Growth promoting and immune response of aqueous extract blends using different concentrations of onion, garlic and chilli on broilers

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Abstract

The current study was designed to evaluate the efficacy in terms of growth performance, gut health and immune response using different concentration of aqueous extract blends of garlic, onion and chilli in comparison with standard Mannan Oligo-Sachharide and known antibiotics. A total of 360 day old broiler chicks Ross-308 were randomly allocated to six treatments groups replicated four times having 15 chicks per replicate. Following groups were designated as A, B and C receiving respective treatments at the rate of 1.5 ml per liter of drinking water having the Garlic:Onion:Chilli concentration as (T-1=37.5:50:12.5, T-2=50:25:25, T-3=50:37.5:12.5).While group D, E and F were designated for receiving standard (T-4) Mannan Oligo-Sacharide, and plain water (T-5) as negative and Zinc Bacitracin antibiotic (T-6) as positive control. Production performance parameters like body weight gain, FCR, feed intake, broiler performance efficiency factor and farm economy index were found significantly higher as compare to control groups in both starter (day 1-21), finisher phase (day 22-35) and over all period. Similarly, carcass traits like dressed weight, leg, thigh, breast, wing weight were found enhanced in groups receiving aqueous extract of garlic, onion and chilli and standard. Dressed weights for T-1 to T-6 were (1334.7, 1319.4, 1374, 1379.6, 1298.2, 1323.6 gm). The immune response for birds receiving aqueous extracts of garlic, onion and chilli for NDV, IB and IBD with increased weight of lymphoid organs. Higher phagocytic activity for PHA-P (1.06, 1.05, 1.02, 0.93, 0.69, and 0.88), C-reactive protein and leuckogram were recorded. These results of the current study revealed that aqueous extract of garlic, onion and chilli could be used as alternative to antibiotics with improved growth performance and immune response when used at 1.5 ml per liter.

Keywords: Garlic, Onion, Chilli, FCR, Growth performance, Immunity, PHA-P, Leuckogram

Introduction

Poultry in comparison to other terrestrial livestock is one of the vibrant sectors having fastest per capita production and the ubiquity of poultry is attributed to its relative short life cycle with robust feed conversion ratio, small body size and better adaptability to the environmental conditions (Elwinger *et al*., 2016). The gut health of poultry plays an important role by digesting the feed, utilizing and absorbing the available nutrients, and providing defense against different harmful microorganism. Any deviation in the gut health by any means impact bird performance and overall productivity. Over the past different antibiotics growth promoters (AGPs) were used in the poultry industry that significantly decreases the load of harmful bacteria and improved gut health with enhanced growth production (Hayden *et al*., 2020). However the extensive misuse of antibiotics in animal farming practices, especially in the low and middle-income countries (LMICs) has become an in-evitable challenge to the containment of global antimicrobial resistance (AMR) leading to the limitations of antimicrobial use (AMU) in poultry because of serious consequences that includes antibiotic residues in the final product, altering gut microbiome and dissemination of antibiotic resistant strains into the environment and further transmissions to human via food chain. The World Health Organization (WHO) is recommending the application of alternative ways to curb antibiotics having less or no harm from consumer and environment point of view (Lowenthal *et al*., 1999).

In the last few decades ban has been imposed on the use of AGPs in poultry production in most of the countries around the globe. Currently the worldwide poultry nutrionists are looking for alternatives to AGP. Different alternatives like phytochemicals, prebiotics, probiotics, organic acids, vaccines, essential oils and in-feed enzymes are being evaluated to address the problems associated with those to antibiotics. Different herbal plants are being investigated having potent therapeutic properties and are naturaly, safe and having centum of different phyochemicals (Puvaca *et al*., 2013). Garlic (Allium sativum), onion (Allium cepa L.) and chilli (Capiscum annum) act as natural growth promoters, immune stimulant and gut health enhancer due to the presence of different phytochemicals and nutraceuticals that help to improve health and

performance leading to increased production of animals ((Masihi, 2002; Okamura *et al*., 2004; Lim *et al.*, 2006; Pourali *et al.*,2010).

Traditionally garlic and onion are used as therapeutic and flavoring agent, belonging to the family Liliaceae and genus Allium. Garlic contains phytochemicals including flavonoids and different organosulphur compounds including Allicin, Inulin, ajoene, DADS (Di Allyl Disulphide), DAS (Di Allyl Sulphide) and S-allyl-cysteine. While onion are enriched with organosuphur (onionin A, cystein), phenols (rutin, quercetin, and quercetin glucosides), saponin, polytsachharides and many other bioactive compounds like cycloalliin, Isoalliin, Methiin and Propiin with proven antimicrobial, antioxidant (Ma *et al*., 2018; Fawole *et al.*, 2020; Xin-Xin Zhao *et al.*, 2021). Chilli (Capsicum annum) belongs to the genus Capsicum and contains a wide range of compounds capsaicinoids sterols, ascorbic acid, saponins, carotenoids and phenolic compounds that are responsible for the biological activities (Sung and Lee, 2016). Capsaicinoids impart the characteristic pungent taste because of the presence of capsaicin, nor-di-hydrocapsaicin, homo-dihydrocapsain, 6-7-dihydrocapsaicin, homo-capsaicin and flavonoids, which make it a good antioxidant (Howard *et al*., 1994). Chilli also constitutes high level of carotenoids like capsanthin, capsorubin, lutein and carotene responsible for cellular activity, vision and growth. It help to reduce the deposition of cholesterol and fats, subsiding the heat stress and maintain the energy balance during adlibitum feeding thus improving the feed utilization resulting in sound performance (Grashorn,2010; Puvaca *et al*., 2014) and act as natural alternative to antibiotics as growth promoter (Demir, 2003).

