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<u>Research Article</u>



EFFECT OF AQUEOUS HERBAL EXTRACTS, PROBIOTICS AND SIGANUS LURIDUS (UNANI DRUG) ON GROWTH PERFORMANCE AND RENAL FUNCTIONS OF BROILER CHICKS

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ABSTRACT:

Aim/Purpose: The aim of this research was to assess the influence of water-based herbal infusions, beneficial bacteria, and SIGANUS LURIDUS on indicators of liver and kidney function in broiler chickens.

Methods: We organized 105-day-old broiler chicks into seven equal groupings, labeled A through G. Each of the initial six groups received a unique water-based herbal infusion alongside their fundamental diet. Aloe vera, Ginger, Siganus luridus, Garlic, Probiotic (Dahi), and Turmeric were given to Groups A through F, correspondingly. Group G served as a control group and was only provided with the basic diet. Every group, comprised of fifteen chicks subdivided into three sets of five, was given their specific herbal infusion (0.2%) two times daily from the 11th day till the conclusion of the study on the 39th day. Serum samples were gathered on the 18th, 25th, 32nd, and 39th days.

Results: The study revealed that ginger (Zingiber officinale) considerably influenced the normalization of urea levels compared to other herbs, signifying that ginger (Zingiber officinale) significantly enhanced kidney function. The Feed conversion ratio was also significantly different for chicks consuming ginger (Zingiber officinale) compared to other groups. The effect of other herbal infusions and probiotics on the serum level of urea in broiler chicks was not significant. Summarizing, the results of this study exhibit a significant relationship between the use of ginger (Zingiber officinale) and improved kidney and liver functions **KEY WORDS**

KEY WORDS

Herbal extracts, Zingiber officinale, urea, kidney function, Feed Conversion Ratio (FCR)



1 | INTRODUCTION

The unchecked inclusion of antibiotics as growth promoters and additives in animal feed has been well-documented, which poses a threat of cross-resistance and multiple resistances among gut pathogens¹. Due to these potential adverse impacts, the European Union has implemented restrictions on the majority of antibiotics in poultry feed. The search for advantageous feed-additives such as probiotics, prebiotics, enzymes, and plant extracts has recently intensified^{2 3 4}. The use of herbs as therapeutic agents in poultry is gaining traction. A growing trend is the incorporation of aromatic plants and their extracts into animal feed⁵. Certain herbs or extracts from them can enhance food intake, stimulate secretion of digestive juices, and bolster immunity in animals⁵. The antibacterial properties of essential oils obtained from herbs, spices, or their purified formulations have been observed⁶. The addition of these herbal essential oils in poultry feed or water can lead to improved feed conversion and weight gain⁷, and also presents antibacterial⁸⁻⁹ and antioxidant benefits¹⁰⁻¹¹. By stimulating the endogenous enzymes and improving nitrogen absorption, the Feed Conversion Ratio (FCR) of poultry can also be enhanced¹² The incorporation of alfalfa, thyme or garlic in broiler feed can lower cholesterol and lipoprotein levels in their meat¹⁴⁻¹⁵. Commercially available herbal products like mentofin, Siganus luridus (union drug), and Suduri have showcased antibacterial activity against urease-producing intestinal bacteria found in poultry and can potentially enhance immunity by reducing ammonia production on farms¹⁶. Given the encouraging outcomes from these herb-based applications, this project aims to investigate the influence of probiotics and plant-based medicinal extracts on the serum biochemistry of broiler chicks. Ginger (Zingiber officinale Roscoe) is a widely used herbal supplement whose significant use for various medical remedies has been established. Ajith et al.,¹⁷ conducted a study to assess ginger (Zingiber officinale) 's protective effect against cisplatin-induced oxidative stress and acute renal failure in mice kidneys. Hence, this study seeks to investigate the impacts of aqueous herbal extracts, probiotics, and SIGANUS LURIDUS (unani drug) on the liver marker enzymes and renal biomarkers in broiler chickens.

