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Blood Chemistry, Liver Function and Serum Glucose Profiles of Broilers Health offered Mixed Herbal Recipes

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Abstract

A research trial was conducted on broiler health to explore the potential role of water based extract from Garlic (*Allium sativum*), Ginger *(Zingiber officinale),* Neem *(Azadirachta indica)* and Barberry *(Berberis lycium).* Their effect was studied upon blood chemistry, liver enzymes and serum glucose level. A total of 240 broilers were randomly allocated into four groups i.e. A, B, C and D. Group A was kept as control, whereas B, C and D received herbal recipes of Garlic, Ginger, Neem and Barberry @ 2, 4, 1 and 8gm / liter of water, 3, 5, 2 and 9gm / liter of water and 4, 6, 3 and 10 gm / liter of water, respectively for 35 days. Blood Chemistry (*Leukocyte*, *Lymphocyte* and *Neutrophil*) showed better response ($P<0.05$) in group B and D, respectively. Serum glucose profiles and hepatic enzymes i.e.; *Alanine Aminotransferase* (ALT), *Alkaline Phosphatase* (ALP) and *Aspartate Aminotransferase* (AST) across the treatment groups remained same. Recipe D did not influence serum glucose

levels and hepatic parameters, but it distinctly affects the concentrations of leukocytes, lymphocytes, and neutrophils in the blood.

Keywords: Herbal recipes, Broilers Health, Haematology, Liver Function Tests (LFT), Serum glucose

Introduction

Herbal recipes are an effective and ancient remedy to many health problems in humans and poultry (Jamil et al., 2022). Among the available herbs, Garlic (*Allium sativum*) is used as diuretic, an emmenagogue and for the treatment of stomach chills, flatulence and colic (Amtaghri et al., 2024). The Ginger *(Zingiber officinale)* screened effectively to reduce blood glucose level (Ghoreishi et al., 2024). Whereas, the combination of both Garlic and Ginger mixed feeding boosted the numbers of white blood cell and Neutrophil (Iheanacho et al., 2024). The plant *Azadirechta Indica,* commonly known as Neem posses an array of complex compounds called “Limonoids" which confined to its bitter taste and carry anti-cancerous, anti-malarial, antibacterial, anti-HIV efficiencies and also capable for the hepatoprotective activity (Roy and Saraf, 2006). The Berberine is a well known active alkaloid usually extracted from *Berberis lycium* (Iqbal et al., 2025), possess a wide range of pharmacological activities, such as vasodilatory and antiarrhythmic activity (Behl et al., 2022), preventive and curative role in hepatotoxicity (Ahmad et al., 2024) reduce oxidative stress in the liver (Naz et al., 2022) and also possess blood glucose lowering potential (Xia et al., 2022).

In the light of the aforementioned important roles of these plants, present experiment have been designed to determine the combined efficacy of aqueous extract from Garlic, Ginger, Neem and Barberry in different proportions and investigate their effect on blood cell count, liver performance and serum glucose in broilers.

2. Materials and Methods

The trial was carried out in an open sided conventionally covered and deep littered poultry house for a period of 35 days.

Experimental design

The experiment was carried out in Randomized Completely Block Design (RCBD). A total of 240 Hubbard strains of broilers were divided into four groups (A, B, C and D). Group A was control while group B, C and D received aqueous extract of four plants i.e. Garlic, Ginger, Neem and Barberry @ 2, 4, 1 and 8gm / liter of water, 3, 5, 2 and 9gm / liter of water and 4, 6, 3 and 10 gm / liter of water, respectively for 35 days. Feeds were commercially prepared as per NRC 1994 formulation (Table 1). Both feed and the herbal extract were offered ad libitum.

Table.1: Ingredients and composition of basal diet

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

Preparation of aqueous extract mixture

Fresh Garlic bulbs, Ginger rhizomes, Neem leaves and root-bark of Barberry were purchased from local wet market. The required grams for each recipe were crushed through metallic grinder, poured with one liter of hot boiling water and kept at room temperature overnight (Liela, 1977; Nidaullah f 2010).

