

# HAEMATOLOGICAL AND IMMUNOLOGICAL EFFECTS OF YOGURT AND GARLIC SUPPLEMENTATIONS ON BROILER CHICKS VACCINATED AGAINST NEWCASTLE DISEASE

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**ABSTRACT:** One hundred twenty-six day old broiler chicks were divided into three experimental groups A, B and C, each having 42 chicks per group. Group B was subdivided into 3 groups; y1, y2 and y3 each having 14 chicks per group and fed 50, 100 and 200 gm yogurt/kg of feed respectively. Similarly group C was subdivided into three groups; g1, g2 and g3 offered grinded garlic @ 10, 20 and 40gm garlic/kg of feed respectively. Group A was kept as a control and fed basal diet having no supplementation. Group y3 showed immense potential in increasing HI titer until last day of experiment but garlic showed no effect upon HI titer. The heterophils population and Heterophils/Lymphocyte (H/L) ratio was also improved in both yogurt and garlic supplemented groups, however g3 garlic fed group showed a better response in increasing heterophil population over the range of garlic fed groups. TLC remained normal in both yogurt and garlic fed groups throughout the experiment. It was concluded that both yogurt and garlic supplementations have beneficial effects to improve immunity.

**Key words:** Yogurt, garlic, immunostimulatory effects, Newcastle disease.

## INTRODUCTION

Yogurt can be used as an effective probiotic. It contains lactobacilli and other beneficial bacteria that have strong positive health effects, aid digestion and inhibit the pathogens by improving the balance of microbes in the digestive tract (Metchnikoff, 1998). Antibody production against Newcastle disease virus (NDV) in a group of broiler chicks treated with probiotic has been reported to be significantly higher 10 days post immunization as compared to untreated group (Khaksefidi and Ghoorchi, 2006). Use of probiotic PrimaLac (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium bifidum*) results in a significantly ( $P<0.05$ ) enhanced broiler performance by improving body weight, decreasing the feed conversion ratio and improvement in antibody responses to Newcastle disease (Talebi *et al.*, 2008). A significant increase in the count of erythrocytes, leukocytes (L), lymphocytes, heterophils (H), H/L ratio and the antibody titer against Sheep red blood cells (SRBC's) was also found in supplementation of biological additives to broiler diet (Riad *et al.*, 2010). Garlic (*Allium sativum*) has several beneficial effects for human and animals, exhibiting antimicrobial, antioxidant, and antihypertensive properties (Konjufca *et al.*, 1997; Sivam 2001). S-allyl cysteine, present in the crushed garlic, was found to enhance the immune response (Sumiyoshi, 1997). The allium species show immune enhancing activities that include promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer cell activity (Kyo *et al.*, 1998). In-water application of a liquid mixture of feed acidifiers, garlic and microbial cell extracts augmented the serological response of chickens to the vaccines prepared from inactivated ND and IBD viruses (Gabor *et al.*, 1998). Keeping in view the above reports, the present study was conducted to evaluate the effect of different concentrations of yogurt and garlic supplementation on haematological and immunological values of broiler chicks.

## MATERIALS AND METHODS

A total of 126 day-old broiler chicks obtained from a commercial hatchery were used in the experiment. Avinew ND vaccine was used and the birds were vaccinated intra-ocularly at day 6 and boosted through drinking water at day 28.

At day 1, 14, 35 and 45 of age 2ml of blood was

collected from all the chicks in a clean and sterilized test tube containing anti-coagulant (1mg/ml blood EDTA) for DLC (Benjamin, 1978) and TLC (Natt and Herrick, 1952). For serum separation blood was collected aseptically from jugular vein on day-1 and from wing vein on days 14, 35 and 45 in non heparinized collecting tubes. The blood was allowed to clot and centrifuged at 3,000rpm at 4°C for 20 minutes to harvest serum. The serum was stored at -20°C till further analysis.

**Statistical Analysis:** The experiment was laid out in accordance to completely randomized design and the data so obtained was subjected to statistical analysis through ANOVA technique using SPSS for windows version 13.0. The differences between the groups were determined with subsequent LSD test to find out the significant difference among various levels of yogurt and garlic.

## RESULTS AND DISCUSSION

In the current study, different graded levels of yogurt and garlic were used to know their effect upon DLC, TLC and HI titer of broiler.

