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THE EFFECT OF USING KOMBUCHA ON BLOOD ANTIBODY LEVEL AND PROVENTRICULUS AND GIZZARD TISSUE CELLS IN BROILER CHICKS

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ABSTRACT
To evaluate the effect of Kombucha and Kombucha with vitamin E- selenium 0.1% on blood metabolites, performance and morphology of some of the organs, of 140 baby broiler chicks' strain Ross 308 of male and female ones were applied for 42 days in 7 treatments and 4 replications, each replication including 5 chicks. Dietaries were equal in all treatments and the difference was about water and Kombucha and Kombucha with vitamin E- selenium s 0.1%. Finally, the performance showed that the increase of body weight in 1-7 and 8-14 day-age was significantly under the influence of experimental treatments. According to the results during 1-7 day-age and 22-28 day-age, there was significant effect on the feed and the best conversion ratio was in 29-35 day-age and there was significant difference ($P<0.05$). From morphological aspects there was significant difference between Bursa offabrisious weight and gizzard in various periods. Morphology of Proventriculus, crypt depth (micron), and goblet cells was significantly different and there was no significant difference aboutvillus height (micron) and Epithelium thickness (micron) ($P>0.05$). there was no significant difference in blood metabolites of liver enzymes concentration and antibody titers against SRBC antigens but antibody titer against Newcastle antigens tested during 21, 25 day-age showed no significant difference ($P<0.05$).

KEYWORDS: Blood metabolites, Bursa of Fabrisious, Gizzard, Kombucha, Proventriculus, Selenium 0.1%, SRBC, Vitamin E.

INTRODUCTION

In recent years, keeping poultry farming is of great importance based on the important role in providing required animal protein of human being as it is turned from traditional to big industrial process. In recent years, new additives are used widely in poultry farming and useful results are reported regarding manufacturing performance and poultry health (Wegener *et al*., 1998; EU, 2006). The studies showed that long-term use of some of the antibiotics in livestock and poultry feed leads to the creation of resistant strains of various harmful microorganisms and this resistance is transferred to other species namely human and livestock (Menten, 2001). The removal of antibiotics of poultry dietary increases mortality rate regarding intestinal infection (Bogaard, 2001). Thus, we should find a good alternative for antibiotics (Bywater *et al*., 2005).

Probiotics are live organisms (fungus or bacteria) added directly to the livestock and poultry diet and they have good effect on their health and performance (Gibson *et al*., 2000; Frits *et al*., 2000; Schrezenmeir *et al*., 2001; Murugesan *et al*., 2009). The mechanism is as the probiotics by affecting intestine micro flora and avoiding pathogenic factors improves the host health (Jane *et al*., 1998). Amir Ghofran *et al*., (2010) investigated the effect of Mirzayani Salviaon immune system and cell deathin blood lymphocytes of human being and showed that low level of this extract motivated immune system and high amount of this extract suppressed immune system. Kombucha is one of the examples and it was used for a long time and recently researchers applied it in livestock and poultry diet. Kombucha is the result of co-habiting of some species of bacteria and yeasts and they are the block of this fungus (Blanc, 1995). Due to specific compounds as Gluconic acid, Acetic acid and Lactic acid, B vitamins have therapy effects including prevention and control in some diseases including diabetes, cancer, fat, blood pressure and helping liver and kidney in removing toxic materials of body and increasing body resistance to diseases on human being and animal (Hoffman, 1998; Malini *et al*., 2010; Perron, 1992; Steinkraus 1997) beside antibacterial has anti-oxidant feature (Depiti *et al*., 2003).

Kombucha is a type of fermented tea with a mass of yeasts and bacteria (Acetobacterxylinum) with anti-microbial compound (Adriany *et al*., 2009). In addition to anti-bacterial feature, it can protect livery tissue (Murugesan *et al*., 2009). Kombucha is consisting of vitamin B, C and some of enzymes (Williams *et al*., 2006).