Blood samples collection

At the trial termination, six birds were randomly selected from each group and blood samples (5 - 6 ml) were collected in EDTA coated vacutainers. Blood parameter including, Packed Cell Volume (PCV) was determined using plain capillary micro-hematocrit tubes (75 mm x 1.0 mm) (Benjamin, 1981). Hemoglobin concentration by Sahli's method (Benjamin, 1981) and Leukocytes were counted by hemocytometeric method (Benjamin, 1981). Differential Leukocytes Count (DLC) was carried out as per protocol described by Benjamin (1981). For this purpose, blood smears were prepared using a drop of fresh blood, air dried and fixed with methanol. The fixed smears were stained with Giemsa stain and studied under microscope for leukocytic count. Serum glucose and hepatic enzymes were determined using digital chemistry analyzer (Hitachi 902 Japan).

Data was statistically analyzed by standard procedure of analysis of variance (two-factorial) using Randomized Completely Block Design (RCBD) described by Steel and Torie (1996). The statistical package (SPSS, 2000) used and Duncan's multiple range tests was applied for the separation of means, where significant differences were noted among them.

3. Results and Discussion

Findings pertaining to blood cells performance, liver enzymes, and serum glucose levels are presented under various sections as follows.

Blood Chemistry

Mean (±SE) Packed Cell Volume (PCV %) was 19.33 (±2.08), 18.33 (±1.36), 20.17 (±1.70) and 23.17 (±1.49) for A, B, C and D groups respectively (Table 2). The obtained levels of PCV % were not framed within the normal reference range as provided by Bounous and Stedman (2000), Kokore et al., (2021) and Alagbe et al., (2023). In our findings, though group D had higher numerical values yet birds under all treatments behaved alike for PCV %. Our recipes consisted a combination of four herbs, however both garlic and ginger given in powder form showed better PCV% in poultry birds (Abd El-Ghany, 2024). But without any response by feed-mixed Garlic (Jimoh et al., 2012) or feed-mixed Ginger (Zomrawii et al., 2012) yet significant improvement by feed-mixed Neem (Ansari et al., 2012) for the subject trait in broilers, which in our case did not clicked by Neem plant in all the three recipes. Even so, Ademola et al., (2009) offered Garlic and Ginger individually and Garlic-Ginger mixed ration in broilers without any better yield in PCV % concentrations. Besides, the mixed-extract of Garlic, Barberry with other herbs together enhanced PCV% in broilers (Javed et al., 2012). Since both Garlic and Neem as individual plant proved to improve PCV% but such potential role were antagonized when mixed together with Ginger and Barberry plants in our recipes.

Mean (±SE) Hemoglobin (Hb) concentrations for group A, B, C and D were 7.42 (±0.70), 7.08 (±0.44), 7.50 (±0.32), 7.92 (±0.30) g/dl respectively (Table 2). Non significant differences were found among experimental groups. However, group D had slightly better concentration of Hb, probably because they received higher ratios of medicinal herbs in the extract. The obtained Hb concentrations across the experimental groups were within the normal blood physiology of chicken as indexed by Horhoruw and Kewilaa (2024). The lack of any enriched performance may probably be due to the existence of hemolytic compounds and the concerned metabolites in Garlic (Iheanacho et al., 2024). A similar findings also been observed by Dolle et al., (2020) whom used Garlic and Ginger separately and in combination as well. Similarly, Zomrawii et al., (2012) and Martha et al., (2012) offered Ginger mixed feed, while Onu (2010) offered feed mixed Garlic and Ginger, both separately and the mixed amalgam but all were concluded without any distinct improvement in Hb concentrations. Fatema et al., (2014) used three herbs (Neem, Ginger and Black pepper) but without any change in Hb concentrations. From the discussed literature, It is apparent that, Garlic, Ginger and Neem single applications or mixed amalgams in feed or water extract, have no impact on the blood Hb concentration, however the Barberry plant, if included in certain herbal recipes, possess the potential to improve Hb level in broilers (Ur-Rehman et al., 2011). Yet, we did not find any study in which Barberry plant administered singly (feed-mixed or extracted) and investigated its impact on Hb concentration.

Mean (±SE) of Total Leukocytes Count (TLC) for group A, B, C and D was 5166.67 (±781.74), 27500.00 (±683.13), 24666.67 (±1122.99) and 23666.67 (±614.64) cu / mm, respectively (Table. 2). The TLC in this study were within the normal reference range as stated by Jian (1993) and Simaraks et al., (2004). Significant differences among groups were recorded. Group B showed better TLC compared with other groups. Our results are in line with Ademola et al., (2004) using Garlic and Ginger in broilers. However, Gowda et al., (1998) used Neem, while Elagib et al., (2013) used Garlic mixed feedings but both failed to observe any variations in TLC in birds, which contradicted our findings. Individual feedings of Garlic and Ginger by Micheal et al., (2009) caused decrease in TLC by former and an increase by latter herb. Surprisingly, Obikaonu et al., (2011) noted a negative impact of Neem but high concentrations were consistent with control group. The Barberry plant contains Berberine, which improve leukocytes in blood (Khushdil et al., 2012). Our findings supported by this statement and we concluded that the plant Barberry if offered in combination with Garlic, Ginger and Neem to the broilers would positively stimulate the leukocytes which ultimately improve the immune performance of broilers (Nidaullah et al., 2010).

