

PHYTOBIOTICS AS AN ALTERNATIVE FEED ADDITIVE

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ABSTRACT

The identification and development of new and effective alternatives to antibiotics that do not hinder productivity assumes paramount importance in the context of antimicrobial resistance and food safety. In recent years, phytobiotics have been used as natural growth promoters in ruminants, poultry and swine, to improve growth and health. A wide variety of herbs, spices and essential oils have been used individually or as blends in poultry for their potential application as alternative growth promoters. In nursery and growing pigs supplemented with phytobiotics, intestinal pathogens were inhibited and beneficial microbial population that contributes to improving digestion and absorption of nutrients were increased resulting in increased weight gain and decrease in diarrheal syndrome. The inclusion of essential oils in the diet of finishing pigs, improved the productive yield and carcass characteristics. It also reduced the incidence of therapeutic treatment and mortality. Natural plant products have the potential to improve rumen fermentation, reduce loss of feed energy, improve animal health and productivity, increase animal lifetime performance, and reduce greenhouse gases production in ruminants. Phytobiotics with wide range of activities, is projected as a feed additive for higher market growth compared to other alternatives to antibiotics in the feed industry.

Key words: Phytobiotics, poultry, swine, ruminants, feed efficiency, methane mitigation

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The global consumption of antibiotics for human use and animal production is 1×10^5 and 2×10^5 tonnes respectively (Manzett and Ghisi, 2014). The use of antibiotics as growth promoters in livestock and poultry production

is negatively viewed, due to the development of antibiotic resistance mechanisms in pathogenic bacteria of both humans and animals, which can be easily spread within microbial communities (Diarra and Malouin, 2014). The Bureau of Indian Standards (2007) on Poultry feed has recommended to stop the use of antibiotics as growth promoters in feed.

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Chicken meat samples obtained from retail meat shops in Chhattisgarh revealed the prevalence of *Salmonella* in 7% of samples. The *Salmonella* were highly sensitive to ciprofloxacin, sensitive to gentamicin (96.8%), but were resistant to erythromycin (93.7%) (Naik *et al.*, 2015).

Helicobacter pullorum isolates from both free-range and broiler chicken in Hyderabad were resistant against fluoroquinolones, cephalosporins, sulphonamides and macrolides (Qumar *et al.* 2017). Poultry farms in Punjab revealed a high prevalence (87% and 42% in broilers and layers, respectively) of ESBL- producing Enterobacteriaceae. A high prevalence of *E. coli* resistant to nalidixic acid (86.1%) and antibiotics such as tetracycline, ciprofloxacin and ampicillin (45%) were observed (Brower *et al.*, 2017). Chicken meat (66.7%) procured from retail outlets harbored methicillin resistant *Staphylococcus aureus* (MRSA) (Ruban *et al.*, 2017).

The emergence of antibiotic related environmental pollutants and the rapid spread of drug resistant pathogens is of global concern. The National Action Plan on Anti-Microbial Resistance (NAP - AMR, 2017 - 2021), India, has identified six strategic priorities (GOI, 2018). The main focus areas are to develop better awareness and understanding of Anti-Microbial Resistance by providing training, strengthening the education on Anti-Microbial Resistance, reducing the incidence of infection, promoting investments and strengthening India's leadership on Anti-Microbial Resistance. Hence, the identification and

development of new and effective alternatives to antibiotics that do not hinder productivity is imperative (Gayatri *et al.*, 2017).

Alternatives to antibiotics as feed additive

Intensive research on the development of alternatives to antibiotics is under progress for the past two decades. The most widely researched alternatives include probiotics, prebiotics, acidifiers, plant extracts and nutraceuticals such as copper and zinc (Thacker, 2013). The alternatives to antibiotic growth promoters include enzymes, organic acids, probiotics, prebiotics, synbiotics, bacteriocins, antimicrobial peptides, natural antimicrobial products and essential oils such as carvacrol, thymol, cinnamaldehyde, eugenol, and eucalyptol (Bajagai, 2020). Alternative feed additives exhibit their positive effects by increasing the numbers of beneficial bacteria in intestinal tract and promoting gut health (M'Sadeq *et al.*, 2015). Colonization of the intestine with beneficial microbes not only prevents pathogenic bacteria-related intestinal disorders, but also improves intestinal maturation and integrity (Lan *et al.*, 2005).