2 | MATERIAL AND METHODS

The process of creating water-based infusions is detailed herein Separately, the fresh leaves of the Aloe vera plant, Zingiber officinale (Zingiber officinale) rhizomes, garlic bulbs, and dehydrated turmeric powder (100 grams each) were individually blended in 50 milliliters of distilled water, left to soak overnight and then run through a centrifuge. The resulting supernatant was used to create an aqueous extract¹⁸. Aqueous extract stock solutions of 10% were prepared and stored. The broiler chicks received 0.2% dilutions of these extracts twice daily from the 11th day until the study's conclusion. Bi-weekly, we produced stock solutions of Probiotic (Dahi) at a 10% concentration, and we prepared SIGANUS LURIDUS (Unani drug) stock solutions on a weekly basis. Both types of solutions were made using water that had been thoroughly filtered. In broilers, we collected blood samples through the method of puncturing the ulnar vein. For this process, we employed plastic syringes with a capacity of 5 ml and needles measuring 25 x 7 mm.

Blood serum samples from SB- broilers (Hubbard classic breed) were collected weekly, specifically on days 18 (G1), 25 (G2), 32 (G3), and 39 (G4). The broilers were provided with a diet that was a combination of corn and soybean meal, meeting the nutritional recommendations. Commercial Cromatest kits from Linear Chemicals S.L. Spain were used to ascertain the serum levels of AST, ALT, ALP, Bilirubin, Creatinine, and Urea, using the standard method outlined by the German society for clinical chemistry (5). Spectrophotometry was used to read the samples (Chemistry Analyzer, Metro Lab, Model: 1600 plus), with each test having a specific wave length. The data that was studied underwent examination using ANOVA and Duncan's multiple range tests, with the

assistance of the SPSS statistical program, specifically version 15.0. The constituents of the serum were articulated as mean values and standard deviations. The statistical analysis involved conducting comparisons of means through the utilization of Duncan's multiple range test, with a significance threshold set at 5%.

2.1 | PRINCIPLE

Alkaline phosphatase (ALP) plays a pivotal role in the disintegration of 4-nitrophenylphosphate (4-NPP), which leads to the formation of two by-products: 4-nitrophenol and inorganic phosphate. This process takes place in an alkaline buffer that absorbs the phosphate group. By observing the reaction at 405 nm, one can track the rate at which 4-nitrophenol is formed, which is directly related to the ALP activity in the given specimen. 4-Nitrophenylphosphate + H2O \rightarrow 4-Nitrophenol + Pi. The test has been developed in accordance with the standardized methodology outlined by DGKC.1



2.2 | CLINICAL SIGNIFICANCE

Serum ALP levels are particularly relevant when examining two categories of conditions: diseases of the bone and hepatobiliary disorders. Paget's disease and osteogenic bone cancer patients exhibit the highest ALP levels among bone illnesses, while osteomalacia and rickets display moderate increases – the latter normalizing upon vitamin D treatment. ALP in serum naturally elevates during children's bone growth and can also temporarily spike during the healing of bone fractures. Conditions such as cretinism, vitamin D deficiency, and hypophosphatasia, a genetic bone disorder, can cause a decrease in plasma ALP levels. The liver reacts to any bile duct obstruction by producing more ALP. An increase in serum ALP can occur due to intrahepatic bile flow obstruction from cancer invasion or medication. Any drug that is harmful to the liver or induces cholestasis can significantly raise serum ALP levels. This has been observed with over 200 drugs in vulnerable patients.

3 | RESULTS

Table-I distinctly reveals a statistically significant effect (p <0.05) of Zingiber officinale on broilers' creatinine serum level on the 32nd day with a value of 0.8 ± 0.1 . The control group, which was not supplemented with any herbal extracts, showed a noticeable variation in the creatinine serum level (0.20 ± 05) on day 18. On the other hand, According to the data presented in Table-I, there was no statistically significant impact of Aloe vera on the serum level of creatinine in broilers (p > 0.05) on the 18th (0.6 ± 0.3), 25th (0.3 ± 0.05), and 39th (0.4 ± 0.1) days. Throughout the duration of the trial, the effects of Aloe vera, Siganus luridus (Unani drug), Allium sativum, Probiotic, and Turmeric were not statistically substantial. The control group, which had a basal diet devoid of any herbal extracts, exhibited consistent values throughout the duration of the study, as seen in Table-I and II.

