

Efficacy of Phytobiotics of *Achyranthes splendens* (Linn) against Bacterial Pathogens of Male Broiler Chickens in Namakkal, India

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Abstract

Phytogenic alternatives drawn a lot of attention because they are natural, non-toxic and residue free. "Phytogenic Alternatives" are the buzz word in livestock field to avoid the use of the antibiotics and chemical growth hormones resulting in health concerns. In the present study *Achyranthes splendens* (Linn) a medicinal plant was selected for its phytogenic property against bacterial pathogens of broiler chickens.

Effect of selected antibiotics of poultry and leaf extract powder of *Achyranthes splendens* L., against the poultry pathogens were tested. Collection of pathogens on swab from the intestine on 7, 14 and 21 days was done aseptically and cultured in the lab as a pretreatment study. The results of pretreatment study showed that there is positive trend of pathogenic control by Achyrabioc from 86.7% to 100% when compared to the antibiotics. Efficacy of achyrabioc in antibacterial assay revealed the phytobiotic nature of the *Achyranthes splendens* L.

Based on the protein profile of *Achyranthes splendens* (Linn) by High performance Liquid Chromatography study, the phytogenic characteristics were used for the growth study of broiler chicks. Leaf extract in the form of dry powder was used in the formulations of phytogenic poultry feed. There is no mortality in the organic achyrabioc fed male broiler chicks. Growth performance parameters such as body weight gain (from 24% to 32%), feed intake (2% to 21%) and Feed conversion ratio (from 26% to 58%) revealed the synergistic effect of phytobiotic and anti-pathogenic properties of achyrabioc on poultry growth.

Keywords: Antibacterial activity; Phytogenic alternative; *Salmonella typhus*; Resistance profile; Organic poultry feed; Weight gain.

Introduction

Phytobiotics represent a wide range of bioactive compounds that can be extracted from various plant sources. Phytobiotics are classified as: (i) Herbs-product from flowering, non-woody and non-persistent plants; (ii) Botanical-entire or processed parts of a plant, e.g., root, leaves, bark; (iii) Essential oils- hydro distilled extracts of volatile plant compounds; (iv) Oleoresins- extracts based on non-aqueous solvents.

Plant-derived products (phytobiotics) are added to the feed in order to improve performance of agricultural livestock are well documented [1-5].

A variety of antibiotics in high dose are used to control many pathogens are increasingly resistant to many commonly used antibiotics including penicillin, macrolides and aminoglycoside [6-8].

Various herbal supplements are used as alternative antibiotic growth promoters in the starter and grower feeds. Better farm management practices are also an essential criteria to sustain the health of livestock [9-15].

Hence with this background the present study has been focused to achieve the following objectives.

- i) To improve the quality of poultry feed
- ii) To control the pathogens and
- iii) To curtail the usage of antibiotics as growth promoters.

A trial on the effect of organic poultry feed along with achyriabiot powder as feed additive on growth performance of male broiler chickens was conducted.

Materials and Methods

Collection and preparation of "achyriabiot"

Achyranthes splendens (Linn) is a small herb, grows as weed up to one meter in tropical and subtropical regions and available in various places of Tamilnadu. It is medicinally valuable and used for various ailments in humans and an organic feed preparations in aquaculture[5].

The leaves of *Achyranthes splendens* (Linn) collected from various areas of Namakkal of Tamilnadu were crushed and extract was taken in sterilized petridishes. Air dried crude extract powder of *Achyranthes splendens* (mentioned in the present study as "achyriabiot") was taken in different concentrations of 0.2, 0.4, 0.6, 0.8 and 1 mg for the study. The antibacterial activity of the extracts was analyzed and compared with the commercial antibiotics Albac, Tylon and Terramycin which are used as growth hormones.

Experimental diets

The organic feed formulation for grower is presented in the table 1.

Table 1. Organic feed composition for Growers (11weeks).