Increasing concerns about the increase of superbugs and limited development of new drugs for livestock necessitates the timely development of alternatives to antibiotic growth promoters. So, the trend in the use of phytobiotics in animal feed has increased during the last two decades. The health benefits and growth-promoting effects of phytobiotics may be dependent on several mechanisms based on their various biological activities (Cyrille and Kuitich, 2023).

Phytobiotics as feed additives

Plant derived products added to the feed to improve livestock and poultry performance are called as phytobiotics. Stem, leaves, roots, tubers, fruits, flowers and seeds of plants / herbs / spices can be used as phytobiotics. They are available as solid, dried and ground forms or as their extracts (Panda, 2009). The composition of bioactive substances present in phytobiotics varies widely based on the plant parts (flowers, fruits, seeds, stem, leaves, roots, tubers *etc.*) used, place of collection, and season of harvest (Burt, 2004; Bakkali *et al.*, 2008; Windisch *et al.*, 2008).

The use of phytobiotics as natural growth promoters in ruminants, swine and poultry has been increasing in the recent years. Mohsen *et al.* (2018) reported that many phytobiotics in the form of herbs, spices, essential oils and extracts are used to improve growth, antibacterial activity, antioxidant activity and anti-inflammatory activity. The antimicrobial and antioxidant properties of phytobiotics, contribute to their beneficial effects. Phytobiotics also have immunomodulatory properties. Increased proliferation of immune cells, modulation of cytokines, and increased antibody titers have been observed on phytobiotic supplementation (Lillehoj *et al.*, 2018). The EU approved the first botanical feed additive for improving performance in poultry and livestock, it was a commercial blend of phytonutrients (carvacrol, cinnamaldehyde and Capsicum oleoresin), that enhanced innate immunity and reduced the negative effects of enteric pathogens (Lee *et al.*, 2010 and Lee *et al.*, 2011).

Phytobiotics as feed additives in Poultry

A wide variety of herbs and spices *viz.*, cinnamon, cumin, coriander, star anise, ginger, turmeric, garlic, rosemary, basil, caraway, lemon, sage, thyme, oregano, marjoram, yarrow, green tea, black and essential oils such as thymol, carvacrol, cinnamaldehyde and eugenol, have been used individually or as combinations in poultry as antibiotic growth promoter alternatives (Gadde *et al.*, 2017).

Phytobiotics as feed additives in Broilers

Encapsulated garlic and *P. niruri* L. mixture in the diet of broilers (0, 0.4, 0.8 and 1.2%) significantly ($P < 0.05$) increased the body weight gain, villi length and lactic acid bacteria number and decreased intestinal viscosity and *E.coli* (Natsir *et al.*, 2013).

Dried peppermint (DPM; *M. longifolia*) in broiler diets at inclusion levels not exceeding two per cent, had antioxidant properties and altered ammonia production, promoted abdominal fat reduction (Khempaka *et al.*, 2013). Supplementing different concentrations of cinnamon powder in the diet of broiler chickens increased their final body weight (Shirzadegan, 2014). Neem leaves powder when added to broiler diet at a rate of 2 g / kg caused significant improvement in the average body weight and feed intake of birds Shihab *et al.* (2017).

M. oleifera (MO) leaf aqueous extracts (MOLE) at different concentrations (0.5, 1.0 and 1.5% w/v), and MO leaf meal (MOLM) at 0, 0.5, 1.0 and 1.5 per cent w/w were tested in the diet of broilers. Broilers fed 0.5 per cent w/w MOLM exhibited enhanced meat

quality and antioxidant status when compared with other groups. (Govindarajan *et al.*, 2015).

