(Trigonella foenum graecum), fennel (Foeniculum vulgare) and cumin (Cuminum cyminum) as phytobiotics on haemoglobin content and serum biochemical parameters in turkey poults. A total number of 150 straight run day old turkey poults of Nandanam turkey variety belonging to single hatch were utilised for the study. The poults were weighed individually and wing banded. Poults were randomly divided into five treatment groups with three replicates of 10 poults each. The experimental birds were reared under identical management conditions up to 8 weeks of age. Mean body weight was recorded between 0 and 8 weeks at biweekly intervals. The dietary treatments included the basal diet as control (T_1), supplemented experimental groups each receiving 5g/kg (0.5%) thyme seeds (T_2) or fenugreek seeds (T_3) or fennel seeds (T4) or cumin seeds (T5) added to the basal diet. These experimental feeds were fed to the respective treatment groups ad libitum from 0 to 4 and 5 to 8 weeks of age. Two milliliters of blood was collected from jugular vein of four poults in each treatment at fourth, sixth and eighth weeks of age using a 2ml syringe for estimation of haematological and serum biochemical parameters. A sample of 0.5ml of blood was taken in vial containing 0.5mg of EDTA (Ethylene Diamine Tetra Acetic Acid) as an anticoagulant. The remaining blood was kept undisturbed for two hours and the serum was separated.

2.1 Estimation of haemoglobin

Haemoglobin (Hb) content was estimated as per the Sahli's acid hematin method (Sahli, 1909).

2.2 Total Serum Protein

The total proteins level in the serum samples were estimated in A15 Biosystem auto analyzer by using commercially available AGAPPE kit based on Direct Biuret method (Gornall et al., 1949) and BCG method (Doumas et al., 1970).

2.3 Serum Calcium

The calcium in the serum samples was estimated in the A15 Biosystem auto analyser using commercially available AGAPPE kit based on the OCPC method (Kessler and Wolfman, 1964).

2.4 Serum Cholesterol and Triglycerides

Cholesterol and triglycerides in the serum were estimated using A15 Biosystem auto anlyser using commercially available kits from AGAPPE diagnostics Ltd. based on CHOD-PAP methodology (Allain et al., 1974) and GPO-method (Buccolo and David, 1973) respectively.

2.5 Statistical Analysis

All the statistical analyses were performed by using SPSS software (version 7.5) as per Snedecor and Cochran (1994). The means were compared by one way ANOVA for significant differences among treatments. The percentage in data was converted into arc sin values before statistical analysis.

3. Results

The data recorded in biological experiment were grouped, analyzed statistically and presented.

3.1 Haemoglobin Content

Effect of four herbal seeds as phytobiotics feed supplementation on haemoglobin (g/dl) at fourth, sixth and eighth weeks of age in turkey poults are presented in table 1. There was a highly significant (P<0.01) difference for haemoglobin content at fourth and sixth week of age with highest haemoglobin content in fenugreek and cumin supplemented group were evident. Haemoglbin content in overall and males at eighth week of age were significant (P<0.05) with highest values recorded in fenugreek and cumin while insignificant difference was noted in females. Irrespective of treatment, consistent lower haemoglobin content was observed in all age groups in thyme groups.

Table 1: Effect of four herbal seeds as phytobiotics feed supplementation on mean (± SE) haemoglobin (g/dl) between 0 and 8 weeks of age in turkey poults (n=4)

Treatments	Age in weeks				
	4	6	8		
			Male	Female	Overall
T1	11.15^{a}± 0.36	10.70^{ab}± 0.31	11.40^{ab}± 0.31	11.60± 0.23	11.50^{a}±0 .18
T2	10.31^{b}± 0.13	9.80^{b}±0. 18	10.27^{b}±0 .07	10.87± 0.59	10.57^{b}±0 .30
T3	11.45^{a}± 0.13	11.65^{a}±0 .33	11.93^{a}±0 .47	11.47± 0.27	11.70^{a}±0 .26
T4	10.25^{b}± 0.10	10.80^{ab}± 0.41	10.93^{ab}± 0.66	11.13± 0.64	11.03^{ab}± 0.41
T5	11.25^{a}± 0.15	11.55^{a}±0 .33	12.17^{a}±0 .35	11.30± 0.25	11.73^{a}±0 .27
F value	8.148**	5.449**	3.393*	0.437^{NS}	2.879*

a, b, c - means within column bearing different superscripts differ significantly (P<0.05)
* - Significant (P<0.05), ** - Significant (P<0.01), NS - Non-significant (P>0.05)

