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## Enhancing immunity level by using phytogetic feed additives in animal diets (review)

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It is established that antibiotics are one of the most important medical discoveries of 20th century and will remain an utmost way of treating disease of animals as well as of human beings. We, the human beings, get meat, milk, egg, etc. from animals being very important and inevitable part of our daily nutrition. The irrational, consecutive and extensive use of antibiotics in food producing animals particularly for growth promotion has lead to antibiotic resistance, microbial resistance and possible the drug residual threat for human beings due to consumption of milk, meat, egg, etc. It was found out that during the normal physiology the animals undergo various types of stresses mainly including environmental, pathogenic, etc which suppress the immune system as well as the GIT of animals and due to this the animals become vulnerable to different diseases which leads to immunosuppression, poor health status, increased mortality and decreased production. Due to this ban on using synthetic antibiotics in animals as antibiotic growth promoters (AGP), an alternative approach is to use phytochemicals in animals' feed for growth promotion, optimum production and enhancing or modulating the immunity level of animal. These phytogetic feed additives are important and have many properties i.e. anti-fungal, antibiotic, anti-inflammatory, antioxidant, antiviral, etc which, when supplemented with diet and fed to animals, ensure the protection and improvement of health condition, integrity of GIT and enhance the immunity level of animal. This review illustrates the importance of plant-based feed additives supplemented with other feedstuff and fed to animals particularly their role in immunomodulation to boost the immunity level on animal under stress conditions.

**Key words:** feed additives, phytobiotics, alternative to antibiotics, animal feeding, immunity.

### Introduction

The phytogetic feed additives (PFAs), also known as phytobiotics or botanicals, are bioactive compounds extracted from plants and their products and added to animal feed to boost growth and production of food producing animals. The important bioactive compounds of PFAs are polyphenols and composition and concentration of these compounds depends on extraction technique, environmental condition, harvesting season, storage conditions, geography, plants and

part of the plant being used for this purpose. This term includes a variety of herbs or spices which are utilized in solid, dried or powder form in crude or concentrated form. Apart from these procedures to extract the bioactive ingredient, PFAs are divided into essential oil (obtained by cold extraction, steam or alcoholic distillation) and oleoresins (extracted by non aqueous solvents) [3, 39, 40]. The mechanism of action of PFA is still not very clearly understood but is largely similar to the antioxidant, antimicrobial, antibiotic, anti-inflammatory

characteristics of the bioactive ingredients being extracted from PFAs. When mixed with animal feed, it alters the gut microbiota, reduce the microbial toxins and restore the intestinal integrity and immune status of animal which leads to optimum growth and production. PFAs impart the immunomodulatory effects e.g. increases the proliferation of lymphocytes, increases the exposure of cytokines and high antibody titer [22, 28, 32, 36, 41].

Since the time when ban was imposed on using synthetic antibiotics in food producing animals for the purpose of optimum production, growth promotion and enhancing immunity level, the search for alternate phytogetic and plant-based feedstuffs, having no side effects and considered safe to be used in animal feed, has gained a huge attraction. The main reason of this paradigm shift was antibiotic resistance, antimicrobial resistance and a possible drug residual effect in human beings. So it was a matter of public health concern which made it more sensitive and a ban was put to curtail the residual effects of antibiotics which were used in food producing animal. These phytogetic feed additives enhance the immunity level of animals and make them strong enough to bear the stress. Apart from this immunomodulation, the phytogetics also improve health status and consequently increase the production of animals [14]. Flavonoids, a group of polyphenolic compounds found in fruits and vegetables, includes two naturally occurring compounds genistein and hesperidin found in soybean and citrus, respectively, and put positive effects on health and egg production of poultry birds [37]. In some other research trials soybean isoflavones and flavonoids rich alfalfa extract were found to be beneficial on growth and production, B and T lymphocyte proliferation in broiler chicken [8, 19]. In another study it was reported that flavonoids and their extracts had some immunomodulatory effects as well as also affected surface area, villus length of small intestine and improved the overall gut health of poultry birds [14, 17, 38].