Kombucha is obtained as the result of cohabitation of bacteria and yeasts and it is applied mostly. Kombucha tea is a fermented product produced by Kombucha mushroom. Many scientific studies regarding Kombucha mushroom showed that there is a strong compound in Kombucha removing toxics of the body (Afsharmansh and Sadeghi, 2013; Murugesan *et al*., 2009). Kombucha tea is a supplement improving defensive system of body and avoids diseases. Kombucha culture has been used for hundreds of years by the Asiatic people of his homeland because of its surprising success as the most effective natural folk remedy for fatigue, lassitude, nervous tension, incipient signs of old age, hardening of the arteries, sluggishness of the bowels, gout and rheumatism, hemorrhoids and diabetes (Ahmad 2005; Al-Hosseini, 2002; Murugesan 2009).

Some of the studies have been conducted on the effect of Kombucha on liver, its cellular tissue and enzymes in mouse and showed various results. In one of the studies, the effect of Kombucha on resistance of liver to toxics is investigated and the result showed the effect of protection of this matter on liver tissue (Murugesan *et al*., 2009). Also, another study showed that Kombucha had positive effect on oxidative stress in kidney cells and it led to the reduction of unsuitable effects of this stress (Gharib, 2009) (Yaman *et al*., 2006; Haghighi *et al* ., 2005; Mountzouris *et al*., 2007). Vitamin E is one of fat soluble vitamins. This vitamin has antioxidant feature and it eliminates the destructive chemical effect on body tissues (Liu *et al*., 1995). The experiment showed that Vitamin E supplement increased Humoral immunity response and antibody secretion (Vakili and Daliri, 2010).

Selenium is one of non-metal rare chemical elements. This element is found as compound and is rarely seen as pure. Selenium with vitamin E protects animals' tissues against oxidation. Vitamin E keeps selenium at its active form and selenium increases vitamin E absorption of intestine and keeps it in blood flow and body tissues (Singh *et al*., 2006). Some of the roles of Vitamin E are as follows:

- Avoiding cellular oxidative damage
- Active in cellular immunity performance
- Body protection against heavy metal toxicity (Singh *et al*., 2006).

In an experiment on broiler chicks for 6 weeks to investigate the effect of selenium, it was found that selenium is effective to improve performance and rapid growth and increasing weight of broiler chicks (Yang *et al*., 2009).
Singh *et al* (2006) in a study evaluated the dietary effects of selenium and vitamin E on broiler chicks for 42 days. They found that Vitamin E as 20mg and Selenium as 0.2 mg in Kg diet improved immunity system.

MATERIALS AND METHODS

All safety issues for tests including health, fumigation, hall ventilation were considered as standard.

Transferring the chicks to hall

140 one-day age broiler chicks strain Ross 308 were purchased. The mean weight of chicks was 41.4g. After the chicks went to farming hall, after initial weighting, Bronchitis vaccine was sprayed on chicks in the box and then the chicks were randomly selected in experiment group.

Hall temperature and humidity

Table 1 shows temperature program of the hall.

Table 1. Hall temperature program (℃)

Raising week	Temperature of hall
First	33-35
Second	30
Third	27
Fourth	24
Fifth	22
Sixth	18-21

Humidity at the beginning of raising was 50-60% and it was 60-65% at the end of raising. The lighting hours of hall were 24 hours. There were 8 low-consuming lamps for 2 rows of 4 lamps at the height of 3m from the floor as the entire place was light.

Vaccination program

To prevent some diseases including Bronchitis Newcastle and Gumboro, vaccination done in accordance to Table 2.

Table 2. Vaccination program

No	Vaccine	Vaccination age (day)	Method
1	Bronchitis	0	Spray-respiratory
2	Newcastle (B1)	8-day age	Eye-drop
3	Gumboro	12-day age	Oral
4	Newcastle (Lasota)	18-day age	Oral
5	Gumboro (repetition)	21-day age	Oral

Experimental dietary

From the first day of raising, the chicks were distributed based on weight randomly and uniformly between the treatments and replications.