Coriander seed at the rate of 2% in broiler feed improved the overall performance of birds without any harmful effect on haemato-biochemical profile and was hence recommended to be included in the diet of broilers up to 42 days to maximize the profit (Barad *et al.*, 2016). Dried coriander and turmeric powders as feed additives each at level of 0.75 per cent alone or as mixture of both of them had an immuno-modulatory effect in broilers and could be used in areas with high risk of viral infection as it will help in maximizing the benefits of vaccines (Zaki *et al.*, 2016). Amla fruit powder can be effectively supplemented as an alternative to antibiotic growth promoters in poultry ration and significantly ($P < 0.05$) best results were obtained at 0.75 per cent amla supplementation level with regard to gut morphology, ecology and immune modulating activity and also resulted in significantly ($P < 0.05$) improved weight gain and FCR (Dalal *et al.*, 2018).

A study in broiler chicken fed diets supplemented with curry leaves (*Murraya koenigii*), drumstick leaves (*Moringa oleifera*) and pepper leaves (*Piper nigrum*) powder revealed that supplementation with drumstick (1%) and pepper leaves (1%) significantly ($P < 0.05$) improved the body weights of broilers. Feed conversion ratio was significantly lower ($P < 0.05$) in drumstick and curry leaf (1%) powder supplemented birds (Jayathilaka *et al.*, 2018).

Ginger supplementation at the rate of one per cent significantly ($P < 0.05$) showed improvement in the body weight

gain (g/bird), lower feed conversion ratio and improved sensory parameters (Karangiya *et al.*, 2016). Gaikwad *et al.* (2019) reported that supplementation of ginger (*Zingiber officinale*) in birds led to significantly ($P < 0.05$) higher body weight and weekly gain with better feed conversion. Dietary addition of standardized formulation of lipophilic turmeric extract containing curcumin and turmerones named TF-36, at an optimized dosage of one per cent (w/w) of broiler feed, safely enhanced the growth performance and health status of the chicken. It provided a better body weight gain and feed conversion ratio during the study period of 42 days. TF-36 fed chicks showed improved antioxidant effect and better detoxification potential to the birds, along with a significant ($P < 0.05$) reduction in lipid peroxidation (Naeem *et al.*, 2018).

The major benefit from garlic powder in improving growth rate and feed conversion of broilers was due to its stimulatory effect on feed intake, up to a level of three per cent in the diet, beyond which both growth rate and feed conversion deteriorated (Mulugeta *et al.*, 2019). Depressive effects on feed intake of too high a level of garlic in the diet were ascribed to the presence of antinutritional substances and repulsive odour and taste. Dietary addition of fresh and dry Tulsi leaves powder at the rate of 0.5 per cent in the diet of broiler chickens significantly ($P < 0.05$) improved body weight gain, feed conversion ratio, return over feed cost and profit per bird (Gohel *et al.*, 2019).

Layers

Significant ($P < 0.05$) improvement in the body weight gain of layers were observed when neem leaves powder was added to the

layer diet at a rate of 1, 2 and 3 g / kg (Singh *et al.*, 2015). Melo *et al.* (2016) reported that black pepper (0.6%) can be used in diets of laying hens as phytogetic additive without affecting their performance. However, the inclusion of more than 0.3 per cent level caused an impairment in egg shell quality and an increase in the level of triglycerides in the blood stream.

The feeding of herbal mixture powder comprising 90% plantain (*Plantago lanceolata* L.), 5% ivy-gourd (*Coccinia grandis*), 3% garlic (*Allium sativum*) and 2% spearmint (*Mentha spicata*) leaves improved the feed intake, HDEP, egg mass and FCR and egg yolk color of Lohman Brown, laying hens (Rahman *et al.*, 2021). Incorporation of moringa leaves at 0, 1, and 3% in laying hen diet showed highest egg production in 1% group (Voemesse *et al.*, 2019). Inclusion of 0.5% fennel and hot red pepper seed improved the laying hen performance including egg weight, egg mass, hen day egg production and feed conversion ratio (Abou-Elkhair *et al.*, 2018). Addition of garlic powder at 1, 2 and 3% in laying hen diet led to improved hen day egg production and egg quality (Asrat *et al.*, 2018).

