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Effects of Various Type of Bentonite (Montmorillonite) on Ascites-Related Physiologic and Metabolic Factors in Broilers

Research Article

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ABSTRACT

This study was conducted to investigate the effect of ascites-related physiological and metabolic parameters in broiler chickens fed with different components of bentonite. Two hundred and seven days old Arian male chicks were allocated into five dietary treatment groups including 1) basal diet (control); 2) basal diet + 1% sulphated sodium bentonite (SSB); 3) basal diet + 1% non-processed sodium bentonite (SB); 4) basal diet + 1% sulphated calcium bentonite (SCB) and 5) basal diet 1% non-processed calcium bentonite (CB). Forty birds in each group were assigned to four replicates (10 birds in each). The ratio of right ventricle to total ventricles weight (RV/TV) was significantly (P<0.05) lower than in the control group (0.29). T_3 and T_4 were increased by use of SSB in comparison to other groups (P<0.05). However, the mortality rate had a tendency to decrease in SSB (P<0. 01). Dietary supplementation of SSB could be of value in lowing ascites index and improving their associated parameters. In conclusion, dietary inclusion of bentonite had some beneficial effect on ascites-related physiologic and metabolic factors in broilers which might be related to better intestinal absorption of nutrients.

KEY WORDS ascites, bentonite, broiler, metabolite, thyroid hormones.

INTRODUCTION

Genetic selection to increase growth rate and meat production of broiler causes some metabolic and physiological disorders such as ascites syndrome during the last decades of rearing (Julian, 2000). Ascites is a metabolic syndrome accounting cause of over 25% of overall mortality, and non-infectious cause of loss in the broiler industry (De Smit *et al.* 2005). Some factors such as rapid growth rate, high altitude, limiting lung volume, high energy rations and pelleted diets, cold weather, poor ventilation, respiratory disease, high sodium and low dietary phosphorus levels, hepatotoxins, mycotoxins and furazolidone in the feed, vitamin E and Se deficiencies, high levels of thyroid hormones (T_3 and T_4) and stress are the main reasons of this syndrome (Vanhooser *et al.* 1995; Diaz-Cruz *et al.* 1996; Gordon, 1997). Proper environmental conditions and optimal nutritional strategies are needed to prevent the development of pulmonary hypertension, or ascites syndrome (PHS) in highly susceptible broilers. As O_2 demands increase, the pulmonary hypertension which is the result of high blood flow or increase of pulmonary impedance causes higher right ventricle hypertension and damage of tricuspid valve (Julian, 1993). Although ascites is known as the most important factor resulting in economy waste, there was no deep attention on these problems (Olkowski *et al.* 1996; Olkowski and Classen, 1998). Bentonite is aluminosilicate (natural clay) adjusting gut structure and has beneficial effects on feed conversion ratio and growth rate of broilers (Galvano *et al.* 2001; Damiri *et al.* 2011).

The gastrointestinal tract (GIT) consumes approximately 20% of dietary energy and has 50 to 75% protein turnover rate per day (Cant *et al.* 1996). Nearly 25% of daily protein synthesis is secreted into the GIT to support digestive and barrier functionality.

In addition, GIT has been a primary focus for action of phytogenic feed additives. Bentonite has beneficial effect on the detoxifying of the diet (Miazzo *et al.* 2000; Damiri *et al.* 2011) and increases the integrity of gut cells. It has been postulated that bentonite derivatives are potential feed additives to decrease gut protein turn over, energy and oxygen demands in broilers. Betonite acts as excellent adsorptive materials of heavy metals or bacteria (Hassen *et al.* 2003) and toxic and antinutritive agents (Schell *et al.* 1993a; Schell *et al.* 1993b; Abdel-Wahhab *et al.* 1999; Phillips *et al.* 2002).

