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Effect of phytochemicals on production performance and immunity of commercial broilers

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Abstract
The objective of the study was to evaluate the effects of dietary supplementation of *Aloe vera*, Tulsi, and Amla on growth rate, feed conversion ratio, immunity, and carcass characteristics in broiler chickens. Four hundred day-old commercial Vencobb 400 broiler chicks were purchased and randomly allotted to four treatment groups with five replicates of twenty chicks each. The control group T_1 was fed a basal diet formulated as per BIS (2007) while in treatment groups T_2 T_3 T_4 fed basal diet with 1% *Aleo vera* (T_2), basal diet with 1% Tulsi (T_3), basal diet with 1% Amla (T_4). The experiment lasted for six weeks period during which the following parameters were recorded body weight, feed conversion ratio, immune response and carcass yield. The highest body weight (in g) was noticed in T_4 (2375±21.36) and T_3 (2272±37.26) followed by T_2 (2216±62.38) and T_1 (2145±74.48) at sixth week age of broilers. Lowest feed conversion ratio was noticed in T_4 (1.80±0.022) followed by T_3, T_2 and T_1 for 1.82±0.020, 1.85±0.026 and 1.91±0.031 respectively. The highest carcass yield (in g) was noticed in T_2 (1700±47.36) followed by T_3, T_2 and T_1 for 1602±42.25, 1567±32.46 and 1483±48.21 respectively. Similarly, the highest dressing percentage was noticed in T_4 (73.15±0.48) followed by T_3, T_2 and T_1 for 72.45±1.41, 71.93±0.98 and 70.43±0.65 respectively. The treatment groups did not have a significant difference in serum biochemical parameters (serum total protein and serum albumin). The serum cholesterol significantly reduced in treatment groups compared to control. The immune response (HI titre) to Newcastle disease non-significant in treatment groups. But numerical improvement observed in all treatment groups compared to control. It could be concluded that *Aloe vera*, Tulsi and Amla act as a feed additive and improve production performance of broilers.

Keywords: Broiler, *Aleo vera*, tulsi, amla, body weight, FCR, carcass yield, immune response

Introduction
Phytochemicals, or photobiotic or phytogenic, are plant-derived bioactive compounds that are added to animal or poultry feed to improve the productivity. Policy makers and consumers have voiced strong opposition to the use of antibiotic growth promoters in the poultry industry because to the development of microbial resistance to these drugs and the possible negative impact on public health. (Botsoglu and Fletouris, 2001; Williams and Losa, 2001) [4, 26]. In order to prevent infections and enhance the performance of birds, organic poultry growers must resort to non-antibiotic therapies. Phytochemicals have gained popularity as a replacement for antibiotic growth promoters.

Phytochemicals are bioactive non nutrient compounds of plant origin and typically help resist bacterial, viral and fungal infection, stress and physical harm. Phytochemicals can be categorised into six main categories: phenolic compounds, alkaloids, nitrogen-containing compounds, organo sulphur compounds, phyto sterols, and carotenoids, and they can be subdivided into various subcategories.

Herbs are natural, less toxic than antibiotics and usually contain no residues. Many of these are usually certified as safe (GRAS) by the Food and Drug Administration (FDA), making them ideal for use as feed additives in organic poultry farming. Herbs have been used as an immune protective agent and as a growth enhancer. (Khetmalis *et al.*, (2018) [12] reported the use of herbs in poultry feed to prevent the residual effects of various chemicals and drugs on chickens. Herbs have antibacterial, antiseptic, antiviral, antifungal, anti-inflammatory, and immunomodulatory properties. Herbs such as *Aloe vera* (*Aloe barbadensis miller*), tulsi (*Ocimum sanctum*), and amla (*Phyllanthus emblica*) have long been used as anti-stress agents in human and veterinary medicine with a proven track record.

Aloe vera (*Aloe barbadensis* Miller) is a recognized medicinal herb that has been utilized commercially and therapeutically in various parts of the globe. *Aloe vera* contains a number of active compounds, including vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acid and amino acids. *Aloe vera* was the most commonly used medicinal plant in poultry farming because it was used to treat various diseases and was a comprehensive therapy in poultry health care. The benefits of adding *Aloe vera* to broiler chicken diet are huge in terms of growth rate, bone structure, hemo-biochemical parameters, gut health, immune system function, and production cost Tulsi (*Ocimum sanctum*) is considered as the 'queen of herbs' due to its important medicinal value. In Ayurveda, it is regarded as an elixir of life; and probably prolongs life. (Puri and Harbans Singh. 2002) [19]. Tulsi is known for its antiseptic, immunomodulatory, anti-inflammatory, antipyretic, asthmatic, diabetic, antihypertensive and analgesic properties. Chaudhary *et al.* (2010) [5] reported that the main components responsible for these properties are eugenol, ascorbic acid, β-carotene, β-sitosterol, palmitic acid and tannins.

