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AQUEOUS EXTRACT FROM DIFFERENT MEDICINAL PLANTS AS ANTICOCCIDIAL, GROWTH PROMOTIVE AND IMMUNOSTIMULANT IN BROILERS

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ABSTRACT

A study was planned to investigate the role of aqueous extract from Garlic (*Allium sativum*), Ginger *(Zingiber officinale),* Neem *(Azadirachta indica)* and Berberry *(Berberis lycium)* fed in-mix for their effect upon growth performance, immunomostimulant and anticoccidial in broilers. Two hundred and fourty chicks were randomly allocated in to four groups A, B, C and D, and further into two subgroups, each consisted of three replicates of 10 chicks per replicate. All the birds were raised in individual pens beneath the same house operating under conventional deep litter, open sided housing system. Group A was treated as control, whereas group B, C and D were watered with the corresponding recipe: a mixture of aqueous extract from Garlic (*Allium sativum*), Ginger *(Zingiber officinale),* Neem *(Azadirachta indica)* and Berberry *(Berberis lycium).* Recipe B consisted of aqueous extract of 2, 4, 1, 8; recipe C of 3, 5, 2, and 9 and recipe D of 4, 6, 3, and 10 g of Garlic (*Allium sativum*), Ginger *(Zingiber officinale),* Neem *(Azadirachta indica)* and Berberry *(Berberis lycium)* respectively, mixed per liter drinking water. The experiment lasted for 35 days. Growth rate, immune performance against Newcastle disease, Infectious Bronchitis, Infectious bursal disease and Coccidiosis were response parameters. At the end of research trail, weight gain, feed consumption and feed conversion ratio (FCR) were non-significantly varied. Better Immune performance ($P<0.05$) was found in recipe-D treatment against ND, IB and IBD. A significant decrease in coccidial oocysts per gram of feaces was registered in recipe-D. Mean gross return was non significantly varied. Present findings suggest that aqueous mixed extract of medicinal plants is better for immunomodulatory response against ND, IB, and IBD and to reduce coccidial oocysts burden, without affecting growth of the broilers.

Keywords: broilers, medicinal plants, aqueous extract, growth rate, immune performance, diseases.

INTRODUCTION

Pakistan have a wide rang of medicinal herbs scattered over a larg area, due to the favourable climatic condition. All these herbs posses a number of chemical substances for the use in poultry (Akhtar *et al*. 1984). Freshly crushed Garlic (*Allium sativum*) contains allicin, alliin, ajoene, diallylsulfide, dithiin, S-allylcysteine (www. wekipedia.org). Ahsan *et al*. (1999) reported that feed added Garlic and Neem can upgrade immune performance against IBD and ND in poultry. Garlic infusion plays a vital role in the weight gaining efficiency of broilers (Shahriyar and Durrani, 2003). Among medicinal herbs, Ginger *(Zingiber officinale)* possesses a mixed composition of zingerone, shogaols and gingerols. Ademola *et al*. (2004) stated that feed added Ginger with Garlic have no role on the growth performance of broilers, although both significantly effected heamatological activity. Neem (*Azadirachta indica*) is the most useful traditional medicinal plant and a valuable natural product for the development of medicinal recipes against various diseases (Biswas *et al.*, 2002). Neem posses Limonoids, protolimonoids, tetranortriterpenoids, pentanortri-terpenoids, hexanortriterpenoids and some nonterpenoid (Koul *et al*., 2006). Dry leaves of Neem are beneficial in IBD affected broilers (Sadekar *et al*., 1998). *Berberis lycium* is also one of the most potent medicinal herb, contains a number of alkaloids like berberine, berbamine, chinabine, karakoramine, palmatine, gilgitine and jhelumine (www.wekipedia.org). Feed added *Berberis lyceum* significantly reduces serum total cholesterol, triglyceride, LDL and increasing HDL in broilers. The present study was designed to observe the efficacy of aqueous mixed extract mixture of Garlic (*Allium sativum*), Ginger *(Zingiber officinale),* Neem *(Azadirachta indica)* and Berberry *(Berberis lycium)* as anticoccidial, growth promotant and immunostimulant in broilers.