Due to the emergence of microbial resistance to different antibiotics of the animals and human importance and the presence of drug residues in the final product from public health aspect different natural growth promoting feed additives over the past has been evaluated with marked effects to enhance gut microbiota, feed intake, endogenous digestive enzymes and bird performance (Dorman, 2000). Herbal spices like garlic, onion and chilli individually and/or in combination with other substances like ginger, moringa, thyme, lemon, turmeric, honey, and olive etc showed marvelous effect such as antibacterial, antiviral, antiprotozal, anti-thrombotic, anti-diabetic, and so many others due to the presence of phytobiotics having biological properties. After use in poultry feed in different concentration and forms they act as prebiotic with improved feed conversion ratio, carcass traits and gut health integrity with no drug residue

in final product (meat and egg) and antibiotic resistance from consumers aspects (Murate *et al*., 2015; Abd El-Hack M.E *et al*., 2022). They can be used in heat stress broilers to improve the feed and water intake (Awodola-Peters *et al*.,2022). Garlic and onion contain flavonoids contains quercetin, flavonol, catechin and flavones, in which quercetin is the main antioxidant with great potential to combat the free radicals retarding the meat oxidation (Diya Al-Ramamneh, 2018).

Keeping in view the alternative for AGP in poultry production the current project was designed to study the growth promoting impact of aqueous extract blends using different concentrations of onion, garlic and chilli on broilers at the rate of 1.5 ml/liter of drinking water.

Material and Method

Experimental design

Day old chicks of Ross-308 (n= 360) in a complete randomized design (CRD) were randomly assigned to different treatment groups (A, B, C, D and E) each replicated four times having 15 chicks per replicate (n=60/group). Birds in different groups A, B and C were given different aqueous extract of onion, garlic and chilli in different concentration as T1, T2 and T3 according to table 3.5 at a rate of 1.5 ml/liter of drinking water. Chicks in Group D and E were kept as control with provision of plane water (T4) and standard (T5) Manano Oligo-Saccharide (MOS) having proven probiotic and antibacterial activity. Chicks replicated in each group were reared in pens with the provision of feeder, drinker and all other requirements to maintain best possible environmental conditions according to the bird's genetics to show its best potential. The duration of experimental trial was 35 days.

Experimental Layout

The experiment was continued according to the given layout arranged in CRD design given in Table. 1

Table.1 Experimental Layout

Treatments	Groups	Replicate			
		R1	R2	R3	R4
T1	A	15	15	15	15
T2	B	15	15	15	15
T3	C	15	15	15	15
T4	D	15	15	15	15
T5	E	15	15	15	15
T6	F	15	15	15	15

Extract distillation

Onion, garlic and chilli was purchased from local market and sent to local distillation company for preparation of their respective water extracts by the process of hydrodistillation (Dilworth, 2017 and Azmir, 2013).

Different Concentration of onion, garlic and chilli per ml

Aqueous extracts of onion, garlic and chilli were allocated to different treatment groups as T-1, T-2 and T-3 to have the required percent concentration per 100 ml.

Table.2 Different concentration of onion, garlic and chilli per 100 ml

S.No	Treatment	Percent concentration per 100 ml		
		Onion :	Garlic :	Chilli
1	T1	50	37.5	12.5
2	T2	50	25	25
3	T3	37.5	50	12.5

Growth promoting and Immune response of aqueous extract blends using different concentrations of onion, garlic and chilli on broilers

A. Bird Performance

Different bird performance parameters were studied to evaluate the impact of aqueous extracts blends of garlic, onion and chilli that includes body weight gain, feed and water intake, feed consumption ratio, livability, Broiler Performance Efficiency Factor (BPEF), Broiler Farm Economy Index (BFEI) as per given given formulas.

Birds were weighed for live body weight. After slaughtering different organs were separated carefully and weighed accordingly. Dressed weight, dressing percentage, weight of chest, leg, thigh, neck, wing, lungs, gizzard, heart, gall bladder, liver and abdominal fat percentage were recorded. Different accessory organs including gizzard, gall bladder, spleen, heart, lungs, bursa and thymus were removed and weighed on a digital balance.

