



Research Article

Effect of Aqueous Extracts Blends of Garlic, Onion and Chilli on Immunity and Ileal Histomorphometry in Broilers

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Abstract | The current study was designed to evaluate the impact of different concentrations of aqueous extract blends of garlic, onion, and chilli on gut performance and immunity of broilers in comparison to Mannan Oligo-Saccharide and plain water. A total of 300 days old chicks of broilers were randomly allocated to five treatment groups each replicated four times having 15 birds per replicate. Groups A, B, and C were receiving respective treatments at the rate of 1.5 ml per liter of drinking water having the garlic, onion, and chilli concentration as (T-1=37.5:50:12.5, T-2=25:50:25, T-3=50:37.5:12.5). While group D and E were designated for receiving (T-4) Mannan Oligo-Saccharide and (T-5) plain water. The gut health parameters including gut pH and ileal histomorphometry including villus height, width, crypt depth and surface area were significantly affected by the treatment groups. The villus surface area for ileum for T-1 to T-5 groups recorded were 129497, 129013, 135655, 137894, 120535 μm^2 . A significantly ($p<0.05$) lower pH value was recorded for T-3 group for crop, small intestine and gizzard having the aqueous concentration of garlic, onion and chilli as (50:37.5:12.5). The weight of lymphoid organs like the weight of bursa for T1 to T5 groups were (4.25, 4.31, 4.23, 4.07 and 3.09 gm) with the phagocytic activity for PHA-P were 1.06, 1.05, 1.02, 0.93 and 0.69 mm. C-reactive protein values were 0.69, 0.70, 0.72, 0.73, 0.68 mg/d, showing best immune response for groups receiving aqueous extract blends of garlic, onion and chilli. The results of the current study revealed that an aqueous extract of garlic, onion, and chilli when used in the concentration of 50:37.5:12.5 could be used as gut health and immune booster at 1.5 ml per liter.

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Keywords | Gut health, Immunity, PHA-P, Extract, C reactive protein



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Introduction

The poultry gut plays an important role by digesting the feed, utilizing and absorbing the available nutrients, and providing defense against

harmful microorganisms. Any deviation in the gut health by any means impact bird performance and overall productivity. Over the past, different antibiotic growth promoters (AGPs) were used in the poultry industry that significantly decreased 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 inevitable challenge to the containment of global antimicrobial resistance (AMR) leading to the limitations of antimicrobial use (AMU) in poultry because of serious consequences that include 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 environmental points of view (Grajek *et al.*, 2005).

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 (Khan *et al.*, 2021; Islam *et al.*, 2024). Different herbal plants that are being investigated have potent therapeutic properties and are naturally, safe and having centum of different phytochemicals (Puvaca *et al.*, 2013). Garlic (*Allium sativum*), onion (*Allium cepa* L.) and chilli (*Capsicum annum*) act as natural growth promoters, immune stimulants and gut health enhancers due to the presence of different phytochemicals and nutraceuticals that help to improve health and performance leading to increased production of animals (Masihi, 2002; Lim *et al.*, 2006; Pourali *et al.*, 2010). 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. It helps to reduce the deposition of cholesterol and fats, subsiding the heat stress and maintain the energy balance during ad-libitum feeding thus improving the feed utilization resulting in sound performance (Jongkwan *et al.*, 2009) and acts as natural alternative to antibiotics as growth promoter (Demir, 2003).

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 effects such as antibacterial, antiviral, antiprotozoal, anti-thrombotic, anti-diabetic, and so many others due to the presence

of phytochemicals having biological properties. After use in poultry feed in different concentrations and forms they act as prebiotics with improved gut health integrity with no drug residue in final product (meat and egg) and antibiotic resistance from consumers aspects (Abd El-Hack *et al.*, 2022). Keeping in view the current project was designed to study the gut and immune response of aqueous extract blends using different concentrations of garlic, onion and chilli on broilers at the rate of 1.5 ml/liter of drinking water with following specific objectives

Objective

To study the immune stimulant and gut health impact of aqueous extract blend using different concentrations of onion, garlic and chilli on broilers at 1.5 ml/liter of drinking water.

Materials and Methods

Experimental design

Day old chicks of Ross-308 (n= 300) 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 blends of garlic, onion, and chilli in different concentration as T-1, T-2 and T-3 according to Table 1 at a rate of 1.5 ml/liter of drinking water. Chicks in Group D and E were kept as control with provision of (T-4) Manano Oligo-Saccharide (MOS) at 1.5 gm per liter of drinking water having proven probiotic activity and plain water (T-5), respectively. 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.

Table 1: Different concentration of onion, garlic and chilli per 100 ml.