Table 3. The combination of basic diet components (based on percent of diet)

Feed (%)	Early period (1-10 day age)	Groth period (11-28)	Final period (29-42)
Corn	49.1	58.7	64.8
Soybean	43.7	34.8	29.1
Fat	2.8	2.6	2.4
D-LMethionine	0.26	0.21	0.18
L- Lysine	0.06	0.06	0.07
Decalcium phosphate	2	1.8	1.6
Oyster powder	1.24	1	1
Salt	0.34	0.33	0.35
Vitamin-mineral supplement	0.5	0.5	0.5
Total %	100	100	100
Metabolism energy (Kcal/Kg)	2900	3000	3050
Crude protein	24.4	21.1	19.1
Calcium	1.03	0.88	0.83
Phosphorus	0.49	0.44	0.41
Lysine	1.38	1.16	1.02
Methionine	0.61	0.52	0.47
Methionine+Cystine	1	0.86	0.79

1-Each kg vitamin supplement including 360000 international unit vitamin A, 800000 international unit vitamin D3, 7.2g vitamin E, 0.8g vitamin K, 0.71 g vitamin B1, 2.64 g vitamin B2, 11.88 g vitamin B3, 3.92G Vitamin pantothenate, 1.176g vitamin B6, 0.4g vitamin B9, 6mg vitamin B12.

1- Each kg mineral materials supplement including 100gCholinChloride, 39.64g Manganese (oxide), 33.688g zinc, 20g iron, 4g copper, 397g iodine, 0.2g cobalt and 80mg selenium.

The evaluated features in experiment

Measuring the mean feed

After weighting, the chicks were fed, during 7, 14, 21, 28, 35, 42 day-age, the feed in the seed dish is returned into treatment feed after weighting and the difference of feed weight was calculated at the beginning and end of period.

Calculation of the mean daily feed of each chick

$$\text{The mean of daily feed (g)} = \frac{\text{periodical feed}}{HD=(Nf*42)+\sum di \ \text{day-hen in each experiment pan})}$$

In formula(Hen-day) N_f, is the number of live chicks of experiment at the end of period, di is the number of live daysof i^{th} slaughtered chick in the experiment at the end of period.

Measuring body weight mean

Weighting was done at the first day of chicks raise by digital scale with precision 0.01g. During 7, 14, 21, 28, 35 and 42, weighting was done as collective. Before weighting, chicks feed was stopped for three house to be equal in terms of gastric system condition.

Calculation of the mean daily weight increase of chicks

$$\text{The mean daily weight increase (g)} = \frac{\text{increasing the weight of each pen in each perod}}{\text{number of hen-day}}$$

Measuring feed conversion ratio

Feed conversion ratio was calculated during 1-7 day age, 8-14 day age, 15-21 day age, 22-28 day age, 29-35 day age, 36-42 day age and 1-42 day age. The conversion ratio is calculated of di viding the mean feed by the mean of chicks weight increase for each period.

$$\text{The periodical mean of feed conversion ratio} = \frac{\text{The mean feed of each pan during the period}}{\text{the mean weight increase of each pen during the period}}$$

Slaughtering and blood sampling

Based on the purpose of the present experiment in day 42 of experiment, for slaughtering and blood sampling, a hen closer to weight mean of group was selected for slaughtering among any experiment unit (replication).

Biochemical blood parameters

To determine biochemical parameters of blood, blood sampling was performed in some stages. First stage was done in 21 day age of flock and 3 days after Newcastle vaccine of flock. Second stage was done in 25day age of flock and 7 days after Newcastle vaccination (the distance of two blood sampling was 4 days) and it was used to evaluate vaccine titer and comparing the treatments and their resistance to increased titer. Another blood sampling was done in 42 day age of flock (before slaughtering) and 6 days after SRBC Sheep Red Blood Cell injection to measure antibody titer and evaluate liver enzymes.blood sampling was done from wing vein and of each repetition, 1 chick receiving SRBC underwent blood sampling. SRBC was injected in 36day age as 0.1 cc in chick breast and wing vein was used for blood sampling in 42 day age.

Biochemical blood experiments

Based on the experiment purpose, liver enzymes including GGT (Gamma GlutamylTransferase), CPK (CreatinePhosphoKinase), ALP (Alkaline Phosohatase), AST(Aspartate amino Transferase), ALT(Alanine amino Transferase) were measured in 42day age. Blood serum was used. After transferring the blood samples to lab, the samples were kept for some hours in lab to make blood clot to provide serum.

