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Ajwain as non-antibiotic growth promoter in Broiler industry: A review

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Abstract
Ajwain (*Trachyspermum ammi*) is an aromatic, grassy and annual medicinal plant belonging to Apiaceae (Umbelliferae) family. It is mostly grown in areas like in Egypt, Iran, Iraq, Pakistan and eastern India. In India mostly it is grown in Rajasthan, Gujarat and Madhya Pradesh. The name Ajwain originated from Sanskrit word Yavanaka or Ajomoda. It is known by various vernacular names such as Bishop's weed (Sanskrit), Carom or Thyme seed (English name) and Ajowan or Ajwain or Omum (Indian name). Ajwain is highly esteemed as a remedial digestive agent for flatulence, flatulent colic, atonic dyspepsia, diarrhoea and as an antiseptic. The phytochemical studies on Ajwain have revealed the presence of alkaloids, steroids, fixed oils glycosides, tannins, saponin and flavonoids, cumene, thymene, amino-acids and dietary fiber essential oils like thymol, carvacrol, c-terpinene, p-cymene, etc. Thymol, the major phenolic compound present in Ajwain, has been reported to be germicide, antispasmodic and antifungal agent. Oil of Ajwain contains thymol and its specific gravity and odour resembles the volatile oil. The oil contains a liquid hydrocarbon, 1-methyl-4-isopropylbenzol, and another hydrocarbon which is isomeric with oil of turpentine. Ajwain, is reported to have platelet aggregation inhibitory action, antifungal potency and blood pressure lowering action. Ajwain can be promoted as a non-antibiotic growth promoter (NAGP) in broiler industry for producing quality meat without antibiotics in a very cost effective way.

Keywords: Broiler, Ajwain, non-antibiotic growth promoter

Introduction

During the past four decades, poultry industry in India has transformed itself from the age-old backyard farming into a dynamic Agri-based industry. Growth promoters are agents added to poultry feeds in order to enhance the feed conversion efficiency and body growth and broadly can be categorized as Antibiotic growth promoters (AGP) and Non–Antibiotic growth promoters (NAGP). In the past the major growth promoters were antibiotics. Antibiotic growth promoters have been helpful in improvement of growth performance and feed conversion ratio in poultry (Miles *et al.*, 2006; Dibner and Buttin, 2002 and Izat *et al.*, 1990) [33, 14, 25]. However, constant treatment of poultry by antibiotic may result in residues of these substances in poultry products and bacteria resistance against treatments in human body. Due to such threats to human health, use of antibiotics in poultry is banned in many countries (Owens *et al.*, 2008; Alcicek *et al.*, 2004; Botsoglou and Fletouris 2001 and Hinton, 1988) [40, 4, 8, 24]. On the other hand use of NAGP is commonly regarded as favourable alternatives to AGP in poultry production. The main advantage of NAGP over AGP is that they usually do not bear any risk regarding bacterial resistance or undesired residues in meat. Addition of NAGP to feeds of poultry may have a number of beneficial effects, including rapid development of a healthy gut microflora and stabilization of digestion along with improved feed efficiency. NAGP include predominantly organic acids, probiotics, prebiotics, synbiotics, phytogenics, feed enzymes and immune stimulants. Among these alternatives, phytogenics are drawing much attention now-a-days. Phytogenics are derived from herbs, spices or aromatic plants and have shown antimicrobial, antifungal, antiviral, antioxidant or sedative properties. A complex mixture of bioactive compounds present in them is known for their appetizing effects, since they increase the palatability of the feed and stimulate endogenous digestive enzymes. Moreover, phytogenics have a pronounced impact on the gut microflora. Many studies have been carried out on using additives including herbs, as alternatives to antibiotics, with direct or indirect effects on intestinal microflora in poultry products (Taylor, 2001) [50]. Several studies have shown antimicrobial properties of herb extracts (Cowan, 1999 and Hammer *et al.*, 1999) [9, 22]

which can improve intestinal microflora population and enhance health in bird's digestive system through reduction in number of disease making bacteria (Mitsch *et al.*, 2004) [34]. In addition, modified harmful microbial population in intestines will change intestinal morphology. Intestinal health is of great importance in poultry for improved performance and reduced feed conversion ratio (Montagne *et al.*, 2003) [35]. However, other properties of herbs, such as antioxidant, antiviral, immune-modulatory properties and their effects on performance and digestive health cannot be ignored.