In a study trial it was observed that *Morinda citrifolia* plant juice extracts enhanced the proliferation of CD4 and CD8 T cell in newly born calves and it positively modulated the immunity level of animals [6]. In a research study performed by [35] the immunomodulatory effects of *Morinda citrifolia* was observed in poultry birds when fresh juice of the plant was supplemented 5% mixed in water and it boosted both the humoral (B cell mediated) and cellular (T cell mediated) immunity level in broiler chickens. It was also observed that humoral immunity response was statistically significant ( $p < 0.05$ ) in the treated birds as compared to that of control group and the peak response was seen in 1<sup>st</sup> week of post infection.

The pathogenic organisms and other environmental stressor can destroy the animal tissues and cells and if this destruction keeps on going, a time will come when concentration of reactive oxygen species (ROS) is increased which causes lipid peroxidation and oxidative damage of cell membranes and it challenges the immunity status of animal. If there is no antioxidant to neutralize ROS it triggers the inflammation in which various types of cells are sent to the inflammation site after the cytokines and chemokines are secreted. It is normal physiological response of body towards pathogenic microbes entering the body and to fight against the infection [4, 7, 25]. GIT is the first site where food is degraded and absorbed and pathogens reach there by destroying the mucus membranes. So the protection of GIT is of great importance to ensure the optimum health and production of animals and it also boosts the immunity level of the animal [7]. The animals react to the external stressors by decreasing feed intake which decreases body weight; these stressors whether are of exogenous or endogenous nature depress the immune response, damage the gut integrity and leads to low growth and production of animals that poses economic losses every year. The antibiotics are used for growth promotion as well as to enhance the immunity level of the animals but due to the detrimental effects of antibiotics, ban was imposed, in 2006 by European Union, on the use of antibiotics in food producing animals for growth promotion purpose. So an alternative approach is made by using phytogetic feed additives in the diets of animals for the purpose of growth promotion, optimum production as well as enhancing the immunity level of animals [7, 29].

#### **Effect of phytochemicals on immune response**

The active constituents of plants, phytochemicals, are broadly categorized into two main groups: terpenes and terpenoids. These compounds are extracted from different parts of plant by adopting different procedures however the powder form is most widely and commonly used [16, 27].

Oregano belongs to botanical family of Labiate and includes widely distributed plant species *Origanum vulgare*, *O. onites*, etc containing a very important bioactive compound carvacrol and these are commonly used as feed additives in animals. A study was conducted by [30] in which oregano essential oil (OEO) was supplemented 300 ppm along with basal diet of broiler chickens and resulted in production of higher antibody titer; more specifically high titer of immunoglobulin G (IgG). Another study revealed that high doses (500 and 1000 ppm) of OEO supplementation resulted in enhanced immunity level in the broiler

birds which were vaccinated with new castle disease and avian influenza virus [13]. In swine higher thymus lymphocytic immune cell concentration was observed on 14<sup>th</sup> day of lactation when OEO was supplemented 250 ppm in the diet which resulted in positive immunomodulatory effects [13]. The concentration of coccidian oocysts in excreta was lowered, improved growth and a better immune response was observed when the broiler diet was supplemented with OEO 300 ppm or mixture of carvacrol and thymol was added 300 ppm [2].

A research trial was performed by [31] in which cinnamon was supplemented in broiler diet 0.4% and 0.8% (doses of 100 and 200 ppm essential cinnamon oil) led to improved body weight from 1-6 week, improved FCR, increased hemoglobin and leukocytes concentration in blood of broiler chicken [1]. On the other hand positively modulated immune response was observed in 21 day old broilers which were given 5 g/L of drinking water [33]. The table shows some recommendations for the use of phytogetic feed additives in animal nutrition.

The phytogetic feed additives are composed of herbs, spices, essential oils, extracts, bioactive compounds which have an overall positive effect on growth promotion, optimum production and enhancing the immunity level up to extent to make them resistant to disease [38, 40]. After the chickens were immunized and infected with *Eimeria tenella* the phytonutrient supplemented diets were fed to the birds and it resulted in increased body weight, higher antibody titer and an increased lymphocytes proliferation as compared

to the birds who were not fed the supplemented diets [26]. Caprylic acid, an organic acid, when it was supplemented to broiler chickens; it lowered the degree of infection caused by *Salmonella enterica*. This acid down-regulated the gene of bacterium responsible for invasion of epithelial cells and ultimately it ensured the immunomodulation in broiler chickens [23].