In animal diet it also acts as gut protectants (enterosorbent), which rapidly and preferentially bind aflatoxins from the digestive tract and thus reduces their absorption into the organism (Grant and Phillips, 1998; Phillips *et al.* 2002). In that manner, adverse effects of aflatoxins on efficiency and liver function are minimized without marked defects in mineral metabolism of the animals (Schell *et al.* 1993a; Schell *et al.* 1993b; Santurio *et al.* 1999). Finally, it can improve gut health and reduce the turnover of proteins and energy in the gut, which is one of the main factors of oxygen demands. Thus, in this study, the effect of ascites-related physiological and metabolic parameters in broilers fed with different components of bentonites was investigated.

MATERIALS AND METHODS

Birds and diets

Two hundred and seven days old Arian chicks were allocated in a complete random design including five treatments, four replicates (cages) and 10 birds in each. Treatments included: 1) basal diet (control); 2) basal diet + one percent sulphated sodium bentonite (SSB); 3) basal diet + one percent non-processed sodium bentonite (SB); 4) basal diet + one percent sulphated calcium bentonite (SCB) and 5) basal diet + one percent non-processed calcium bentonite (CB). Table 1 shows the diets' composition during three different feeding periods of broiler. Diets were provided according to the Arian guidelines and the experimental period lasted for six weeks.

Growth performance and carcass measurements

Body weight and feed intake were measured weekly. The percentage of mortality, heart weight, and the RV/TV ratio or artery pressure index (API) were evaluated after slaughtering on day 35 and 42 of the experiment.

The feed conversion ratio (FCR) and production index (PI) were evaluated during the experimental period.

PI= viability (%) × average body weight (g) / FCR × expreimental period × 10

Biochemical, hormonal and enzymatic measurements

Blood sampling was done on day 28, 35 and 42 of the experiment for measuring blood metabolites, enzymes and thyroid hormones. The samples were immediately maintained at 4 ˚C for 24 h and the serum was harvested by centrifugation (3000×g for 15 min) and was maintained at -18 ˚C until chemical and hormonal parameters measurements. As the higher incidence of ascites in Arian broiler occur at the age of 28-38 days, the percentage of hematocrit was measured at 5^{th} weeks of experiment.

Blood glucose, cholesterol and triglyceride (TG) were measured by enzymatic, colorimetric-endpoint Pars Azmoon kit (15000107, 1500010 and 1500032 Iran, respectively) and spectrophotometric method. Blood total protein was assayed according to biuret procedure, albumin was determined with bromocresolgreen; serum total globulin was calculated by extracting albumin from total protein, blood urea by Berthelot colour assay and blood creatinine by Jaffe colour and their related kit of Pars Azmoon Company. Activities of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were measured using kinetic photometric (DGKC) Pars Azmoon kit. Blood uric acid was assayed using direct colorimetric test with phosphotungstate colour and measured using diagnosis kit of Zist-Chimi Company (10-522, Iran).

Thyroid hormones determinations were performed by single assays, using the Coat-A-Count solidphase125I radioimmunoassay (Denmark) kit. Diagnostic for the kits produced by the Institute of Isotopes of the Hungarian Academy of Science (Budapest, Hungary) with catalog number of RK-6CT1 and having been validated for sheep plasma. The intra-assay coefficients of variation for T_4 and T_3 were 8% and 7.8%, respectively.

Statistical analysis

Data were analyzed with SPSS 19 by one way Anova analysis and Duncan's new multiple range tests (SPSS, 2011). The mathematical model was:

$$y_{ij}= \mu + H_i + e_{ij}$$

Where:

y_{ij}: observations.

μ: mean of observations.

H_i: effect of treatments.

e_{ij}: residual effects.