Ajwain reported as a digestive aid and as an antiseptic (Bentely & Wrimen, 1999) [7]. Thymol, the major phenolic compound present in Ajwain, has been reported to be germicide, antispasmodic and antifungal agent (Murthy *et al*., 2009) [37]. Ajwain is reported to have platelet aggregation inhibitory action (Srivastava *et al.*, 1988) [47], antifungal potency (Dwivedi and Dubey, 1993) [16] and blood pressure lowering action (Aftab *et al*., 1995) [2]. Aflatoxicosis can be treated using Ajwain in diet (Shruti *et al*., 2005) [45]. Zahin *et al*. (2010) [57] showed that Ajwain has antioxidant activities which play an important role in biological systems by suppressing the formation of active oxygen species like hydrogen peroxide. Ajwain seed extract has flavonoids which has antioxidant activity (Saxena *et al*., 2012) [44]. Ajwain supplementation could improve blood cholesterol profile and gastrointestinal microbial population on broiler chicken (Valiollahi *et al*., 2014) [52]. The intestinal epithelium acts as a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen. The efficiency of utilization of dietary protein in poultry depends partly on the features of the gastrointestinal tract (Swatson *et al*., 2002) [49]. The small intestine, especially crypts and villi of the absorptive epithelium, plays a significant role in the final phase of nutrient digestion and assimilation (Wang and Peng, 2008) [55]. Intestinal development can be accessed through measurements of the crypt, a region in which new intestinal cells are formed, as well as villus height and surface area, to determine the area available for digestion and absorption (Swatson *et al*., 2002 and Franco *et al*., 2006) [49, 18]. According to Yamauchi (2002) [55], the morphological changes of the intestinal villi in broilers are dependent on the presence of digested nutrients in the small intestinal lumen.

Effect of Ajwain on performance parameters of broilers

Growth performance

Greater body weight and better feed conversion ratio (FCR) are among important economic goals in broiler farming. The bans on application of AGP have affected this goal, resulting in poor growth performance of broilers. Many studies have examined potential effects of feed additives, like prebiotics, probiotics, organic acids, and herbs, on growth performance compared to those of antibiotics. Valliolahi *et al*. (2014) investigated the effect of sumac (*Rhus coriaria* L.) and ajwain (Trachyspermum copticum) on performance of broiler chicks. Chicks were fed a basal diet (control group), basal diet + 0.02% sumac powder, basal diet + 0.02% ajwain powder and basal diet + 0.02% virginiamycin powder. Data showed that feed intake (FI) increased significantly in treated groups in comparison to control. Body weight gain and total body weight were also significantly ($P<0.05$) higher in treated groups. Dinodiya (2014) [15] studied the broiler performance of chicks on diet fortified with herbal feed Herbiotic-FS @ 250-500g/ton of feed (Indian Herbs Limited) Versus synthetic antimicrobial feed supplements Neftin200 @ 500g/ton of feed (Pfizer Ltd. India) containing Furazolidone (20%w/w). Herbiotic-FS contained Trachyspermum amin (Ajwain), Rheum Emodii (Indian rhubarb), Curcuma longa (Turmeric), Zingiber officinale (Ginger) and Piper nigrum (Black pepper). The chicks supplemented with both herbal and synthetic antimicrobials achieved significantly ($P<0.05$) higher body weight at 6 weeks of age and total gain in body weight (0-6 weeks) than the control, while the differences between the treatment groups were non significant. The body wt. (g) of the chicks at 6 weeks of age was highest in Herbiotic-FS 250g/ton of feed as compared to 500g/ton of feed and Neftin200. But the feed conversion efficiency was highest in Herbiotic-FS 500g/ton, while the differences between the groups were significant ($P<0.05$).