Prebiotics are macromolecules defined as “nonviable feed components which are beneficial for host after the gut’s microbial modulation” [10]. These are either taken from plant or synthesized by the microbes. The mannanoligosaccharide (MOS), extracted from the outer cell wall layer of *Saccharomyces cerevisiae*, is used as a prebiotic supplement in broiler chicken diets and is reported to enhance immunity level [18, 34].

#### Challenges of using phytogetic compound in animal feed

Due to the complex composition, sometimes it becomes difficult to systematically and comprehensively evaluate the phytochemicals to use them in feedstuff. There can be some challenges regarding how to use these compounds in feed due to possible side effects (toxic, unpleasant odor), regularity and legal affairs and their possible interaction (good or bad) with rest of the other ingredients of feedstuff [12, 15]. There is a dire need to have a state-of-the-art and well developed analytical method to quantify the phytochemicals before being added to the animal feedstuff. The phytonutrients are natural and organic substitute to the synthetic antibiotics and are considered safe by FDA USA but an authentic and complete as-

Table 1 – The recommendations for animal nutrition

Phytogetic material	Species in which can be used	Supplemented form	Dose rate	Immune response	Reference
Echinacea purpurea L.	Broiler,	Ariel part of plant, powder	5-10 g/kg diet	Higher antibody titer against ND	[24]
	Laying hens	Juice	0.25 ml.kg BW	Higher WBC cells	[5]
Oregano	Broilers	Essential oil	300 ppm in diet	Higher IgG titer	[30]
Cinnamon		Powder	4 and 8 g/kg diet	High lymphocytes	[31]
Turmeric		Rhizome powder	2.5, 5 and 7.5 g/kg diet	Higher IgA, IgG and IgM titer	[9]
Thyme		Oil extract	100 and 200 ppm in diet	Higher WBC cell	[1]

assessment protocol, encircling all the procedures to analytically ensure and quantify the bioactive compounds before being used in animal feed, is still needed [11].

#### Relevant literature

A research study was performed on 720, 1 day old Arbor Acres broiler chicks by adding two flavonoid compounds genistein and hesperidin found in soybean and citrus in the diets. The objective of the trial was to check the effects of these flavonoids on immunity and intestinal morphology of poultry birds. On 16<sup>th</sup>, 18<sup>th</sup> and 20<sup>th</sup> day one half of the birds from each group were injected *Escherichia coli* intraperitoneally 250 µg/kg of body weight to induce immunological response. The samples were collected on 21<sup>st</sup> and 42<sup>nd</sup> day of trial and it was observed that the compounds enhanced the immunity level by improving phagocytosis and due to which statistically significant immunomodulatory effect ( $p < 0.05$ ) was observed. Other parameters were also improved but no effect was seen on feed intake, body weight gain and FCR of broilers. In conclusion it was observed that the phytogetic feed additives boosted the immunity level and improved gut health of broiler chickens [20].

An experiment was conducted on 336, one day old broiler (Ross 308) chicks to observe the comparative impact of *Echinacea purpurea* (EP) with standard antibiotic on growth promotion, carcass quality and immune level. The chicks were divided into 7 different treatment groups. Group 1 was control and only fed the basal diet, group 2 was given basal diet + antibiotic (4.5 mg flavophospholipol/kg diet), group 3 was fed basal diet + powder of dried aerial parts of EP (5 g/kg diet), group 4 was given basal diet + powder of dried aerial part of EP (10 g/kg diet), group 5 was supplemented with basal diet + powder of dried aerial part of EP (0.25 g/kg diet), group 6 was fed basal diet + powder of dried aerial part of EP 5 g/kg diet but EP was supplemented continuously for 3 days followed by break of 11 days and last group was given basal diet + powder of dried aerial part of EP 10 g/kg diet but EP was supplemented continuously for 3 days followed by break of 11 days. At 28<sup>th</sup> and 31<sup>st</sup> day the blood sampling was done to analyze the immune status. The overall results showed that supplementation of EP 5 g/kg diet had statistically significantly better ( $p < 0.05$ ) daily feed intake, daily weight gain and higher antibody titer against Newcastle virus and sheep red blood cell (SRBC) as compared to rest of other treatments groups [24].