Table 1The Impact of Aqueous Herbal Extracts on Serum Creatinine level (measured in milligrams per one hundred milliliters) in broilers

S. No	Aqueous Extract	18 th day	25 th day	32 nd day	39 th day
1	Aloe Vera	0.3, 0.2, 0.4	0.4, 0.4, 0.3	0.3, 0.5, 0.1	0.3, 0.6, 0.4
		(0.3±0.1) ^a	(0.36±0.05) ^a	(0.3±0.2) ^a	(0.4±0.1) ^a
2	Zingiber officinale	0.2, 0.8, 0.9	0.3, 0.4, 0.3	0.8, 1.0, 0.7	0.4, 0.3, 0.6
2		(0.6±0.3) ^a	(0.33±0.05) ^a	(0.8±0.1) ^b	(0.4±0.1) ^a
3	Garlic (Allium	0.2, 0.3, 0.5	0.3, 0.4, 0.5	0.3, 0.6, 0.8	0.6, 0.5, 0.4
	sativum)	(0.3±0.1) ^a	(0.40±0.10) ^a	$(0.5\pm0.2)^{a,b}$	(0.5±0.1) ^a
4	Turmeric	0.5, 0.2, 0.3	0.3, 0.4, 0.3	0.3, 0.3, 0.8	0.4, 0.7, 0.9
		(0.3±0.1) ^a	(0.33±0.05) ^a	$(0.4\pm0.2)^{a,b}$	(0.6±0.2) ^a
5	Control	0.3, 0.2, 0.3	0.3, 0.4, 0.4	0.8, 0.3, 0.5	0.5, 0.3, 0.9
		(0.2±0.05) ^a	(0.36±0.05) ^a	(0.5±0.2) ^{a,b}	(0.5±0.3) ^a

Values enclosed in parentheses in each column represent the mean and standard deviation of the creatinine concentration in the serum of broilers. Distinct superscripts within the same columns in parentheses indicate a significant difference, with a probability level less than 0.05 (p < 0.05).

Table 2 Effect of Probiotic and Siganus luridus (Unani drug) on the Serum Level of Creatinine (mg/100 mls) in broilers

S.No.	Aqueous Extract	18 th day	25 th day	32 nd day	39 th day
1	Safi Siganus	0.7, 0.2, 0.8	0.3, 0.4, 0.4	0.7, 0.5, 0.4	0.3, 0.9, 0.7
	luridus	(0.5±0.3) ^a	(0.36±0.05) ^a	$(0.5\pm0.1)^{a.b}$	(0.6±0.3) ^a
2	Probiotic	0.7, 0.2, 0.3	0.3, 0.3, 0.4	0.6, 0.4, 0.5	0.9, 0.5, 0.4
		(0.4±0.2) ^a	(0.33±0.05) ^a	$(0.5\pm0.1)^{a,b}$	(0.6±0.2) ^a
3	Control	0.3, 0.2, 0.3	0.3, 0.4, 0.4	0.8, 0.3, 0.5	0.5, 0.3, 0.9
		(0.2±0.05) ^a	$(0.36\pm0.05)^{a}$	$(0.5\pm0.2)^{a,b}$	(0.5±0.3) ^a

Data values in identical columns that bear unique superscripts exhibit significant differences, with a probability value less than 0.05 considered statistically significant. The numbers enclosed within brackets in every column represent the average Creatinine concentration in the serum of broilers, accompanied by the standard deviation.



3.1 | UREA

The impact of Aloe vera on the serum Urea levels of broilers was shown to be statistically significant (p < 0.05) on the 18th day, as presented in Table III. Significant variations in values were noted on the 25th day (0.8 ± 0.4) among the control group, which exclusively consumed the basal diet devoid of any herbal extracts. Table III presents the findings on the effect of Aloe vera on the serum Urea levels of broilers. The statistical analysis indicates that there was no significant impact observed (p > 0.05) on the 25th day (3.5 ± 0.6), 32nd day (3.6 ± 0.5), and 39th day (2.5 ± 0.4). The impact of Zingiber officinale, Siganus luridus (Unani drug), Allium sativum, Probiotic, and Turmeric on the experimental outcomes remained statistically negligible, as evidenced by the data presented in Tables III and IV. In the control group, which received simply the baseline diet without any herbal additives, there were no significant differences seen in the measured values on the 18th day (mean \pm standard deviation: 1.7 ± 0.6), 32nd day (mean \pm standard deviation: 1.7 ± 0.6).

