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Research Article
Effects of Dietary Addition of Raw and Treated Calcium Bentonite on Growth, Digesta Characteristics, Blood Profiles and Meat Fatty Acids Composition of Broilers Chicks

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Abstract
Objective: This experiment was conducted to evaluate the effects of addition of Raw (R) and Treated (T) calcium bentonite (CaB) on growth, digesta characteristics, blood profiles and meat fatty acid of broiler chicks. **Methodology:** Two hundred and fifty, 1 day-old male Hubbard ISA broilers were allocated to 5 groups, reared for 50 days, designated as follows: Soy-corn based diet without addition of bentonite as control group (C), 2% CaBR, 5% CaBR, 2% CaBT and 5% CaBT. **Results:** Among the groups treated with bentonite, the CaBR 2% showed the best growth performance. The digesta moisture decreased (81-73%) when the bentonite rate increased from 2-5% of CaB at raw and treated form, which the intestinal viscosity passed of 1.76-2.12 (cP). The serum level of triglycerides, total cholesterol, cholesterol HDL, calcium and sodium were significantly increased in bentonite groups. The bentonite supplementations have increased the intramuscular lipid from 4.04 in control to 5.41% in CaBR group. Saturated fatty acids of chick's meat were similar between the different groups. Among fatty acids, the oleic acid has been the predominant, with a significantly higher content in CaBR groups. The linolenic acid was negatively affected by 2% of bentonite treatment. However, at 5% of bentonite, the linoleic acid was higher compared to the control. **Conclusion:** This experiment suggest that the addition 2% of raw calcium bentonite in broiler diets has the best beneficial effects on the growth performance and carcass parameters, but the meat fatty acid composition is improved by 5% bentonite.

Key words: Calcium bentonite, broiler, growth, digesta, serum, blood profiles, carcass parameters, meat, fatty acids

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

About 50 years ago, scientists rediscovered clay minerals for medical purposes. In fact, the clay consumption was used for hundreds years by animals and indigenous cultures to promote internal healing and the improvement of economic indicators and commercialization use of silicate minerals is recommended as an ingredient in broiler fed[1,2]. Bentonites are among the first binders appeared as supply in animal diet that contains more or less montmorillonite which is clay of 3 sheets associated with colloidal compounds. The lubricating properties are enhanced when the montmorillonite content is elevated. The properties of bentonite are derived from the crystal structure of the smectite group, which is an octahedral alumina sheet between two tetrahedral silica sheets. Variations in interstitial water and exchangeable cations in the interlayer space affect the properties of bentonite and thus the commercial uses of its different types[3-5]. The sheets are bonded by ionic bridges due to the presence of cations, the connection is so weak that it breaks under the simple action of water[6]. Furthermore, the lubrication efficiency of the mixture of bentonite with the animal's food depends of the water addition and temperature[7]. The use of clay supplements in animal and poultry feed manufacturing is not new. Bentonites are the clays having strong colloidal properties and when in contact with water absorb rapidly and by swelling increase its volume giving rise to a thixotropic, gelatinous substance[8]. Bentonite as a feed additive has been used successfully in poultry feeds without any harmful effects[9,10]. Bentonite slowed down feed passage rate for better utilization of feed nutrients[11]. According to Cosialls *et al.*[12], the incorporation of clay in the chicken diet increases the transit time, about 2-3 h in 87.5% of the birds with the feed containing 1.5% clay against 1.5-1.75 h for 62.5% of the birds with the control diet. The bentonite can be used as feed additive in the broiler chicken diet to improve litter characteristics and as results performance[13].

Moreover, the inclusions of mineral clays as feed additive in broiler diet have a beneficial effect on performance, carcass yield, passage rate and chemical composition of meat[14-16]. Little research has been devoted to the composition of meat fatty acids of chicken receiving the bentonite and on the other hand based on previous studies and reports of the positive effects of clay minerals incorporation into diets of chickens and also of availability of bentonite in the Western part of Algeria, we have conducted this study to determine the effects of CaB addition under two forms, the Raw (R) and the Treated (T) form with water injection, on the growth performance, digesta characteristics, blood biochemical parameters and meat fatty acids.

