intestinal tissue against pathogenic attack (Windisch and Kroismayr, 2007). Fallah *et al.* (2013) further stated that phytobiotics play major roles against bacterial, viruses, protozoa, and fungus.

Phenolic substances like phenylpropane, carvacrol, geraniol, thymol, citronellal, and limonene are active extracts that comprise antimicrobial properties (Yang *et al.*, 2015). These herbs have the ability to disrupt pathogenesis which can be used against certain Gram-negative (*Escherichia coli* or *Salmonella* spp.) and Gram-positive (*Streptococcus* spp. or *Staphylococcus* spp.) bacteria by changing the fatty acid composition of the cell membranes, therefore, affecting the hydrophobicity causing ion leakage (Conner, 1993; Suganya *et al.*, 2016). The antimicrobial ability of these phytobiotics is based on the plant's physicochemical compound and the effect on certain bacterial strains (Sari *et al.*, 2006). An *in vitro* study by Bhaisare *et al.* (2016) has examined the effectiveness of essential oils against *S. typhi*, *Pasteurella multocida*, *E. coli*, and *S. aureus* via the disc diffusion method. The result indicated that the mixture of 10 µl per disc of thyme oil has a similar inhibition zone against all types of bacteria as compared to the standard antibiotics, whereas cumin and fennel oils only showed inhibition towards *P. multocida* and *E. coli*.

Currently, plant-based compounds are widely utilised as an alternative to antibiotic growth promoters in poultry diets, due to their ability against certain bacterial infections (Khaksar *et al.*, 2012; Dhama *et al.*, 2015; Karangiya *et al.*, 2016). For instance, carvacrol and thymol demonstrated antimicrobial activity particularly against Gram-negative bacteria (*E. coli* and *S. typhimuruim*) by penetrating the cell wall and damaging the cells by binding to the amine and hydroxylamine groups (Abd El-Hack *et al.*, 2016). Following this, Arsi *et al.* (2014) found out that the supplementation of 0.5% thymol reduced the number of campylobacter in broiler chicken. Cerisuelo *et al.* (2014) observed that the addition of 50 mg/kg of thymol and cinnamaldehyde essential oil mixture in the broiler chicks' diet would decrease the number of *Salmonella*. Furthermore, Corduk *et al.* (2008) and Eleiwa *et al.* (2011) recommended the use of Orego-stim® as a prophylactic agent against *E. coli* infection in poultry. The key ingredients of the product, 2.42% thymol, 81.89% carvacrol, and mixture of other essential oils from *Origanum vulgare* spp. are known for their potent antiseptic properties were proven to reduce the number of pathogens such as *E. coli* in the gut, while increasing the number of beneficial bacteria like *Lactobacillus* in chickens. In meat products, Lu and Wu (2012) noted that the mixture of 0.2 mg/mL thymol and 2 mg/mL acetic acid could act as a natural alternative to chlorine-based washing solution to decrease *Salmonella* contamination on the chicken breast meat. Similarly, Murali *et al.* (2012) suggested that chicken breast fillets treated with 5 ml of turmeric, lemon, and green tea extracts combination were able to kill all *C. jejuni* and *S. enteritidis* within 12 hours of incubation, meanwhile using only single extracts were not effective in the same study.

Table 4. The effect of phytobiotics as antimicrobials in poultry

Plants	Plant compounds	Antimicrobials	References
Cumin and fennel	10 µl per disc of cumin and fennel essential oil	Inhibit the growth of *P. multocida* and *E. coli* via the disc diffusion method	Bhaisare *et al.* (2016)
Thyme and other plant mixture	10 µl per disc of thyme essential oil	Inhibit the growth of *S. typhi*, *P. multocida*, *E. coli*, and *S. aureus* via the disc diffusion method	Bhaisare *et al.* (2016)
	0.5% of thymol	Reduce the number of campylobacter in broiler chicken	Arsi *et al.* (2014)
	50 mg/kg of thymol and cinnamaldehyde mixture	Decrease the number of *Salmonella*	Cerisuelo *et al.* (2014)
	0.2 mg/mL thymol and 2 mg/mL acetic acid mixture	Decrease *Salmonella* contamination on the chicken breast meat	Lu and Wu (2012)
	75 g/kg of vanillin and thymol essential oils mixture	Encourage the growth of *Lactobacillus* population in broilers	Gheisar *et al.* (2015a)
Orego-stim®	2.42% thymol, 81.89% carvacrol, and mixture of other essential oils from *Origanum vulgare* spp.	Reduce the number of *E. coli* in the gut, while increasing the number of *Lactobacillus* in chickens' gut	Corduk *et al.*, 2008; Eleiwa *et al.* (2011)
Turmeric, lemon and green tea	5 ml of extracts combination	Effective against *C. jejuni* and *S. enteritidis*	Murali *et al.* (2012)
Cinnamon	0.24 mg/ml of thymol and 0.33 mg/ml of cinnamaldehyde extracts	Inhibit the growth of *C. perfringens*	Timbermont *et al.* (2010)