Table 1 Composition of diet (percentage on the basis of dry matter) during different phases of growing broiler

Ingredients	d 7-20		d 21-33		d 34-42	
	Control	Treatments*	Control	Treatments*	Control	Treatments*
Corn	60.11	58.6	61.40	59.50	65.23	63.57
Soybean meal	30.03	30.03	29	29	27.87	27.87
Fish meal	4	3.93	2.68	3.00	0	0.15
Sodium carbonate	0	0	0.04	0.04	0.1	0.1
Sodium chloride	0.3	0.3	0.3	0.3	0.32	0.32
Bentonite*	0	1	0	1	0	1
Dicalcium phosphate	1.22	1.22	1.15	1.10	1.46	1.46
Vitamin and mineral premix**	0.5	0.5	0.5	0.5	0.5	0.5
DL-methionine	0.23	0.24	0.25	0.24	0.25	0.28
L-lysine HCl	0.11	0.12	0.11	0.09	0.11	0.11
Oil	2.1	2.64	3.24	3.82	2.91	3.42
Oyster	1.4	1.42	1.32	1.33	1.27	1.27
Calculated analysis						
Metabolizable energy (kcal/kg)	2950	2950	3030	3030	3035	3035
			Percent			
Crude protein	20.7	20.7	19.7	19.7	17.8	17.8
Lysine	1.26	1.26	1.17	1.17	1	1
Methionine + Cysteine	0.94	0.94	0.92	0.92	0.84	0.84
Threonine	0.80	0.80	0.75	0.75	0.67	0.67
Arginine	1.32	1.32	1.25	1.25	1.12	1.12
Calcium	1.02	1.02	0.92	0.92	0.90	0.92
Available phosphorous	0.45	0.45	0.40	0.40	0.40	0.40
Sodium	0.17	0.17	0.17	0.17	0.17	0.17

* Treatments included: 1) basal diet (control); 2) basal diet + one percent sulphated sodium bentonite (SSB); 3) basal diet + one percent non-processed sodium bentonite (SB); 4) basal diet + one percent sulphated calcium bentonite (SCB) and 5) basal diet one percent non-processed calcium bentonite (CB).
** Vitamin mix provided the following per kilogram of complete feed: vitamin A: 9000000 IU; vitamin D_3: 200000 IU; vitamin E: 18000 IU; vitamin K_3: 200000 mg; vitamin B_1: 200000 mg; vitamin B_2: 6600 mg, vitamin B_3: 30000 mg; vitamin B_5: 10000 mg; vitamin B_6: 3000 mg; vitamin B_9: 15 mg; vitamin B_{12}: 100 mg and Antioxidant: 500 mg.
Mineral mix provided the following per kilogram of complete diet: Colin: 400000 mg; Fe: 50000 mg; Mn: 100000 mg; Zn: 85000 mg; Cu: 10000 mg; Se: 200 mg and I: 100 mg.

RESULTS AND DISCUSSION

Serum metabolites

Both sulphated sodium and calcium bentonite (SSB and SCB) increased (P<0.05, Table 2) the serum glucose level as compared to control. Serum TG was higher (P<0.05) in SSB than in the control group at day 35 of the experiment, but at the age of 42 days, this difference was not significant. Serum cholesterol concentration was also higher (P<0.05) in the SSB and SCB groups than other groups only at the age of 35 days old. Serum albumin level was higher and globulin lower in the SB group (P<0.05) than SSB and SCB groups. Serum albumin was higher (P<0.05) in all groups received diet with bentonite supplementations compared to the control group (P<0.05). Serum creatinine was the lowest (P<0.05) in the control group on day 28 of experiment, but at the age of 42 days, CB had the lowest level of creatinine.

LDH activity

There were no significant differences between treatments for LDH activity on days 28 or 42 of the experiment (Table 3).

However, bentonite supplementation decreased LDH activity as compared to control, especially on day 35 of the experiment (Table 3).

Thyroid hormones

Birds in the SSB group showed the increase in T_3 concentration at day 35 of the experiment (P<0.05, Table 4). However, there were no significant differences for T_3 at day 42. The T_4 concentration on day 28 of the experiment was the highest for SSB group and the lowest for SCB group (P<0.05).