MATERIALS AND METHODS

Birds and diets: Two hundred and fifty, 1 day-old male Hubbard ISA broilers were reared conventionally and fed until 12 days of age on a standard starter diet (3.100 kcal kg^{-1}, 22% protein) were allowed free access to water and food. At the 12th day, birds with an initial Body Weight (BW) of 450 ± 42.17 g were divided into five groups (50 in each group): The control group (C) received commercial feed without bentonite, the experimental groups were fed on commercial diets supplemented with 2% CaBR (group 1), 5% CaBR (group 2), 2% CaBT (group 3), 5% CaBT (group 4). The room temperature in experimental house was maintained at 38°C during the first days of experiment and decreased gradually by 3°C in the 2nd and 3rd week to be fixed at 22°C there'after. Chicks were vaccinated against New Castle disease at the 3rd and 30th days, gumboro disease at 12th day, coccidiosis at 21st day, via drinking water. Ingredients and nutrient composition of diets were shown in Table 1. The chickens weight and ingested feed were measured at the 21st, 28th, 35th, 42th and 50th days of age. Animals used in this experiment were reared and slaughtered in compliance with ethics regulations for the humane care and use of animals in research.

Bentonite treatment and preparation: This study was carried out on calcium Bentonite of Maghnia (BentMag) obtained from the western part of Algeria and delivered by the National Company for non-ferrous Mining Products ENOF. The BentMag contains more montmorillonite (Mt) and fewer impurities than BentMostaganem and possesses the ability to retain water beyond 200°C[17]. Chemical composition of calcium bentonite (CaB) was shown in Table 2. For a better adhesion of bentonite particles on food surface, the feeds were humidified in advance by water vapor. The steam was injected into feed using a conditioner-mixer as described by David and Lefumeux[18]. Then, the particles of humidified diet were mixed with bentonite by the mixer at dose of 2 or 5% of diet. In this way, the bentonite adsorbs moisture and form a complex feed-bentonite. In the second treatment, bentonite without previous humidification was mixed with food at the same percentage (2 or 5%).

Blood sampling and serum analysis: At 49th day, blood samples were collected from 5 broilers from each group. The serum was separated by centrifugation and stored at -20°C until further analysis. The serum concentration of glucose (g L^{-1}), triglycerides (g L^{-1}), total cholesterol (g L^{-1}), cholesterol-HDL (high-density lipoprotein) (g L^{-1}), cholesterol-LDL (low-density lipoprotein) (g L^{-1}), total

Table 1: Composition of the experimental diets

Ingredients (%)	Standard diet (C)	CaBR2	CaBR5	CaBT2	CaBT5
Corn	67.0	67.0	66.0	67.0	66.0
Soya beanmeal	27.0	27.0	25.0	27.0	25.0
Wheat bran	4.0	2.0	2.5	2.0	2.5
Vit-min premix*	1.0	1.0	0.8	1.0	0.8
Calcium	0.5	0.5	0.2	0.5	0.2
Phosphorus	0.5	0.5	0.5	0.5	0.5
Calcium bentonite	00.0	2.0	5.0	2.0	5.0
Calculated composition					
ME (kcal kg^{-1})	2960.0	2900.0	2890.0	2900.0	2890.0
Calcium (%)	0.8	0.9	1.1	0.9	1.1
Phosphorus (%)	0.3	0.3	0.2	0.3	0.2
Lysine (%)	1.1	1.1	1.0	1.1	1.0
Sulphur aminoacid (%)	0.8	0.7	0.6	0.7	0.6
Analysed composition (%)					
Crude protein	21.0	20.7	19.6	20.7	19.6
Lipids	3.4	3.8	3.5	3.8	3.5
Ash	5.1	5.2	4.5	4.8	5.3
FA analysis (Percentage of the identified FA)					
C14:0	0.08	0.08	0.07	0.08	0.07
C16:0	13.05	13.24	13.27	13.24	13.27
C16:1 (n-7)	0.12	0.12	0.13	0.12	0.13
C18:0	2.66	2.24	2.25	2.24	2.25
C18:1 (n-9)	24.45	26.46	27.50	25.97	25.53
C18:2 (n-6)	50.51	52.34	51.87	52.44	52.93
C20:0	0.36	1.24	1.27	1.32	1.27
C18:3 (n-3)	2.82	2.24	2.38	2.57	2.54
C20:1 (n-9)	0.46	0.35	0.36	0.40	0.35
ΣSFA	19.59	17.71	17.21	16.77	17.41
ΣMUFA	25.85	27.73	28.88	27.31	26.86
ΣPUFA	54.56	55.06	54.35	55.28	55.82