**HI Titer:** The sera collected from chicks in different groups were evaluated for the presence of antibodies by haemagglutination inhibition test. The HI antibody titer continued to increase up to 35th day of experiment and highest titer originated was 170.66 in y3 yogurt group (Table 1). However a significantly higher ( $P<0.05$ ) HI antibody titer was repeated in y3 yogurt group (Table 1) at day 45 over the range of yogurt fed groups when compared with control group. Although all levels of garlic group improved serological response in term of antibody titer but remain statistically insignificant in increasing HI titer. The findings of present study about HI are in close agreement with the study of Apata (2008) who reported that antibody titer against Newcastle disease vaccine showed a curvilinear response in broilers over the range of *Lactobacillus* concentrations examined. Similarly Ryu *et al.*, (2007) reported that ND vaccine titer of birds fed probiotics were significantly higher ( $P<0.05$ ) than control. In current study garlic groups remain statistically insignificant in increasing HI titer which contradict the result of Nidaullah *et al.*, (2010) who found a better immune performance ( $P<0.05$ ) in broilers at day 35 against ND when supplied garlic @ 10 gm/L of drinking water. This difference in results might be due to supplementation forms, strain of vaccine used, species differences, environmental and managerial factors.

**Table 1. Values of HI titer (Mean±SE) for various treatments levels at various days of interval**

Days	Control	Yogurt Groups			Garlic Groups		
		50gm (Y1)	100gm (Y2)	200gm (Y3)	10gm (G1)	20gm (G2)	40gm (G3)
1	10.4±5.36	13.33±4.61	9.33±6.11	16.00±00	10.66±4.61	13.33±4.61	13.33±4.61
14	20.80±10.7 3	26.66±9.23	21.33±9.23	21.33±9.23	21.33±9.23	26.6±9.23	18.66±12.2 2
35	76.80±28.6 2	128±110.85	149.33±97.7 6	170.66±73.9 0	85.33±36.9 5	74.66±48.8 8	85.33±36.9 5
45	64±39.19	85.33±36.9 5	74.66±48.88	149.33±97.7 6	53.33±18.4 7	64±55.42	74.66±48.8 8

## HAEMATOLOGICAL PARAMETERS

**Differential Leukocytic Count (DLC):** Hematological changes were studied in yogurt, garlic and control groups. All treatment levels of garlic and yogurt showed an increase in Heterophil population at different days of interval, however g3 garlic group showed a significant improvement ( $P < 0.05$ ) in Heterophil population over the range of other garlic fed groups (Table 2). Yogurt and garlic groups remained statistically insignificant in increasing Eosinophils, Basophil and Monocyte population both at treatment and levels which resemble with the study of Farinu *et al.*, (2004) who reported that lymphocyte, monocytes, eosinophils and basophils were not affected by the dietary treatments of garlic. However a significant decline ( $P > 0.05$ ) in count of these cells was observed at different days of interval which may be relative decrease, birds individuals response, vaccination stress, interaction of garlic with immune system or the hemolytic constituent in garlic Parsad *et al.*, (2009). No significant increment in Lymphocyte population was

pragmatic in both yogurt and garlic groups (Table 2) which is in line with the study of Farinu *et al.*, (2004). However, a significant decline ( $P > 0.05$ ) in lymphocyte population was observed both in yogurt and garlic fed groups at different days of interval, which may be relative decrease to Heterophil or due to use of live vaccine. Finding of present study about DLC (Table 2) are closely matched with the study of Parsad *et al.*, (2009) who observed that hematological parameters i.e. Differential Leukocyte Count (DLC) were not significantly affected by garlic supplementation in feed and variation in DLC may be due to the interaction of garlic with immune system. Cetin *et al.*, (2005) also investigated that differential leukocyte counts were not affected by dietary MOS and probiotics supplementation. In our findings heterophils count were increased significantly ( $P < 0.05$ ) in g3 group (Table 2) which are perversely to the result of Parsad *et al.*, (2009) and Cetin *et al.*, (2005). The dissimilarity in DLC results may be due to treatment level, supplementation forms, species differences, environmental and mangamental factors.