Ingredients	Quantity (g/kg)
Maize	500
Rice husk (de oiled)	100
Soya bean meal	100
Achyriabiot powder	100
Fish waste powder	50
Crab shell powder	50
Dried bone powder	50
Dried Liver powder	05
Turmeric powder	05
Salt	15
Garlic powder	10
Ginger powder	05
Coriander seeds powder	05
Asafoetida	05

Pretreatment study

Collection of microbes for antibacterial assay: The enteric pathogens of intestine of male broiler chicks were collected at 7, 14 and 21 days on cotton swabs. Bacterial strains such as *Escherichia coli*, *Salmonella typhus*, *Enterococcus faecalis* and *Shigella dysenteriae* were isolated and maintained in Nutrient agar Broth and cultured in Nutrient Agar medium.

Preparation of medium and inoculums: Nutrient agar medium was prepared by dissolving 2.8 g of nutrient agar in 100 ml of distilled water. The solution was sterilized in an autoclave at

121°C for 15 min. It was cooled and poured into sterile Petri dishes to solidify. Each intestinal microbiota was inoculated on agar by streaking with the swab containing inoculum. Rotate the plate by 60° and repeat the rubbing procedure. This will ensure an equal distribution of the inocula.

Antibiotic discs were kept on the surface of the inoculated plates. The fresh extract of disc was prepared by dipping the 6 mm Whatman No1 Filter paper in the 0.2, 0.4, 0.6, 0.8 and 1 mg aqua extract of achyriabiot discs that were kept in the incubator for complete drying. Maximum of 5 discs were kept in the petriplate.

Management of broilers

The growth performance trial was carried out at a private layer farm at Mohanur, Namakkal, Tamilnadu, India with a total of 60 grower birds Lohmann breed at 20 birds per group for 11 weeks. The birds were reared in cage with controlled feeding and followed vaccination as per the schedule before the initiation of the experiment. The male chicks were kept for seven days for adaptation and were kept in separate cages and given normal drinking water. The brooding temperature was maintained at 35°C initially and it was then gradually lowered by 3°C every week until it reached to room temperature (i.e. 26 ± 1°C).

Body weight gain study and Feed Conversion Ratio (FCR)

Effect of commercial feed (Control-C) and achyriabiot powder along with organic poultry feed as additive (Treatment-T) in gaining weight of male broiler chickens was experimented and recorded. Mean initial weight of the male chicks from week-1 to week-10 was recorded. Weighing average body weight of male broiler chickens were randomly selected from the control (C) and the treatment (T) in a completely randomly design (CRD).

FCR represents the proportion of food that is converted into meat.

FCR can be calculated over a set period and the formula is

$$FCR = \frac{\text{Total quantity of feed consumed/bird in g}}{\text{Mean body weight gain in g}}$$

Statistical analysis

The data collected on various parameters were subjected to Duncan's test as per the method suggested by Snedecor and Cochran [16].

Results and Discussion

Antibacterial assay

Concentrations 0.2, 0.4, 0.6, 0.8 and 1 mg and the same concentrations of standard antibiotics act on intestinal microbiota viz., *Escherichia coli*, *Salmonella typhus*, *Enterococcus faecalis* and *Shigella dysenteriae* were recorded with reference to the zone of inhibition in cm (Table 2 and Figures 1-4). The result was found to be most effective against all tested microorganisms. Maximum inhibition concentration of phytobiotics is 0.8 to 1 mg which is effective against intestinal pathogenic bacteria.

The experimental results of antibiotic assay against the intestinal bacterial pathogens viz., *E.coli*, *Salmonella typhus*, *Enterococcus faecalis* and *Shigella dysenteriae* were compared with the efficacy of antibiotic growth hormones (Figures 1-4). Achyrbiot potentiality in controlling these pathogens was on par with the Terramycin.

Hence from the results 0.8 to 1 mg of Achyrbiot per 10 g of organic feed was recommended for the preparation of organic poultry feed.

The response of *E. coli* to antibiotics Albac, Tylon and Terramycin was maximum upto 2.2 cm as zone of inhibition. Compared to terramycin the efficacy of antibiotic activity of *Achyranthes* was 90% increased. The antibiotic response of other poultry pathogens showed that there is positive trend of pathogenic control by Achyrbiotic, from 86.7% to 100% (Table 2).