The tubes of clot blood were centrifuged. Under centrifugal force for 10 min at 1500rpm. The samples serum was collected by serum sampler after taking out them from centrifuge system and they were transferred to micro tubes being numbered. It was considered that to avoid partial mixture of various serum, sampler head was changed and the serums were kept in refrigerator to be used for each experiment at appropriate time. Commercial kits and Photometer (Auto Analyzer) were used to do the tests.

Making Kombucha mushroom

At first the water is boiled to provide extract. For each liter water, 80-100 g sugar is added to boiling water. Then, for each three liter water, a spoonful black tea without essence is put into a paper towel inside water and sugar mixture to be brewed. Then the tea leaf is taken out and waits until the solution temperature reaches environment temperature.

Then, the solution is poured into a good quality glass or plastic jar and then the mushroom is added. A paper towel covers the container in order that mushroom Scoby can breathe. After putting the paper towel, the container is put in a dark place at temperature 25 degree. Kombucha extract is prepared after one week. Table 4 shows Kombucha extract analysis.

Table 4. The analysis of Kombucha extract (Kombucha beverage)

Value	Compound
4.19	DM%
3.1	Phos(mg/dl)
5.91	Ca(mg/dl)
0.21	Crude Protein%
0.00	Fiber%
0.00	Fat%
0.001	Ash%
0.00	Starch%
16.87	Na(ppm)
40.60	K(ppm)

RESULTS AND DISCUSSION

Increasing body weight

The effect of using treatments on increasing body weight (live and carcass) during various periods is shown in Table 5. The increase of body weight during 1-7 day age was significantly under the influence of experiment treatments ($P<0.05$). The treatment in which 10% Kombucha with E and Selenium are used had the highest weight compared to other experiment groups. In this period, the treatment in with diet 15% Kombucha with vitamin E and selenium had the lowest weight compared to others. As none of the two treatments had significant difference with control group ($P>0.05$), during 8-14 day age, the increase of body weight was significantly under the influence of experiment treatments ($P<0.05$).

In this period, the treatments with 10% Kombucha with vitamin E and Selenium showed the highest weight increase ($P<0.05$) but control groups had 5% Kombucha, 5% Kombucha with vitamin E and selenium and 10% Kombucha had the lowest weight increase ($P<0.05$). It can be said the treatments with 15% Kombucha and 15% Kombucha with vitamin E and Selenium had the highest weight increase compared to control group ($P<0.05$). There was no significant difference of weight increase among various treatments during 8-21, 22-28, 29-35, 36-42 day age ($P>0.05$). The carcass weight also didn't show any significant difference among various treatments ($P>0.05$). The general results of raising period (1-42 day) showed that increasing body weight was under the effect of various diets only in the first and second weeks ($P<0.05$) and the treatments had no influence on animal weight by increasing the chick age.

the fact that increasing body weight is only under the influence of various diets of experiment only during first and second weeks ($P<0.05$) and the treatments had no influence on animal weight by increasing the chick age can be due to the reduction of feed by the chick since three week age and this led to the reduction of energy and protein consumption, weight reduction and reduction of growth (Siregar *et al*., 1989).

The positive effect of Kombucha with Vitamin E and Selenium to 10% in diet can be associated to the increase of feed and increasing protein and energy consumption. The result is increasing weight of chick. The increase of dietary protein absorption (if maximum diet has 10% Kombucha with vitamin E and Selenium) and it can be a factor in improving the appetite of animal and increasing food consumption (Afsharmanesh and Sadeghi, 2013).