3.2 Serum Total Proteins

Influence of four herbal seeds as phytobiotics feed supplementation on serum protein (g/dl) in turkey poults is presented in table 2. Significant (P<0.05) effect on serum total proteins was observed at fourth week of age with highest value spotted in thyme and lowest in fennel supplemented groups. Whereas statistically insignificant effect was noted in total proteins at sixth week of age. At eighth week of age significant (P<0.05) effect was found in total proteins on overall mean and in males with compare to control. Cumin treated birds showed significantly (P<0.05) lower total proteins compared to the other herbal seeds supplemented group. Control birds had intermediatery values which is statistically comparable with all other treatments, in males cumin supplemented birds had significantly lower serum total proteins compared to other herbal seeds supplemented birds which had statistically similar serum total proteins. Except thyme fed birds all the other herbal seeds supplemented birds had similar serum total proteins to that of control.

Table 2: Effect of four herbal seeds as phytobiotics feed supplementation on mean (± SE) serum total proteins (g/dl) between 0 and 8 weeks of age in turkey poults (n=6)

Treatments	Total proteins				
	4th week	6th week	8th		
			Male	Female	Overall
T1	3.47^{bc}±0.05	3.67±0.05	3.27^{bc}±0.03	3.41±0.24	3.34^{ab}±0.11
T2	3.92^{a}±0.05	3.59±0.05	3.69^{a}±0.12	3.45±0.09	3.57^{a}±0.08
T3	3.46^{bc}±0.05	3.62±0.05	3.56^{ab}±0.09	3.44±0.21	3.50^{a}±0.11
T4	3.42^{c}±0.02	3.57±0.05	3.53^{ab}±0.09	3.54±0.13	3.53^{a}±0.07
T5	3.69^{ab}±0.11	3.63±0.05	3.18^{c}±0.11	3.06±0.36	3.12^{b}±0.17
F value	10.982*	0.592^{NS}	5.100*	0.668^{NS}	2.665*

a, b, c, d - means within column bearing different superscripts differ significantly (P<0.05),
* - Significant (P<0.05), ** - Significant (P<0.01), NS - Non-significant (P>0.05)

3.3 Serum Calcium

Serum calcium level variation due to feed supplementation of four herbal seeds as phytobiotics feed supplementation in turkey poults are presented in table 3. Inclusion of herbal seeds had significant (P<0.01) effect on the serum calcium level of turkey poults at fourth and sixth weeks of age. At eighth week, even though males recorded significant (P<0.05) effect for phytobiotic supplementation, the mean values of female and overall average was not altered by phytobiotic supplementation.

Table 3: Effect of four herbal seeds as phytobiotics feed supplementation on mean (± SE) serum calcium (mg/dl) between 0 and 8 weeks of age in turkey poults (n=6)

Treatments	Age in weeks				
	4th	6th	8th		
			Male	Female	Overall
T1	5.96^{c}±0.03	6.06^{c}±0.02	5.18^{b}±0.35	5.23±0.54	5.20±0.26
T2	7.19^{a}±0.05	6.35^{bc}±0.2	5.87^{ab}±0.07	5.88±0.19	5.85±0.11
T3	6.87^{b}±0.05	6.42^{b}±0.01	6.02^{ab}±0.16	6.31±0.04	6.16±0.10
T4	6.77^{b}±0.05	6.06^{c}±0.02	6.24^{ab}±0.65	6.15±0.09	6.19±0.50
T5	7.18^{a}±0.06	7.16^{a}±0.05	6.94^{a}±0.49	5.04±0.04	6.17±0.44
F value	104.971**	22.946**	2.100*	0.769^{NS}	1.198^{NS}

a, b, c - means within column bearing different superscripts differ significantly (P<0.05)
* - Significant (P<0.05), ** - Significant (P<0.01), NS - Non-significant (P>0.05)