Similar kind of experimental results showed the efficacy of bioactive compounds of various herbal medicinal plants against poultry pathogens [4,7,10,17-19].

Table 2. Effect of *Achyranthes splendens* L. against bacterial pathogens of male broiler chickens.

Antibiotics & Phytochemicals	Bacterial pathogens			
	<i>E. coli</i>	<i>S. typhus</i>	<i>E. faecalis</i>	<i>S. dysenteriae</i>
Albac	2.2 ± 0.2	1.9 ± 0.1	1.8 ± 0.4	1.8 ± 0.3
Tylon	2.0 ± 0.3	1.8 ± 0.3	2.0 ± 0.1	2.0 ± 0.2
Terramycin	2.2 ± 0.1	1.9 ± 0.2	2.1 ± 0.3	2.1 ± 0.4
Achyrbiotic	2.2 ± 0.1	1.9 ± 0.2	2.1 ± 0.3	2.1 ± 0.4

*Results are expressed as the mean value of triplicates. (Diameter of inhibition zone in cm)

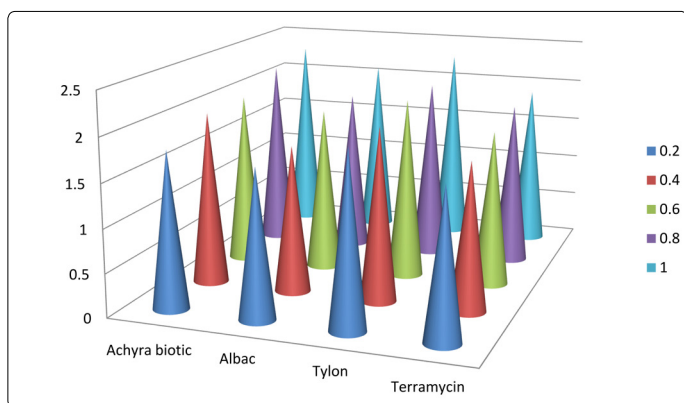


Figure 1. Effect of chemo and phytochemicals on *E.coli*. (concentration in mg Vs Zone of inhibition in cm).

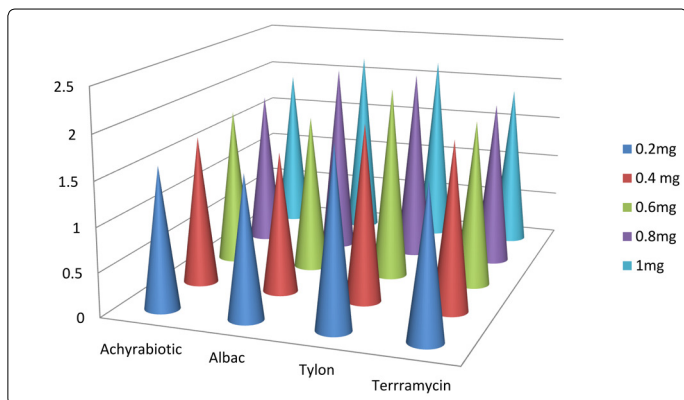


Figure 2. Effect of chemo and phytochemicals on *Salmonella typhus*. (Concentration in mg Vs Zone of inhibition in cm).

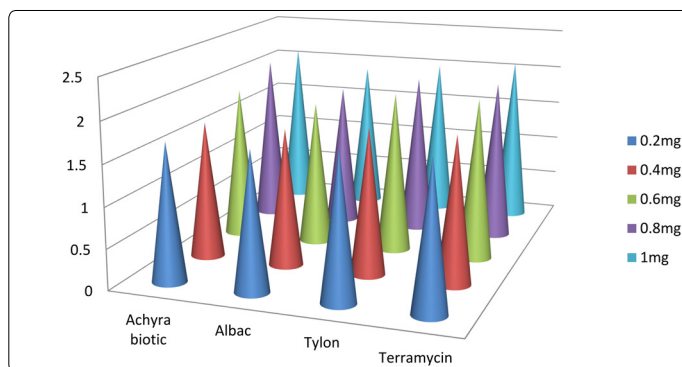


Figure 3. Effect of chemo and phytochemicals on *Enterococcus faecalis*. (Concentration in mg Vs Zone of inhibition in cm).