Table 5. The effects of various experiment treatments on the mean of body weight increase (gram for each chick during raising period)

Treatments / Period (Day	1-7	8-14	15-21	22-28	29-35	36-42	Carcass weight (g)
Control	149.00^{ab}	417.33^{b}	661.83	1,061.17	1,806.00	2,238.00	1,750.00
5% Kombucha	146.66^{ab}	421.00^{b}	671.33	1,067.00	1,810.66	2,241.33	1,766.00
5% Kombucha with vitamin E and selenium	147.00^{ab}	418.33^{b}	654.66	1,028.67	1,776.66	2,176.33	1,755.00
10% Kombucha	146.29^{ab}	420.05^{b}	660.15	1,054.87	1,805.00	2,209.74	1,743.00
10% Kombucha with vitamin E and selenium	150.33^{ab}	435.00^{a}	633.66	1,017.67	1,850.66	2,224.00	1,757.00
15% Kombucha	146.66^{ab}	425.00^{ab}	669.66	1,066.67	1,829.00	2,256.33	1,761.00
15% Kombucha with vitamin E and selenium	145.66^{b}	424.00^{ab}	629.66	1,010.50	1,813.33	2,212.00	1,760.00
SEM	1.23	3.88	23.34	30.22	23.92	34.57	24.22
P value (%5)	*	*	NS	NS	NS	NS	NS

The means in each column with similar alphabets are different at the level 5% denoted by * and NS indicates the lack of significance of the difference between treatments mean.

Table 6. The effects of various experiment treatments on the mean of feed (gram for each chick)

Treatments / period (Day)	1-7	8-14	15-21	22-28	29-35	36-42
Control	133.33^{a}	424.67	1,222.33	1,934.66^{ab}	3,019.66	4,606.33
5% Kombucha	125.66^{ab}	405.67	1,212.00	1,918.00^{ab}	3,032.00	4,396.00
5% Kombucha with vitamin E and selenium	109.33^{ab}	408.33	1,160.33	1,860.66^{ab}	2,925.66	4,306.66
10% Kombucha	123.33^{ab}	430.33	1,207.67	1,858.33^{ab}	2,983.66	4,246.00
10% Kombucha with vitamin E and selenium	144.33^{ab}	393.00	1,206.00	1,954.66^{a}	3,036.33	4,363.00
15% Kombucha	110.00^{b}	388.00	1,106.33	1,786.00^{b}	2,885.00	4,248.00
15% Kombucha with vitamin E and selenium	113.20^{ab}	397.00	1,223.41	1,879.21^{ab}	3,004.54	4,329.65
SEM	5.74	16.32	44.05	47.59	51.93	124.51
P value (%5)	*	NS	NS	*	NS	NS

The means in each column with similar alphabets are different at the level 5% denoted by * and NS indicates the lack of significance of the difference between treatments mean.

Feed

The results of the effect of diets on the mean feed are shown in Table 6. According to the table, various experimental diets during 1-7 day age and 22-28day age had significant effect on feed ($P<0.05$). During 1-7 day age, the highest

mean of feed was dedicated to control group and had significant difference with treatment 5% of Kombucha with vitamin E and Selenium (P<0.05) but had no difference with other levels.

During 22-28 day age, the lowest mean of feed was about treatment 15% of Kombucha with significant difference with treatment 10% of Kombucha with vitamin E and Selenium (P<0.05) but had no significant difference with other treatments (P>0.05). There was no significant difference in other periods regarding feed among various treatments.

Feed conversion ratio

The mean feed conversion ratio of various treatments in various periods is shown in Table 7. The results showed that there was significant difference between experiment treatments only during 29-35 day age in terms of feed conversion ratio (P<0.05). However, feed conversion ratio didn't show any significant difference in other periods among experiment treatments (P>0.05). The best food conversion coefficient in treatment 15% of Kombucha was during 29-35 day age and it showed significant difference with control group and the treatment with 5% Kombucha (P<0.05).

These results showed that these two treatments (5, 15% Kombucha) had good effect on conversion ratio of chick and the best conversion ratio was among the birds feeding these two Kombucha values. This showed that broiler chicks can accept Kombucha to 15% in diet and conversion coefficient of feed to mean is done well in these two levels (Forutan and Forudi, 2011).