MATARIALS AND METHODS

The trial which spread over a period of 35 days was carried out in an open sided and deep litter house at N.W.F.P Agricultural University Peshawar.

Experimental design

The experiment was carried out in Randomized Completely Block Design (RCBD). A total of 240 broiler chicks, obtained from the local commercial market, were divided into four groups, where group A was control, and groups B, C and D received herbal extract from four herbs i.e., Garlic (*Allium sativum*) bulb, Ginger *(Zingiber officinale)* rhizomes, Neem *(Azadirachta indica)* leaves and Berberry *(Berberis lycium)* roots barks at different ratios. Each group was further divided into two subgroups:

where one received normal vaccination schedule against the *ND, IB and IBD*, and the later left non-vaccinated. Subgroup represented by three replicates of 10 chicks each. Herbal recipe B consisted of aqueous extract from 2, 4, 1, 8; recipe C of 3, 5, 2, 9 and recipe D of 4, 6, 3, and 10 g of Garlic (*Allium sativum*) bulb, Ginger *(Zingiber officinale)* rhizomes, Neem *(Azadirachta indica)* leaves and Berberry *(Berberis lycium)* roots barks per liter of drinking water, respectively. Composition of the commercial diet offered to the broilers during the entire research trail is presented in Table-1.

Table-1. Ingredient and composition of basal diet.

Ingredient and composition	Starter ration	Finisher ration
Yellow corn (%)	63.8	72.2
Soybean meal (44% CP)	28	21.5
Fish meal (72% CP)	5	3
Lime stone (%)	1.6	1.6
Dicalcium phosphate (%)	1	1.2
Vitamins and minerals (%)	0.1	0.1
DL. Methionine (%)	0.2	0.1
Sodium chloride (%)	0.3	0.3
Coccidiostat (5%)	0.05	0.05
Lysine (%)	1.19	0.93
Methionine (%)	0.55	0.33
Methionine and Cysteine (%)	0.89	0.62
Calcium (%)	1.09	1.08
Total phosphate (%)	0.98	0.68
Crude protein (%)	21.4	18.1
Metabolizing energy (Kcal/kg)	2921	2994

Preparation of aqueous extract mixture

Fresh Garlic bulbs, Ginger rhizomes, Neem leaves and root bark of *Berberis lycium* were purchased from local vegetable market. Three mentioned levels were trodden into small pieces with the help of metallic grinder and taken in separate non-metallic jar and were added one liter of hot boiling water, kept at room temperature overnight following the procedure mentioned by Liela (1977). The aqueous extract thus collected was mixed in drinking water.

Samples collection

At the end of research study blood samples were collected from each replicate in test tubes. Test tubes were centrifuge at the rate of 4000 rpm for ten minutes to separate the serum. Serum was taken in a pendrof tubes, were properly labeled for identification, placed in iceboxes and was sent to SB Poultry Lab, Rawalpindi for the determination of antibody titer against ND, IB using HI by Synder *et al*. (1984) and IBD using ELISA Kit (Marquardt *et al*. 1980). Feacal samples were collected from the coccidial challenged groups on day-28 and day-35, on weekly basis post infection to determine oocysts count per gram of sample by Mc Master Technique as described by Theinpont *et al*. (1979). The data were statistically analyzed by the standard procedure of analysis of variance using Randomized Completely Block Design (RCBD) described by Steel and Torie (1981). The statistical package (SPSS 2000) was used to perform the analysis on computer. Duncan's multiple range tests was applied for the separation of means, where significant differences were noted among them.

RESULTS AND DISCUSSIONS

Findings pertaining to growth rate, immune performance against ND, IB and IBD and anticoccidial efficacy are presented under various sections as follows:

Growth performance

Mean body weight gain (g) at week 1, 2, 3, 4 and 5 was 120.63 (1.02), 288.88 (3.83), 565.00 (2.96), 1084.75 (0.50) and 1322.83 (16.85), respectively (Table-2). Day old body weight {43.83g (0.12)} was similar among the experimental groups. Significant variation had been registered at week-1, slower weight gain in recipe-D treatment, however, non-significant changes had been found in the weeks thereafter. Upon termination of the third week reduced weight gaining efficiency had been registered with increasing concentration of the medicinal plants in the respective recipes. Increasing trend of medicinal plants in the extract but the variation was non significant. At the end of fourth week, approximately similar weight gaining efficiency had been observed in all treated recipes. Once again, at the termination of fifth week, reduction in weight gaining efficiency had been registered with increased concentration of medicinal herbs in their respective recipe. Findings of Shahriyar and Durrani (2003) are contradictory to the present study; they reported significant effect of Garlic *(Alum Sativum)* infusion on the weight gain. Like present findings, feed supplemented Garlic and Ginger had no effect in weight gaining performance of broilers (Ademola *et al*., 2004). Similarly, feed added *B. lycium* showed non-significant correlation in the weight gaining efficiency of broilers (Chand *et al*., 2005). Contradictory findings to the present study had been reported by Sarang and Durrani (2005) offered Neem leaves infusion to broilers, Niazi and Durrani (2006) used aqueous extract of *B. lycium* in broilers production; Atiq and Durrani (2007) administered Ginger and Aniseed aqueous extract to broilers.

Feed consumption and feed conversion ratio

Average feed consumption by the birds received recipe A, B, C and D was 2619.80 (49.02), 2578.31 (57.14), 2496.2 (46.21) and 2439.35 (57.83) grams per chicks, respectively (Table-3). Significant variation had been

observed during the early three weeks. Low feed intake per bird had been registered in the recipe-D at first three weeks. However, total feed intake per bird during the entire trail, especially during the last two weeks, were non-significantly varied. Non significant variations were also found by Atiq and Durrani (2007) in the Feed Conversion Ratio (FCR) of the birds. Present findings support the findings of Chowdhury *et al*. (2002), they fed sun-dried Garlic and reported non-significant differences in the feed consumption and feed efficiency of the laying hens. Similarly Ademola *et al*. (2004) observed such like finding by supplementing feed added Garlic and Ginger in broilers ration. Like same, Atiq and Durrani (2007) also observed non-significant correlation in feed intake by supplementing Aniseed and Ginger infusion in broilers diet.

Antibody titer against IBD, IB and ND

Significance differences had been observed in all treated recipes. i.e., A, B, C and D. and between each recipe treated subgroups against Infectious Bursal Disease (IBD), Infectious Bronchitis (IB) and New Castle (ND) (Table-4). All vaccinated treated recipes resulted in significant immune response to their respective non-vaccinated recipes (Figure-1). Better immune performance had been registered with increased concentrations of medicinal herbs in their corresponding recipes. Sadekar *et al*. (1998) fed Neem dry leaves to the broilers and observed significant effect on the immune performance against IBD. Contradictory findings have been reported by Waihenya *et al*. (2001), who offered crude extract of *Aloe secundiflora* in chickens and observed non-significant response against *Newcastle disease*. Like present findings, Neem leaves infusion is better to enhance immunomodulatory response against IBD (Sarang and Durrani, 2005). Garlic infusion have better ($P<0.05$) immunostimulant efficacy against Infectious Bursal Disease (IBD) and Infectious Bronchitis (IB) reported by Shahriyar and Durrani (2006). Similarly Anissed and Ginger aqueous extract have significant effect on the immune performance of broilers against IBD, IB and ND (Atiq and Durrani, 2007).

Response to coccidial challenge

Mean oocysts counts at the end of first week of coccidial challenge (Day-28) were 7586.16 (350.90), 3817.33 (63.79), 2749.16 (190.59) and 1248.16 (37.05) per gram of feaces for recipe A, B, C and D treated groups, respectively (Table-5). Significant variation in all treated recipes has been observed. Reduction in oocysts count was better ($P<0.05$) with increased concentration of herbal plants in their respective recipe. On termination of the second week (Day-35) oocysts count was significantly lower in recipe-D treated group. Similar approach had been done by Murtaza *et al*. (2002) they fed Neem fruit to broilers observed reduction in coccidial oocysts count per gram of feaces. Like same findings Mushtaq and Durrani (2007) observed significant reduction in cocidial oocysts count by supplementing aqueous extract of *Withania Somnifera* in broilers production. Mean morality during the entire coccidial period was 0.33 (0.21), 0.00 (0.00), 0.17 (0.17) and 0.17 (0.17) in treated recipe A, B, C and D respectively, showed non-significant variations (Table-5). Guha *et al*. (1991),- reported that herbal anticoccidial show less mortality in birds, which is evident from our results that group B, C and D showed less mortality as compared to control.