A study trial was conducted in Ferdowsi University of Mashhad, Iran, on 200, 1 day old Ross broiler chicken to check the immunomodulatory effect of turmeric rhizome powder (TRP). The

chicks were divided into 4 main treatment groups and 5 replicates which were composed of 10 birds each and these groups were supplemented with corn-soybean meal having TRP at the concentration of 0%, 0.25%, 0.50% and 0.75%. On 14<sup>th</sup> day one bird from each replicate was infected with 0.2 ml of 5% SRBC while the blood sampling was done at 21<sup>st</sup> and 42<sup>nd</sup> day. The results showed that supplementation of TRP significantly increased IgA, IgM, and IgG as well as TRP also significantly decreased monocytes ratio. In a conclusion it was reported that TRP had positively modulated the immunity response in broiler chickens [9].

The supplementation of herbal oil has been in practice since so many years as a part of ethnoveterinary practices in animal nutrition; in fact it is a way to boost immunity and strategically control the viral diseases. An experiment trial was performed on 120 broiler chicks which were randomly divided into 4 treatment groups. Group A was not vaccinated being control group while other 3 groups were vaccinated with inactivated avian influenza and live Lasota vaccine. Oregano essential oil (OEO) was orally administered 0.005 and 0.01% to group C and group D respectively. Results showed that the oral supplementation of OEO had positive effects on performance of birds and it also positively modulated the humoral and innate immunity in birds [13].

The immunomodulatory effects of proanthocyanidins rich extract (PAE) from *Pinus radiata* bark, proanthocyanidins are natural compounds present in fruits, flowers, seeds, barks, and vegetables, were observed in specific pathogen free (SPF) White Leghorn chickens. The proliferation of mononuclear cells was increased in birds which were treated 20 mg/kg PAE for 2 weeks. On the other hand proliferation of splenocytes and bursal cells was also increased in the birds treated 5, 10 and 20 mg/kg PAE for the period of 5 weeks. The thymocytes cell proliferation was increased in birds which were treated 5 and 10 mg/kg PAE for the period of 5 weeks. The PAE enhanced the expression of T helper 1 cytokines (interferone- $\gamma$ ) and lowered the expression of T helper 2 cytokine (interleukins-6). So it was concluded that the PAE had an effective immunomodulatory effects on SPF white leghorn chicken [21].

In an experiment the chicks were orally injected the virulent oocysts of *Eimeria tenella* and were supplemented the mixture of two phytobiotics, VAC (carvacrol, capsicum oleoresin and cinnamaldehyde) and MC (turmeric oleoresin and capsicum oleoresin) to observe the immunomodulatory response after immunization with *Eimeria* profilin protein. After being orally infected with

*Eimeria oocysts* and immunized with profilin protein, the chicks were given VAC or MC supplemented diets which increased BWG, higher antibody titer and increased proliferation of lymphocytes as compared to control group. Prior to the oral infection, the MC fed immunized chickens expressed reduced interferon- $\gamma$  (IFN- $\gamma$ ) and interleukin-6 (IL-6). After the chickens were infected they showed increased levels of IFN- $\gamma$  and IL-6. On the other hand, decreased IL-17F and TNFSF15 was shown only in infected chickens when VAC supplemented diet was fed. In conclusion the VAC or MC supplemented diets showed immunomodulatory impact against avian coccidiosis [26].

An experiment was performed to comparatively analyze two commercially available oregano essential oils (OEO) mixed in broilers diets to check growth productivity and immune response of 200, 1 day old Ross 308 broiler chickens. The dietary protocol included: (1) control group, no phytobiotics, (2) mixture of phytonutrients 150 ppm, (3) OEO 300 ppm, (4) OEO 500 ppm. The results showed that higher antibody titer particularly the IgG ( $p < 0.05$ ) was seen in the broiler which were fed OEO 300 ppm as compared to the control group [30].

### Conclusion

The immunological system of animals is the first line of defense whenever the animals are subjected to heat, toxin, external, internal, environmental, pathological stress, etc. In this regard the defense system plays a pivotal role in protecting the body from these stresses and it ensures optimum health, growth and production of animal. The animal obtains energy from type of feed and the function of immune system depends on the quality of diet being fed to the animals. It also depends on how much the feed is natural and organic. The phytogenic feed additives are natural, organic and are aligned with the physiology of animal; these plant based bioactive compounds are safe for animals, do not have side effects and are also safe for the end consumers, the human beings. Since majority of the phytogenic feed stuffs have the properties of antioxidant, anti-inflammatory, so these react accordingly to modulate and boost the immunity level. The antibody titer is increased, the concentrations of leukocytes, T lymphocytes, B lymphocytes, immunoglobulins are increased which modulate and enhance the immunity level and consequently the animals become strong enough to bear the detrimental situation. In this way the GIT of animal is protected which ensures favorable site for probiotics, good health, growth, optimum production and better immunity level.