Table 7. The effects of various experimental treatments on conversion coefficient mean of feed

Treatments \ period (Day)	1-7	8-14	15-21	22-28	29-35	36-42
Control	1.27	1.14	1.98	1.90	1.71[a]	2.10
5% Kombucha	1.23	1.08	1.94	1.88	1.71[a]	2.00
5% Kombucha with vitamin E and selenium	1.06	1.10	1.90	1.89	1.69[ab]	2.02
10% Kombucha	1.16	1.03	2.05	1.92	1.65[ab]	1.94
10% Kombucha with vitamin E and selenium	1.12	1.02	1.93	1.91	1.70[ab]	1.97
15% Kombucha	1.09	1.02	1.89	1.85	1.63[b]	1.96
15% Kombucha with vitamin E and selenium	1.28	1.06	1.90	1.87	1.61[b]	1.95
SEM	0.062	0.049	0.071	0.053	0.023	0.046
P value (%5)	NS	NS	NS	NS	*	NS

The means in each column with similar alphabets are different at the level 5% denoted by * and NS indicates the lack of significance of the difference between treatments mean.

Carcass weight

The weight of carcass in various treatments during 42day age is shown in Table 8. The results of Table showed that Bursa of fabrisious and Gizzard were under the influence of experiment diets during 42 day age (P<0.05), but other parameters were not influenced (P>0.05). based on the results, in control treatments 5% Kombucha and 5% Kombucha with vitamin E and selenium had highest weight compared to other treatments (P<0.05). The treatments with 10% Kombucha and 10%Kombucha with vitamin E and selenium had the lowest weight of Bursa of fabrisious**.**

Regarding gizzard, the results of table showed that gizzard in treatment of 10%Kombucha showed the lowest weight (P<0.05). There was no significant difference in gizzard weight in other treatments. The thickness of gizzard (fine and thick) were not influenced by experiment diets during raise (P>0.05).

Table 8. The effects of various experiment treatments on the mean of carcass weight (gram) and Gizzard thickness (mm)

Treatments / Carcass	Bursa of fabrisious weight (g)	Gizzard weight (g)	The thickness of fine part of gizzard (mm)	The thickness of thick part of gizzard (mm)
Control	5.29^{ab}	50.97^{ab}	2.22	15.24
5% Kombucha	6.05^{a}	49.94^{ab}	2.25	16.37
5% Kombucha with vitamin E and selenium	6.26^{a}	53.60^{ab}	2.19	16.29
10% Kombucha	4.27^{b}	47.43^{b}	2.27	15.26
10% Kombucha with vitamin E and selenium	4.37^{b}	59.45^{ab}	2.25	14.88
15% Kombucha	5.82^{ab}	61.26^{a}	2.71	15.38
15% Kombucha with vitamin E and selenium	5.79^{ab}	58.33^{ab}	2.37	16.05
SEM	0.52	3.88	0.65	1.89
P value (%5)	*	*	NS	NS

The means in each column with similar alphabets are different at the level 5% denoted by * and NS indicates the lack of significance of the difference between treatments mean.

Proventriculus morphology

Proventriculus morphology including villus height, crypt depth, and goblet cells and Epithelium thickness in various treatments during 42 day age is shown in Table 4-5. The results of the table showed that crypt depth and goblet cells during 42-day age underwent experiment diet ($P<0.05$) but villus height and Epithelium thickness were not influenced ($P>0.05$). Based on the results of crypt depth and goblet cells in treatments 10% Kombucha with vitamin E and Selenium, 15% Kombucha and 15%Kombucha with vitamin E and Selenium had the highest crypt depth and goblet cells than other treatments ($P<0.05$) but other treatments had the lowest crypt depth and goblet cells.

Table 9. The effects of various experiment treatments on Proventriculus morphology

Treatments / carcass	villus height (micron)	crypt depth (micron)	Goblet cells	Epithelium thickness (micron)
Control	1375.29	250.97^{ab}	2.00^{b}	48.24
5% Kombucha	1396.05	239.94^{ab}	3.00^{b}	51.37
5% Kombucha with vitamin E and selenium	1356.26	203.60^{b}	2.00^{b}	46.29
10% Kombucha	1364.27	247.43^{ab}	4.00^{ab}	39.26
10% Kombucha with vitamin E and selenium	1374.37	359.45^{a}	6.00^{a}	54.88
15% Kombucha	1385.82	361.26^{a}	7.00^{a}	45.38
15% Kombucha with vitamin E and selenium	1395.79	358.33^{a}	6.00^{a}	52.05
SEM	30.52	17.23	1	7.87
P value (%5)	NS	*	*	NS

The means in each column with similar alphabets are different at the level 5% denoted by * and NS indicates the lack of significance of the difference between treatments mean.