S.No.	Aqueous Extract	18 th day	25 th day	32 nd day	39 th day
1	Aloe Vera	3.0, 3.8, 3.6 $(3.4 \pm 0.4)^{d}$	4.1,3.7,2.9 (3.5 ± 0.6) ^b	3.1,4.2,3.7 (3.6 ± 0.5) ^b	2.1,2.5,2.9 (2.5 ± 0.4) ^b
2	Zingiber officinale	3.4,3.4,2.3 $(3.0 \pm 0.6)^{c,d}$	1.1, 2.3, 1.9 (1.7 ± 0.6) ^b	1.9, 2.3, 1.1 $(1.7 \pm 0.6)^{a}$	1.6, 2.6, 1.4 $(1.86 \pm 0.64)^{a,b}$
3	Garlic (Allium sativum)	$\begin{array}{c} 3.1,2.3,2.2\\ (2.5\pm0.4)^{\text{b,c,d}} \end{array}$	$\begin{array}{c} 1.9, 0.9, 1.3 \\ (13\pm 0.5)^{a} \end{array}$	1.9, 2.3, 2.7 $(2.2 \pm 0.5)^{a}$	1.8, 1.9, 1.4 $(1.4 \pm 0.4)^{a}$
4	Turmeric	2.7, 2.1, 1.9 $(2.2 \pm 0.4)^{a,b,c}$	2.1, 1.7, 1.5 (1.7 ± 0.3) ^a	1.5, 1.5, 1.9 (1.6 ± 0.2) ^a	1.4, 2.9, 2.1 (2.1 ± 0.7) ^{a,b}
5	Control	1.1,1.7,2.3 (1.7 ± 0.6) ^{a,b}	1.0, 0.4, 1.2 $(0.8 \pm 0.4)^{a}$	2.6, 0.9, 1.8 $(1.7 \pm 0.8)^{a}$	1.7, 2.9, 2.3 $(2.30 \pm 0.60)^{a,b}$

Table 3 The impact of aqueous extracts of herbs on the concentration of urea in the serum (in milligrams per one hundred milliliters) in Broilers

Table 4 Effect of Probiotic and Siganus luridus on the serum level of Urea (mg/100 mls) in Broiler

S.No.	Aqueous Extract	18 th day	25 th day	32 nd day	39 th day
1	Siganus luridus	2.6, 3.7, 3.0 $(3.1 \pm 0.5)^{c,d}$	0.9, 1.3, 1.7 $(1.3 \pm 0.4)^{a}$	3.5, 2.5, 1.6 $(2.5 \pm 0.9)^{a,b}$	1.2, 1.9, 1.6 $(1.5 \pm 0.3)^{a,b}$
2	Probiotic	(0.1 ± 0.0) 1.5, 1.9, 1.1 $(1.5 \pm 0.4)^{a}$	(1.0 ± 0.1) 0.9, 1.5, 1.8 $(1.4 \pm 0.4)^{a}$	(1.4, 2.1, 2.6) $(2.0 \pm 0.6)^{a}$	(1.0 ± 0.0) 1.1, 1.3, 1.7 $(1.3 \pm 0.3)^{a}$
3	Control	1.1,1.7,2.3 (1.7 ± 0.6) ^{a,b}	1.0, 0.4, 1.2 $(0.8 \pm 0.4)^{a}$	2.6, 0.9, 1.8 (1.7 ± 0.8) ^a	1.7, 2.9, 2.3 (2.30 ± 0.60) ^{a,b}

4 | DISCUSSION

The impact of Zingiber officinale on the blood Creatinine levels of broilers was found to be statistically significant (p < 0.05) on day 32, as indicated in Table-I. However, the effects of Aloe vera, Siganus luridus (an ingredient used in Unani medicine), Allium sativum, Probiotic, and Turmeric were not found to be statistically significant (p > 0.05), as shown in Tables I and II. The study conducted by Otuechere et al.¹⁹ yielded comparable findings on turmeric, suggesting that the inclusion of curcumin (a constituent of turmeric) in rat diets did not have a statistically significant impact (p > 0.05) on serum levels of Creatinine. The impact of Allium sativum was partially consistent with the findings of Dieumou et al.²⁰ who observed that administering Allium sativum essential oil to broilers did not have a statistically significant effect (p > 0.05) on blood Creatinine levels. An inconsistency was seen in comparison to the study conducted by Dieumou et al.²⁰ on the administration of Zingiber officinale essential oil to broilers did not have a statistically significant effect (p > 0.05) on the administration of Zingiber officinale essential oil to broilers did not have a statistically significant effect (p > 0.05) on blood Creatinine levels. An inconsistency was seen in comparison to the study conducted by Dieumou et al.²⁰ on the administration of Zingiber officinale essential oil to broiler chickens. It was found that this treatment did not result in a significant change in serum levels of Creatinine. A notable inconsistency was observed regarding the impact of Aloe vera, in contrast to the findings of Saka et al.²¹ who reported that Aloe vera led to a substantial rise (p < 0.05) in Creatinine serum levels in Sprague-Dawley rats.