The T_3/T_4 ratio was the highest for SCB treatment and the lowest for CB treatment on day 28 (P<0.05).

Heart and ventricles weight

Weight of heart and total ventricles was the lowest in broilers fed with the diet supplemented with SB and the highest in the CB supplemented group on day 42 (P<0.05, Table 5). In addition, weight of heart and total weight of ventricles was lower in the SB than the CB supplemented group (P<0.05). We found no effect of bentonite on RV/TV ratio and TV/H.

Table 2 The effect of supplementation of the diet with different compounds on serum metabolites in broiler chicken at different days of experimental period

Metabolites / (d of exp.)	Treatments				
	Control	SSB	SB	SCB	CB
Glucose (mmol/L*)					
d 28**	9.55±0.51[b]	11.36±0.48[a]	10.45±0.63[ab]	11.85±0.69[a]	9.28±0.40[b]
d 35	12.54±0.45	11.36±0.36	11.92±0.36	13.09±0.47	12.45±1.03
d 42	11.62±0.96	11.61±0.34	12.76±0.65	11.66±0.75	11.41±0.81
Triglycerides (mmol/L)					
d 28	1.41±0.21	0.95±0.36	1.08±0.18	1.08±0.11	1.43±0.23
d 35	0.54±0.03[b]	0.81±0.02[a]	0.82±0.07[a]	0.65±0.11[ab]	0.64±0.08[ab]
d 42	0.93±0.18	0.69±0.08	0.72±0.16	0.70±0.19	0.56±0.07
Cholesterol (mmol/L)					
d 28	3.47±0.32	3.45±0.38	3.24±0.10	3.24±0.41	3.20±0.42
d 35	3.47±0.10[ab]	3.80±0.09[a]	3.44±0.09[ab]	3.71±0.15[a]	3.28±0.18[b]
d 42	3.83±0.33	3.38±0.51	3.70±0.36	3.31±0.28	3.00±0.03
Total protein (g/L)					
d 28	38.00±1.59	40.50±5.20	35.40±0.62	38.80±2.46	36.80±3.56
d 35	34.80±9.30[f]	39.90±0.41[e]	37.00±1.54[ef]	39.80±2.03[e]	36.20±2.15[ef]
d 42	43.90±7.57	44.90±2.33	37.70±4.54	40.10±2.83	36.60±2.56
Albumin (g/L)					
d 28	17.40±3.06[ab]	14.90±4.70[b]	22.40±2.30[a]	13.70±1.05[b]	18.30±1.23[ab]
d 35	13.60±0.16[b]	14.90±0.63[a]	15.90±0.89[a]	16.20±1.15[a]	16.00±0.93[a]
d 42	15.60±2.24	14.30±1.81	12.50±1.19	15.10±1.56	12.30±0.73
Globulin (g/L)					
d 28	20.60±4.47[ab]	25.60±6.27[a]	13.10±2.56[b]	25.00±1.67[a]	18.50±3.43[ab]
d 35	21.50±1.73[ef]	25.00±0.45[e]	21.20±1.80[ef]	23.60±1.01[e]	20.20±2.14[f]
d 42	28.30±7.90	30.60±9.30	25.20±4.67	25.00±2.67	26.40±2.78
Uric acid (mmol/L)					
d 28	0.246±0.037	0.250±0.015	0.256±0.043	0.221±0.017	0.236±0.027
d 35	0.206±0.039	0.284±0.005	0.249±0.020	0.250±0.025	0.265±0.034
d 42	0.257±0.013	0.234±0.20	0.256±0.055	0.280±0.042	0.202±0.026
Creatinine (mmol/L)					
d 28	0.033±0.002[c]	0.049±0.003[a]	0.050±0.003[a]	0.044±0.003[ab]	0.040±0.003[bc]
d 35	0.041±0.005	0.042±0.003	0.034±0.003	0.034±0.004	0.036±0.004
d 42	0.005±0.011[ab]	0.066±0.015[a]	0.039±0.008[b]	0.049±0.009[ab]	0.038±0.005[b]