Blood metabolites of liver enzymes

The effect of experiment treatments on liver enzymes includes GGT, CPK, ALP, AST, ALT during 42 day age is shown in Table 10. The results showed that in terms of liver enzymes among various treatments, there was no significant difference (P>0.05). It means that the application of various levels of Kombucha extract alone or with Vitamin E and selenium in diet had no effect on liver enzymes and there was no difference between the consumption or non-consumption of Kombucha extract alone or with vitamin E and Selenium in chick diet.

Table 10. The effect of treatments on liver enzymes in broiler chicks during 42 day age (U/L)

Treatments factors	GGT	CPK	ALP	AST	ALT
Control	29.92	655.84	19.98	300.00	50.76
5% Kombucha	32.31	750.67	22.00	363.73	32.19
5% Kombucha with vitamin E and selenium	25.18	749.81	21.04	294.50	35.85
10% Kombucha	35.64	659.05	17.94	289.69	40.54
10% Kombucha with vitamin E and selenium	24.31	647.48	25.36	276.78	43.51
15% Kombucha	22.06	719.79	20.42	307.54	47.66
15% Kombucha with vitamin E and selenium	37.05	688.54	16.92	327.22	39.48
SEM	7.62	49.21	5.21	33.08	10.74
P value (%5)	NS	NS	NS	NS	NS

NS indicated the insignificance of difference between treatments mean.

Anti-body titer against Newcastle antigens and SRBC

The effect of experiment treatments on Newcastle antibody titer during 25, 21 day age and SRBC in 42day age is shown in Table 11. The results showed that there was significant difference in terms of Newcastle titer among the various treatments in 21, 25 day age (P<0.05) but it was ineffective on SRBC during 42day age. The application of 15% Kombucha extract alone and with vitamin E and selenium in diet was effective on Newcastle titer and increased this parameter and it showed positive effect on immunity system and increasing resistance of chicks to Newcastle disease (Afsharmanesh and Sadeghi, 2013).

Table 11. The effect of treatments on Newcastle during 21, 25 day age and SRBC during 42 day age among broiler chicks

Factors / Treatments	21 day	25 day	SRBC
Control	3.00^{b}	3.95^{b}	7.00
5% Kombucha	3.01^{b}	3.89^{b}	8.00
5% Kombucha with vitamin E and selenium	2.09^{b}	3.85^{b}	8.00
10% Kombucha	2.89^{b}	4.04^{b}	8.00
10% Kombucha with vitamin E and selenium	2.88^{b}	4.11^{b}	7.00
15% Kombucha	3.84^{a}	4.95^{a}	7.00
15% Kombucha with vitamin E and selenium	3.92^{a}	4.76^{a}	7.00
SEM	0.28	0.30	1.80
P value (%5)	*	*	NS

The means in each column with similar alphabets are different at the level 5% denoted by * and NS indicates the lack of significance of the difference between treatments mean.

CONCLUSION

The treatment in the diet with 10% Kombucha with vitamin E and Selenium had the highest weight compared to other experiment groups. The results of raising period showed that increasing weight is under the influence of various diets in the first and second weeks and by increase the age of chick, the treatments had no influence on animal weight. The best feed conversion ratio is in treatment 15% Kombucha during 29-35 day-age. Bursa of fabrisious in control treatments**,** 5% Kombucha and 5% Kombucha with vitamin E and Selenium had highest weight compared to other treatments. The effect of experiment treatments on Newcastle during 12, 25 day age showed that there was significant difference in terms of Newcastle titer. Thus, the results showed that for some parameters, 10% Kombucha with vitamin E and Selenium and for others 5% Kombucha and 5% Kombucha with vitamin E and Selenium are suitable. This extract can show positive effect on increasing the resistance of chicks to Newcastle disease.

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