Mean (±SE) count of Neutrophils for groups A, B, C and D were 28.17 (±1.01), 26.33 (±1.56), 25.83 (±1.04) and 30.67 (±1.17), respectively (Table 3). Group D given better percentage of Neutrophil, followed by group A, B and C respectively. All the obtained values were framed within the normal referenced range as suggested by Jian (1993), Ghergariu et al., (2000) and Simaraks et al., (2004). A likelihood performance also perceived by Ademola et al., (2004) while feedings Garlic and Ginger to broilers and Elagib et al., (2013) using

Garlic supplemented feed in broilers production. Moreover, Esonu et al., (2006) observed improved Neutrophils while feeding Neem mixed rations to layers. Micheal et al., (2009) reported that Garlic reduces Neutrophils but Ginger gives better Neutrophils number in rats. Neem plant also improved Neutrophils in broilers (Obun et al., 2013) which supported our work. Further, Neem leaves posses certain active ingredients (Nagano et al., 2021) for example *Azadirachtin* which modifies blood physiology (Bui et al., 2009) But the usage is solely depend on the mode of application. The extract of four plants (Garlic, Ginger, Neem and Barberry) in our recipes positively correlated to express an agonistic effect which ultimately improved Neutrophils in broilers.

Mean (±SE) Lymphocyte count in groups A, B, C and D were 60.33 (±2.17) %, 66.50 (±2.01) %, 65.00 (±2.16) % and 57.83 (±1.40) %, respectively (Table 3) and have been affected by the respective treatment ($P<0.05$). The observations were within the normal reference range across the studied groups as compared with Jian (1993), Ghergariu et al., (2000), Simaraks et al., (2004) and Trinca et al., (2012). Our findings are in line with Jafari et al., (2008) whom fed Garlic mixed ration to broilers. Also, Iranloye et al., (2002) supported our observations by feedings Garlic to broilers. Najafi et al., (2014) and Micheal et al., (2009) failed to notice any apparent variations in Lymphocytes, offered Ginger to broiler. Esonu et al., (2006) suggested that a low quantity of Neem in feed have no impact on Lymphocyte in layers, however an increased ratio of Neem in feed i.e. 10% or more, cause negative job and reduced lymphocytes in blood. However this trait (lymphocyte) in our study was potentially affected by our suggested recipes, fenced the negative impact of Neem plant and protagonisted Garlic to improve Lymphocyte concentrations across the studied groups.

Mean (±SE) Monocyte counts were 4.50 (±0.67), 3.17 (±0.48), 3.50 (±0.56) and 4.67 (±0.49) percent for group A, B, C and D respectively (Table 3) and was same across all treatment groups ($P>0.05$). All the values were within the normal reference range as suggested by Simaraks et al., (2004) and Trinca et al., (2012). A likelihood finding was also concluded by Ademola et al., (2004) using Garlic in broiler ration. But, Samson et al., (2012) offered the aqueous extract of Garlic with a significant improvement in Monocytes and opposed our results. Further, the feed mixed Garlic has no role to improve Monocytes concentration in broilers (Elagib et al., 2013). Further, Daniel et al., (2012) used both Garlic and Ginger in equal ratio and perceived significant variations in Monocytes. Moreover, Bui et al., (2009) infused Neem plant extract and got better Moncyte concentrations and opposed our study. It is apparent from the discussed literature, that both Garlic and Neem carry the potential to boost Monocytes which solely based upon their individual extract used in broilers than mixed with feed. However, their individual potencies were antagonized when individual extract was mixed with other herbal extracts like Ginger (Ademola et al., 2004 and Daniel et al., 2012), Onion (Samson et al., 2012) or Barberry in our present study.