3.4 Serum Cholesterol and Triglycerides

Mean serum cholesterol and triglyceride (mg/dl) in different herbal seeds supplemented groups in turkey poults is presented in table 4. Serum cholesterol level was significantly (P<0.05) affected at fourth week of age with hyper cholesterol in thyme while hypo cholesterol in fenugreek supplemented groups were seen, while no significant difference was observed at sixth weeks of age. Whereas at eighth week of age, significant (P<0.05) difference was observed both in males and in females leading to a significant (P<0.01) effect on overall values also. The serum cholesterol level was elevated in males due to fennel supplementation whereas cumin supplementation has resulted in significant (P<0.05) decrease in overall values. Apart from this, serum cholesterol level in males, females and both the sex together has not been affected by supplementation of herbal seeds. There was highly significant (P<0.01) difference in serum triglycerides level at fourth and sixth weeks of age. At fourth week except thyme supplementation, addition of other phytobiotics (P<0.05) depressed serum triglyceride level. The magnitude of depression was higher in fenugreek and fennel supplemented birds making it significantly (P<0.05) lower than cumin fed birds. At the sixth week also all the phytobiotic herbal seeds except thyme had significant (P<0.05) reducing effect of serum triglyceride in turkey poults. However at eighth week males, females and both the sex together registered significantly (P<0.05) lower triglyceride in turkey poults due to supplementation of herbal seeds. The decrease was strongly noticed in thyme, fenugreek and fennel supplemented birds making it significantly (P<0.05) lower than cumin fed birds.

Table 4: Effect four herbal seeds as phytobiotics feed supplementation on mean (± SE) serum cholesterol and triglycerides (mg/dl) between 0 and 8 weeks of age in turkey poults (n=6)

Treatments	Cholesterol					Triglycerides				
	4th week	6th week	8th week			4th week	6th week	8th week		
			Male	Female	Overall			Male	Female	Overall
T1	126.5^{ab} ±3.5	120.5 ±1.5	135.05^{b} ±5.50	136.50^{ab}±0.50	136.00^{ab}±2.27	178.50^{a} ±2.06	173^{a} ±50	160.00^{a} ±10	162.05^{a} ±4.50	161.25^{a} ±2.02
T2	138.0^{a} ±50	134.0 ±50	130.33^{b} ±1.76	128.33^{ab}±7.54	129.33^{bc}±3.49	174.75^{a} ±2.06	172^{a} ±30	97.33^{c} ±2.60	99.33^{c} ±5.24	98.33^{c} ±2.65
T3	114.0^{b} ±50	124.0 ±50	139.67^{ab}±6.33	141.67^{a} ±2.73	140.67^{ab}±3.12	142.50^{c} ±1.26	138^{b} ±5	96.33^{c} ±6.44	91.00^{c} ±2.08	93.67^{c} ±3.25
T4	128.0^{ab} ±50	128.0 ±50	150.00^{a} ±1.53	145.00^{a} ±4.16	147.05^{a} ±2.28	143.00^{c} ±1.08	137^{b} ±50	99.67^{c} ±3.71	100.00^{c} ±2.08	99.83^{c} ±1.90
T5	123.0^{ab} ±50	117.0 ±50	127.00^{b} ±3.79	114.00^{b} ±11.15	120.05^{c} ±6.02	148.00^{b} ±1.08	139^{b} ±50	143.67^{b} ±4.26	120.33^{b} ±10.84	132.00^{b} ±7.37
F value	3.354*	2.137^{NS}	5.164*	3.332*	7.588**	127.457**	16.408**	42.751**	17.630**	39.797**

a, b, c - means within column bearing different superscripts differ significantly (P<0.05)
* - Significant (P<0.05), ** - Significant (P<0.01), NS - Non-significant (P>0.05)