FI = Feed Offered in grams -Feed Refused in grams

W.I = Water offered in liters – Water remains in liters

Body Weight Gain= Final Body Weight (g) – Initial Body Weight (g)

FCR= Feed consumed (Kg) ÷ Total body weight gain (Kg)

Percent Livability = No of birds sold ÷ No of birds present at the beginning x 100

BPEF= Live weight of bird (Kg) ÷ FCR x 100

BFEI= [Average live weight (kg) x Percent livability] ÷ [FCR x Growing period (days)]

Dressing percentage = Dressed Weight / Live Weight x 100

B. Immune Response

Humoral immunity

Haemagglutination (HA) and Haemagglutination Inhibition (HI) test was performed according to the method of Olsen *et al*., (2003) to evaluate the antibody titers for ND, IB and IBD of broilers on day 21 and 35.

Cell-mediated immunity

a) Lympho-proliferative response to phyto-hemagglutinin (PHA-P)

On day 14 of experimental trial 2 birds per replicate were randomly injected at intra digital space with PHA-P and the thickness were measured at 24 Hr and 48 Hr duration at 3^{rd} and 4^{th} digit (Corrier, 1990).

Skin thickness was measured at 3^{rd} and 4^{th} digit on left and right foot with the help of screw gauge.

PHA-P 50 µg in 0.1ml of normal saline was injected under the skin between 3rd and 4th digit of right foot.

Normal saline as control at 0.1 ml was injected in left foot.

After 24 and 48 Hr the skin thickness was recorded using digital already corrected screw gauge.

Hyper sensitive cutaneous basophilic response was recorded from the skin thickness of right foot and compared with the control i.e left foot

b) Carbon Clearance Assay

Mononuclear phagocytic system ability of chicken against carbon clearance (CCA) was evaluated according to the modified method of Lamount and Cheng (1988).

The phagocytic ability of chicken circulatory macrophages were determine by the carbon clearance assay (CCA) based on the method of Cheng and Lamont (1988) with some modification.

The supernatant fraction of the black India ink (Pelikan-4001) was harvested after centrifugation at 3000 rpm for 30 minutes.

2 birds from each replicate was randomly selected and injected in the right brachial vein with 1ml/Kg body weight.

Blood samples (200µl/bird) were then taken from the left brachial vein at an interval of 0, 3 and 15 minutes.

The blood samples were immediately transferred to 4ml (1%) aqueous sodium citrate solution and were centrifuged for 4 minutes at 50 rpm.

The optical density (OD) of supernatant was evaluated at 640 nm wave-length.

Phagocytosis of the carbon particles (CC) of ink was determined by the percent absorption of ink by the following formula

Percent absorbance= Abs. of particular time - Abs. at time 0/Abs. at time 0 x 100

c) C - reactive protein (CRP) Test

2 birds per replicate were randomly selected on day 30, 35 and 42 of experimental trial and after blood collection from brachial vein the serum was analyzed for CRP (C-reactive protein) using the Hitachi 912 chemistry analyzer (USA).

Results

Effect As Growth Promoter

Following parameters were investigated to show growth promoting impact of aqueous extract blends using different concentrations of onion, garlic and chilli on broilers

a. Bird Performance Parameters

The effect of aqueous extract blends of garlic, onion and chilli when used in broilers at the rate of 1.5 ml per liter of drinking water having different concentration to that of standard, positive and negative control as per table 3.5 are illustrated in Table 3.1. Data analysis revealed statistical significant difference ($P<0.05$) among the treated groups for the performance parameters in started, finisher and over all period of experiment. In started phase (day 1-21) improved average body weight gain and better FCR was observed in the group receiving T-4 (797gm and 1.44)

followed by T-3 (793.3g and 1.45), T-1 (789.6g and 1.46), T-2 (7887.7g and 1.46), T-6 (784.3g and 1.47) and T-5 (778.6 and 1.48) group. The feed intake was non-significant ($p>0.05$) among all the treatments groups. In finisher phase, (day 22-35) similar trend was recorded. The maximum average body weight gain with improved feed conversion ratio was recorded for group receiving T-4 (1109.8g and 1.61) followed by T-3 (1101.1g and 1.62).

For the overall experimental period (day 1-35) a significant difference was recorded among all the treated groups in body weight gain, FCR and feed intake to that of standard and both negative and positive control. Maximum body weight gain was recorded for group receiving T-4 (1906.9g), followed by T-3 (1894.4g), T-1 (1879.5g), T-2 (1871.7g) as compared to both the control groups. Feed intake among the treated groups to that of controls were higher for T-2 (2949.3g) and T-3 (2947.4g) respectively. There were slight differences recorded for water intake among all the groups. No significant difference ($P<0.05$) was recorded for percent livability and broiler farm economy index (BFEI). Broiler performance efficiency factor (BFEF) was significantly higher ($P<0.05$) for group receiving T-4 standard (MOS) followed by T-3, T-1, T-2 as compared to positive and negative control respectively.