Tripathi *et al*. (2013) [51] determined the effect of Ajwain (0.5%), hot red pepper(0.5%) and black pepper (0.25%) on broiler Japanese quail reared in deep litter system. There was a significant ($P<0.05$) improvement in feed conversion ratio (FCR) and body weight gain in Japanese quail of hot red pepper supplemented group followed by black pepper treated group and then Ajwain treated group. It may be concluded that feeding of hot pepper, black pepper and ajwain significantly ($P<0.05$) improved the performance of broilers, thereby improving the profitability of broiler industry. Srivastava *et al*. (2012) proposed the effect of indigenous herbal drug (Withania somnifera, Asparagus racemosus, Mucuna pruriens) supplementation in diets on performance in broilers. All the birds were fed basal diets and in treatment group, birds were further supplemented with 2% of indigenous herbal drug per kg feed. It may be concluded that broilers that fed indigenous herbal drug in diets show the significant ($P<0.05$) effect on body weight gain and dressing percentage. It may also be concluded that broilers that fed indigenous herbal drug in diets show the significant ($P<0.05$) effect on feed conversion ratio and decreased feed intake. (Javed *et al*. 2006) [27] studied the comparative antihyperlipidaemic efficacy of Trachyspermum ammi extract in chloroform, methanol, petroleum ether and water in albino rabbits. Thus, petroleum ether extract of T. ammi most potent in antihyperlipidaemic action in albino rabbits. (Javed *et al*. 2009) [28] investigated the antihyperlipidaemic efficacy of *Trachyspermum ammi* (L.) Sprague, seed (Ajowan) powder in albino rabbits. Hyperlipidaemia was induced with butter ad libitum and oral intubation of cholesterol 400 mg/kg body weight. Simvastatin, (Tablet survive®) was used as a synthetic cholesterol lowering drug. The results suggest that 2 g/kg T. ammi seed powder produced hypolipidaemic activity. Nonetheless, 0.5 g/kg and 1 g/kg T. ammi seed powder did not show antihyperlipidaemic activity. T. ammi seed powder at the rate of 2 g/kg and simvastatin (0.6 mg/kg body weight) were equally effective in treating hyperlipidaemia in albino rabbits. (Khan *et al*. 2010) studied the anti-inflammatory and antioxidant activity of Trachyspermum ammi seeds in collagen induced arthritis (CIA) in rats. Trachyspermum ammi extract (TAE) at a dose of 100mg/kg was orally administered to rat for 21 days after immunization. The estimation of levels of oxidant products and the activities of antioxidant enzymes were carried out in the joints. The induction of arthritis significantly ($P<0.05$) increased the levels of oxidative stress markers like thiobarbituric acid reactive substances and inflammation markers like elastase. The level of non-enzymatic antioxidant, reduced glutathione (GSH) and the activities of enzymatic antioxidants like superoxide dismutase and catalase decreased. The study

revealed that the treatment with TAE was effective in bringing significant changes on all the parameters studied as compared with CIA rat. Supplementation with T. ammi reversed the oxidative changes in all the parameters suggesting either termination of cellular infiltration or limiting the generation of oxidants following CIA in rats and might have potential value in the treatment of inflammatory disease. Singh *et al*. (2012) observed that a significant (P<0.05) difference in formulated and marketed herbal (Herbstone) nutraceuticals fed @ 2% of concentrate ration respectively, on growth performance, dry matter intake (DMI) and digestibility coefficient in crossbred calves. The. herbal formulation was made with Pudina (Mentha piperita Linn), Ajwain (Trachyspermum ammi (Linn) Sprague), Harada (Terminalia chebula), Kalmegh (Andrographis paniculata), Amla (*Phyllanthus emblica*), Chirayita (Swertia chirata Buch Ham), Dry Zinger (Zingiber officinale), Black Salt and marketed herbal nutraceuticals (Herbstone). As a result of the administration of the two different nutraceuticals given to calves; had showed significantly (P<0.05) higher body weight gain, length, girth circumferences, DMI and digestibility coefficient than control group but body height had not shown any significant difference amongst treatments and periods. DM digestibility was significantly lower (p<0.05) in control group.

Several studies have been conducted to examine the effects of ajwain powder on growth performance of broilers. Ram Niwas *et al*. (2014) [39] concluded that the enhancement of parameters such as feed intake, growth rate, cure of diseases of broiler production with eco-friendly by using the organic bio-stimulator. Muhammad *et al*. (2000) [36] observed that the effect of Digestarcom (a herbal feed additive) on the performance of broiler chicks fed different levels of rapeseed cake. A higher weight gain per bird and feed intake and better feed to gain ratio was observed for all the levels of rapeseed treated with Digestarcom as compared to to non-supplemented group. Kaur *et al*. (2010) [29] reported that the antilithiatic activity of Trachyspermum ammi anticalcifying protein (TAP) in urolithiatic rat model. Further, evaluated ability of to 2mg/kg body weight TAP to inhibit the attachment of calcium oxalate (CaO(x)) crystal in kidney tissue and studied the consequences of CaO(x) adhesion on renal functioning and tissue integrity. The antilithiatic potential of TAP was confirmed by its ability to maintain renal functioning, reduce renal injury and decrease crystal excretion in urine and retention in renal tissues. Guo *et al*. (2010) [21] supplemented Chinese herbal medicine (CHM) formulation (0.25, 0.5, 1 and 2 g/kg), as an alternative for virginiamycin (VRG), on growth performance in broilers. The CHM dietary treatments produced increased body weight gain at 7 to 21 d of age but not at 21 to 28 d of age compared with the non-supplemented and VRG groups. The CHM groups had a higher feed intake and a higher feed conversion ratio (FCR) than the VRG group between 21 and 28 d. It was concluded that the birds of the CHM group had better growth performance from d 7 to 21 but not thereafter.