Economics of the recipes

Mean gross return per chick of the current research study was 56.50 (3.40), 47.83 (3.16), 47.83 (2.60) and 44.17 (3.14) rupees per chick for recipe A, B, C and D treated groups respectively, showed non-significant variations (Table-6). Shahriyar and Durrani (2003) fed Garlic infusion to broilers and observed significant variations in mean gross return per chicks, which did not support present findings. Current trail support Atiq and Durrani (2007) statement, they offered Aniseed and Ginger extract in broilers production and recorded non-significant differences in the average gross return per chick.

Table-2. Mean (S.E) body weight gain (g) of the experimental chicks fed different recipes.

Groups	Day-old weight	Week-1	Week-2	Week-3	Week-4	Week-5
A	44.17 (0.31)	123.83 [b] (0.87)	293.00 (5.27)	571.00 (6.54)	1086.17 (1.99)	1376.33 (38.78)
B	43.67 (0.21)	122.17 [b] (2.29)	288.33 (10.68)	570.83 (6.33)	1084.33 (0.21)	1315.33 (28.49)
C	43.67 (120.83)	120.83 [b] (1.83)	293.83 (5.59)	565.00 (5.05)	1084.17 (0.17)	1301.00 (27.10)
D	43.83 (0.17)	115.67 [a] (1.54)	280.33 (8.60)	553.17 (3.29)	1084.33 (0.33)	1298.67 (37.1)
Total body weight gain (g)	43.833 (0.12)	120.63 (1.02)	288.88 (3.83)	565.00 (2.96)	1084.75 (0.50)	1322.83 (16.85)
Level of significance	NS	**	NS	NS	NS	NS

A = control
B = (Garlic-2g, Ginger-4g, Neem-1g, Berberry-8g)
C = (Garlic-3g, Ginger-5g, Neem-2g, Berberry-9g)
D = (Garlic-4g, Ginger-6g, Neem-3g, Berberry-10g)
*** = High-high significant (P = 0.00-0.01); ** = highly significant (P = 0.02-0.04);
* = significant (P = 0.05); NS = Non-significant.
Mean (S.E) in the same column bearing different superscript are significantly different (P<0.05).

Table-3. Mean (S.E) feed consumption (gm) of the experimental groups fed different recipes.

Feed groups	Feed consumption (g)					Total feed consumed
	Week-1	Week-2	Week-3	Week-4	Week-5	
A	128.72 [b] (2.24)	271.93 [b] (5.40)	513.85 [b] (8.58)	774.60 (16.52)	930.70 (33.94)	2619.80 (49.02
B	125.57[b] (1.65)	268.30[ab] (10.79)	490.82[ab] (14.34)	721.97 (22.33)	971.65 (34.62)	2578.31 (57.14)
C	117.50 [a] (1.20)	278.22 [b] (3.78)	476.80 [a] (5.49)	741.90 (20.38)	881.77 (24.06)	2496.20 (46.21)
D	114.67 [a] (1.63)	251.12 [a] (3.25)	463.33 [a] (8.86)	735.08 (29.35)	875.15 (22.42)	2439.35 (57.83)
Level of significance	***	*	**	NS	NS	NS

A = control
B = garlic-2g, ginger-4g, neem-1g, berberry-8g
C = garlic-3g, ginger-5g, neem-2g, berberry-9g
D = garlic-4g, ginger-6g, neem-3g, berberry-10g
*** = High-high significant (P = 0.00-0.01); ** = highly significant (P = 0.02-0.04);
* = significant (P = 0.05); NS = Non-significant.
Mean (S.E) in the same column bearing different superscript are significantly different (P<0.05)

Table-4. Mean (S.E) antibody titer against IB, ND and IBD of the experimental chicks fed different recipes.