4. Discussion

4.1 Haemoglobin Content

Haemoglobin content was favourably changed at fourth, sixth and eighth week of age by supplementation of thyme seeds in feed of turkey poults. Haemoglobin content in males at eighth week of age was significantly low. These could be due to effect of some bioactive principles present in thyme seeds against haemopoietic process in the body. The results of present study was found different with Demir et al. (2008) who indicated that feeding thyme powders did not change the blood haemoglobin level but Rahimi et al. (2011) observed the improved hemoglobin concentration, but not significantly due to inclusion of 0.1% aqueous extracts of thyme in the drinking water however in broiler chickens. The statistical analysis revealed highly significant (P<0.01) difference for haemoglobin content at fourth and sixth week of age with higher haemoglobin content in fenugreek supplemented group. Haemoglobin content in overall and males at eighth week of age were significantly influenced by fenugreek supplemented groups with increased level recorded in fenugreek. These results indicated that certain bioactive principles in fenugreek seeds have positive effect on haemoglobin level. Haemoglobin content at fourth, sixth and eighth week of age was positively influenced by addition of fennel seeds at 0.5% level in feed and also in males at eighth week of age. In fennel supplemented groups lesser values were observed compared to control. This result suggests that these plants may contain substances which involved in the derangement of the haemopoietic process. In contrary with present findings, Abdullah and Rabia (2009) who observed significant (P<0.05) improvement in haemoglobin in broiler chickens fed fennel seeds as compared to control. Higher haemoglobin content at fourth, sixth and eighth week of age in cumin supplemented group was evident. The results was in disagreement with Al-Kassi (2010) who showed that broiler chicks fed with 1.5% cumin powder developed a significant (P<0.05) decrease in the levels of haemoglobin. Further AL-Kassie et al. (2011) revealed lower level of haemoglobin in broiler which fed with cumin powder at 0.25, 0.50, 0.75 and 1% level.

4.2 Serum Total Proteins

Thyme supplementation elevated serum total proteins level at fourth week and in males at eighth week. The overall serum total proteins of thyme fed birds were insignificantly higher than control at eighth week. In accordance with present results Toghyani et al. (2011) reported that 5g/kg thyme powder feed supplementation significantly (P<0.05) increased serum total proteins and globulin concentration. Addition of fenugreek has not resulted in any change in total proteins of turkey poults at 4th, 6th, 8th weeks of age. Similar to the findings of this experiment, Abbas (2010) has also reported no significant effect on total proteins in diet of broiler containing 3g/kg fenugreek seeds. Addition of fennel seeds in the turkey feed has no influence on serum total proteins level up to eighth week of age. No previous literature could be cited to explain the findings of this experiment. Inclusion of cumin seeds has in no way connected with a change in serum total proteins of turkey poults up to eight weeks of age. However, Shabaan (2012) recorded lower values of total proteins in an experiment containing cumin seeds at 0.15 and 0.30% in diet of sbroilers. But in contrary with present findings Elagib et al. (2012) found that in broiler chick fed with diets containing cumin showed increase in total proteins fraction of the blood proteins.

4.3 Serum Calcium

Perusal of literature has indicated that the research on supplementation of phytobiotics on serum calcium level has not been studied adequately as evidenced by scanty literature. Inclusion of thyme seeds elevated serum

calcium level at fourth week of age in turkey poults. However during the rest of the period (sixth and eighth week of age), the numerical increase observed in this group was not statistically significant.

Feeding of turkey poults with fenugreek seeds has significantly (P<0.05) enhanced the serum calcium level at four and six weeks of age. However the increase at eighth week was statistically not significant. The turkey poults receiving fennel seeds in feed recorded significantly (P<0.05) higher serum calcium level at fourth week and same value at sixth week of age compared to control. Increase in serum calcium level at eighth week was not significantly higher than control. The addition of cumin seeds in the feed of turkey poults has resulted in increased level of serum calcium at four and six weeks of age. At eighth week even though males recorded significantly (P<0.05) higher serum calcium in males, females recorded insignificantly lower values. The overall serum calcium level at eighth week compared to control was numerically higher but statistically significant.