On the other hand, Murali *et al*. (2012) indicated that certain plant compounds are also effective against Gram-positive bacteria while encouraging the proliferation of beneficial gut bacteria. In another *in vitro* study, plant extracts like thymol and cinnamaldehyde have shown effective results in constraining the growth of *Clostridium perfringens* at minimum inhibitory concentrations (MICs) of 0.24 and 0.33 mg/ml respectively which could lead to the growth of beneficial bacteria in the gut (Timbermont *et al*., 2010). This is consistent to Gheisar *et al*. (2015a), who confirmed that adding 75 g/kg of essential oils such as vanillin and thymol to the broiler's feed can encourage the growth of *Lactobacillus* population. In another study, supplementing thymol by 100 mg/kg in the diet can reduce the incidence of infectious diarrhoea and mortality rate in newly weaned pigs indicating a healthier gut environment (Li *et al*., 2012). Alternatively, cinnamon oil can limit and control the growth and colonisation of several bacterial intestines by unsealing and disrupting their cell membrane, which eventually leads to the disintegration of the cells (Mehdipour and Afsharmanesh, 2018). Reis *et al*. (2018) asserted that cinnamaldehyde from cinnamon oil can be used to balance the microbial population in poultry to enhance their intestinal health since they can selectively inhibit the growth and improvement of commensal and pathogenic intestinal bacteria. On top of that, cinnamon oil also possesses the capability of enterocytes which decrease the oxidative damage to the intestinal epithelium (Krauze *et al*., 2019). In addition, Rusdi *et al*. (2019) also posited that coconut husk extracts could increase the growth of L. acidophilus bacteria as they have the potential to be a useful phytobiotics in the poultry industry. The effects of phytobiotics which have been used as antimicrobials in poultry industry are summarised in Table 4. Overall, with the application of plant-based products, it will create an opportunity to avoid the usage of artificial chemicals as prophylactic in the livestock and food industry.

The effect of phytobiotics on immunity of poultry

Besides production performance and antimicrobial activity, plant products have been widely used as an immune enhancer in the poultry industry to prevent disease outbreak provided that they are supplemented at the optimum percentage. Generally, Vitamin C, carotenoids, and flavonoids are the most significant compounds in herbs and spices for the immune system owing to their ability to improve the activity of macrophages, lymphocytes, and NK cells, which increase phagocytosis and motivate interferon synthesis. For example, eucalyptus oil from the leaves of the eucalyptus has the ability to stimulate the immune system in poultry by affecting the phagocytic capacity of monocyte-derived macrophages (Serafino *et al*., 2008). The addition of 1 to 1.4% of black cumin seed would enhance the immune responsiveness in broiler chickens (Al-Mufarrej, 2014). In addition, supplementing *Ganoderma lucidum*, a wild mushroom powder at 2 g/kg was able to enhance the immune response of chickens during vaccination (Ogbe *et al*., 2008). The compounds extracted from *G. lucidum* can motivate and activate dendritic cells, which function to present antigens to the T-cells (Wang *et al*., 2002). Furthermore, it has been demonstrated that garlic powder in chicken diets could enhance the immunity of broiler birds (Khan *et al*., 2014). In this study, garlic powder supplemented at 3 g/kg in the broiler diets for 42 days' study trial demonstrated a higher antibody titer against Newcastle disease, Infectious bronchitis, and Infectious bursal diseases, as opposed to the other groups, supplemented with a lower level of garlic powder. Confirming to the study, El-Latif *et al*. (2013) also indicated that supplementing 100 mg/kg of garlic essential oil was able to stimulate innate immunity by increasing phagocytic capacity of heterophils in broilers.

On top of that, phytobiotics are also shown to increase humoral immunity through the production of immunoglobulins (Ig) (Kong *et al*., 2006; Khaligh *et al*., 2011). This is because, certain plant extracts can stimulate T-cell which results in interferon (IFN)-ɣ secretion and also enhance the function of antigen-presenting cells that lead to the stimulation of the immune response (Chung *et al*., 2020b; Wang *et al*., 2002). For instance, angelan compound isolated from Angelica gigas could increase the T-cell population for antibody production. Zhang *et al*. (2002) reported the same results by isolating proteoglycan (GLIS) from the fruiting bodies of *G. lucidum*, which induces B-cell activation. Besides, *Astragalus membranaceus* could be used as an immune enhancer and modulator to im-