Amla (*Phyllanthus emblica*), also known as Indian gooseberry, is an important herb in Ayurvedic medicine of India. Amla has a higher value in traditional indigenous medicine. (Variya *et al*., 2016) [25]. Amla fruit is rich in quercetin, gallic acid, flavonoids, pectin, phyllaemblic compounds and vitamin C and also contain variety of polyphenol compounds. Sunil Kumar *et al*. (2018) [24] reported that amla contain phytochemical compounds such as terpenoids, alkaloids, flavonoids, and tannins that have high biological effects.

Amla fruit contain high amount of ascorbic acid, minerals, amino acids, tannins and phenolic compounds. Due to their high content of ascorbic acid, the amla fruit have a therapeutic potential acid (Eevuri and Putturu, 2018) [7]. In addition to ascorbic acid, gallic and tannic acids are phenolic acids found in amla that contribute to its antioxidant effect. Amla fruit powder has several advantages when fed as a feed supplement to broilers, including anti stress and antioxidant properties, growth promotion, immune system stimulation, and improved feed conversion efficiency. Hence a biological experiment was conducted by adding *Aloe vera*, tulsi and amla powder to the diet to study the growth performance, carcass characteristics, serum biochemical parameters and immune responses of broilers.

Materials and Methods

Experimental birds

The biological experiment was carried out at the Department of Veterinary and Animal Sciences, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Day-old straight-run broiler chicks (Vencobb 400) were procured from Hatcheries. Birds were used in an experiment and reared in deep litter housing under standard management practices until six weeks of age.

Experimental design

Day-old four hundred numbers of broiler chicks were randomly divided into four treatment groups of five replicates of twenty chicks each.

Experimental diet

According to the recommendation of the Bureau of Indian Standards (BIS, 2007) a basal diet for control group T_1 to meet the requirements of the broilers. The proximate composition of the basal diet for the pre-starter, starter and finisher broiler diet was analyzed by the Association of Official Analytical Chemists (AOAC, 2016) [2] and is shown in Table 1. Experimental birds were fed *ad libatum* during the study period. Broilers received pre-starter feed 0-2 weeks and starter feed 2-4 week and finisher feed 4-6 week of age.

The proximate composition of *Aloe vera*, tulsi and amla powder was analyzed by Association of Official Analytical Chemists (AOAC, 2016) [2] method and their values are shown in Table 2. The *Aloe vera*, Tulsi and Amla were supplemented as mentioned below.

Treatment Groups	Experimental diets
T_1	Basal diet (Control)
T_2	Basal diet + 1% *Aloe vera*
T_3	Basal diet + 1% Tulsi
T_4	Basal diet 1% Amla

The chickens were provided with a uniform space for floor, feeders, and drinker's. The chickens were kept under standard management conditions for 42 days of the experiment. *Ad libitum* feed and water were given to the experimental birds. The birds were vaccinated with Newcastle disease vaccine at 7th and 21st day of age, and Infectious Bursal disease vaccine on 14th 28th day of age. The body weight was determined by weighing individual chickens weekly until the age of six weeks. The daily feed intake was recorded regularly. The incidence of mortality was observed.

Blood was randomly drawn from each treatment group after vaccination (days 14 and 28). Samples were allowed to clot and centrifuged at 1500 rpm/20 min to separate sera. Separated serum samples were used in a hemagglutination inhibition (HI) test (Alexander, 1998) [1] to detect the level of immunity developed against new castle disease.

At the end of the experiment, two males and two females were randomly selected from each replicate and slaughter studies were performed. Birds were received 12 hours before slaughter, feeding was stopped, but drinking water was provided in sufficient quantity. The birds had to be killed. At slaughter, blood was collected from all selected birds via the jugular vein into a heparinized tube. Samples were allowed to clot and centrifuged at 1500 rpm/20 min to separate sera. Serum samples were stored for analysis of total serum protein, serum albumin, and serum cholesterol. The body weight, feed consumption, livability, and carcass traits data were collected and statistically analyzed utilizing methods suggested by Snedecor and Cochran (1995) [21].