4.4 Serum Cholesterol and Triglycerides

It was observed from the study that supplementation of thyme seeds has no effect on serum cholesterol level. However earlier workers (Isa, (2011) at 0.02 and 0.04%; Navid and Nezhady, (2011) at 1.5% and Toghyani et al. (2011) at 0.5%) recorded significant decrease in serum cholesterol level due to thyme supplementation. High levels of fibers of these seeds can increase the excretion of bile and this can decrease the cholesterol level of blood (Zargari, 2001). Even though thyme supplementation had no significant effect on serum triglycerides during four and six weeks of age, significant decrease was observed at eighth week in overall and sex separate values. The results was supported by Isa (2011) who showed that broilers fed diet supplemented with thyme powder at 0.02 and 0.04% up to 6 weeks resulted in significant reduction (P<0.05) in the levels of serum triglycerides. The main reason is these substances have effect on cholesterol and triglycerides and decrease these harmful parameters in blood serum. The reduction in triglyceride and cholesterol level noticed by thyme has been attributed to the lowering effect of thymol or carvacrol on HMG-Co A reductase the rate limiting enzyme of cholesterol synthesis (Lee et al., 2003).Similar to thyme supplementation, the addition of fenugreek seeds in the diets of turkey poults also showed no significant change in serum cholesterol level during entire experiment period. These results often related to the mode of action of fenugreek in bird metabolism which includes competition with cholesterol at binding sites or interference with the cholesterol biosynthesis in the liver. Hypocholesterolaemic effects of fenugreek are owing to increased conversion of hepatic cholesterol to bile salts due to loss in the faeces of complexes of these substances with fenugreek fiber and saponins (Lee et al., 2003).However Abbas (2010) ascertained the effect of fenugreek seeds on broiler performance at the rate of 3g per kg of diet and showed significant (P<0.05) reduction in serum cholesterol level. Addition of fenugreek to the diets of turkey poults revealed the decreasing trend in serum triglyceride level compared to control throughout the study period. There are no earlier research findings is available in the literature to corroborate the findings of this experiment. The dietary inclusion of fennel in the diet of turkey poults resulted in consistent numerical increase throughout the experiment period. However, the increase was high in magnitude in males at eighth week making it statistically significant (P<0.05).Addition of fennel in the diet of turkey poult revealed a decreasing trend in serum triglyceride level compared to control throughout the experimental period. No meaningful comparison could be possible as there is no traceable literature available on this parameter. Even though the dietary inclusion of cumin seeds had depressive effect on serum cholesterol during entire study period, the difference was statistically significant (P<0.05) only for overall mean at eighth week. The decrease in the level of cholesterol is expected to be due to the active compound that found in cumin which acts as inhibitors to the active enzyme hepatic 3-hydroxyl-3 methylglutaryl coenzyme A (HMG-CoA) that synthesized the cholesterol (Crowell, 1999). This reduction in blood cholesterol could be contributed in some cases to the reduction in some harmones secreted by the cortex of the adrenal glands which in turn causes the reduction in the secretion of fatty acids from the adipose tissues or reduction of fat oxidation which leads to the reduction of the level of fatty acids including blood cholesterol.

A similar trend of decreasing effect on serum triglyceride has been noticed in supplementation of cumin seeds throughout the study period similar to fenugreek and fennel seeds supplementation in turkey poults. Similar finding have already been obtained by Al-Kassi (2010) at 1.5% and AL-Kassie et al. (2011) at 0.25, 0.50, 0.75, and 1% observed a significant (P<0.05) decrease of serum cholesterol and triglyceride in broiler chicks fed with cumin powder in support of the current findings.

5. Conclusion

Haemoglobin content was constantly lower in thyme supplemented group while it was recorded higher in case of cumin supplemented group. The serum total proteins was significantly higher in thyme, fennel and fenugreek supplemented group compared to cumin supplemented group. The highly significant difference was observed in serum cholesterol level at eighth week of age in turkey poults with highest level in fennel supplemented group and lowest was in cumin supplemented group. The difference in serum triglyceride level was highly significant at eighth week of age with highest triglyceride level in control group while lower level was recorded in fenugreek, thyme, fennel and cumin supplemented groups. Among all treatments supplementation of cumin seeds in feed of turkey poults showed better performance in respect of haemoglobin content, serum calcium and cholesterol level. Further, it is necessary to carry out studies to validate the findings of these at different concentrations of seeds and at different duration.

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