In addition, improvement in FCR was observed for broilers treated with ajwain compared to the control group, but the difference was not significant. Haselmeyer *et al*. (2015) [23] supplemented thyme (Thymus vulgaris) herb consisting of leaves and flowers without stems in the diet for broilers. The herb was fed for 35 days to five groups of broilers (0, 0.1, 0.2, 0.3, and 1% w/w in the diet). Animal performance and the concentrations of the main essential oil component from thyme, thymol, were measured in gut contents, plasma and liver and muscle tissues using solid phase microextraction and gas chromatography/mass spectrometry. There were no differences between the groups in feed intake, daily weight gain, feed conversion and slaughter weight. Khosravi *et al*. (2003) [31] studied the effects of some feed additives on the performance of broilers. For preparing other diets, control diet supplemented with propionic acid, Protexin and nettle extract at the levels of 2, 0.1 and 1g/kg of diet, respectively. Diet contained propionic acid resulted in significant increase in BWG during the starter and from 0-42 periods. The birds fed control diet had more FI and PI during the finisher period than those fed propionic acid. When diet supplemented with propionic acid and protexin, a significant improvement in FCR and PER was observed among this diets and other diets. Demir *et al*. (2003) [11] investigated the effects of five herbal natural feed additives as alternative to an antibiotic growth promoter. The treatment groups were supplemented with antibiotic growth promoter, oregano, du-sacch, quiponin, and garlic and thyme powder, respectively. Differences in body weight gain feed intake and feed efficiency of broilers fed diets supplemented with antibiotic growth promoter and five herbal natural feed additives were not significant from 0 to 42 d of age. Omar *et al*. (2016) [41] studied the effects of a natural herb extract on the general performance, carcass parameters and mortality of broiler chicks. The herbal extract was supplemented in drinking water at rate of 300 ml/cubic meter. Results showed that weights and feed conversion ratios (FCR) increased (P< 0.05) in birds supplemented with herb extract compared to control birds. Mortalities and sudden deaths were minimized via herbal supplementation. It can be concluded that feeding the herbal extract has significant positive effects on broilers general performance as feed efficiency is increased by 11% and on mortality and sudden death.

Since coccidiosis may compromise growth performance, researchers have examined potential effects of ajwain on improving growth performance in broilers with coccidiosis. Gugolek *et al*. (2006) [20] studied the effects of a herbal preparation on body weight gains, mortality rates and coccidiosis incidence in rabbits. The animals were kept indoor, in standard cages for broilers. Rabbits of both groups were fed the same pelleted feed. The diet for the control group (I) was supplemented with the anticoccidial drug Robenidine. The experimental factor was the herbal preparation DIAROAK added to the diet for the experimental group (II). It was found that DIAROAK had a positive, statistically significant effect on the body weights of rabbits aged 90 days. The number of oocysts per g of feces was comparable in both groups, whereas mortality rates were twofold lower in the experimental group. Arczewska-wlosek *et al*. (2012) evaluated that treatment with a herbal extract blend comprising Allium sativum, Salvia officinalis, Echinacea purpurea, Thymus vulgaris and Origanum vulgare at a dose of 1 g/kg partly alleviates the negative impact of Eimeria infection in broiler chickens. The beneficial effect of the herbal extract blend on the growth performance and number of oocytes per gram of excreta of the infected chickens was comparable to that exhibited by diclazuril. Zaman *et al*. (2012) [58] studied the anticoccidial effect of different concentrations of the herbal complex of 4 plants (leaves of Azadirachta indica and Nicotiana tabacum, flowers of Calotropis procera and seeds of Trachyspermum ammi) in broiler chickens in comparison with Amprolium anticoccidial. Among herbal complex medicated groups, the maximum

anticoccidial effect was seen in the group medicated with 6 g herbal complex followed by 4 g and 2 g herbal complex medicated groups. Treatment with 6 g of the herbal complex significantly (P<0.05) reduced the negative performance and pathogenic effects associated with *Eimeria tenella* challenge at a level that was comparable with amprolium when using a largely susceptible recent field isolate. Thus concentration dependent anticoccidial activity of herbal complex suggests its use as an alternative anticoccidial agent to chemotherapeutic drugs for Eimeria tenella control.