*Vitamin premix: Provided (in mg kg^{-1} of diet), Vitamin E: 6, Vitamin K3: 0.80, Vitamin B1: 1, Vitamin B2: 3, Pantothenate of Ca: 6, Vitamin B6: 1.5, Vitamin B12: 0.006, Folic acid: 0.2, Nicotinic acid: 12, Copper: 5, Cobalt: 0.65, Manganese: 65, Zinc: 65, Selenium: 0.25, Iron: 50, Iode: 0.8, Magnesium: 100, ME: Metabolisable energy

Table 2: Chemical composition of calcium bentonite (CaB)

	SiO_2	Al_2O_3	Fe_2O_3	Na_2O	K_2O	CaO	MgO	TiO_2
Composition (%)	65.20	17.25	2.10	2.15	0.20	3.10	1.20	0.20

proteins (g dL^{-1}), albumin (g dL^{-1}), calcium (mg dL^{-1}), magnesium (meq L^{-1}), sodium (meq L^{-1}) and potassium (meq L^{-1}) were determined by Spinreact Kits (Santa Coloma, Spain) using a clinical auto-analyzer.

Measurement at slaughter: At the 50th day, 10 birds from each batch were selected, slaughtered at the jugular vein processed and eviscerated in a local commercial slaughterhouse. After evisceration, the carcasses were apportioned by hand. Meat samples were taken from the left thigh and frozen at -20°C. Liver and Abdominal Adipose Tissue (AAT) were also removed and weighed individually.

The digesta from the jejunum and ileum were recovered by finger pressure, weighed and immediately centrifuged (3200xg for 5 min). Precipitate and supernatant were separated, weighed, cooled on ice and immediately placed at -20°C for digesta analysis.

Analysis of meat and diet: Mineral content was determined by ashing at 550°C for 4 h. Nitrogen was determined by the Kjeldahl method (CP = N×6.25). The Total Lipids (TL) of each sample (diet or meat) were extracted by chloroform:methanol (2:1) according to the method of Folch *et al.*[19]. Fatty acids were freed by saponification (NaOH) and then methylated by methanol-BF3[20]. The methyl esters of FA were separated and quantified by gas chromatograph (Perkin-Elmer AutoSystem XL) equipped with flame ionization detector and a capillary column (30 m×0.25 mm internal diameter). The operating conditions of the gas chromatograph were as follows: Injector and detector temperature of 220 and 280°C, respectively; the oven temperature was programmed to increase from 45-240°C at 20-35°C min^{-1}; aliquots of 1 µL were injected with bicyanopropyl phenyl silicone as a stationary phase; hydrogen was used as conductor gas; FA peaks were identified by comparison with retention times of methyl fatty acid standards; quantification was made by reference to an internal standard (C17:0).

Digesta analysis: Dry matter and moisture content of digesta were measured by drying an aliquot of 2 g at 105°C in an oven for 24 h. From each jejunal digesta sample, two Eppendorf tubes were filled and centrifuged at 12000xg for 3 min. The intestinal supernatant viscosity was measured at 20-22°C using a Rheonalyser (viscometer Rheoma, R180) as previously described by Carre *et al.*[21].