Result and Discussion

Effect on growth performance

The effect of *Aloe vera*, tulsi and amla supplementation on average body weight (g) of broiler chickens is shown in Table 3. The highest body weight of broilers (in g) was noticed in T_4 (2375±21.36) followed by T_3 (2272±37.26) and T_2 (2216±62.38) and control group T_1 (2145±74.48) at sixth week age. The present study indicate that dietary supplementation broilers with *Aloe vera*, tulsi, and amla had a significant (p<0.05) effect on their body weight in the fifth and sixth week of age, but no significant change was shown in their body weight in the first, second, third, or fourth week of age.

These results are agreed with findings of Mereole (2011) [17] with *Aloe vera* supplementation, Swathi *et al.* (2012) [23] and Hasan *et al.* (2016) [11] with tulsi leaf powder supplementation and Sujatha *et al.* (2010) [22] and Kumar *et al.* (2013) [13],

Gaiwad *et al.*, (2016) [9] and Patel *et al* (2016) [18] by using Amla powder respectively. Tirupathi Reddy (2010) [27] reported that broilers fed with Amla, Tulsi and amla either alone or in combination @ 0.25% and 0.5% levels resulted in better feed efficiency.

Aloe vera may be the cause of weight gain in broilers because it can increase digestive enzymes, liver bile production, has antioxidant effects and inhibits pathogenic micro flora in the small intestine. The presence of anti-stress agents in the addition of tulsi leaf powder in broilers may be the reason for the increased body weight.

Feed conversion ratio (FCR)

The effect of *Aloe vera*, tulsi and amla supplementation on the average feed conversion ratio of broiler chickens of different ages is shown in Table 4. This result showed that a significant (p<0.05) difference between the treatment groups was observed at the fifth and sixth weeks of life, but no significant difference was observed at the first, second, third and fourth weeks of broiler age. Only slight numerical differences are observed. Similarly, the lowest feed conversion ratio was observed in T_4 (1.80±0.022) followed by T_3, T_2 and T_1 at 1.82±0.020, 1.85±0.026 and 1.91±0.031 respectively. This study showed that birds fed amla Tulsi leaf powder and *Aloe vera* utilized their feed more efficiently than birds fed without the supplement. These findings are consistent with Fadllalla *et al*. (2010) and Swathi *et al.* (2012) [23] on the addition of tulsi to Ghavate *et al*. (2009) [10] with amla addition. Tirupathi Reddy (2010) [27] reported that broilers fed amla, tulsi and amla either alone or in combination at 0.25% and 0.5% levels increased feed efficiency.

Dressing percentage (%)

The effect of *Aloe vera*, tulsi and amla supplementation on the average dressing percentage (%) of broiler chickens is shown in Table 5. The results showed that the highest carcass yield (g) was observed at T_4 (1700±47.36), followed by T_3, T_2, and T_1 at 1602±42.25, 1567±32.46, and 1488±48.21, respectively. Similarly, the highest dressing rate was found in T_4 (73.15±0.48), followed by T_3, T_2, and T_1 with 72.45±1.41, 71.93±0.98, and 70.43±0.65, respectively. Differences between all treatment groups were not significant. In treatment T_4, the proportion of bandages was numerically higher (73.15±0.48). Jameel *et al.* (2019) [28] found that there were no significant differences in carcass yield regardless of treatment group, which he observed across his four treatments in broilers fed *Aloe vera* at levels of 1.0, 1.5, and 2.0%.

Immune response

The effect of supplementation of *Aloe vera*, Tulsi and Amla on the hemagglutination inhibition (HI) titer against Newcastle disease in broilers is shown in Table 6. This study showed that it had no effect (p>0.05) on hemagglutination inhibition (HI) titers against Newcastle disease in broilers. Numerical improvements in antibody titers against NDV were observed in all treatment groups compared to the control group. This result is due to the fact that the active ingredients of the herbs (*Aloe vera* and amla) improve digestion and stimulate the immune function of broilers. The results are agreed with Darabighane *et al.*, (2012) [6], Fallah (2014) [8], and Reddy *et al.* (year 2012) [20]. They reported that dietary intake of *Aloe vera* and amla significantly increased antibody titers against Newcastle disease virus (NDV).