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

Extract distillation

Onion, garlic and chilli were purchased from local market and sent to local distillation company for

preparation of their individual stock of onion, garlic and chilli. The individual stock of aqueous extracts was prepared by the process of hydro-distillation using 1 unit (Kg) of each onion, garlic and chilli to 3 units (Liter) of water (Dilworth *et al.*, 2017; Azmir, 2013).

Different concentration of garlic, onion and chilli per ml
The aqueous extracts individual stock of onion, garlic and chilli were allocated to different treatment groups as T-1, T-2 and T-3 to have the required concentration per 100 ml according to Table 1.

Gut health

The gut pH was measured by a digital pen type pH meter. In a consecutive method, the pH values for various parts (crop, gizzard and small intestine) of the gastrointestinal tract was measured by inserting a sensing electrode directly in the opening made in the organs filled with digesta during the slaughter and pH was recorded (Mabelebele *et al.*, 2017).

Histopathological examination of intestinal sections were performed according to the method described by (Slaoui and Fiette, 2011). 3 birds per treatment were slaughtered on day 35 to find out villus height through high resolution microscope. A 3 cm part of mid of ileum was incised, removed and these parts was rinsed by physiological saline solution and fixed in buffer formalin. Then every part was embedded in paraffin and 2 mm part of every sample were fixed for inspection on a glass slide and stained by eosin and haematoxylin. Histological parts were studied and examined through microscope. Height of Villus was measured from top of villus to the top of Lamina Propria. For this parameter 15 measurements per bird were taken. Crypt depth was measured from Villus-Crypt junction to the bottom of Crypt and noted as the average of 10 fields for every specimen.

Immunity

Humoral immunity: Haemagglutination (HA) and Haemagglutination Inhibition (HI) tests were performed according to the method of Olsen *et al.*, (2003) to evaluate the humoral immune response of broilers on day 21 and 35 for antibody titre against NDV.

Cell-mediated immunity

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 h and 48 h duration at 3rd and 4th digit (Corrier, 1990).

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

C-reactive protein (CRP) test

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

Data collection and analysis

Data was arranged in Excel sheet and was statistically analyzed for ANOVA, Means and LSD via CRD design through Statistix 10.0 latest software.

Table 2: Determination of pH for gastro intestinal tract using different concentration of garlic, onion and chilli in comparison to MOS and negative control.

pH	Treatments						P-value
	T-1	T-2	T-3	T-4	T-5	SEM	
Crop	4.58 ^b	4.46 ^{bc}	4.39 ^c	4.46 ^{bc}	5.20 ^a	0.04	0.00
S. intestine	6.44 ^b	6.31 ^d	6.13 ^c	6.36 ^c	6.86 ^a	0.09	0.00
Gizzard	3.27 ^b	3.27 ^b	3.20 ^c	3.25 ^b	3.77 ^a	0.01	0.00

T1=Onion:Garlic:Chilli (50:37.5:12.5), T2= Onion:Garlic:Chilli (50:25:25), T3= Onion:Garlic:Chilli (37.5:50:12.5) T4= Manan Oligo Saccharide, T5= Plain water. 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. A at the rate of 1.5 ml per liter of drinking water in comparison to (T4) Manan Oligo-Saccharide (MOS), (T5) plain water. The values are presented with mean with a probability value of < 0.05.

Results and Discussion

Gut health

pH of gastro intestinal tract: The effect of aqueous extract blends of garlic, onion and chilli in broilers at different concentrations with that of standard and negative control to determine the pH at different parts of gastrointestinal tract are presented in Table 2. Significantly a lower pH value (P<0.05) on the average was recorded in crop for the groups that received garlic, onion and chilli in different concentration. The lowest pH value in crop was recorded for group receiving T-3 (4.39), followed by T-2, T-4 with mean average pH

of (4.46) and T-1 (4.58) as compared to the negative control group T-5 (5.20). Lowest pH for small intestine was recorded for T-3 (6.13) while highest recorded for negative control T-5 (6.86). Similar results were estimated for gizzard where lowest pH value was recorded for group receiving garlic, onion and chilli T-3, followed by T-4, T1 and T-2, while high pH was recorded for T-5 negative control group.