Phytobiotics as feed additives in Swine

Piglets

Weaning poses a challenge in swine production worldwide. A high incidence of entero-toxigenic *E. coli* proliferation causes postweaning diarrhoea, resulting in dehydration, growth retardation, sudden death leading to economic losses. Phytobiotics, in the swine industry have been developed

as potential tools to mitigate the abovesaid effects (Fresno Rueda *et al.*, 2021). Dietary supplementation of blend extracts of cinnamon, thyme, and oregano inhibited colonization of pathogenic *E. coli* in the intestine of nursery pigs (Namkung *et al.*, 2004). Blend of plant extracts (oregano, cinnamon, and Mexican pepper), decreased total microbial mass and increased Lactobacilli to Enterobacteria ratio in the ileum of nursery pigs (Manzanilla *et al.*, 2004). Phytobiotics in swine nursery diets improved post-weaning growth performance compared to pigs fed of diets without antibiotics. However, the improvement in growth rate was intermediate between diets fed with and without in-feed antibiotics. Further research is needed to elucidate specific modes of action that caused positive effects in post-weaning growth and efficiency (Sulabo *et al.*, 2007). Improved weight gain of nursery pigs was observed when supplemented with aged garlic extract, with allicin as the functional component (Tatara *et al.*, 2008).

Phytochemical ingredients can reduce coliform bacteria in gastrointestinal tract (GIT) and decrease the diarrheal frequency or mortality rates among young pigs. Phytochemical additives also play an important role in deterring diarrhoea and oedema in piglets during the weaning process (Li *et al.*, 2011). Increased weight gain and decreased diarrhoea occurrence were observed in nursery and growing pigs by using the extracts of *Macleaya cordata* (Wild) R. Br. as feed additives at the concentration ranging from 15 to 50 mg/kg (Kantas *et al.*, 2015 and Liu *et al.*, 2016). Moita *et al.* (2021) reported that the use of a blend of plant-based functional oils can modulate the mucosa-

associated microbiota and enhance intestinal health by reducing oxidative stress and enhancing jejunal morphology in nursery pigs.

By adding medicinal plant powders to the pigs' diet, in the digestive tract, the development of intestinal pathogens (*E. coli*, *Bacteroides* spp. and *Clostridium* spp.) is inhibited and increases the beneficial microbial population that contributes to improving digestion and absorption of nutrients, with increased weight gain, as well as a decrease in diarrheal syndrome in post-weaning piglets (Segarra, 2016).

Growing and finishing pigs

Feeding growing-finishing pigs with ration supplemented with thyme, rosemary, oregano extracts caused significant improvement in the average daily gain and feed conversion (Yan *et al.*, 2010). The pigs fed diets supplemented with essential oils had higher weight gain (10.3%) and improved digestibility of dry matter and crude protein by 10.3, 2.9 and 5.9 per cent, respectively. The improved performance of pigs was as a result of improvement of the intestinal morphology and consequently improvement of nutrients digestibility (Li *et al.*, 2012).

Lactating sows

Hall *et al.* (2021) conducted a study in lactating sows supplemented with oregano essential oils (EO). These authors obtained heavier piglets at one week of age, 10 weeks after weaning and at the slaughter time. Also, health records showed that piglets from litters supplemented with EO significantly reduced the incidence of therapeutic treatment and

mortality. In another study, with male and female fattening pigs, the inclusion of oregano EO in the diet improved the productive yield and carcass characteristics of the animals (Janacua-Vidales *et al.*, 2018).