Treatments included: 1) basal diet (control); 2) basal diet + one percent sulphated sodium bentonite (SSB); 3) basal diet + one percent non-processed sodium bentonite (SB); 4) basal diet + one percent sulphated calcium bentonite (SCB) and 5) basal diet one percent non-processed calcium bentonite (CB).
The means within the same row with at least one common letter, do not have significant difference (P>0.01) and (P>0.05).
* (concentration according to S.I. unit).
** d: day.

Table 3 Effect of different form of dietary bentonite supplementation on LDH activity (IU/L) of Arian broiler chickens (Means±SE)

Age (d of exp.)	Treatments				
	Control	SSB	SB	SCB	CB
d 28	1000.5±211.45	1408.5±631.19	1487.5±187.40	1575.6±338.16	1150.2±247.60
d 35	1309.1±52.44[e]	1092.7±131.11[ef]	1122.1±142.88[ef]	1257.5±102.61[ef]	991.2±76.21[f]
d 42	1063.3±126.47	1056±290.93	897.7±88.74	965.8±143.65	1058±417.65

Treatments included: 1) basal diet (control); 2) basal diet + one percent sulphated sodium bentonite (SSB); 3) basal diet + one percent non-processed sodium bentonite (SB); 4) basal diet + one percent sulphated calcium bentonite (SCB) and 5) basal diet one percent non-processed calcium bentonite (CB).
The means within the same row with at least one common letter, do not have significant difference (P>0.01).

Average daily weight gain

Average daily weight gain was the highest in the SB and the lowest in the SCB fed birds during 14-21 d of age (P<0.05, Table 6).

Average daily feed intake

Average daily feed intake was increased (P<0.05, Table 7) in the non-processed bentonite (either sodium or calcium form) than that of SSB on days 21 or 28 of age. The average feed intake was the highest in the SCB and the lowest in the SSB supplemented group in 28 to 35 and 21 to 42 periods of age (P<0.05, Table 7).

Feed conversion ratio (FCR)

Birds fed with SSB showed the lowest FCR relative to other groups fed with bentonite supplemented diets (P<0.05, Table 8).

Serum metabolites

The information about the effect of bentonite on serum metabolites and their relationship with ascites symptoms is limited; however bentonite increased the concentration of most metabolites indicating improvement of metabolic status of broiler chicken. It may be the result of more effective absorption of nutrient in the gastrointestinal tract (Damiri *et al.* 2010). However, more investigations are needed to clarify the relationship of serum metabolite with ascites indexes.

LDH activity

Broilers having a large proportion of muscles compared to visceral organs like heart, lung and kidney which are not proportionally developed, leading to inadequate supply of oxygen resulting in hypoxia and lack of aerobic metabolisms (Siddiqui *et al.* 2009). SDS and ascites have different degrees of metabolic condition but cardiac involvement and edema are common in both conditions. When the condition is acute, it results in SDS and if chronic, it results in ascites. In this situation, production of lactate from pyruvate in presence of lactate dehydrogenase gives rise to increase the production of lactic acid resulting in the systemic acidosis, change in blood pH, cardiovascular system disturbances and cardiac arrhythmia (Siddiqui *et al.* 2009). On the other hand, lower LDH activity, was an index for lower lactate production, better metabolism and finally lower incidence of ascites in SSB, SB, SCB, and CB groups in comparison with control group**.**

Thyroid hormones

Luger *et al.* (2002) showed that thyroxin concentration was significantly lower in broilers suffering from ascites. In addition, they showed that broilers which died due to ascites, had no increase in thyroxin concentration in response to cold stress. The concentration of T_3 and T_4 was lower in chickens suffering from ascites since one week before death. Another study by Scheele *et al.* (2003) demonstrated that the broilers that were insensitive to ascites had higher T_4 value at 5 weeks of age. Gonzales, *et al.* (1999) found the highest T_3 and the lowest T_4 concentration in naked neck strain which had the best RV/TV ratio. On the other hand, higher T_3/T_4 ratio is associated with lower RV/TV ratio and lower risk of ascites.