Statistical analysis: Data were analyzed by two ways of analysis of variance (ANOVA) using the general linear models procedure of SAS software[22]. The means differences were compared by Duncan's multiple range tests. Statements of statistical significance are based on $p<0.05$.

RESULTS AND DISCUSSION

Productive performance: Body Weight (BW), Weight Gain (WG) and Feed Conversion (FC) are presented in Table 3. At 14th, 21st and 28th days no significant differences were observed between the different groups. At 35th, 42th and 50th days, the groups treated with bentonite displayed the highest performances, especially in the CaBR2 treated group whose BW was about 13% ($p<0.05$) higher compared to the control group. At 50th day, the BW was increased ($p<0.05$) in broilers chicks fed with CaBR2 and CaBR5 and less augmented in the CaBT2 group. However, the BW of chickens fed with diet supplemented with CaBT5 was decreased ($p<0.05$) by about 13-25% compared to the C and CaBR2 groups, respectively. This result demonstrates that when the raw or treated bentonite is added at 5%, a negative effect on the body weight gain was observed. The same tendency was observed for WG. The CaBR2 increased the chicks growth ($p<0.05$) from 18-24% compared to those receiving control and CaBT5 diets. On the other hand, at 50th day among the five groups of animals, chickens that received the diet supplemented with CaBT5 showed comparable weight gain to control group. The same observation was noticed regard to the feed efficiency. There were not any significant differences between treatments regarding FCR from 14-21th day. However, from 35-50th day and in comparison with control and treated bentonite diets, the FCR was significantly increased ($p<0.05$) by adding 2 and 5% of raw bentonite (1.60 vs 1.80 of FCR).

Characteristics of digesta: Dry matter and moisture of digesta are presented in Table 4. Significant differences ($p<0.05$) between the control and bentonite treatments were observed. The CaBT5 diet decreased significantly ($p<0.05$) the moisture proportion of digesta when compared to the control and CaBR2 groups (79 vs 87%, respectively). The slow intestinal transit of broilers of CaBT groups at 2 and 5% has been

Table 3: Effects of experimental diets on growth performance of broilers (accumulative results)

	Days					
Parameters	14	21	28	35	42	50
BW (g)						
CaBR2	471.25	605.00	962.95	1425.00	1977.50	2464.00
CaBR5	485.83	596.75	1014.75	1471.75	1985.00	2381.80
CaBT2	462.92	568.50	930.75	1347.00	1990.50	2128.25
CaBT5	452.08	595.50	960.75	1278.00	1697.50	1848.00
C	464.17	664.85	1099.50	1560.50	1920.50	2146.00
SEM	53.01	84.05	11.88	16.85	23.96	22.64
WG (g)						
CaBR2	256.43	367.57	481.24	631.43	736.81	842.19
CaBR5	249.52	431.10	420.48	595.81	675.62	780.95
CaBT2	253.22	446.90	531.43	660.24	674.76	779.99
CaBT5	250.89	483.10	520.57	587.57	598.48	639.45
C	257.68	437.29	466.43	561.19	618.14	676.11
SEM	17.61	17.70	17.35	18.14	19.75	17.89
CaBR2	1.76	1.82	1.69	1.69	1.79	1.80
CaBR5	1.89	1.92	1.92	1.73	1.85	1.86
CaBT2	1.91	1.96	1.74	1.65	1.70	1.79
CaBT5	1.62	1.65	2.03	1.69	1.46	1.58
C	1.65	1.67	1.70	1.68	1.62	1.65
SEM	0.41	0.47	0.57	0.60	0.60	0.58
Effect of bentonite rate	NS	NS	p<0.05	NS	p<0.05	p<0.05
CaB form effect	NS	NS	p<0.05	NS	p<0.05	p<0.05

For each group n = 40, results expressed as Mean and Standard Error of the Mean (SEM), BW: Body weight, WG: Weight gain, FC: Feed conversion, p<0.05: Significant effect, NS: Not significant

Table 4: Effects of experimental diets on dry matter, moisture and viscosity of digesta