Intestinal morphometry: Table 3 depicts the effects of aqueous extract blend of garlic, onion and chilli in different concentration were recorded for the intestinal histo-morphometry of broilers. Results showed that aqueous extract of garlic, onion and chilli in different concentrations exhibited a significant ($p < 0.05$) increase in histo-morphometrical dimensions including height, width, crypt depth and surface area. The villus height for ileum were found higher for T-3 and T-4 having, respective villus height of (836.87 and 834.37 μm), followed by T-1 (829.93 μm) and T-2 (822.27 μm) while lowest was recorded for T-5 (783.03 μm). Highest value for width was recorded for group receiving T-3 and T-4 (165.2 μm and 162.1 μm). The highest crypt depth of the ileal segment were found higher for T-3 and T-4 having the value of (119.4 and 119.1 μm) while lowest for T-5 (115.9 μm). Highest surface area was recorded for T-4 and T-3 followed by T-1 and T-2 while lowest for T-5,

respectively.

Immunity

Antibody titre 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 titre against Newcastle Disease (ND), and weight of lymphoid organs of broilers as compared to standard, positive and negative control and are presented in Table 4. The antibody titre against ND was accomplished through HI test ($\log_2$ value). The mean antibody titre during starter phase (day-21) was significantly higher against ND for group receiving T-4 (5.17), followed by T-2 (5.15) T-3 and T-1 having (5.14), respectively with the lowest mean antibody titre for T-5 (4.95). In contrast at finisher phase (day-35) a slight increase was recorded for antibody titre among the treated groups and the highest antibody titre for ND was observed for T-4 (5.94) followed by T-3 and T-2 (5.89 and 5.87) with the lowest for T-1 and T-5 (5.77 and 4.98), respectively. 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 other 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 (4.31 g), respectively.

Table 3: Effect on histomorphometry of broilers using different concentration of garlic, onion, chilli in comparison to MOS and negative control.

Variables		Treatments						P-Value
		T-1	T-2	T-3	T-4	T-5	SEM	
Ileum villus	Height (μm)	829.93 ^b	822.27 ^c	836.87 ^a	834.37 ^a	783.03 ^d	0.88	0.00
	Width (μm)	156.03 ^c	156.90 ^c	162.10 ^b	165.27 ^a	153.93 ^d	0.68	0.00
	Crypt depth (μm)	118.6 ^{ab}	118 ^b	119.4 ^a	119.1 ^a	115.9 ^c	0.33	0.00
	Surface area HxW (μm^2)	129497 ^c	129013 ^c	135655 ^b	137894 ^a	120535 ^d	556.2	0.00

Table 4: Effect on antibody titre and weight of lymphoid organs of broilers using different concentration of garlic, onion, chilli in comparison to standard and negative control.

Antibody titre		Treatments						P-value
		T-1	T-2	T-3	T-4	T-5	SEM	
ND	Day-21	5.14 ^b	5.15 ^{ab}	5.14 ^b	5.17 ^a	4.95 ^c	0.08	0.00
	Day-35	5.77 ^d	5.87 ^{bc}	5.89 ^b	5.94 ^a	4.98 ^c	0.09	0.00
Weight of lymphoid organs								P-value
Spleen, g		2.56 ^a	2.57 ^a	2.58 ^a	2.54 ^a	2.42 ^b	0.01	
Thymus, g		2.93 ^c	2.90 ^{cd}	3.06 ^a	2.97 ^b	2.78 ^c	0.01	
Bursa, g		4.25 ^a	4.31 ^a	4.23 ^a	4.07 ^b	3.09 ^c	0.02	

T1=Onion:Garlic:Chilli (50:37.5:12.5), T2= Onion:Garlic:Chilli (50:25:25), T3= Onion:Garlic:Chilli (37.5:50:12.5) T4=Manan Oligo Saccharide, T5=Plain water. 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. A at the rate of 1.5 ml per liter of drinking water in comparison to (T4) Manan Oligo-Saccharide (MOS), (T5) plain water. The values are presented with mean with a probability value of < 0.05 .