In the present study, SSB treatment increased T_3 concentration at 35 days of age. In addition, the T_4 concentration was the highest at 28 days of age.

The T_3/T_4 ratio in the SCB group was the highest at 35 days of age and all bentonite fed birds showed tendency to increase relatively to the control group. These results were consistent with the obtained RV/TV index which was better in SSB group. The most likely reason of the observed increase in T_3 and T_4 values could be related to the positive effect of SSB on better absorption of trace elements, such as iodine and selenium, in intestine and their effect on thyroid function and peripheral denomination. However, further studies are needed to find the effect of bentonite on mineral digestion and absorption in the intestine.

Artery pressure index

Ascites is a prevalence syndrome in broilers with high growth rate and is associated with the increase in volume and weight of right ventricle as a consequence of high pulmonary blood pressure (McGovern *et al.* 1999). Higher (>0.25) RV/TV ratio so called artery pressure index (API) shows progressing ascites in broilers (Silversides *et al.* 1997). The API is a common index to examine ascites and relating factors (Gonzales *et al.* 1998; Malan *et al.* 2003; Scheele *et al.* 2003).

In the present study, only the SSB group showed API < 0.25 (0.24). Gonzales *et al.* (1998) showed API higher than 0.25 in a study with seven strains of broiler chickens, except for naked neck strain (API=0.18) after 4 weeks of age. The effect of genetic background on ascites outbreak may be related to lower thyroid activity and limited pulmonary perfusion.

At the critical age for ascites (35 days of age), the RV/TV ratio was 0.24 for SB group showing its beneficial effects on decreasing RV/TV ratio, cardiac disorder, and sudden death syndrome in Arian broiler chicken.

Growth rate and FCR

In the present study, SB increased the average daily weight gain on day 7 to 21 and 14 to 21 of age. As these periods are not critical on ascites incidence, such growth rate could not be of value. However, birds of SSB group showed the decrease in the weight gain between 28 and 35 days of age which was consistent with the decreased API and increased thyroid hormone values in these treatments. Arce *et al.* (1992) suggested that the incidence of ascites can be decreased by the feed restriction and lowering of growth rate of broilers. However, in the present study the lower growth rate in SSB was associated with lower feed intake. Overall, FCR had no significant difference in this treatment as compared to others and also increased in day 21 to 28 showing better utilization of nutrient as was found by Malane *et al.* (2003).

Broilers, which are sensitive to ascites experiencing high growth rate and low FCR with higher body temperature per kg live weight; however, based on metabolic weight, these birds produce lower body temperature than those having lower growth rate and are insensitive to ascites (Malan *et al.* 2003). According to Leeson and Summers (2001), the density of nutrients, manipulation of feed ingredients and change in electrolytes in the diet which may affect the growth rate, can increase the incidence of ascites.

Table 4 The effect of dietary inclusion of different bentonite form (Mean±SE) on thyroid hormones (ng/L) of Arian broilers

Parameter	Treatments				
(d of exp.)	Control	SSB	SB	SCB	CB
T_3					
d 28	1406±260	1551±199	1696±257	1474±289	1315±278
d 35	1601±148[b]	2514±50[a]	1815±215[b]	1830±81[b]	1730±219[b]
d 42	2460±404[e]	2309±231[ef]	1810±2774[f]	2241±405[ef]	1664±93f
T_4					
d 28	6068±1146[ab]	8271±2797[a]	6081±204[a]	3920±370[b]	6750±1077[ab]
d 35	7305±722	4315±709	7586±1067	5616±2122	5149±1808
d 42	4770±545	5595±2014	4240±331	5172±417	6163±959
T_3/T_4					
d 28	0.24±0.023[ab]	0.22±0.057[ab]	0.22±0.057[ab]	0.41±0.125[a]	0.19±0.012[b]
d 35	0.23±0.043	0.62±0.103	0.62±0.103	0.49±0.238	0.48±0.153
d 42	0.61±0.135	0.52±0.125	0.52±0.125	0.46±0.114	0.30±0.051