Parameters	CaBR2	CaBR5	CaBT2	CaBT5	C	SEM	CaB rate effect	CaB form effect
Dry matter (%)	18.34	23.32	20.67	26.20	12.78	5.62	p<0.05	p<0.05
Moisture (%)	81.66	76.68	79.33	73.80	87.22	7.47	p<0.05	p<0.05
Viscosity (cP)	1.76	1.89	1.82	2.12	1.45	0.24	p<0.05	p<0.05

For each group n = 5, SEM: Standard error of mean, p<0.05, Significant effect, NS: Not significant

Table 5: Effects of experimental diets on blood profiles

Parameters	CaBR2	CaBR5	CaBT2	CaBT5	C	SEM	CaB rate effect	CaB forme effect
Glucose (g L^{-1})	1.13	0.98	1.01	1.03	0.10	0.01	NS	NS
Triglyceride (g L^{-1})	2.61	2.06	2.02	2.88	1.13	0.14	p<0.05	NS
Cholesterol (g L^{-1})	1.67	1.49	1.40	1.53	1.33	0.08	p<0.05	NS
Cho-LDL (g L^{-1})	0.51	0.79	0.78	0.22	0.82	0.07	p<0.05	p<0.05
Cho-HDL (g L^{-1})	0.64	0.30	0.31	0.74	0.29	0.01	p<0.05	p<0.05
Protein (g dL^{-1})	5.07	5.25	5.83	5.96	5.58	0.57	NS	NS
Albumin (g dL^{-1})	3.83	3.85	3.84	3.82	3.73	0.07	NS	NS
Calcium (mg dL^{-1})	16.09	15.96	14.60	14.73	13.02	1.13	p<0.05	p<0.05
Magnesium (meq L^{-1})	2.52	2.53	2.51	2.48	2.46	0.11	NS	NS
Potassium (meq L^{-1})	11.39	12.93	12.52	12.18	11.19	0.08	p<0.05	NS
Sodium (meq L^{-1})	94.09	95.81	100.69	139.59	88.56	27.48	p<0.05	p<0.05

For each group n = 5, results expressed as Mean and Standard Error of the mean (SEM), p<0.05: Significant effect, NS: Not significant

Table 6: Influence of experimental diets on carcass characteristics of broilers

Parameters	CaBR2	CaBR5	CaBT2	CaBT5	C	SEM	CaB rate effect	CaB form effect
EW (g)	1796.50	1794.50	1627.00	1623.33	1707.50	18.79	p<0.05	p<0.05
AAT (g)	17.00	16.00	15.00	16.25	15.00	3.05	NS	NS
TW (g)	353.50	344.00	322.00	290.00	323.00	41.52	p<0.05	p<0.05
BrW (g)	359.00	359.00	343.50	341.11	360.00	53.94	NS	NS
LW (g)	51.00	52.00	50.00	51.00	53.00	2.23	NS	NS

EW: Eviscerated weight, AAT: Abdominal adipose tissue, TW: Thigh weight, BrW: Breast weight., LW: Liver weight, For each group n = 10, results expressed as Mean and Standard Error of the mean (SEM), p<0.05: Significant effect, NS: Not significant

affected by the decrease in digesta moisture. In fact, a high viscosity has been noticed (p<0.05) in this groups compared to the control (2.21 vs. 1.45).

Blood profiles: Blood profiles are presented in Table 5. The glucose, protein and albumin concentrations were not affected by bentonite supplementation in all treatments. However, the chickens receiving respectively treated and raw bentonite have increased serum concentration of triglycerides (p<0.05) about 2.3-2.5 fold compared to the control group. Moreover, a high significant difference (p<0.05) about 13-20% of cholesterol concentrations were observed in CaB2R and CaB5T compared to the control group. The LDL cholesterol was higher (p<0.05) in the control compared to bentonite groups. So, the lowest concentration was observed in chickens group receiving an addition of CaBT5. Concerning HDL cholesterol concentration, the situation is reversed with a higher concentration of 0.74 g L^{-1} (p<0.05) that noticed in the group receiving the treated bentonite.