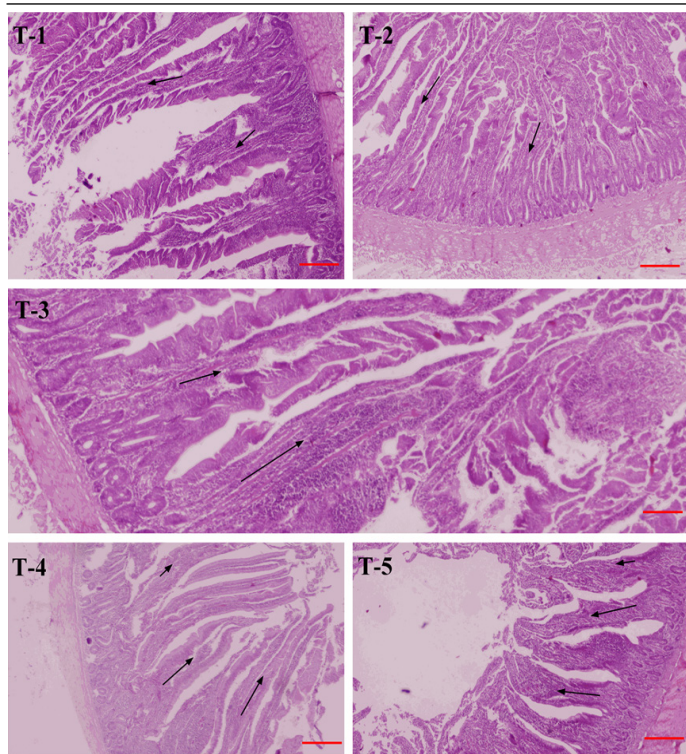


Figure 1: Histomorphometry of small intestine (Ileum section 10X) collected from broilers in experimental groups A, B, C, D and E. Following slides T-1, T-2, T-3, T-4 and T-5 shows the villus from broilers ileum section with red scale bar in micrometer (100 μ m).

Leauckocytes index: Table 5 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 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 showed high absorption for carbon clearance with marked ($p < 0.05$) phagocytic activity after 15 minutes post injection due to increase in the number of mononuclear cells with passage of time that engulf the carbon particles. The highest response for mean thickness for PHA-P 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) showed marked suppression in response. The C-reactive protein value was numerically different ($p < 0.05$) among the treated group. The highest CRP was recorded for T-4 (0.73 mg/dL) while lowest for T-5 (0.68 mg/dL), respectively.

The leukogram showed a significant difference ($p < 0.05$) among all the treated groups. The highest Heterophile to Lymphocyte (H/L) ratio was recorded for T-1, T-2 having numerical values of (40.01 and

37.70). 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-4 with a mean value of (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 5: Effect of humoral and cell mediated response in broilers using different concentration of garlic, onion, chilli in comparison to MOS and negative control.

Variables	Treatments					SEM	P value
	T-1	T-2	T-3	T-4	T-5		
PHA-P (mm)	1.06 ^a	1.05 ^{ab}	1.02 ^b	0.93 ^c	0.69 ^e	0.08	0.00
CRP (mg/dL)	0.69 ^c	0.70 ^{bc}	0.72 ^{ab}	0.73 ^a	0.68 ^c	0.08	0.00
%CCA-3 Min	45.8 ^a	41.8 ^b	45.7 ^a	42.6 ^b	27.8 ^d	0.50	0.00
%CCA-15 Min	75.5 ^b	79.5 ^a	80.05 ^a	79.8 ^a	62.7 ^d	0.31	0.00
Heterophil%	19.5 ^{ab}	18.7 ^{bc}	18.4 ^{bc}	17.8 ^{cd}	16.9 ^d	0.42	0.001
Lymphocyte%	48.8 ^{cd}	49.6 ^{bc}	50.5 ^{ab}	48.6 ^d	50.7 ^a	0.30	0.001
H/L Ratio	40.01 ^b	37.7 ^{bc}	36.4 ^c	36.7 ^c	33.4 ^d	0.82	0.00
Basophile%	9.52 ^b	8.8 ^c	9.7 ^b	10.7 ^a	6.8 ^d	0.11	0.00
Eosinophil%	7.44 ^b	8.43 ^a	7.13 ^b	7.13 ^b	8.60 ^a	0.10	0.00
Monocyte%	14.6 ^{cd}	14.3 ^d	14.1 ^d	15.6 ^b	16.7 ^a	0.24	0.00

T1=Onion:Garlic:Chilli (50:37.5:12.5), T2= Onion:Garlic:Chilli (50:25:25), T3= Onion:Garlic:Chilli (37.5:50:12.5) T4=Manan Oligo Saccharide, T5=Plain water, PHA-P (Phytohaemagglutinin-P) CRP (C-reactive protein) %CCA (Carbon clearance assay percent absorption). 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. A at the rate of 1.5 ml per liter of drinking water in comparison to standard (T4) Manan Oligo-Saccharide (MOS), negative (T5) plain water. The values are presented with mean with a probability value of < 0.05 .

Antibiotic growth promoters for many years have been used in livestock and poultry industry for high meat production at relatively cheaper cost. But with the global threat of increased 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 have led to ban of antibiotics as non-therapeutic agent and prophylactic use in animal production (Hayden *et al.*, 2020; 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 (Mak *et al.*, 2022; Raquel *et al.*, 2023).