Table.3.1. Effect of garlic, onion, chilli on performance of broiler in comparison to standard, positive and negative control

Performance Traits	Treatments						SEM	P-Value
	T-1	T-2	T-3	T-4	T-5	T-6		
	Starter Phase (d 1-21)							
BWG, g	789.6^{c}	787.7^{c}	793.3^{b}	797.1^{a}	778.6^{e}	784.3^{d}	0.78	0.00
F.I, g	1152^{b}	1154.3^{b}	1156.2^{b}	1155.1^{c}	1152.4^{a}	1156.9^{a}	2.22	0.58
FCR	1.45^{b}	1.46^{b}	1.45^{b}	1.44^{c}	1.48^{a}	1.47^{a}	0.02	0.00
	Finisher Phase (d 22-35)							
BWG, g	1089.9^{c}	1084^{d}	1101.1^{b}	1109.8^{a}	1058.1^{f}	1070.9^{e}	0.79	0.00
F.I, g	1776.2^{b}	1773.8^{bc}	1791.2^{a}	1794.3^{a}	1753^{d}	1764.4^{c}	3.51	0.00
FCR	1.629^{cd}	1.63^{c}	1.626^{d}	1.61^{e}	1.65^{a}	1.64^{b}	0.027	0.00

	Overall Period (d 1- 35)							
BWG, g	1879.5^{c}	1871.7^{d}	1894.4^{a}	1906.9^{b}	1836.8^{f}	1855.2^{e}	0.89	0.00
F.I, g	2928.3^{b}	2928.1^{b}	2947.4^{a}	2949.3^{a}	2905.4^{c}	2921.3^{b}	3.91	0.00
FCR	1.55^{cd}	1.56^{c}	1.55^{d}	1.54^{e}	1.58^{a}	1.57^{b}	0.02	0.00
WI	6352.4^{a}	6305.1 ^{ab}	6386.9 ^{a}	6350.3 ^{a}	6248.6 ^{b}	6351.6 ^{a}	27.33	0.47
Livability%	95.0	93.33	96.66	95.0	93.3	95.0	2.63	0.94
BFEF	123.4^{c}	122.4^{d}	124.5^{b}	126.1^{a}	118.8^{f}	120.6^{e}	0.19	0.00
BFEI	3.32^{ab}	3.24^{ab}	3.40^{a}	3.39^{a}	3.11^{b}	3.21^{ab}	0.08	0.21

Superscripts $^{a, b, c, d, e}$ are the mean difference among various treated groups of broilers having different aqueous extract of onion, garlic and chilli in different concentration as T1, T2, T3 according to table 3.5 at the rate of 1.5 ml per liter of drinking water in comparison to standard (T4) Manan Oligo-Saccharide (MOS), negative (T5) plane water and positive (T6) having known antibiotic.

The values are presented with mean with a probability value of < 0.05.

Carcass traits determination

The impact of aqueous extract blends of garlic, onion and chilli in comparison to standard, positive and negative control in broilers are illustrated in Table 3.2 for carcass traits determination. The effect of aqueous extract blend of garlic, onion and chilli when used in different concentration showed significant ($P<0.05$) increased among different carcass traits as compared to standard and both positive and negative controls respectively. Mean live body weight was higher for group receiving T-4 (1950.4g) followed by T-3 (1938g) with lowest recorded for control among the all treated groups. Dressed carcass weight and dressing percentage was higher for T-4 (1379.6g and 70.7%) followed by T-3 (1374.7g and 70.9%), T-1 (1334.7g and 69.4%) and T-6 (1323.6g and 69.7%) as compared to control groups. Mean organs weight like breast, thigh, wing, leg was higher for T-4. No significant difference were recorded among different treatment groups for the relative weight of different visceral organs like gizzard, lungs, gall bladder and neck while liver was slightly different having highest value for T-4 (49.06g). Significant lower abdominal weights were found among the treated groups receiving different aqueous concentration of garlic, onion and chilli like T-1,T-2 and T-3 as compared to standard, negative and positive control.

Table.3.2 Carcass traits determination using different concentration of garlic, onion, chilli in comparison to standard, positive and negative control