The findings reported in Table III shows that Aloe vera had a statistically significant effect (p < 0.05) on the serum levels of Urea in broiler chickens on day 18. During the investigation, it was noted that the effects of Zingiber officinale, Siganus luridus (Unani medicine), Allium sativum, Probiotic, and Turmeric did not show a statistically significant difference (p > 0.05) when compared to the control group. The discovery is displayed in Tables III and IV. The results of Saka et al.²¹ are in opposition to the observed effects of Aloe vera, since they found no statistically significant influence (p > 0.05) on the levels of Urea serum in Sprague-Dawley rats. The findings of Oleforuh-Okoleh et al.²² align to some degree with this observation regarding the impacts of Zingiber officinale and Allium sativum. They found that administering broilers with aqueous extracts of these substances did not result in any statistically significant differences (p > 0.05) in serum urea levels compared to the control group.

When compared to other herbal extracts, Probiotic, and the control group, Zingiber officinale had a significant impact (p < 0.05) on the feed conversion ratio (FCR) of broilers on the 25th, 32nd, and 39th days. This was observed in comparison to the control group. At each and every step of the research project, it was discovered that the influence of a number of herbal extracts and probiotics on the feed conversion ratio (FCR) of broilers was statistically negligible (p > 0.05). Earlier research carried out by Moorthy et al.²³⁻²⁴ and Onimisi et al.²⁵ is congruent with this discovery about the effects of Zingiber officinale, at least to a certain extent. When compared to the control group, the feed conversion ratio (FCR) of broiler chicks that were given Zingiber officinale showed a statistically significant improvement (p < 0.05). This improvement was observed in comparison to the group serving as the control. When compared to the control group, broilers that were fed a diet that contained 2% dry red Zingiber officinale meal exhibited a considerably lower feed intake (p < 0.05). This conclusion contradicts the findings of Herawati²⁶ Scumann et al²⁷ who revealed that the control group consumed much more feed. Zingiber officinale significantly increased broiler development performance in comparison to other medicinal herbs and probiotics that were utilized in the study.

5 | CONCULUSION

In conclusion it was revealed all of the medicinal herbs and probiotics did not exhibit any significant differences on the serum levels of Creatinine and urea. Equally negligible were the outcomes for the control group. After conducting an exhaustive study, it is possible that the metabolic role that Zingiber officinale plays at the molecular level in broiler chicks could show to be a good feed supplement.

CONFLICT OF INTEREST

All authors declare no conflict of interest

REFERENCES

- 1. Schwarz S, Kehrenberg C, Walsh TR. Use of antimicrobial agents in veterinary medicine and food animal production. International journal of antimicrobial agents. 2001 Jun 1;17(6):431-7.
- 2. Demir E, Sarica Ş, Özcan MA, Sui Mez M. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. British Poultry Science. 2003 Mar 1; 44(S1):44-5.
- 3. Sarica S, Ciftci A, Demir E, Kilinc K, Yildirim Y. Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. South African Journal of Animal Science. 2005; 35(1):61-72.
- 4. Hernandez F, Madrid J, Garcia V, Orengo J, Megias MD. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry science. 2004 Feb 1;83(2):169-74.
- Mikulski D, Zdunczyk Z, Jankowski J, Juskiewicz J. Effects of organic acids or natural plant extracts added to diets for turkeys on growth performance, gastrointestinal tract metabolism and carcass characteristics. Journal of Animal and Feed Sciences. 2008 Apr 8; 17(2):233.
- 6. Faleiro ML, Miguel MG, Ladeiro F, Venancio F, Tavares R, Brito JC, Figueiredo AC, Barroso JG, Pedro LG. Antimicrobial activity of essential oils isolated from Portuguese endemic species of Thymus. Letters in applied microbiology. 2003 Jan 1; 36(1):35-40.
- 7. Bassett DR, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. Medicine and science in sports and exercise. 2000 Jan 1; 32(1):70-84.