**Table 2. Values of DLC (Mean±SE) for various treatments levels at various days of intervals**

Parameter	Day	Control	Yogurt Groups			Garlic Groups		
			50gm (Y1)	100gm (Y2)	200gm (Y3)	10gm (G1)	20gm (G2)	40gm (G3)
<b>Heterophils</b>	1	26±1.00	26.33±1.5 2	26±1.00	26±2.00	29±1.00	28.66±1.5	28.33±1.5 2
	14	26.2±1.78	25.33±1.5 2	28±1.00	27.66±1.1 5	27.33±2.0 8	26.66±1.5 2	28.33±1.5 2
	35	25.8±1.30	27.66±1.5 2	29.33±0.5	29.66±2.3	28±2.00	29±2.00	32.00±1.0 0
	45	25.8±1.48	25.66±2.0 8	30.33±1.5	31±1.00	29±2.00	31.33±1.5 2	33.33±2.0 8
<b>Eosinophils</b>	1	5.8±0.83	6.66±0.57	5.66±1.52	5.66±0.57	6.00±1.00	5.33±1.52	5.66±1.52
	14	6.00±1.00	5.33±0.57	6.66±0.57	5.33±0.57	5.33±1.52	6.00±1.00	5.33±0.57
	35	6.00±1.00	5.66±1.15	5.00±1.00	6.66±1.52	5.33±0.57	6.66±1.15	5.33±0.57
	45	6.2±0.83	6.00±1.00	5.33±0.57	5.33±0.57	6.66±1.15	5.00±1.00	4.33±0.57
<b>Basophils</b>	1	1.2±0.83	1.00±1.00	1.33±0.57	1.33±0.57	1.33±0.57	1.00±.00	1.33±0.57
	14	1.4±0.54	1.33±0.57	1.33±0.57	1.33±0.57	1.00±.00	1.66±0.57	1.33±0.57
	35	1.00±0.70	1.00±.00	1.00±.00	1.33±0.57	1.33±0.57	1.00±.00	0.66±0.57
	45	1.2±0.44	1.66±0.57	0.66±0.57	1.66±0.57	1.66±0.57	1.00±.00	1.33±0.57
<b>Lymphocytes</b>	1	66.2±0.83	65.00±2.6 4	67.00±1.7	66.33±1.5 2	63.00±1.00	64.33±0.57	64.00±1.0 0
	14	65.4±3.13	67.00±2.0 0	63.33±1.5	64.66±1.1 5	65.33±3.2 1	65.00±2.0 0	64.00±1.7 3
	35	66.2±1.30	64.66±2.0 8	64±1.73	61.6±1.52	64.6±2.08	64.3±1.52	61.00±1.0 0
	45	65.4±2.50	67.00±4.3 5	63±1.00	61.00±1.0 0	61.66±0.5 7	62.00±1.0 0	60.33±0.5 7
<b>Monocytes</b>	1	1.00±0.70	1.00±.00	0.66±0.57	0.66±0.57	0.66±0.57	0.66±0.57	0.66±0.57
	14	1.00±0.70	1.00±1.00	0.66±0.57	1.00±.00	1.00±.00	0.66±0.57	1.00±.00
	35	1.40±0.54	1.00±.00	0.66±0.57	0.66±0.57	0.66±0.57	0.66±0.57	1.00±.00
	45	1.40±0.54	1.66±0.57	0.66±0.57	1.00±.00	1.00±.00	0.66±0.57	0.66±.57

**Total Leukocytic Count (TLC):** In our experiment no significant ( $P < 0.05$ ) increment in TLC was observed in yogurt and garlic fed groups and also among their various treatment levels (Table 3) when compared with control group. Our finding are in line with the results of Parsad *et al.*, (2009) who reported that Total Leukocyte Count were not significantly affected by garlic supplementation in feed. Cetin *et al.*, (2005) also investigated that Total Leukocyte Count were not significantly affected by

dietary MOS and probiotics supplementation. This alliance in TLC results may be due to same technique used for leukocyte count in same species.

In conclusion, yogurt as a probiotic and garlic as a immunostimulant agent displayed an efficacy in increasing HI titer and heterophils population. The yogurt group y3 and garlic group g3 showed an immense potential in improving above traits, so their use in broiler diet is recommended for healthy and profitable farming.

**Table 3. Values of TLC/mm<sup>3</sup> (Mean±SE) for various treatments levels at various days of interval**

Day	Control	Yogurt Groups			Garlic Groups		
		50gm (Y1)	100gm (Y2)	200gm (Y3)	10gm (G1)	20gm(G2)	40gm (G3)
1	28218±2677	28883±2225	30266±4379	29626±2798	30366±2798	30366±1699	31850±1695
14	28602±3784	29626±4626	31846±3573	30736±5481	29896±4174	31106±3335	30740±1281
35	30440±4202	31850±1695	30366±5251	29996±2942	29253±5010	30366±1699	28043±2483
45	30884±3183	30266±4652	32216±2942	28786±3112	29896±4595	30636±4795	27030±2310

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