Gut health

Plant extracts contain numerous phytochemicals also known as phytobiotics that have proven biological activities like antioxidant, antiparasitic, antimicrobial and can be used as alternative to antibiotics. The phytochemicals like organosulphur, phenols, saponin and polysaccharides help in the gut health and birds performance with increased carcass traits (Karangiya *et al.*, 2016; Akyildiz and Denli, 2016). Garlic and onion have different phytochemicals substances especially organ Sulphur compounds like allicin, alliin, inulin, onionin A and quercetin etc. with marked biological activities. The current results showed marked decrease in gut pH for the groups receiving garlic, onion and chilli as well as standard Mannan Oligo-Sachharide as compared to control group. Phytochemical like Inulin lower the pH of gut digesta due the production of higher volatile fatty acids and help in the increased colonization of useful microbes in the gut (Grajek *et al.*, 2005). Harmful bacteria like *Salmonella* and *E. coli* colonization to the intestinal mucosa is done through type-1 fimbria containing Mannose specific lectins FimH. Mannose Oligo-Saccharide reduces this type of attachment by binding to FimH and the bacteria move out without colonization in the gut (Hooge *et al.*, 2003; Fernandez *et al.*, 2002). Use of phytochemicals in conjugation with vaccination improved the gut health and bird performance by having low lesion score when challenged with *Eimeria*, *Salmonella* *Clostridium* and *campylobacter* (Ritzi *et al.*, 2016; Saint-Cyr *et al.*, 2016). Gastrointestinal tract especially the small intestine is a vital organ for nutrient absorption and the development of this piece is important for bird health and performance (Kawalilak *et al.*, 2010). Our study results provided significant improvement of gut parameters by using aqueous extracts of onion, garlic and chilli like villus height, width and surface area, which are standard tools used to evaluate the nutrient absorption and GIT physiology. Live bird performance is correlated with the villus height and crypt depth (Chacher *et al.*, 2017; Yakhkeshi *et al.*, 2011). Supplementation of onion powder significantly increased villus height, width and crypt depth. Antimicrobial activity of the

onion powder might be the cause of increased villus height, crypt depth, and villus surface area, resulting in better intestinal health of the broiler chicks. Another probable cause of increased gut health (villus height, crypt depth, and villus surface area) may be attributed to the presence of Sulphur, phytochemicals, and quercetin compounds resulted from the onion supplementation (Ayssiwede *et al.*, 2011; Goodarzi *et al.*, 2014). Prebiotics are not eventually absorbed in the small intestine rather the act as selective substrate for beneficial bacteria of the colon and activate their metabolic function and activity in favor of colon micro flora resulting in healthier GIT condition (Gibson *et al.*, 2000). The aqueous extract blend of garlic, onion and chilli and that of group having MOS significantly increase the villus height, crypt depth, villus surface area in our study which is in accordance with those of (Goodarzi *et al.*, 2014). Higher villus length is related to larger surface area and lower crypt depth which helps in the absorption of nutrients. Crypt depth are area known for the enterocyte multiplication. Shallow crypt depth indicates less epithelial turn over, placing energy toward animal growth (Markovic, 2009). Probiotics help in the gut health improvement and lower damage was recorded after probiotic investigation which alleviated villus height, width and surface area (Awad *et al.*, 2006).

Immunity

Garlic, onion and chilli contain different phytochemicals which are useful for boosting the immunity in broilers. Our study results are in accordance with those of Marefati *et al.* (2021) who used onion extract help in the proliferation like natural killer cells and the phytochemical compound Onionin-A showed marked potential for the multiplication of lymphocytes and help in the prevention of immunosuppressive effect in result of macrophages. Similarly, Adeel and Ahrar (2018) found increase antibody titre 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 leukogram. 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 lymphocyte 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 microbicidal activity and reactive oxygen species. Antibiotics may increase the immunity by inhibiting the peroxidation of non-enzymes by increasing the membrane protection, production of particular antibodies or effect 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, 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 and Nanekarani \(2013\)](#) also reported the increased level of antibodies titre 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 i.e., allicin to broilers ([El-Katcha et al., 2016](#)).

Conclusions and Recommendations

It was concluded from the above experimental data that using garlic, onion and chilli aqueous extract blends in different concentration has marked impact on gut histo-morphometry and birds immunity. It was concluded that T-3 having garlic, onion and chilli in the concentration of 50:37.5:12.5 was effective treatment in broilers for enhanced performance with better gut health and improved immunity when used at 1.5 ml per liter of drinking water.

From the results data, it is recommended that the treatment containing garlic, onion and chilli at a concentration of 50:37, 5:12.5 should be further evaluated at different dosage level of 1, 1.5 and 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 with improved gut health and immunity.

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Novelty Statement

This research presents a pioneering evaluation of onion, garlic, and chili extracts as immune stimulants and their effects on gut histomorphometry in broilers when used at a dose rate of 1.5 ml per liter of drinking water. Our findings demonstrate that this triad significantly enhances immune response and improves gut morphology, offering innovative strategies for optimizing poultry health and performance.