Carcass traits (gm)	Treatments						SEM	P-Value
	T-1	T-2	T-3	T-4	T-5	T-6		
Live Bwt	1923.2^{c}	1915.5^{d}	1938^{b}	1950.4^{a}	1880.5^{f}	1898.9^{e}	0.91	0.00
Dressed Wt	1334.7^{b}	1319.4^{b}	1374.7^{a}	1379.6^{a}	1298.2^{c}	1323.6^{b}	5.65	0.00
Dressing %	69.4^{b}	68.8^{b}	70.9^{a}	70.7^{a}	69.03^{b}	69.7^{b}	0.27	0.00
Leg (g)	177.53^{d}	181.9^{c}	186.6^{b}	191.2^{a}	155.2^{f}	171.3^{e}	1.20	0.00
Thigh (g)	176.83^{b}	179.67^{ab}	189.87^{a}	190.67^{a}	152.13^{c}	173.73^{b}	3.66	0.00
Breast (g)	395.06 ^{c}	400.84^{b}	415.88^{a}	418.78^{a}	393.9^{c}	395.5^{c}	1.55	0.00
Wing Wt (g)	129.9^{a}	129.27 ^{a}	129.23 ^{a}	130.5 ^{a}	123.4^{b}	127.6 ^{a}	1.19	0.012
Abd.Fats (g)	22.3^{c}	23.5^{c}	23.2^{c}	33.9^{ab}	34.1^{a}	32.1^{b}	0.60	0.00
Neck (g)	66.17	69.05	70.83	70.4	68.2	68.3	0.87	0.29
Lung (g)	7.17	7.336	7.330	7.33	7.01	7.16	0.30	0.96
Liver (g)	45.2^{bc}	44.3^{bc}	47.8^{ab}	49.06^{a}	44.06^{c}	44.7^{bc}	1.14	0.043
Heart (g)	12.93^{ab}	12.69^{b}	13.3^{a}	13.2^{ab}	12.1^{c}	13.0^{ab}	0.18	0.004
Gall Bladder (g)	1.322	1.321	1.337	1.334	1.318	1.314	0.016	0.88
Gizzard (g)	41.41	40.41	41.9	42.2	40.3	41.19	0.50	0.11

Superscripts $^{a, b, c, d, e,f}$ are the mean difference among various treated groups of broilers having different aqueous extract of onion, garlic and chilli in different concentration as T1, T2, T3 according to table 3.5 at the rate of 1.5 ml per liter of drinking water in comparison to standard (T4) Manan Oligo-Saccharide (MOS), negative (T5) plane water and positive (T6) having known antibiotic.

The values are presented with mean with a probability value of < 0.05.

b. Immunity

Following parameters were investigated to show growth promoting impact of aqueous extract blends using different concentrations of onion, garlic and chilli on broilers

Antibody Titer and weight of lymphoid organs

The impact of aqueous extract blends of garlic, onion and chilli has significant effect (P<0.05) on the antibody titer against Newcastle Disease (ND), Infectious bronchitis (IB) and Infectious Bursal Diseases (IBD) and weight of lymphoid organs of broilers as compared to standard, positive and negative control and are presented in Table 4.1. The antibody titer against ND was accomplished through HI test (log2 value). The mean antibody titer during starter phase (day-21) was significantly higher against ND for group receiving T-4 (5.17), followed by T-6 (5.16), T-2 (5.15) T-3 and T-4 having (5.14) respectively with the lowest among T-5. In contrast at finisher phase (day-35) a slight increased was recorded for antibody titer among the treated groups and the highest antibody titer for ND was observed for T-4 (5.94) followed by T-3, T-2, T-6 (i.e. 5.89,5.87, 5.84) with the lowest for T-1 and T-5 (5.77 and 4.98) respectively. The mean antibody titer for IB and IBD was accomplished through ELISA. The antibody titer for IB was not numerically increased for the starter and finisher phase among the same treatment groups while a significant difference (p< 0.05) was recorded between the treated ones. Maximum antibody was observed for T-3 (871.2), followed by T-1 (868.1),T-2 and T-4 with (866.3) while lowest was recorded for T-5 (861.5). A higher value (843.5) with no significant difference for antibody titer against IBD at starter phase was recorded among groups receiving difference concentration of aqueous extract of garlic, onion and chilli (i.e. T-1,T-2 and T-3) followed by T-4 (841.4), T-5 and T-6 with a lower value of (839.9). The antibody titer for IBD in finisher phase was not significant among T-1, T-3, T-4 and T-6 with average maximum values of (1519.3) which are significantly different (P<0.05) from group receiving T-2 and T-5 with an average value of (1505.5). The relative weights of lymphoid organs including spleen, thymus and bursa are significantly different (P<0.05) among the treatment groups. The lowest weight of spleen was recorded for T-5 while others groups show no significant difference among them but only in figures. Similarly the maximum relative weight for thymus and Bursa was recorded for T-3 (3.06 g) and T-2 (4.31g) respectively.