Findings on the effects of ajwain on growth performance are inconsistent and these discrepancies can be attributed to the form of supplement (leaf powder, gel powder, or fresh gel), dosage, or whether ajwain is added to feed or drinking water. However, particular attention must be paid to anti-bacterial activities and improvement in immune response as these two factors may contribute to better growth performance in broilers (Yang *et al*., 2009) [56], and previous studies confirm these two properties (anti-bacterial effect and improvement in immune response) for ajwain. In fact, anti-bacterial properties of ajwain improve intestinal microflora and reduce pathogens, thereby changing intestinal morphology and improving growth performance. On the other hand, by improving immune response in broilers and increasing body resistance, ajwain indirectly affects growth performance.

Kumar *et al*. (2002) [48] studied ethanolic extract of Trachyspermum ammi fruits are tested on male reproductive system of rats. Rats are pretreated with ethanolic extract of Trachyspermum ammi at four different doses such as 100mg/kg, 200mg/kg and 400mg/kg for a period of 60 days with the recovery group animals for 120 days at the dose of 400mg/kg. Parameters such as testes weight, sperm count, sperm motility, sperm morphology and histopathological examination of the testis are carried out. The study revealed that the drug posses significant male anti-fertility effect dose dependently. The recovery group reverts back the elevated parameters by increasing the decreased testis weight, sperm count, sperm motility, decrease in production of abnormal sperms and restoring the cellular pattern of the testis. These findings indicate that Trachyspermum ammi fruit extract is a very good choice of male anti-fertility activity drug which can be formulated as a male contraceptive formulation.

GIT health characteristics

Pelicano *et al*. (2005) [42] evaluated the use of probiotics and prebiotics on the histological and morphological indexes of the intestinal mucosa of broilers at 21 days of age. Greater villus height was obtained in duodenum, jejunum and ileum with the use of probiotics and prebiotics and greater crypt depths with the use of probiotics, in relation to the control group. There was no difference in villus density between birds fed diets without additives or diets containing probiotics and prebiotics. De verdal *et al*. (2010) [12] studied that two lines of broilers divergently selected for a high (D+) or a low (D−) AMEn (Apparent metabolizable energy) on a wheat-based diet were studied for morphological and histological characteristics of the digestive tract. Intestinal villi were larger and longer in D− birds (P< 0.001), mainly in the jejunum (14 to 16%), and crypts were 10 to 15% deeper for the 3 intestinal segments in D− birds (P< 0.001). Muscle layers of the intestine were 17 to 24% thicker (P< 0.001) and goblet cells were 27 to 34% more numerous in the jejunum and ileum of D− birds (P = 0.027). This new characterization of the 2 lines shows that divergent selection based on AMEn modified the morphology of the proventriculus and gizzard, suggesting greater activity of this compartment in D+ than in D− birds. Nasrin *et al*. (2012) [38] studied anatomy and histology of different segments of the digestive tract in postnatal growing broiler chickens with regard to their location, shape, size and weight. A group of four chickens, each at day 1 (D1), days 14 (D14) and days 28 (D28). The apical parts of villi of the duodenum were slightly pointed and the basal parts of the villi were thicker than jejunum and ileum, whereas, the villi of the jejunum and ileum became shorter and broader than duodenum and most of the villi had blunt apical part and the basal parts were wider. The numbers of goblet cells were numerous in number in ileum than duodenum and jejunum. The average lengths and widths of villi of small intestine were significantly higher (P<0.01) in chickens at D28 than that at D14 and at D1. The number of goblet cells in lamina epithelium and intestinal glands of the lamina propria were numerous in number at D28 than the chickens at D14 and at D1.