Author's Contribution

Muhammad Bilal Islam: Research idea implementation, Conceived and designed the study, performed experiments, analyzed data, and drafted the manuscript.

Sar Zamin Khan: Provided expertise on histomorphometry techniques, interpreted results, and reviewed the manuscript for scientific accuracy.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

- Abd El-Hack, M.E., T. Mohamed, El-Saadony, H.M. Salem, M.E Amira, M.S. Mohamed, B.A. Gehan, Youssef, E.T. Ayman, M.S.A. Soliman, E. Ahmed, F.E. Attalla, M.A Khalid and A.S. Ayman. 2022. Alternatives to antibiotics for organic poultry production: Types, modes of action and impacts on bird's health and production. *Poult. Sci.*, 101: 101696. <https://doi.org/10.1016/j.psj.2022.101696>
- Abdel-Azeem, F., 2002. Digeston, neomycin and yeast supplementation in broiler diets under Egyptian summer conditions. *Egypt. Poult. Sci. J.*, 22: 235-257.
- Adeel, S. and K. Ahrar. 2013. Immunopathology of Newcastle Disease in Arsenic treated Broiler

- Chicks. Lambert academic publisher.
- Akyildiz, S. and M. Denli. 2016. Application of plant extracts as feed additives in poultry nutrition. *Anim. Sci.*, 59: 2285–5750.
- Al-Harthi, M.A., A.A. El-Deek, M. Osman, F.A. Jassas and R. Nassar. 2011. Hot pepper (*Capsicum annum*) as an alternative to oxytetracycline in broiler diets and effects on productive traits, meat quality, immunological responses and plasma lipids. *Arch. Gef.*, 76(2): 73–80.
- Awad, W.A., J. Böhm, E. Razzazi-Fazeli, K. Ghareeb and J. Zentek. 2006. Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens *Poult. Sci.*, 85: 974–979. <https://doi.org/10.1093/ps/85.6.974>
- Ayssiwede, S.B., J.C. Zanmenou, Y. Issa, M.B. Hane, A. Dieng, C.A.A.M. Chrysostome, M.R. Houinato, J.L. Hornick and A. Missohou. 2011. Nutrient composition of some unconventional and local feed resources available in Senegal and recoverable in indigenous chickens or animal feeding. *Pak. J. Nutr.*, 10: 707–717. <https://doi.org/10.3923/pjn.2011.707.717>
- Azmir, J., I.S.M. Zaidul, M.M. Rahma, K.M. Sharif, A. Mohamed, F. Sahena and A.K.M. Omar. 2013. Techniques for extraction of bioactive compounds from plant materials. *J. Food Eng.*, 117(4): 426–436. <https://doi.org/10.1016/j.jfoodeng.2013.01.014>
- Castanon, J.I.R., 2007. History of the use of antibiotic as growth promoters in European poultry feeds. *Poult. Sci.*, 86: 2466–2471. <https://doi.org/10.3382/ps.2007-00249>
- Chacher, M.F.A., K. Zahid and A. Umair. 2017. Use of mannan oligosaccharide in broiler diets: An overview of underlying mechanisms. *World's Poult. Sci. J.*, 73(4): 831–844. <https://doi.org/10.1017/S0043933917000757>
- Cheng and S.J. Lamont. 1988. Genetic analysis of immuno competence measures in a white leghorn chicken. *Breed. Genet.*, 67: 989–995. <https://doi.org/10.3382/ps.0670989>
- Corrier, D. And J.R. Deloach. 1990. Evaluation of cell-mediated, cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. *Poult. Sci.*, 69: 403–408. <https://doi.org/10.3382/ps.0690403>
- Demir, E., S. Sarica, M.A. Ozcan and M. Suicmez. 2003. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. *Br. J. Poult. Sci.*, 44: 44–45.
- Dilworth, L.L., C.K. Riley and D.K. Stennett. 2016. Plant constituents: Carbohydrates, oils, resins, balsams and plant hormones. *Pharmacognosy*. pp. 61–80. <https://doi.org/10.1016/B978-0-12-802104-0.00005-6>
- El-katcha, M.I., M.A. Soltan, M.M. Sharaf and A. Hasen. 2016. Growth performance, immune response, blood serum parameters, nutrient digestibility and carcass traits of broiler chicken as affected by dietary supplementation of garlic extract (Allicin). *Alex. J. Vet. Sci.*, 49: 50–64. <https://doi.org/10.5455/ajvs.219261>
- Fernandez, F., M. Hinton and B. Van Gils. 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to salmonella enteritidis Colonization. *Avian Pathol.*, 31: 49–58. <https://doi.org/10.1080/03079450120106000>
- Gibson, G.R. and R. Fuller. 2000. Aspects of *in vitro* and *in vivo* research approaches directed toward identifying probiotics and prebiotics for human use. *J. Nutr.*, 130: 391–395. <https://doi.org/10.1093/jn/130.2.391S>
- Goodarzi, M. and S. Nanekarani. 2014. Effect of dietary supplementation with onion (*Allium cepa* L.) on performance, carcass traits and intestinal microflora composition in broiler chickens. *Asia Pac. J. Trop. Dis.*, [https://doi.org/10.1016/S2222-1808\(14\)60459-X](https://doi.org/10.1016/S2222-1808(14)60459-X)
- Goodarzi, M. and S. Nanekarani. 2013. Effect of onion (*Allium cepa* L.) as an antibiotic growth promoter substitution on performance, immune responses and serum biochemical parameters in broiler chicks. *Health.*, 5(8): 1210–1215.
- Grajek, W., A. Olejnik and A. Sip. 2005. Probiotics, prebiotics and antioxidants as functional foods. *Acta Biochim. Pol.*, 52: 665–671. https://doi.org/10.18388/abp.2005_3428
- Hanieh, H., K. Narabara, M. Piao, C. Gerile, A. Abe and Y. Kondo. 2010. Modulatory effects of two levels of dietary Alliums on immune responses. *Anim. Sci. J.*, 81: 673–680. <https://doi.org/10.1111/j.1740-0929.2010.00798.x>
- Hayden, D., K. Hedman, A. Vasco and L. Zhang. 2020. A review of antimicrobial resistance in poultry farming within low-resource. *Animals*, 10(8): 1264. <https://doi.org/10.3390/ani10081264>
- Hooge, D.M., M.D. Sims, A.E. Sefton, A. Connolly