Treatments included: 1) basal diet (control); 2) basal diet + one percent sulphated sodium bentonite (SSB); 3) basal diet + one percent non-processed sodium bentonite (SB); 4) basal diet + one percent sulphated calcium bentonite (SCB) and 5) basal diet one percent non-processed calcium bentonite (CB).
The means within the same row with at least one common letter, do not have significant difference (P>0.01) and (P>0.05).

Table 5 The effect of dietary inclusion of different betonies form on heart weight, ventricles, and its ratio of the Arian broiler chickens (Means±SE)

Parameter (days of exp.)	Treatments				
	Control	SSB	SB	SCB	CB
Heart weight (g)					
d 35	10.23±0.49	10.33±0.61	10.80±1.21	10.85±0.06	9.2±0.60
d 42	12.18±0.40[ab]	10.75±0.52[bc]	10.10±0.90[c]	10.95±0.75[bc]	12.90±0.61[a]
Total weight of ventricles(g)					
d 35	7.68±0.42	7.98±0.29	7.58±0.62	8.05±0.58	7.35±0.40
d 42	9.30±0.44[ab]	8.25±0.71[bc]	7.43±0.73[c]	8.78±0.71[bc]	10.38±0.70[a]
Weight of right ventricles (g)					
d 35	2.20±0.15	1.95±0.14	2.05±0.17	2.20±0.25	2.25±0.06
d 42	2.55±0.01[ef]	2.23±0.33[ef]	1.90±0.31[f]	2.53±0.36[ef]	2.78±0.22[e]
Ratio of right to total ventricles weight					
d 35	0.29±0.11[a]	0.24±0.24[b]	0.27±0.181[ab]	0.27±0.12[ab]	0.31±0.23[a]
d 42	0.28±0.02	0.27±0.023	0.25±0.020	0.28±0.02	0.27±0.01
Ratio of ventricles weight to heart weight					
d 35	0.75±0.03[ef]	0.78±0.03[ef]	0.72±0.03[f]	0.74±0.03[ef]	0.80±0.03[e]
d 42	0.77±0.04	0.76±0.04	0.74±0.05	0.80±0.03	0.81±0.05

Treatments included: 1) basal diet (control); 2) basal diet + one percent sulphated sodium bentonite (SSB); 3) basal diet + one percent non-processed sodium bentonite (SB); 4) basal diet + one percent sulphated calcium bentonite (SCB) and 5) basal diet one percent non-processed calcium bentonite (CB).
The means within the same row with at least one common letter, do not have significant difference (P>0.01) and (P>0.05).

Table 6 The effect of dietary supplementation with different forms of bentonite on Arian broiler live weight gain (Mean±SE) at different age

Age	Treatments				
(days of exp.)	Control	SSB	SB	SCB	CB
d 7-14	25.31±2.42	26.48±0.55	24.89±0.58	24.68±1.35	26.17±0.59
d 14-21	45.11±1.90[ab]	46.67±1.66[ab]	50.57±2.64[a]	43.83±1.95[b]	45.78±1.74[ab]
d 7-21	31.57±1.91[ef]	33.13±0.58[ef]	35.08±1.97[e]	30.93±1.47[f]	32.60±0.67[ef]
d 21-28	64.85±2.87	65.31±1.73	63.37±1.67	61.19±3.38	65.10±1.07
d 28-35	61.22±1.72[e]	49.04±0.58[f]	55.78±5.38[ef]	49.04±4.74[ef]	61.22±1.7[ef]
d 7-42	44.54±1.73	39.65±2.51	43.74±3.24	42.69±1.83	44.71±2.13

Treatments included: 1) basal diet (control); 2) basal diet + one percent sulphated sodium bentonite (SSB); 3) basal diet + one percent non-processed sodium bentonite (SB); 4) basal diet + one percent sulphated calcium bentonite (SCB) and 5) basal diet one percent non-processed calcium bentonite (CB).
The means within the same row with at least one common letter, do not have significant difference (P>0.01) and (P>0.05).