Table 5. The effect of phytobiotics on immunity of poultry

Plants	Plant compounds	Immune responses	References
Black cumin	1 to 1.4% of seed	Enhance the immune responsiveness in broiler chickens	Al-Mufarrej (2014)
Wild mushroom (*G. lucidum*)	2 g/kg of powder	Enhance the immune response of chickens during vaccination	Ogbe *et al. (*2008)
Garlic	3 g/kg of powder	Demonstrate a higher antibody titer against ND, IB, and IBD	Khan *et al.* (2014)
	100 mg/kg of essential oil	Increase phagocytic capacity of heterophils in broilers	El-Latif *et al.* (2013)
A. membranaceus	2 g/kg of polysaccharide	Increase serum IgG, IgM, and IgA in broilers	Wu (2018)
	300 mg/kg of root powder	Increase the serum total protein, A/G ratio, albumin, and IgG	El-Shafei *et al*. (2013)
	100, 200 and 300 mg/kg of root powder	Increase the immune organs weight and IgG level	Farag and Alagawany (2019)
Fructo-oligosaccharide	4.0 g/kg	Enhance the growth of *Bifidobacterium* and *Lactobacillus spp*. while inhibiting *E. coli* in the small intestinal and cecal digesta	Xu *et al.* (2003)
	0.50%	Enhance intestinal histomorphology, IgY cytokines, and mRNA in the ileum of broilers	Shang *et al.* (2015)
	0.75%	Decrease in the level of *S. typhimurium* present in the ceca	Bailey (1991)

prove the innate immunity or specific immunity (both humoral and cell-mediated immunity) in broiler chickens (Mitsch *et al*., 2004; Zhang *et al*., 2013). Referring to Wu (2018), 2 g/kg of polysaccharide from *A. membranaceus* was able to increase serum IgG, IgM, and IgA in broilers. Similar trends were reported by El-Shafei *et al*. (2013) and Farag and Alagawany (2019) who stated that 100, 200, and 300 mg/kg of *A. membranaceus* root powder supplementation could increase the immune organs weight and the level of IgG significantly.

In another study, Prabakar *et al*. (2016) proved that dietary fructo-oligosaccharide could alter the intestinal microflora environment and cause it to regulate the secretion of IgA in murine Payer's patches cell. This is supported by Xu *et al*. (2003) who investigated the effect of fructo-oligosaccharide on intestinal microflora by adding different concentrations of the extract in broiler chick diet. The results revealed that the concentration at 4.0 g/kg enhanced the growth of *Bifidobacterium* and *Lactobacillus* spp. while inhibiting *E. coli* in the small intestinal and cecal digesta. On the other hand, Shang *et al*. (2015) have proven the effectiveness of dietary fructo-oligosaccharide supplementation on the growth performance, intestinal morphology, and immune responses in broiler chickens challenged with *S. enteritidis* lipopolysaccharides. In their study, the addition of 0.5% fructo-oligosaccharide in broiler diets was able to enhance crypt depth, villus height, and total mucosa thickness in the ileum; reduced heterophil but stimulated monocyte count and specific IgY; as well as increased the expression of interleukin (IL)-1ß, IL-10, IFN-γ, and mRNA in the ileum of broilers despite being injected with 2 mg/kg of immunogen intraperitoneally. Correspondingly, birds that were treated with 0.75% fructo-oligosaccharide had a fourfold decreased in the level of *S. typhi*murium present in the ceca (Bailey *et al*., 1991). According to Chotikatum *et al*. (2009), the supplementation of fructo-oligosaccharide in the chicken's diet could lead to a shift in the intestinal gut microflora, and under some environments may lead to decreased susceptibility to *Salmonella* colonisation.

In summary, the effects of different phytobiotics on the immune response of poultry are presented in Table 5. Many studies have proven the effectiveness of plant-based compounds in stimulating the immunity of commercial birds. Generally, those compounds were able to enhance overall immunity during vaccination, stimulate the production of specific Ig, and influence intestinal microflora environment. Nevertheless, the use of specific plant extract to stimulate immunity against specific diseases is still unclear. For that reason, further investigation such as disease challenge in poultry treated with plant extracts could shed light on the effectiveness of those phytobiotics in replacing antibiotics.

Conclusion

There are significant reasons to seek alternative feed additives to replace synthetic antibiotics in poultry diets to prevent the incident of antibiotic resistance or the development of superbugs. Based on the information discussed in this review paper, it is clear that phytobiotics or plant extracts would offer a better solution to solve these issues as they are proven to have beneficial properties on growth performance, carcass and meat quality, plus immune responses of the poultry. Although phytobiotics are considered as better alternatives, several issues such as the optimum dosages, complex composition, stability, weather conditions, harvesting time, extraction method, storage conditions, antagonistic effects, antinutritional factors, as well as microbial contamination requires further investigation. Understanding from these studies should be able to convince the industry to utilise phytobiotics confidently as alternative feed additives instead of using antibiotics for disease prevention and growth promotion. This will also ultimately improve the food safety of poultry products for the consumers.

Acknowledgement

The authors would like to thank the Ministry of Higher Education for providing funds for some of the studies conducted in this review. The research was funded by the Fundamental Research Grant Scheme (FRGS). FRGS reference code: FRGS/1/2020/WAB04/UPM/02/1. FRGS project code: 07-01-20-2256FR.

Conflict of interest

All authors declare no competing interests.

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