- 8. Azaz D, Demirci F, Satıl F, Kürkçüoğlu M, Hüsnü K, Başerb C. Antimicrobial activity of some Satureja essential oils. Zeitschrift für Naturforschung C. 2002 Oct 1; 57(9-10):817-21.
- 9. Dorman HD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of applied microbiology. 2000 Feb 1; 88(2):308-16.
- 10. Botsoglou NA, Christaki E, Florou-Paneri P, Giannenas I, Papageorgiou G, Spais AB. The effect of a mixture of herbal essential oils or α-tocopheryl acetate on performance parameters and oxidation of body lipid in broilers. South African Journal of Animal Science. 2004 Jan 1; 34(1):52-61.
- 11. Botsoglou NA, Florou-Paneri P, Christaki E, Fletouris DJ, Spais AB. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. British poultry science. 2002 May 1; 43(2):223-30.
- 12. Gill C, Safe and sustainable feed ingredients. Feed Int. 2001; 22, 40-45.
- 13. Dehkordi SH, Moghadam AZ, Maghsoudi N, Aali, E, Gerami R, Dehsadeghi E, The effects of fresh garlic on the serum concentration of total cholesterol, total triglyceride and adipose tissues of broilers. *Comparative clinical pathology*. 2010; 19: 363-365.
- 14. Ponte PI, Mendes I, Quaresma M, Aguiar MN, Lemos JP, Ferreira LM, Soares MA, Alfaia CM, Prates JA, Fontes CM. Cholesterol levels and sensory characteristics of meat from broilers consuming moderate to high levels of alfalfa. Poultry science. 2004 May 1; 83(5):810-4.
- 15. Yasmeen R, Hashmi AS, Anjum AA, Saeed S, Muhammad K. Antibacterial activity of indigenous herbal extracts against urease producing bacteria.
- 16. Ajith TA, Usha S. and Nivitha V, Ascorbic acid and α-tocopherol protect anticancer drug cisplatin induced nephrotoxicity in mice: a comparative study. Clinica chimica acta, 2007; *375*(1-2): pp.82-86.
- 17. Babayi H, Kolo I, Okogun JI, Ijah UJ. The antimicrobial activities of methanolic extracts of Eucalyptus camalctulensis and Terminalia catappa against some pathogenic microorganisms.
- 18. Otuechere CA, Abarikwu SO, Olateju VI, Animashaun AL, Kale OE. Protective effect of curcumin against the liver toxicity caused by propanil in rats. International scholarly research notices. 2014; 2014.
- 19. Dieumou FE, Teguia A, Kuiate JR, Tamokou JD, Fonge NB, Dongmo MC. Effects of ginger (Zingiber officinale) and garlic (Allium sativum) essential oils on growth performance and gut microbial population of broiler chickens. Livestock research for rural development. 2009 Aug 26; 21(8):23-32
- 20. Saka WA, Akhigbe RE, Popoola OT, Oyekunle OS. Changes in serum electrolytes, urea, and creatinine in Aloe vera-treated rats. Journal of Young Pharmacists. 2012 Apr 1;4(2):78-81.
- Oleforuh-Okoleh VU, Ndofor-Foleng HM, Olorunleke SO, Uguru JO. Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. Journal of Agricultural Science. 2015 Apr 1;7(4):167.
- 22. Moorthy M, Ravi S, Ravikumar M, Viswanathan K, Edwin SC. Ginger, pepper and curry leaf powder as feed additives in broiler diet. International Journal of Poultry Science. 2009 Sep 30;8(8):779-82.
- 23. Moorthy M, Saravanan S, Mehala C, Ravi S, Ravikumar M, Viswanathan K, Edwin SC. Performance of single comb white leghorn layers fed with Aloe vera, Curcuma longa (turmeric) and probiotic. International Journal of Poultry Science. 2009 Sep 30; 8(8):775-8.
- 24. Onimisi PA, Dafwang II, Omage JJ. Growth performance and water consumption pattern of broiler chicks fed graded levels of ginger waste meal. Journal of Agriculture, Forestry and the Social Sciences. 2005;3(2):113-9.
- 25. Herawati H. The effect of feeding red ginger as phytobiotic on body weight gain, feed conversion and internal organs condition of broiler.
- 26. Schumann G, Klauke R. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clinica chimica acta. 2003 Jan 1;327(1-2):69-79.