The mean percent Eosinophil in group A, B, C and D were 7.00 (±1.46), 4.00 (±0.45), 5.67 (±1.26) and 6.83 (±0.48), respectively. The obtained values were within the normal reference range reported by Simaraks et al., (2004). Treatment did not affect the Eosinophilic percentage. However, numerically better Eosinophilic percentage was found in group A while a lower percentage in group B. Our findings were supported by Elagib et al., (2013) who offered Garlic mixed feed to broilers without any significant improvement in Eosinophils. Likely, Ademola et al., (2004) and Micheal et al., (2009) offered Garlic and Ginger individually or mixed together, during broiler production but failed to get any significant variations in Eosinophils. Moreover, species like rats, also exhibited similar findings as our's, when offered Garlic juice (Iranloye et al., 2002). The plant Neem given to poultry in different concentrations also had no influence on Eosinophilic performance (Esonu et al., 2006). Contrarily, Bui et al., (2009) noticed significant increase in Eosinphilic percentages with increasing dosage of Neem aqueous extract in broilers. However in our study, the mixed extract of Neem with other herbs like Garlic, Ginger and Barberry masked the respective potency of Neem plant to improve Eosinophils in broilers.

Table 2: Haematological findings of the experimental group A, B, C and D

Groups	**PCV %**	**HB g/dl**	**TLC cu/mm**
A	19.33±2.08	7.42±0.70	25166.67^{ab} ± 781.74
B	18.33±1.36	7.08±0.44	27500.00^{b} ± 683.13
C	20.17±1.70	7.50±0.32	24666.67^{a}±1122.99
D	23.17±1.49	7.92±0.30	23666.67^{a} ± 614.64
Significance level	NS	NS	***

*** = Significant (P<0.05), NS= Non-significant. Values bearing different superscript are significantly different.

Table 3: Blood cells observations of the experimental broilers.

Groups	Neutrophile %	Lymphocyte %	Monocyte %	Eosinophil %
A	28.17^{ab} ±1.01)	60.33^{ab} ±2.17	4.500±0.67	7.00±1.46
B	2 6.33^{a}±1.56	66.50^{c} ±2.01	3.17±0.48	4.00±0.45
C	25.83^{a} ±1.04	65.00^{bc} ±2.16	3.50±0.56	5.67±1.26
D	30.67^{b} ±1.17	57.83^{a} ±1.40	4.67±0.49	6.83±0.48
Significance Level	***	***	NS	NS

*** = Significant ($P<0.05$), NS= Non-significant. Values bearing different superscript are significantly different.

Serum Glucose

Mean (±SE) serum Glucose levels were 171.67 (±17.2), 170.83 (±11.85), 164.67 (±21.10) and 170.00 (±14.65) $mgdl^{-1}$ for group A, B, C and D respectively (Table 4). Our findings for the studied parameter were within the normal reference range as described by Simaraks et al., (2004) and Hernawan et al., (2012). A likelihood judgment have also been reported by Horton et al., (1991) and Mansoub et al., (2011) by feeding Garlic mixed rations to experimental broiler and perceived similar Glucose level across the treated groups. Also, Saeid et al., (2010) found the same response like our's when infused Ginger extract orally in different ratios to broilers. A decrease in serum Glucose level was observed by mixing Ginger in broilers rations (Mohamed et al., 2012), however, Zomrawii et al., (2012) supported our findings with a constant Glucose levels among the experimental groups upon subjecting birds to Ginger supplemented feed. Feeding Neem to laying hens exhibited a constant Glucose level across experimental groups (Gowda et al., 1998) but obikaonu et al., (2011) suggested that a lower concentration of Neem in broiler production significantly enhance serum Glucose, which contradicted our results. Nnenna et al., (2013) confirmed the aqueous extract of Neem leaves possess a potential Glucose lowering efficacy in broilers. The plants Barberry blessed with a Glucose lowering properties (Sood et al., 2012 and Iqbal et al., 2025). Further, the presence of tannin compounds and alkaloids usually stimulate the antidiabetic role inside Barberry (Liu et al., 2005). A range of extracts of Barberry i.e; water, methanol and chloroform based, have been studied by Ahmed et al., (2009) and found an extended Glucose lowering potential in rabbits. They further concluded that antidibetic compounds are maximally extracted in water than those of methanol and chloroform. In our study we gained a constant range of Glucose, here the antidibetic potential of Barberry in experimental birds have been masked by the member plants in each respective recipe. A similar and unaltered pattern of serum Glucose in this experiment and the discussed literature without any significant variation have been explained by Liukkonen-Anttila (2000) that the birds usually maintain a relatively constant blood sugar level even in low feed intake.