Table 4.1 Effect on antibody titre and weight of lymphoid organs of broilers using different concentration of garlic, onion, chilli in comparison to standard, positive and negative control

Antibody Titer		Treatments						SEM	P-Value
		T-1	T-2	T-3	T-4	T-5	T-6		
ND	Day-21	5.14^{b}	5.15^{ab}	5.14^{b}	5.17^{a}	4.95^{c}	5.16^{ab}	0.083	0.00
	Day-35	5.77^{d}	5.87^{bc}	5.89^{b}	5.94^{a}	4.98^{e}	5.84^{c}	0.09	0.00
IB	Day-21	867.07^{ab}	866.2^{b}	869^{a}	865.9^{b}	855.5^{d}	863.4^{c}	0.75	0.00
	Day-35	868.1^{b}	866.4^{c}	871.2^{a}	866.3^{c}	861.5^{e}	865.1^{d}	0.26	0.00
IBD	Day-21	842.6^{a}	843.5^{a}	842.7^{a}	841.4^{b}	839.9^{c}	838.9^{c}	0.34	0.00
	Day-35	1518.7^{a}	1505.8^{b}	1519.8^{a}	1520.1^{a}	1505.3^{b}	1518.9^{a}	0.45	0.00
Weight of Lymphoid Organs									
Spleen, g		2.56^{a}	2.57^{a}	2.58^{a}	2.54^{a}	2.42^{b}	2.53^{a}	0.01	0.0005
Thymus, g		2.93^{c}	2.90^{cd}	3.06^{a}	2.97^{b}	2.78^{e}	2.87^{d}	0.01	0.00
Bursa, g		4.25^{a}	4.31^{a}	4.23^{a}	4.07^{b}	3.09^{c}	4.12^{b}	0.02	0.00

Superscripts $^{a, b, c, d, e,f}$ are the mean difference among various treated groups of broilers having different aqueous extract of onion, garlic and chilli in different concentration as T1, T2, T3 according to table 3.5 at the rate of 1.5 ml per liter of drinking water in comparison to standard (T4) Manan Oligo-Saccharide (MOS), negative (T5) plane water and positive (T6) having known antibiotic.

The values are presented with mean with a probability value of < 0.05.

Leauckocytes Index

Table 4.2 illustrates the data for humoral and cell mediated response by using different aqueous extract blends of garlic, onion and chilli in different concentration in comparison to standard, positive and negative control. Lymphoproliferative response to phytohaemagglutinin-P(PHA-P) was used as tool for cell mediated immunity which were found significantly higher ($p<0.05$)

among the groups receiving different concentration of garlic, onion and chilli. In comparison to control group all other groups should high absorption for carbon clearance with marked ($p<0.05$) phagocytic activity after 15 minute post injection as compare to 3 minute due to increased in the number of mononuclear cells with passage of time that engulf the carbon particles. The highest response for mean thickness was for T-1 (1.06) followed by T-2 (1.5), T-3 (1.02) and T-4 (0.93) while the negative T-5 (0.69) and positive T-6 (0.88) show marked suppression in response. The C-reative protein value was numerical different $p<0.05$) among the treated group. The highest but not significant value for C-reative protein was record for T-4, T-3, T-2 with the mean value of 0.73.

The leukogram showed a significant difference ($p<0.05$) among all the treated groups. The highest Heterophil to Lympocyte (H/L) ratio was recorded for T-6 (43.3) receiving know antibiotics followed by T-1, T2 having numerical values of (40.01 and 37.70) respectively. The group receiving T-3 and T-4 showed no numerical difference with an average of (36.7). Lowest H/L ratio was recorded for group receiving no treatment T-5 (33.4). The highest percentage basophile was recorded for T-6 and T-4 with no significant difference between the two groups having mean value of (11.07 and 10.7), followed by T-3 and T-1 having (9.7 and 9.52). Lowest percent basophile mean count was recorded for T-5 (6.8). The highest mean percent eosinophile and monocytes count was higher for group receiving no treatment.

Table 4.2 Effect of humoral and cell mediated response in broilers using different concentration of garlic, onion, chilli in comparison to standard, positive and negative control

Variables	Treatments						SEM	P-Value
	T-1	T-2	T-3	T-4	T-5	T-6		
PHA-P	1.06^{a}	1.05^{ab}	1.02^{b}	0.93^{c}	0.69^{e}	0.88^{d}	0.08	0.00
CRP	0.68^{ab}	0.71^{a}	0.71^{a}	0.73^{a}	0.52^{c}	0.60^{bc}	0.02	0.001
CCA-3 Min	75.5^{b}	79.5^{a}	80.05^{a}	79.8^{a}	62.7^{d}	71.09^{c}	0.31	0.00
CCA-15 Min	45.8^{a}	41.8^{b}	45.7^{a}	42.6^{b}	27.8^{d}	39.7^{c}	0.50	0.00
Heterophil%	19.5^{ab}	18.7^{bc}	18.4^{bc}	17.8^{cd}	16.9^{d}	20.4^{a}	0.42	0.001

Lymphocyte%	48.8^{cd}	49.6^{bc}	50.5^{ab}	48.6^{d}	50.7^{a}	47.2^{e}	0.30	0.001
H/L Ratio	40.01^{b}	37.7^{bc}	36.4^{c}	36.7^{c}	33.4^{d}	43.3^{a}	0.82	0.00
Basophile%	9.52^{b}	8.8^{c}	9.7^{b}	10.7^{a}	6.8^{d}	11.07^{a}	0.11	0.00
Eosenophile%	7.44^{b}	8.43^{a}	7.13^{b}	7.13^{b}	8.60^{a}	5.96^{c}	0.10	0.00
Monocyte%	14.6^{cd}	14.3^{d}	14.1^{d}	15.6^{b}	16.7^{a}	15.3^{bc}	0.24	0.00

Superscripts $^{a, b, c, d, e,f}$ are the mean difference among various treated groups of broilers having different aqueous extract of onion, garlic and chilli in different concentration as T1, T2, T3 according to table 3.5 at the rate of 1.5 ml per liter of drinking water in comparison to standard (T4) Manan Oligo-Saccharide (MOS), negative (T5) plane water and positive (T6) having known antibiotic.