Serum Biochemistry

The effect of *Aloe vera*, Tulsi and Amla supplementation on serum biochemical parameters of broiler chickens is shown in Table 7. The present study showed no effects on total serum proteins and albumin, except Serum cholesterol level. The result showed that the significant decrease (p>0.05) in serum cholesterol level in the treatment groups compared to the control group. Similarly, Lanjejar *et al*. (2008) [15] reported that a significant decrease in serum total cholesterol was observed in broilers fed with tulsi leaf powder. Mehala and Moorthy (2008) [16] reported non-significant differences in serum total protein, serum albumin, serum globulin, albumin-globulin ratio, glucose, cholesterol and triglycerides in broiler diets fed *Aloe vera*. Kylie Amber *et al*. (2020) [14] found that in a group of birds fed 1.5% *Aloe vera* gel dissolved in drinking water, blood levels of total cholesterol, triglycerides and low density lipoprotein were significantly reduced compared to the control group.

Table 1: Ingredient (%) and Nutrition composition (% DM basis) of Pre starter, Starter and Finisher feed for broilers

Feed ingredients (percent)	Pre starter feed (0-2 weeks)	Starter feed (3-4 weeks)	Finisher feed (5-6 weeks)
Maize	51	53	55
Cumbu/Bajra	7	9	10
Soyabean meal	40	36	33
Mineral Mixture [1]	2	2	2
	100	100	100
Feed Additives (g/100 kg)			
Vitamin Supplements [2] AB_2D_3K (gm)	20	20	20
B- Complex vitamins [3]	25	25	25
Trace Minerals [4]	50	50	50
Aminoacids	100	100	100
Lysine	50	50	50
DL- methionine-80 g	80	80	80
Nutrient Composition (% DM basis)			
Metabolizable Energy (Kcal/kg)	3015	3142	3256
Dry matter %	91.20	91.50	90.20
Crude protein %	22.25	21.25	19.50
Crude fibre %	3.75	3.50	3.40
Ether Extract %	5.27	6.89	7.57
Calcium %	0.99	0.96	0.92
Available Phosphours %	0.45	0.45	0.43
Lysine %	1.33	1.27	1.21
Methionine %	0.64	0.62	0.61

[1] Mineral mixture supplied per kg of feed: Calcium 6.4 g, Phosphorus 1-2 g, Manganese- 55 mg, Iodine 2 mg, Zinc52 mg, Copper 2 mg and Iron 20 mg

[2] One gram of Vitamin AB2D3K supplement contained 82500 IU of vitamin A, 50 mg of vitamin B2, 12,000 IU of vitamin D3 and 10 mg of vitamin K

[3] One gram of B-Complex supplement contained 8 mg of vitamin B1, 16 mg of vitamin B6, 80 mg of vitamin B12, 80 mg of vitamin E, 120 mg of niacin, 8 mg of folic acid, 80 mg of calcium pantothenate, 120 mg of calcium and 300 mg of phosphate.

[4] One gram of trace minerals contained 54 mg of manganese, 52 mg of zinc, 20 mg of iron, 2 mg of iodine and 1 mg of cobalt

Table 2: Proximate composition of *Aleo vera* (*Aloe barbadensis Miller*), Tulsi (*Ocimum sanctum*) and Amla (*Phyllanthus emblica*)

	***Aloe vera* (*Aloe barbadensis Miller*)**	**Tulsi (*Ocimum sanctum*)**	**Amla (*Phyllanthus emblica*)**
Dry matter %	82.02	86.70	87.56
Crude protein %	8.78	7.65	8.65
Crude fibre %	16.58	16.72	11.82
Ether extract %	2.4	6.25	3.92
Total ash %	11.25	6.82	9.62

Table 3: Effect of supplementation of *Aloe vera*, Tulsi and Amla on the body weight (g) of broilers

Treatment Groups	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
T_1 Basal diet (Control)	149±4.58	401±10.27	813±19.61	1259±47.99	1737±54.49^{a}	2145±74.48^{a}
T_2 Basal diet + 1% *Aloe vera*	146±6.75	401±18.65	813±18.12	1280±40.33	1746±64.21^{b}	2216±62.38^{b}
T_3 Basal diet + 1% Tulsi	149±4.76	403±20.54	815±17.19	1290±25.34	1832±47.36^{bc}	2272±37.26^{b}
T_4 Basal diet 1% Amla	149±4.76	405±9.64	821±13.76	1351±26.60	1875±41.22^{c}	2375±21.36^{c}