Anti-body titer	IB (Infectious bronchitis)	ND (New-castle disease)	IBD (Infectious bursal disease)
A	64.00 [a] (20.24)	9.67 [a] (2.20)	2431.83 [b] (886.98)
B	80.00 [a] (21.47)	19.00 [a] (4.30)	1432.83 [a] (819.28)
C	85.33 [a] (19.67)	42.67 [ab] (9.83)	2570.67 [bc] (807.72)
D	133.33 [b] (40.83)	64.00 [b] (21.47)	4904.33 [c] (1461.01)
Level of significance	*	***	**

A = control; B = garlic-2g, ginger-4g, neem-1g, berberry-8g; C = garlic-3g, ginger-5g, neem-2g, berberry-9g; D = garlic-4g, ginger-6g, neem-3g, berberry-10g
*** = High-high significant (P = 0.00-0.01); ** = highly significant (P = 0.02-0.04);
* = significant (P = 0.05); NS = Non-significant.
Mean (S.E) in the same column bearing different superscript are significantly different (P<0.05)

Table-5. Mean (S.E) Cocccidial oocyts count of the coccidiosis challenged birds.

Cocci-oocyts count	28-day	Day-35	Mortality
A	7586.166[d] (350.90)	40553.00[c] (505.49)	0.33 (0.21)
B	3817.33[c] (63.79)	4046.833[b] (195.199)	0.00 (0.00)
C	2749.166[b] (190.59)	1544.833[a] (43.01)	0.17 (0.17)
D	1248.166[a] (37.05)	1925.33[a] (123.11)	0.17 (0.17)
Level of significance	***	***	NS

*** = High-high significant (P = 0.00-0.01); ** = highly significant (P = 0.02-0.04);
* = significant (P = 0.05), NS = Non-significant.
Mean (S.E) in the same column bearing different superscript are significantly different (P<0.05)

Table-6. Economics of the experimental chicks fed different recipes.

Economics	Gross return per chick
A	56.50 (3.40)
B	47.83 (3.16)
C	47.83 (2.60)
D	44.17 (3.44)
Level of Significance	NS

A = control
B = garlic-2g, ginger-4g, neem-1g, berberry-8g
C = garlic-3g, ginger-5g, neem-2g, berberry-9g
D = garlic-4g, ginger-6g, neem-3g, berberry-10g
*** = High-high significant (P = 0.00-0.01); ** = highly significant (P = 0.02-0.04);
* = significant (P = 0.05); NS = Non-significant.
Mean (S.E) in the same column bearing different superscript are significantly different