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#### Підвищення рівня імунітету за допомогою фітогенних кормових добавок у раціоні тварин

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Встановлено, що антибіотики є одним із найпотужніших відкриттів у медицині ХХ століття і залишаються найефективнішим способом лікування багатьох хвороб як тварин, так і людей. Люди отримують м'ясо, молоко, яйця тощо від тварин, що є важливою і невід'ємною частиною щоденного харчування. Нераціональне, систематичне та широке використання антибіотиків для сільськогосподарських тварин, особливо для стимулювання росту, спричинило розвиток резистентності до антибіотиків, мікробної стійкості та можливої залишкової загрози для людей через споживання молока, м'яса, яєць та ін. З'ясовано, що під час нормального перебігу фізіологічних процесів тварини зазнають різних стресів, зокрема екологічного, патогенного походження тощо, які пригнічують імунну систему, а також діяльність шлунково-кишкового тракту тварин. Унаслідок цього тварини стають уразливими до різних захворювань, що призводить до імуносупресії, поганого стану здоров'я, збільшення смертності та зниження продуктивності. Через заборону на використання синтетичних антибіотиків – стимуляторів росту у тваринництві, є альтернативний підхід, який полягає у використанні фітопрепаратів у кормах тварин для стимуляції росту, оптимізації виробництва та підвищення або корекції рівня імунного статусу тварини. Ці фітогенні кормові добавки як складники раціону є важливими і мають багато властивостей, зокрема протигрибкові, антибіотичні, протизапальні, антиоксидантні, противірусні та ін., забезпечують захист та поліпшення стану здоров'я, цілісність ШКТ та підвищення рівня імунітету тварини. Цей огляд ілюструє важливість рослинних кормових добавок, що додаються до кормосумішей і згодуються тваринам, особливо їх значення в імуномодуляції для підвищення рівня імунітету у тварин в стресових умовах.

**Ключові слова:** кормові добавки, фітобиотики, альтернатива антибіотикам, годівля тварин, імунітет.

#### Повышение уровня иммунитета с помощью фитогенных кормовых добавок в рационе животных

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Установлено, что антибиотики являются одним из самых мощных открытий в медицине ХХ века и остаются эффективным способом лечения многих болезней как животных, так и людей. Люди получают мясо, молоко, яйца от животных, что является важной и неотъемлемой частью повседневного питания. Нерациональное, систематическое и широкое использование антибиотиков для сельскохозяйственных животных, особенно для стимулирования роста, привело к развитию резистентности к антибиотикам, микробной устойчивости и возможной остаточной угрозе для людей через потребление молока, мяса, яиц и т.д. Установлено, что во время нормального протекания физиологических процессов животные испытывают разные стрессы, в частности экологического, патогенного происхождения, которые подавляют иммунную систему, а также деятельность желудочно-кишечного тракта животных. Вследствие этого животные становятся уязвимыми к различным заболеваниям, что приводит к иммуносупрессии, плохому состоянию здоровья, увеличению смертности и снижению производительности. Из-за запрета на использование синтетических антибиотиков-стимуляторов роста в животноводстве, рассматривается альтернативный подход, который заключается в использовании фитопрепаратов в кормах животных для стимуляции роста, оптимизации производства и повышения или коррекции уровня иммунного статуса животного. Эти фитогенные кормовые добавки, как составляющие рациона, важны и имеют много свойств, в частности противогрибковые, антибиотические, противовоспалительные, антиоксидантные, противовирусные и другие, обеспечивают защиту и улучшение состояния здоровья, целостность ЖКТ и повышение уровня иммунитета животного. Этот обзор иллюстрирует важность растительных кормовых добавок, которые прилагаются к кормосмесям и скармливаются животным, особенно их значение в иммуномодуляции для повышения уровня иммунитета у животных в стрессовых условиях.

**Ключевые слова:** кормовые добавки, фитобиотики, альтернатива антибиотикам, кормление животных, иммунитет.



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