Abdullah *et al.* (2010) [1] concluded that the chicks were fed rations supplemented with different levels (0%, 0.25%, 0.50%, and 1.0%) of dried garlic powder. In duodenum, villus length was the highest (P<0.05) in birds fed with diets containing 1% garlic powder, and villus and epithelial width were the highest (P<0.05) in chicks fed with diets containing 0.5 percentage of dried powder. In jejunum, the villus length was the highest (P<0.05) in birds fed with diets containing 0.25% and 1% garlic powder, while villus and epithelial width were the lowest (P<0.05) in chicks fed with diets containing 0.5% of dried powder. As a conclusion, this study shows that garlic at a 0.5% level might be of beneficial effect on intestinal morphymetry parameters. Awad *et al*. (2008) [6] studied on broiler chickens the effects of the synbiotic BIOMIN IMBO [a combination of Enterococcus faecium, a prebiotic (derived from chicory) and immune modulating substances (derived from sea algae)], with a dose of 1 kg/ton of the starter diets and 0.5 kg/ton of the grower diets on the intestinal morphometry and nutrient absorption. The addition of synbiotic increased (P< 0.001) the villus height/crypt depth ratio and villus height in ileum. However, the ileal crypt depth was decreased by dietary supplementation of synbiotic compared with control. In conclusion, dietary inclusion of synbiotic BIOMIN IMBO improved intestinal morphology and nutrient absorption.

Haemato- biochemical parameters

Srivastava *et al*. (2012) proposed the effect of indigenous herbal drug (Withania somnifera, Asparagus racemosus, Mucuna pruriens) supplementation in diets on performance in broilers. All the birds were fed basal diets and in treatment group, birds were further supplemented with 2% of indigenous herbal drug per kg feed. It may be concluded that there was no significant difference for SGOT, SGPT, and serum protein serum glucose and serum urea between the treatments. (Javed *et al*. 2009) [28] Investigated the antihyperlipidaemic efficacy of *Trachyspermum ammi* (L) Sprague, seed (Ajowan) powder in albino rabbits. The results suggest that 2 g/kg T. ammi seed powder produced hypolipidaemic activity, rendering 49%, 53%, 71% and 63% reduction in total lipids, triglycerides, total cholesterol and LDL-cholesterol, respectively. However, at this dosage level 62% increase in the value of HDL-cholesterol was induced by T. ammi seed powder. Moreover, this dosage level also significantly reduced the cholesterol content of liver tissue. It

can be anticipated that the lipid lowering mechanism may involve enhanced removal or catabolism of lipoproteins, inhibition of liver.