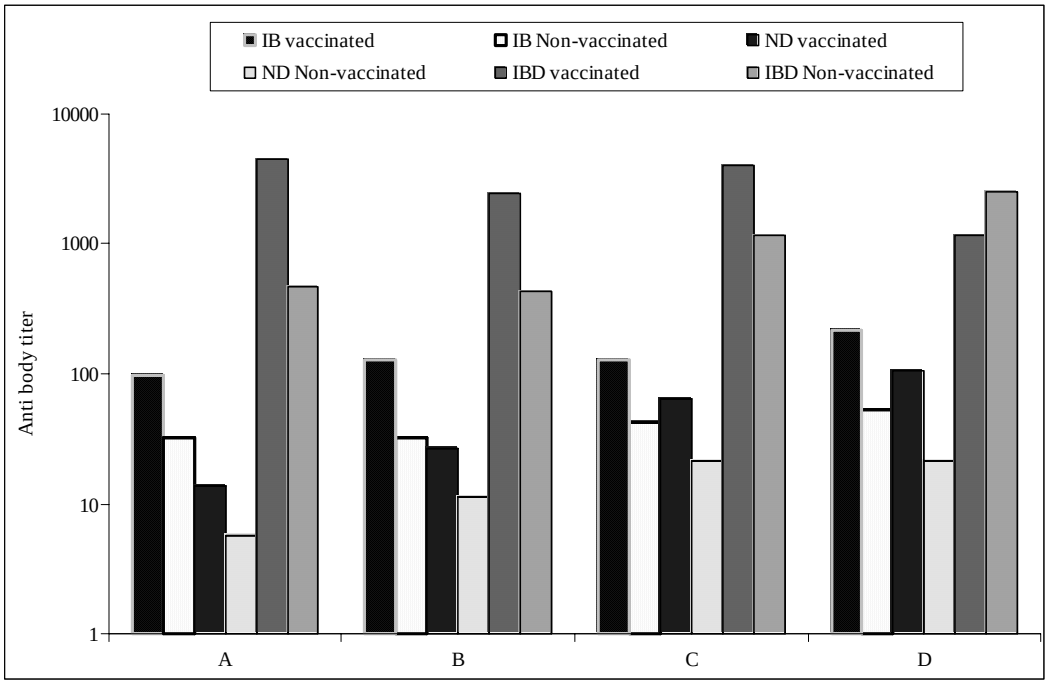

Figure-1. Mean (S.E) antibody titer against ND, IB and IBD of treated group A, B, C and D (vaccinated and non-vaccinated) fed different recipes.

A = control
B = (garlic-2g, ginger-4g, neem-1g, berberry-8g)
C = (garlic-3g, ginger-5g, neem-2g, berberry-9g),
D = (garlic-4g, ginger-6g, neem-3g, berberry-10g)

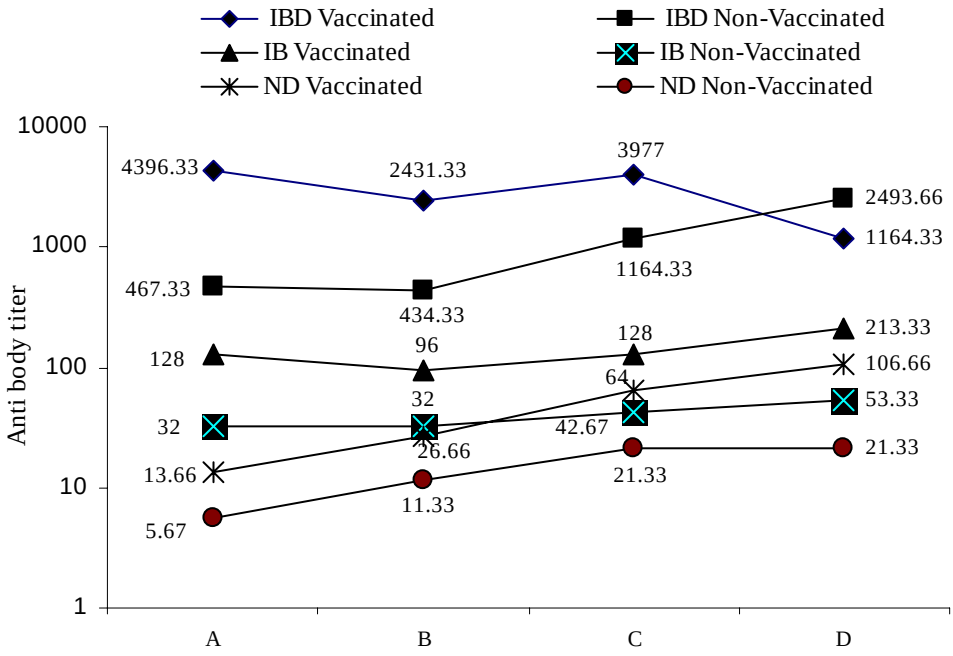

Figure-1. Mean antibody titer against ND, IB and IBD of treated group A, B, C and D (vaccinated and non-vaccinated) fed different recipes.

A = control
B = (garlic-2g, ginger-4g, neem-1g, berberry-8g)
C = (garlic-3g, ginger-5g, neem-2g, berberry-9g)
D = (garlic-4g, ginger-6g, neem-3g, berberry-10g)

CONCLUSIONS

In conclusion, the results of present study suggests aqueous extract of Garlic (*Allium sativum*) bulb, Ginger *(Zingiber officinale)* rhizome , Neem *(Azadirachta indica)* leaves and Berberry *(Berberis lycium)* stem barks mixed in 4, 6, 3 and 10 gl^{-1} of water play better performance as immunstimulant against ND, IB and IBD Coccidiosis.

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