The values are presented with mean with a probability value of < 0.05.

Discussion

Antibiotics growth promoters for many years have been used in livestock and poultry industry for high meat production at relative cheaper cost. But with the global threat for increase antimicrobial resistance to many different antibiotics and failure of treatments, drug residues in final product and altering the gut microbiota and emergence of multi drug resistance bacteria has led to ban of antibiotics as non-therapeutic agent and prophylactic use in animal production (Hayden et.al 2020 and Castanon,2007). WHO (2015) adopt global action plan to address antimicrobial resistance and focus on search for other alternative instead AGP. Many alternate approaches were made includes use of nano particles, phagetherapy, prebiotics, probiotics, essential oils, phytochemicals, vaccines, organic acids and in-feed enzymes (Abd El-Hack *et al*.,2022; Mak *et al*., 2022;Raquel Abreu *et al.*, 2023).

Growth Performance

The current study was designed to investigate the growth promoting impact of aqueous extract blends using different concentrations of different phytochemical plants like onion, garlic and chilli in broilers when used at 1.5ml per liter of drinking water in comparison to Manna Oligo-saccharide as standard, known antibiotics as positive control and negative control group was provided with plain water with no treatments. Overall significant effect (P>0.05) was observed in

broiler during starter and finisher phase and it was concluded that garlic, onion and chilli in different concentration can be used as alternative to antibiotics having no antimicrobial resistance and less harmful effect by having no drug residues in the final product. Improved performance parameters were recorded for groups receiving different extracts of garlic, onion and chilli as compared to negative control group. Phytobiotic herbs like garlic and onion broadly contain different bioactive organosulphur compounds, phenols, saponins and polysaccharides. The widely used phytochemical in garlic is Allicin and that of onion is cysteine sulfoxides and onionin-A while that for chilli is capsacin (Loleny *et al*., 2021; Xin-Xin. 2021; Awodola-Peters. 2021) exhibit different including antioxidant, anticancer, anti-inflammatory, cardiovascular protective, antifungal, anti-diabetic, antibacterial ,immunomodulatory, anti-obesity. These phytochemicals attributes to better gut health, growth, carcass traits and feed efficiency. Our study is in accordance with findings of that was reported by (Raeesi *et al.*, 2010; Goodarzi *et al.*, 2013) using aqueous extract of onion resulted in marked feed intake leading to increased body weight gain which may be due to lowering the blood glucose level by the organosulphur (allyl propyl disulfide) compounds by competing with insulin (a Disulfide) in liver for insulin-inactivation sites resulting in increased free insulin which triggers the nervous system to cope with hypolglycemia by taking high intake of feed (Kumari *et al.,* 1995;Andallu *et al.*,2001). Similar results were reported by (Jo Jongkwan *et al,* .2014) and An *et al.*, 2015) who recorded significantly improvement in the performance parameters i.e body weight gain, feed and water intake. High feed intake and weight gain was recorded in different trails by using garlic at the rate of 150 ppm by (Petrolli *et al.*,2012) and 0.1 ml (Rahimi *et al.*, 2011) respectively. Garlic when used as dried powder, garlic meal, fermented garlic powder or as bulb showed high feed intake and weight gain with improved performance traits (Abdullah *et al.*, 2010; Jimoh *et al.*, 2013; Ao *et al.*, 2011; Varmaghany, 2015). In accordance to our study findings garlic in combination with turmeric showed improved performance which might be due to lowering the digesta pH of crop, cecal content and proventriculus and improved ileal digestibility Olukosi and Dono (2014). The lower pH indicates the prebiotic activity that results in the production of volatile fatty acid that results in the proliferation of beneficial microflora in the gut and help in the reduction of harmful microbial colonization (Yang *et al*., 2009; Varmaghany *et al*., 2015).The high performance may be attributed to positive changes in the small intestine by increasing the villus height and surface area enhancing the nutrient absorption capacity, while the