- and P. Spring, P., 2003. Effect of dietary mannan oligosaccharide, with or without bacitracin or virginiamycin, on live performance of broiler chickens at relatively high stocking density on new litter. *J. Appl. Poult. Res.*, 12: 461-467. <https://doi.org/10.1093/japr/12.4.461>
- Islam, Z., A. Sultan, S. Khan, K. Khan, A. Jan, U. Aziz and A.F. Alasmari. 2024. Effects of an organic acids blend and coated essential oils on broiler growth performance, blood biochemical profile, gut health, and nutrient digestibility. *Ital. J. Anim. Sci.*, 23(1): 152-163. <https://doi.org/10.1080/1828051X.2023.2297562>
- Jafari, R., M. Razi, M. Ghorbanpoor and S. Marashian. 2008. Effect of dietary garlic on immune response of broiler chicks to live Newcastle disease vaccine. *Pak. J. Biol. Sci.*, 14: 1848-1851. <https://doi.org/10.3923/pjbs.2008.1848.1851>
- Jongkwan, J., Y. Seyoung, K. Jinsu, K. Youngwoo, Y. Ku, K. Illkhyung and C. Byunglo. 2009. Effect of garlic extract supplementation on growth performance, nutrient digestibility, carcass characteristics and meat composition. *Korean J. Poult. Sci.*, 36(4): 287-292. <https://doi.org/10.5536/KJPS.2009.36.4.287>
- Karangiya, V.K., H.H. Savsani, S.S. Patil, D.D. Garg, K.S. Murthy, N.K. Ribadiya and S.J. Vekariya. 2016. Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. *Vet. World*, 9(3): 245-250. <https://doi.org/10.14202/vetworld.2016.245-250>
- Kawalilak, L., A.U. Franco and G. Fasenko. 2010. Impaired intestinal villi growth in broiler chicks with unhealed navels. *Poult. Sci.*, 89: 82-87. <https://doi.org/10.3382/ps.2009-00284>
- Khan, K., K. Aziz, N.A. Khan, S. Khan and T. Ayasan. 2021. Effect of enzyme and yeast-based feed additives on growth, nutrient digestibility, meat quality and intestinal morphology of fattening rabbits. *J. Hellenic Vet. Med. Soc.*, 72(4): 3511-3518. <https://doi.org/10.12681/jhvms.29404>
- Lampe, J.W., 1999. Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. *Am. J. Clin. Nutr.*, 70: 475-490. <https://doi.org/10.1093/ajcn/70.3.475s>
- Lee, S.H., H.S. Lillehoj, S.I.K. Jang, D.K. Kim, C. Ionescu and D. Bravo. 2010. Effect of dietary Curcuma, capsicum, and lentinus on enhancing local immunity against, *Eimeria* a Cevilian infection. *J. Poult. Sci.*, 47: 89-95. <https://doi.org/10.2141/jpsa.009025>
- Lim, K.S., S.J. You, H.K. An and C.W. Kang. 2006. Effects of dietary garlic powder and copper on cholesterol content and quality characteristics of chicken eggs. *Asian-Australas. J. Anim. Sci.*, 19: 582-586. <https://doi.org/10.5713/ajas.2006.582>
- Mabelebele, M., D. Norris, D. Brown, M.M. Ginindza and J.W. Ngambi. 2017. Breed and sex differences in the gross anatomy, digesta pH and histomorphology of the gastrointestinal tract of gallus gallus domesticus. *Braz. J. Poult. Sci.*, 19(2): 486-490. <https://doi.org/10.1590/1806-9061-2016-0275>
- Mak, H., W. Philip, M.A. Rehman, E.G. Kiarie, T. Edward and M.S. Diarra. 2022. Production systems and important antimicrobial resistant-pathogenic bacteria. *J. Anim. Sci. Biotech.*, 13: 148. <https://doi.org/10.1186/s40104-022-00786-0>
- Marefati, N., V. Ghorani, F. Shakeri, M. Boskabady, F. Kianian, R. Rezaee and M.H. Boskabady. 2021. A review of anti-inflammatory, antioxidant, and immunomodulatory effects of *Allium cepa* and its main constituents. *Pharm Biol.* 59(1): 285-300
- Marković, R., 2009. Effect of different growth promoters on broiler performance and gut morphology. *Arch. Med. Vet.*, 41: 163-169. <https://doi.org/10.4067/S0301-732X2009000200010>
- Masihi, K., 2002. Immunomodulators in infectious diseases: Panoply of possibilities. *Int. Immunopharmacol.*, 22: 1083-1091. [https://doi.org/10.1016/S0192-0561\(00\)00074-6](https://doi.org/10.1016/S0192-0561(00)00074-6)
- Olsen, C.W., A. Karasin and G. Erickson, 2003. Characterization of a swine-like reassortant H1N2 influenza virus isolated from a wild duck in the United States. *Virus Res.*, 93: 115-121. [https://doi.org/10.1016/S0168-1702\(03\)00073-X](https://doi.org/10.1016/S0168-1702(03)00073-X)
- Pourali, M., S.A. Mirghelenj and H. Kermanshahi. 2010. Effects of garlic powder on productive performance and immune.
- Puvaca, N., Stanacev, V. Glamocic, D. Levic, J. Peric, L. Stanacev and D. Milic. 2013. Beneficial effects of phytoadditives in broiler nutrition.