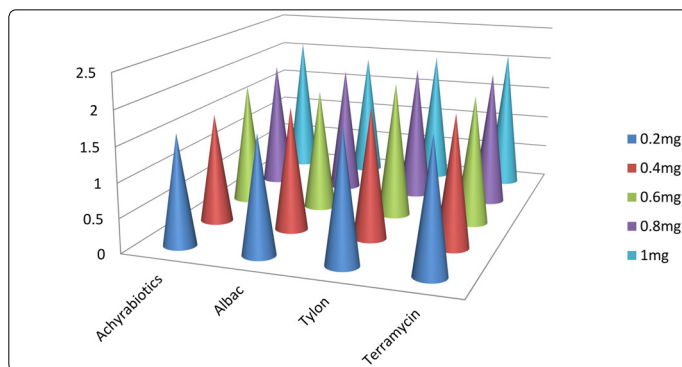


Figure 4. Effect of chemo and phytochemicals on *Shigella dysenteriae*. (Concentration in mg Vs Zone of inhibition in cm)

Feed conversion ratio and body weight gain

The effects of feeding different concentration of achyrbiot and commercial feed on daily body weight gain are shown in table 3. All groups had initial body weight 550 ± 1.4 g.

No mortality was detected in the treatment group throughout the study period, where as 2-3% mortality was observed in the commercial feed with the antibiotic.

These results are in accordance with the experiments of Forder et al. and Brisbin et al. [20,21].

Table 3. Body weight gain of male broiler chicks.

Growth Duration	Commercial feed	Organic feed with achyrbiot powder (g)
Week 1	587.95-NS	589.55-NS
Week 2	687.05-NS	697.00-NS
Week 3	695.53 ± 0.49	721.00 ± 0.40
Week 4	723.00 ± 0.33	785.00 ± 0.51
Week 5	790.75 ± 0.33	850.50 ± 0.51
Week 6	805.25 ± 0.45	915.25 ± 0.54
Week 7	875.00 ± 0.52	995.50 ± 0.51
Week 8	900.05 ± 0.55	1012.25 ± 0.40
Week 9	955.05 ± 0.59	1120.60 ± 0.49
Week 10	1008.50 ± 0.50	1290.75 ± 0.59

*Average weight of 20 birds in a cage of triplicates significant at P<0.05; NS-Not Significant

Feed Conversion Ratio (FCR) of male birds

In every week interval, FCR was calculated where best FCR was found in week-3 to week-10. Feed Conversion Ratio (FCR) is shown in the tables 4 and 5.

Similar kind of experiments and results recorded by Javed et al. and Senthil Kumar et al. [22,23]. Differences were revealed for FCR in all the treatments from week 2 to week 10 of age.

Moderate FCR was observed in commercial poultry feed and better FCR was observed in treatment with achyribiot.

Effective increase in FCR of Group-2 chicks could be due to the quality of feed additive (Figure -5). This view is supported by the experiment done with aquaculture as HPLC study by Thirumal and Laavu [5].

An increasing healthy growth was recorded in the birds fed with achyribiot as additive feed (Tables 1 and 2). The growth response trend of male broiler chickens showed the positive effect of achyribiot on gut health and which might be due to the increase in appetite for feed intake from 2.5% to 21%. This reflects on the body weight gain from 24% to 32% in the organic poultry feed (Tables 3 and 4).

This is in line of data reported by several authors [3,15,17,18,20,24].

Most essential oils have been classified as generally recognized as safe (GRAS), by the US Food and Drug Administration (FDA). These oils from herbs were subjected to antimicrobial activities with positive influence on poultry growth. Several oils, including carvacrol and thymol obtained from oregano and eugenol from the clove plant, have been shown to inhibit a wide range of pathogenic bacteria [17].

Gut is composed of numerous communities of microorganisms like bacteria, fungi, protozoa and viruses, but bacteria are the predominant microorganisms. The colonization of bacteria in the gut is determined by its substrate preferences and the chemical composition of the contents. To combat the enteric infections and to improve the gut health, antibiotic growth promoters were widely used in poultry feed since last 50 years. But the risk associated with the development of resistant organisms made European Union to ban the use of antibiotics since January 2006 [25,26].