high villus crypth depth indicates marked cell turn over during normal cellular sloughing or due to inflammation Adibmoradi *et al*., (2006). Poultry birds lack the spicy effect of spices, which may be due lack of specific receptors for binding of capsaicin or the lack of sensitive receptors to capsaicin (Mason and Maruniak, 1983; Szolcsangi, 1976). Capsaicin the bioactive compound of chilli showed marked increase in the appetite and influenced the feed consumption with marked chemo preventive and chemo-therapeutic benefits (Yoshioka *et al.*, 1999; Munglang and Vidyarthi, 2019). Chilli help to lower the fat percentage due to its hypocholesterolaemic and hypolipidemic action and significantly reduces the deposition of cholesterol and fat in the body, resulted lowering the triglycerides level in the final product (Puvaca *et al.*, 2013). In contrast to our findings different researchers reported promising outcomes which might be due to difference in experimental condition, health status, birds type and genetics and also might be due to type, quality, processing and dosage of the product used in the experiment. Rahmatnejad *et al.*, (2009) reported that garlic had no significant effect on feed intake, weight gain and feed conversion ratio. Similar findings were reported by (Aji *et al.*, 2011) using onion and garlic in broilers resulted in lowered carcass yield. Birrenkott *et al*., (2000), also reported that that garlic did not significantly decrease the cholestrol level. Similar results were concluded by (Lim *et al*., 2006) who reported that the level of HDL was not alter by dietary supplementation of dietary garlic to chickens at different level. Pourali et *al.*, (2010) reported no significant influence of garlic on the carcass parameters like dressed carcass, weight of heart, gizzard and liver. The data reported by

Immune Response

Garlic, onion and chili contain different phytochemicals which are useful for boosting the immunity in broilers. Our study results are in accordance with those of (Marefati N *et al.*,2021) who used onion extracts help in the proliferation like natural killer cells and the phytochemical compound Onionin-A showed marked potential for the multiplication of lymphocyte and help in the prevention of immunosuppressive effect in result of macrophages. Similarly Adeel Sattar (2018) found increase antibody titer for birds with highest PHA-P response. Jafari *et al*., (2008) investigated using garlic as dietary supplement in broilers results in high antibodies production against NDV but with no marked increased in the leuckogram. In parallel to our results (Hanieh *et al*., 2010) concluded using garlic as supplement with increased lymphocyte proliferation and marked increased in the size of spleen and bursa was recorded, that may be attributed to

increased lympocyte proliferation. In another study stimulatory effect in of anti-NDV antibody was produced by garlic in layer chickens. Increased splenocyte and thymocyte was recorded in chicken fed garlic with high level of macrophages activation that control infection and disease progression due to micobicidal activity and reactive oxygen. In a study by Costantini and Moller, 2009;Kyo *et al*. (1999) it was concluded that garlic has antioxidant activity that can improved the humoral immune response by increased cytokines production, IL-12, IL-2, INF-γ and tumor necrosis factor (TNF)-α in stimulated splenocytes. Antibiotics may increase the immunity by inhibiting the peroxidation of non-enzymes by increasing the membrane protection, production of particular antibodies or affect the homopoietic tissue Abdel Azeem (2002). Contrary to our results no significant increase was found in antibody titre against ND, IBD and IB with no increase in weight gain of spleen and bursa (Lee *et al.,* 2010; Zulkifli *et al.,* 2000). Onion and garlic increased the immune response in birds by enhancing the concentration of IgM antibodies and percent phygocytic activity with increase in the thymus and bursa size and weight, which may be due to the presence of bioactive compounds of onion and garlic, which have proven antiviral, anti-inflammatory antioxidant activity (Lampe *et al.,*1999). In another study, it was concluded that garlic, onion and chili had marked effect on the weight of follicles and spleen in birds that are vaccinated against NDV (Hanie *et al.,*2010). Goodarzi *et al.,* (2013) also reported the increased level of antibodies titer against NDV after supplementation of onion to broilers. In contrary there were no consistent increased in the leukogram parameters like neutrophils, basophil, lymphocytes, and eosinophils percentages of broiler chickens were found after supplementation the bioactive compound of onion allicin to broilers El Katcha *et al.,*(2016).

Conclusion and Recommendation

It was concluded from the above experimental data that using garlic, onion and chilli aqueous extract blend in different concentration has marked impact on growth performance and immune response of broilers. Significant results were found as compared to control groups. It can be used as an alternative to antibiotic growth promoter with no harm as antimicrobial resistant to consumers and no drug residue in final product. Marked increased in carcass traits and birds immunity revealed that T-3 at 1.5 ml per liter of drinking water having Garlic:Onion:Chilli in the ratio of (50:37.5:12.5) can be used as an effective treatment in broilers for enhanced performance traits and better immunity. It's recommended that the said treatment should be further evaluated

at different dosage at 1,1.5and 2 ml per liter of drinking water to find the best concentration and dosage of the aqueous extract of garlic, onion and chilli to be used in the broilers as growth promoting and immune stimulant.

Data availability

The data that support the findings of this study are listed in the article and are available from the corresponding authors upon reasonable request.

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Declaration of Interest

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. The authors certified that there are no conflicts of interest associated with this publication, and there has been no significant financial support for publishing this work that could have influenced its outcome. As corresponding Author, I conform that the manuscript has been read and approved for submission by all the named authors.

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