- World's Poult. Sci. J., 69: 27-34. <https://doi.org/10.54865/mjas1331069s>
- Raquel, A., T.S. Lemsaddek, E. Cunha, L. Tavares and M. Oliveira. 2023. Antimicrobial drug resistance in poultry production: Current status and innovative strategies for bacterial control. *Microorganisms*, 11(4): 953. <https://doi.org/10.3390/microorganisms11040953>
- Ritzi, M.M., W. Abdelrahman, K. Van-Heerden, M. Mohnl, N.W. Barrett and R.A. Dalloul. 2016. Combination of probiotics and coccidiosis vaccine enhances protection against an *Eimeria* challenge. *Vet. Res.*, 47: 111. <https://doi.org/10.1186/s13567-016-0397-y>
- Saint-Cyr, M.J., M. Guyard-Nicodème, S. Messaoudi, M. Chemaly, J.M. Cappelier, X. Dousset and N. Haddad. 2016. Recent advances in screening of anti-*Campylobacter* activity in probiotics for use in poultry *Front. Microbiol.* 7: 553. <https://doi.org/10.3389/fmicb.2016.00553>
- Slaoui, M. and L. Fiette. 2011. Histopathology procedures: From tissue sampling to histopathological evaluation. *Clifton, N.J.*, 691: 69-82. https://doi.org/10.1007/978-1-60761-849-2_4
- WHO, World Health Organization, 2015. Global action plan on antimicrobial resistance. Geneva, Switzerland, pp:1–28
- Yakhkeshi, S., S. Rahimi and K.G. Naseri.2011. The effect of comparison of herbal extract, antibiotics, probiotics and organic acids on serum lipids, immune response, git microbial population, intestinal morphology, and performance of broilers. *J. Med. Plant Res.*, 10: 80-95.
- Zulkifli, I., N. Abdulllah, N.M. Azrin and Y.M. Ho. 2000. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and OTC under heat stress conditions. *Br. Poult. Sci.*, 41: 593-597. <https://doi.org/10.1080/713654979>