Aftab *et al*. (1995) [2] concluded that bioassay-directed fractionation of Trachyspermum ammi has resulted in the isolation of thymol (1-10mg/kg) which produce dose-dependent fall in blood pressure and heart rate in rats. These effects were not blocked by atropine (1 mg/kg) and thymol did not modify presser response of norepinephrine, which rules out the possibility of cholinergic stimulation or adrenergic blockade. In spontaneously beating atria, thymol caused decrease in force and rate of atrial contractions. These effects remained unaltered in the presence of atropine. In rabbit aorta, thymol caused relaxation of norepinepherine and potassium induced contractions in a concentration-dependent manner. These relaxant effects remained unchanged after the removal of endothelium. Moreover, atropine, propranolol, indomethacine and glibenclamide did not alter the vasorelaxation by thymol. These results suggest that Trachyspermum ammi contains a calcium channel blocker-like constituent (thymol) which may explain the hypotensive and bradycardiac effects observed in the *in vivo* studies. Ram Niwas *et al*. (2014) [39] concluded that the enhancement of haematological and biochemical parameters of broiler production with eco-friendly by using the organic bio-stimulator. Khosravi *et al*. (2003) [31] studied the haemato-biochemical parameters of some feed additives on the performance of broilers. The total cholesterol, total protein, albumin and globulin of birds were unaffected by feed additives, whereas HDL and LDL were affected significantly ($p<0.05$) by dietary propionic acid. Demir *et al*. (2003) [11] investigated the effects of five herbal natural feed additives as alternative to an antibiotic growth promoter. Further, reported that no significant differences were observed for total protein, albumin, total cholesterol, triglyceride, AST and ALT levels among dietary treatments. Rajput *et al*. (2009) [43] elaborate the comparative effect of Ajwain extract (50mg/kg) and warfarin (0.54mg/kg) on coagulation parameters after 14 days continuous administration of drugs to rats. The antithrombotic effect was assessed by determining aPTT (activated partial thromboplastin time) and PT (prothrombin time). Ajwain extract did not show any significant effect on aPTT as compared to control; however there was highly significant increase in PT i.e. 28.00±1.2 seconds as compared to control i.e.13.57±0.30 seconds. Valliolahi *et al*.(2014) investigated the effect of sumac (Rhus coriaria L.) and ajwain (Trachyspermum copticum) on performance of broiler chicks. Triglyceride and cholesterol level decreased significantly in both groups. Antibody titre increased significantly in ajwain group compared to the control. Maini *et al*. (2009) [32] investigated the effect of supplementation of polyherbal formulation Stresroak@1g per kg of feed to birds under overcrowding stress has lead to normalization of haematological (Hb, TLC and DLC) values in at different intervals. Singh *et al*. (2009) [46] studied the effects of dietary supplementation of probiotics on broiler chiken. However, there was no significant effect on haematobiochemical parameters assessed at six weeks of age except serum cholesterol level (mg/dl) which was significantly ($P<0.05$) lower in probiotic supplemented groups than control group. Yadava *et al*. (2009) [54] observed the effects of dietary supplementation of enzymes on the commercial broiler chicken. No significant (P>0.05) alteration in haemoglobin concentration, total erythrocyte count, total leucocyte count, differential leucocyte count (heterophil, basophil, eosinophil, monocyte and lymphocyte) and packed cell volume were observed between the dietary treatments. AST, ALT and ALP in the serum of the birds did not differ (P>0.05) between control and enzyme treated groups. Eevuri and Putturu (2013) [17] found that herbal supplementation in broilers significantly reduced the serum cholesterol, serum triglycerides and increased the humoral response against NCD vaccine. Das *et al*. (2005) studied that there was no significant difference in haemoglobin content, RBC and WBC count and total albumen content of birds fed on diets with 0, 5, 10 and 15% of TLMC(Tree leaves meal cocktail). However, the total protein and globulin content was more in the groups fed on 5% TLMC. In the groups fed 10 and 15% of TLMC total protein and globulin content was not effected. This might be because of presence of tannins in the tree leaves. Similarly, there was increased cholesterol level accompanied by increased glucose level in the blood fed on 5% TLMC compared to the control diet. Dhanalakshmi *et al*. (2015) [13] studied the toxic effects of the Ochratoxin A (OA) on haemato-biochemical parameters in broiler birds and the effect of the ethanolic extract of rhizome of Picrorrhiza kurroa (PK) to counteract the damage caused by this toxin. In the PK extract treated OA groups the haemoglobin concentration, PCV increased. TEC and TLC showed no significant effect in PK extract fed groups. There was no significant effect in glucose, total serum protein, albumin and globulin, creatinine, uric acid, ALP and BUN levels in PK extract fed groups.

Cost of production

Yadava *et al*. (2008) [54] observed that the enzyme supplementation significantly ($P<0.05$) increased the profit per kg live weight gain and relative profit compared with the control group. It may be concluded that mixture of commercially available enzymes be supplemented at level of 0.015% of the diet for better performance with cost effective production in mixed commercial strain of broiler chicken. Eevuri and Putturu (2013) [17] found that herbal supplementation in broilers decreased the mortality rates and the cost of feed has been decreased from 6.2 to 13.5%. Omar *et al*. (2016) [41] reported that the relative economic efficiency (REE) was up to 13% improved by the herb supplementation. Demir *et al*. (2005) concluded that the feed costs per kg of weight gain in broilers fed diets supplemented with oregano, garlic and thyme was higher than in those given diets supplemented with du-sacch, quiponin and antibiotic growth promoter. Dinodiya *et al*. (2015) [15] inferred that Herbiotic-FS @ 500 g/ ton of feed was a cost-efficient growth promoter in broilers.

Conclusions

It may be concluded that the effects of ajwain on growth performance are inconsistent and these discrepancies can be attributed to the form of supplement (leaf powder, gel powder, or fresh gel), dosage, or whether ajwain is added to feed or drinking water. However, particular attention must be paid to anti-bacterial activities and improvement in immune response as these two factors may contribute to better growth performance in broilers (Yang *et al*., 2009) [56], and previous studies confirm these two properties (anti-bacterial effect and improvement in immune response) for ajwain. In fact, anti-bacterial properties of ajwain improve intestinal microflora and reduce pathogens, thereby changing intestinal morphology and improving growth performance. On the other

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