The value given in each cell is the mean of hundred observations. Values are mentioned as Means ± SE a-c Means within a column bearing one common letter superscript differ significantly (p<0.05) Means bearing same superscript do not differ significantly within the rows otherwise significant at 5% level of significance

Table 4: Effect of supplementation of *Aloe vera*, Tulsi and Amla on the Feed conversion ratio of broilers

Treatment Groups	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
T_1 - Basal diet (Control)	0.14±0.003	0.39±0.002	0.85±0.010	1.38±0.018	1.88±0.031^{a}	1.91±0.031^{a}
T_2 - Basal diet + 1% *Aloe vera*	0.13±0.001	0.38±0.002^{a}	0.81±0.015	1.39±0.025	1.78±0.020^{b}	1.85±0.026^{b}
T_3 - Basal diet + 1% Tulsi	0.14±0.003	0.39±0.003	0.83±0.007	1.37±0.013	1.76±0.020^{bc}	1.82±0.020^{c}
T_4 - Basal diet + 1% Amla	0.14±0.001	0.39±0.005	0.83±0.012	1.37±0.018	1.75±0.022^{c}	1.80±0.022^{c}

The value given in each cell is the mean of hundred observations. Values are mentioned as Means ± SE
a-c Means within a column bearing one common letter superscript differ significantly (p<0.05)
Means bearing same superscript do not differ significantly within the rows otherwise significant at 5% level of significance

Table 5: Effect of supplementation of *Aloe vera*, Tulsi and Amla on the carcass characteristics of broilers

Treatment	**Live weight (g)**	**Carcass weight (g)**	**Giblet weight (g)**	**Dressing %**
Basal diet (Control)	2105±54.49	1483±48.21	0.10±0.48	70.43±0.65
Basal diet + 1% *Aloe vera*	2178±64.21	1567±32.46	0.12±0.48	71.93±0.98
Basal diet + 1% Tulsi	2212±47.36	1602±42.25	0.12±0.48	72.45±1.41
Basal diet 1% Amla	2325±41.22	1700±47.36	0.14±0.48	73.15±0.48

The value given in each cell is the mean of twenty observations. Values are mentioned as Means ± SE

Table 6: Effect of supplementation of *Aloe vera*, Tulsi and Amla on the Hemagglutination - Inhibition (HI) titre (log_2) against Newcastle disease of broiler

Treatment	**14 th Day**	**28 th day**
Basal diet (Control)	4.25±0.14	5.92±0.14
Basal diet + 1% *Aloe vera*	4.65± 0.14	6.72±0.14
Basal diet + 1% Tulsi	4.92± 0.14	6.32±0.14
Basal diet 1% Amla	4.32± 0.14	6.21±0.14

The value given in each cell is the mean of twenty observations. Values are mentioned as Means ± SE

Table 7: Effect of supplementation of *Aloe vera*, Tulsi and Amla on the Serum biochemical parameters of broiler

Treatment	**Total serum protein (g/dl)**	**Serum Albumin (g/dl)**	**Serum Cholesterol (mg/dl)**
Basal diet (Control)	3.89±1.45	1.96±0.25	145.34±1.82^{c}
Basal diet + 1% *Aloe vera*	3.92±1.29	1.93±0.19	128.25±1.23^{a}
Basal diet + 1% Tulsi	3.86±1.34	1.94±0.23	134.14±1.62^{b}
Basal diet 1% Amla	3.88±1.62	1.92±0.27	126.26±1.86^{a}

The value given in each cell is the mean of twenty observations. Values are mentioned as Means ± SE a-c Means within a column bearing one common letter superscript differ significantly (p<0.05)

Conclusion

In conclusion, this study shows that supplementation with *Aloe vera*, tulsi, and amla in broiler diets improves body weight, feed conversion ratio and dressing rate, respectively. *Aleo vera*, Tulsi and Amla at 1% concentration improve growth rate and dressing percentage and reduce feed conversion ratio compared to control. It can be used as a feed additive in broilerfeed. It also strengthens the immune system against Newcastle disease. This study found a positive response to *Aloe vera* tulsi and amla supplementation and concluded that *Aloe vera*, tulsi and amla powder can be used as alternative antibiotics in poultry production.

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