Table 7 The effect of dietary supplementation with different forms of betonite on broiler average daily feed intake (Mean±SE) at different age

Age	Treatments				
(d of exp.)	Control	SSB	SB	SCB	CB
d 7-14	48.15±3.32	46.37±2.46	47.34±2.37	46.64±1.76	46.63±1.06
d 14-21	77.74±3.89	74.37±2.28	77.94±1.30	75.56±3.59	71.95±3.76
d 7-21	57.42±2.17	59.56±2.33	61.88±1.19	60.24±2.37	57.42±2.17
d 21-28	110.63±4.78[ab]	99.40±2.37[b]	124.48±7.22[a]	115.32±7.97[ab]	125.58±4.95[a]
d 28-35	127.31±7.30[ab]	115.86±7.34[b]	128.21±5.17[ab]	144.83±8.35 [a]	126.33±5.03[ab]
d 7-42	94.74±1.08[ab]	98.45±1.46[a]	96.94±3.87[ab]	90.28±1.81 [b]	97.60±1.16[a]

Treatments included: 1) basal diet (control); 2) basal diet + one percent sulphated sodium bentonite (SSB); 3) basal diet + one percent non-processed sodium bentonite (SB); 4) basal diet + one percent sulphated calcium bentonite (SCB) and 5) basal diet one percent non-processed calcium bentonite (CB).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 8 The effect of dietary supplementation different form of bentonite on broiler FCR (Mean±SE) at different age

Age (days of exp.)	Treatments				
	Control	SSB	SB	SCB	CB
d 7-14	1.93±0.12[e]	1.75±0.06[ef]	1.90±0.07[ef]	1.90±0.04[ef]	1.71±0.06[f]
d 14-21	1.73±0.79	1.60±0.100	1.55±0.08	1.73±0.08	1.58±0.09
d 7-21	1.98±0.09	1.80±0.09	1.78±0.07	1.95±0.08	1.76±0.07
d 21-28	1.71±0.03[ab]	1.51±0.12[b]	1.96±0.08[a]	1.90±0.17[a]	1.93±0.09[a]
d 28-35	2.09±0.17	2.40±0.16	2.34±0.15	2.50±0.17	2.26±0.22
d 7-42	2.20±0.07	2.18±0.03	2.23±0.08	2.32±0.10	2.13±0.10

Treatments included: 1) basal diet (control); 2) basal diet + one percent sulphated sodium bentonite (SSB); 3) basal diet + one percent non-processed sodium bentonite (SB); 4) basal diet + one percent sulphated calcium bentonite (SCB) and 5) basal diet one percent non-processed calcium bentonite (CB).
The means within the same row with at least one common letter, do not have significant difference (P>0.01) and (P>0.05).

On the other hand, increasing dietary cobalt and other minerals such as Mn and Na can increase the risk of ascites. High salt diet and increase in water salts is another prevalent cause of ascites as content of high level of Na (Mirsalimi and Julian, 1991). As the literature shows, change in the density of nutrients and mineral density in the diets and their absorption could be considered as main factors in ascites incidence. Therefore, it could be of value to identify the relationship between different types of dietary bentonite and nutrient absorption.

CONCLUSION

In conclusion, results of present study showed that sulphated sodium bentonite had beneficial effects on ascites indexes. Dietary supplementation of one percent SSB in broiler diet could decrease adverse symptoms of ascites.

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