Phytobiotics as feed additives in Ruminants

Natural plant products or phytobiotics are inexpensive and environmentally safe and have been used in methane mitigation strategies. These products have the potential to improve rumen fermentation, reduce loss of feed energy, improve animal health and productivity, increase animal lifetime performance and reduce greenhouse gases production during animals' production (Ugbogu *et al.* 2019). Phytobiotics in ruminant nutrition through their antimicrobial activity mitigate methane emissions and improve ruminal fermentation efficiency (Khiaosa-ard and Zebeli, 2013, Oskoueian *et al.*, 2013 and Petrič *et al.*, 2020). They are superior feed additives and can replace the ionophores and probiotics for controlling methanogenesis in ruminants (Oskoueian *et al.*, 2013).

Tannins are widely distributed in tropical foliage which is consumed by ruminants and they possess the potential in reducing protein degradation in rumen, inhibiting methanogenesis and increasing conjugated linoleic acid content in meat and milk (Patra and Saxena, 2011). Condensed tannins have direct inhibitory effect on methanogens (Jayanegara *et al.*, 2015). Hydrolysable tannins permeate through protozoal membranes and compromise their association with methanogens (Patra and Saxena, 2011) and thus have both direct and indirect effect on methanogens. *Cosmos*

bipinnatus Cav., which is a source of phenols and their derivatives, exerts anti methanogenic effects *in vivo* (Hernández Pineda *et al.* 2018) in dairy cattle.

Supplementation of tamarind seed husk and soap nut at 5.1% (60:40) caused significant reduction in enteric methane emission and was found equally effective to the individual supplementation of tamarind seed husk at similar level. Soap nut supplementation at similar level for short duration was not effective in reducing methane emission significantly (Poornachandra *et al.*, 2019).

Flavonoids (quercetin and naringin) reduce the population of protozoa and methanogens by inhibiting the synthesis of microbial cell walls, cytoplasmic membrane, and nucleic acids, this leads to a decrease in methane production (Oskoueian *et al.*, 2013). Increase in milk yield and lactation performance in dairy cows upon 25-day administration of sylimarín (10 g/d) which mainly consist of flavonolignans was reported by Tedesco *et al.* (2004). Plant extract containing flavonoids at the concentration of 300 mg/kg DM was able to decrease the incidence of acidosis and enhance the animal growth performance in cattle receiving high-concentrate diet (Balcells *et al.*, 2012).

The phytobiotic preparations used in feeding ruminants, can enhance taste sensations and stimulate appetite. As regulators of digestive functions, they also affect gastrointestinal motility and the secretion of digestive juices, reduce the occurrence of diarrhoea and regulate the pH of the gastrointestinal tract (Wójtowski *et al.*, 2019). Petrič *et al.* (2020) have demonstrated

that a mixture of wormwood, chamomile, common fumitory, and mallow has potent antioxidant activity in the rumen and potential to reduce methane, and ammonia emissions and concentrations.

Four herbs, rosemary, marigold, citrus, and grape, kept their antioxidant capacity *in vivo* in sheep when used as a ruminant model (Gladine *et al.*, 2007). In a later study, Gobert *et al.* (2009) demonstrated that combinations of the mentioned herbal extracts, when supplied in association with vitamin E, were able to reduce oxidative stress in lactating cows given a diet rich in n-3 fatty acids. The combination of herbal extracts acting as a hydrophilic antioxidant may synergistically interact with lipophilic antioxidants such as vitamin E and result in an enhanced antioxidant status.

CONCLUSION

With the increase in consumer's awareness and their concern over the presence of antibiotic residues in livestock and poultry products, exploring feed additives as alternatives to antibiotic growth promoters is the need of the hour. Phytobiotics with wide range of activities, is projected as a feed additive for higher market growth compared to other alternatives to antibiotics in the feed industry. The global feed phytobiotics market is expected to grow at a CAGR of 6.5% from 2022 to 2030. Phytobiotics are categorized as Generally Recognized As Safe (GRAS) 91, however, they contain many pharmacologically active components or may have irritating ingredients which may be harmful. The bioactive ingredients or their metabolites may be transferred to tissues leading to undesired residues in a natural

product. Hence, phytobiotics needs to be thoroughly checked for potential safety issues.

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