Liver Function Tests

Mean (±SE) ALT values for A, B, C and D were 22.67 (±5.00), 26.00 (±7.74), 26.50 (±3.85) and 22.67 (±2.61) UL^{-1} respectively and was non-significant among groups (Table 4). The mean (±SE) values of ALP were 6.67 (±6.67), 0.00 (±0.00), 1.67 (±1.67) and 5.00 (±3.42) UL^{-1} for group A, B, C and D respectively (Table 4) and was not affected by the treatments. The third hepatic enzyme was AST, for which the mean (±SE) values were 2010.00 (±73.89), 2033.33 (±159.22), 2075.00 (±81.84) and 1896.66 (±84.49) UL^{-1} for group A, B, C and D respectively. Overall, non-significant variations observed among the four groups. During hepatic damage, the cells lining liver parenchyma dies and a mark increase occur in liver enzymes especially ALP in the bile duct (Sallie et al., 1991 and Jawad et al., 2014). The AST, also called as Serum Glutamic Oxaloacetic Transaminase (SGOT), increase in blood when an injury to hepatic and cardiac cells occur. Usually, a marked increase of AST exhibit hepatic tumor, renal or pulmonary problems. Testing AST is likely more helpful than ALT to perceive hepatocytes problems (Jawad et al., 2014). Chattopadhyay et al., (2005) and Kashaw et al., (2011) challenged experimental rats with lead (Pb) and observed a significant decrease in ALT and ALP level upon treating the effected rats with Garlic. Similarly, Jimoh et al., (2012) used Garlic supplemented feed with a reduced hepatic AST and ALT concentration in plasma. Likely to our findings, Dieumou et al., (2009) confirmed that both the Garlic and Ginger oils carry non significant role in the activities of hepatic AST and ALT. Contrary to us, a significant increase in hepatic ALP recorded by Martha et al., (2012) who offered Ginger mixed ration to broilers. Supplementing Ginger powder in bird's rations confirmed a significant decrease in hepatic AST, ALT (Malekizadeh et al., 2012). A better performance of liver enzymes (ALP and AST) have been observed by Jawad et al., (2014) as they increased Neem leaf concentration in ration, without any toxicity in liver parenchyma of the birds. It is clear from the discussed literature that Garlic plays a vital role to stabilize hepatic enzymes in blood and enhance liver performance. Nevertheless, Ginger posses a complex impact, if mixed with Garlic cause to reduce its efficacy in the form of liver performance. However, Neem plant has no impact on liver enzymes but Barberry in combination with Garlic, Ginger and Aloevera improved liver performance (Ur-rehman et al., 2011). Our findings for AST, ALP and ALT were similar across the control and experimental groups, which clarifies that the mixed recipes of Garlic, Ginger, Neem and Baberry carried non harmful affect to damage liver or interrupt the hepatocytes performance in the term of ALT, ALP and AST enzymes in broiler productions.

Table.4: Serological values (Glucose level, and LFT) of the experimental broilers.

Group	Glucose mg/dl	Liver Function Tests (LFT)		
		ALT u/l	ALP u/l	AST u/l
A	171.67±17.20	22.67±5.00	6.67± 6.67	2010.00±73.89
B	170.83±11.85	26.00±7.74	0.00±0.00	2033.33±159.22
C	164.67±21.10	26.50±3.85	1.67±1.67	2075.00±81.84
D	170.00±14.25	22.67±2.61	5.00±3.42	1896.66±84.49
Level of significance	NS	NS	NS	NS

*** = Significant (P<0.05), NS= Non Significant. Values bearing different superscript are significantly different.

4. Conclusions

Present study suggests aqueous extract inclusion of Garlic bulb, Ginger rhizomes, Neem leaves and Barberry stem barks mixed in 2, 4, 1 and 8 gm / liter of water is an excellent recipe to boost up *Leukocytes* and *Lymphocytes*, whereas, the inclusion of 4, 6, 3, 10 gram per liter of water possess excellent potential to stimulate *Neutrophils* in broilers production. This was a baseline study to explore the combined efficacy of mixed herbals extract based on their individual potential, mixed together and screened. Furthermore, a mixed ethonolic/methanolic extracts of these recipes are suggested with various amalgamation, dosage rates and multiple feeding schedules in broilers performance.

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