There is 2-3% mortality in the Commercial feed (C) group chicks and no mortality in the organic feed treatment (T). Hence the results of our findings obtained on bodyweight gain confirmed the benefits of using botanicals as phytobiotics in influencing the growth performance, in terms of feed intake and feed conversion ratio.

Effect of adding specific botanicals and herb extracts on growth performance/Gut morphology/intestinal microflora were experimented by Chambers and Gong, Wei et al. and Denbow [27-29]. These investigations had shown the beneficial synergistic effect of herbal feed additives in controlling the pathogens and increasing the appetite to increase the body weight gain.

Table 4. Feed intake in male broiler chicks.

Growth Duration	Commercial feed	Organic feed with achyribiot powder (g)
Week 1	587.95 -NS	589.55 -NS
Week 2	687.05 -NS	697.00 -NS
Week 3	695.53 (±0.49)	721.00 (±0.40)
Week 4	723.00 (±0.33)	785.00 (±0.51)
Week 5	790.75 (±0.33)	850.50 (±0.51)
Week 6	805.25 (±0.45)	915.25 (±0.54)
Week 7	875.00 (±0.52)	995.50±0.51
Week 8	900.05 (±0.55)	1012.25±0.40
Week 9	955.05 (±0.59)	1120.60±0.49
Week 10	1008.50 (±0.50)	1290.75±0.59

*Average weight of 20 birds in a cage of triplicates significant at P<0.05; NS-Not Significant

Table 5. Feed Conversion Ratio (FCR) of male broiler chicks.

Growth Duration	Commercial feed	Organic feed with achyribiotic powder
Week 1	1.23 ± 0.33	1.55 ± 0.22
Week 2	1.35 ± 0.22	1.70 ± 0.23
Week 3	1.39 ± 0.29	1.89 ± 0.25
Week 4	1.40 ± 0.33	1.90 ± 0.21
Week 5	1.55 ± 0.23	2.10 ± 0.26
Week 6	1.78 ± 0.25	2.25 ± 0.52
Week 7	1.74 ± 0.24	2.50 ± 0.50
Week 8	1.75 ± 0.25	2.45 ± 0.49
Week 9	1.65 ± 0.29	2.66 ± 0.49
Week 10	1.70 ± 0.25	2.75 ± 0.45

*Average weight of 20 birds in a cage of triplicates -significance level at P<0.05

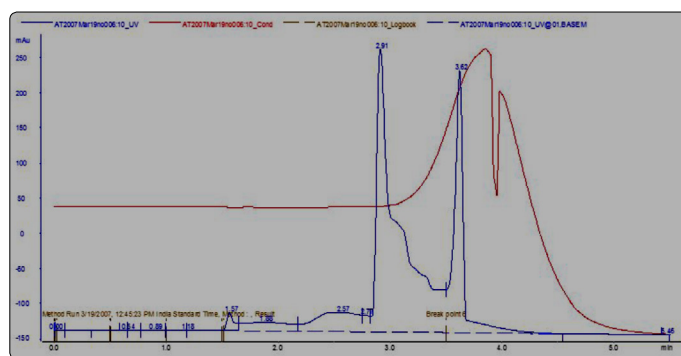


Figure 5. HPLC chromatogram of Protein profile of *Achyranthes splendens* L. [5].

Conclusion

Achyranthes splendens L., a medicinal plant was selected for its phyto-genic property against bacterial pathogens of male broiler chickens. Pretreatment study revealed the phytobiotic nature of that plant also named "**Achyribiot**". Our findings showed 100% sustainability of poultry health throughout the investigation. Body weight experiments on male broiler chicken using Achyribiot along with other organic formulations showed its phytobiotic and anti-pathogenic properties with positive influence on poultry growth. Particularly the aquatic leaf extract powder can be used in the formulations of phyto-genic poultry feed. Further investigations will be carried out on the post treatment study to identify gut health. Comparison of health profile of male and female broiler chickens as influenced by the selected phytobiotics will be the future focus of our research.

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