The bentonite supplementation has no significant effect on magnesium content. However, calcium, sodium and potassium serum concentrations were significantly higher (respectively about, 10, 19 and 36%, p<0.005) in groups receiving CaBT compared to the control one.

Carcass parameters: As body weight, the inclusion of raw Ca bentonite 2 and 5% has significantly increased (p<0.05) the eviscerated carcass and thigh weights (Table 6) compared to the control treatment. However, the group receiving treated CaB 5% showed the lowest eviscerated carcass and thigh weights. There were not significant differences in liver, abdominal fat and breast weights between all treatments.

Fatty acids composition: Total lipids and fatty acids composition of sartorius muscle are shown in Table 7. The intra muscular lipids content has increased (p<0.05) in chickens fed Ca bentonite compared to the control group (5.41 vs 4.04%, respectively). The CaBT5 diet has generated low lipid content, similar to control group. Among saturated fatty acid (SFA), palmitic and stearic acids contents are in equivalent percentages (p>0.05) in meat of all groups.

The monounsaturated fatty acids (MUFA) showed the greatest level in meat for both types of diet, with the oleic acid was the predominant FA especially (p<0.05) in CaBR group. Except CaBR2 diet that generated a low percentage of linolenic acid (n-3C18:3) when compared to the control (0.79 vs 0.92%; p<0.05), the other diets have involved a comparable proportions. However, linoleic acid (n-6 C18:2) in chickens meat receiving the diet supplemented with Ca

Table 7: Total lipid content (%) and fatty acid composition (Percentage of identified FA) of sartorius muscle of broilers fed on experimental diets

Parameters	CaBR2	CaBR5	CaBT2	CaBT5	C	SEM	CaB rate effect	CaB treatment effect
TL (%)	5.41	5.17	4.91	4.23	4.04	1.204	p<0.05	p<0.05
C14:0	0.68	0.66	0.72	0.67	0.67	0.04	p<0.05	NS
C14:n-1	0.20	0.21	0.22	0.22	0.21	0.03	NS	NS
C16:0	26.89	26.35	26.70	26.89	25.95	1.11	NS	NS
C16:1 n-9	0.55	0.56	0.53	0.54	0.54	0.05	NS	NS
C16:1 n-7	6.65	6.69	6.84	6.87	6.76	0.84	NS	NS
C18:0	7.24	6.94	7.10	6.93	7.01	0.64	NS	NS
C18:1 (n-9)	40.06	39.64	38.53	39.70	38.70	1.31	p<0.05	NS
C18:1 (n-7)	2.18	2.29	2.29	2.24	2.33	0.17	NS	NS
C18:2 (n-6)c	13.25	14.18	14.24	13.47	14.99	1.48	p<0.05	NS
C18:3 (n-3)	0.64	0.70	0.65	0.67	0.74	0.08	p<0.05	NS
C20:0	0.09	0.09	0.09	0.08	0.10	0.02	NS	NS
C20:1	0.35	0.32	0.56	0.51	0.34	0.09	p<0.05	p<0.05
C20:2	0.11	0.11	0.12	0.10	0.14	0.03	p<0.05	NS
C20:3	0.14	0.15	0.16	0.15	0.21	0.04	p<0.05	NS
C20:4 (n-6)	0.30	0.37	0.44	0.30	0. 60	0.21	p<0.05	NS
C22:0	0.02	0.01	0.00	0.00	0.00	0.00	NS	NS
C22:11	0.02	0.01	0.01	0.01	0.00	0.02	NS	NS
C22:4 (n-6)	0.08	0.11	0.11	0.08	0.15	0.05	p<0.05	NS
C22:5 (n-3) DPA	0.04	0.03	0.05	0.03	0.07	0.04	NS	NS
C22:6 (n-3) DHA	0.04	0.05	0.06	0.04	0.03	0.03	NS	NS
ΣSFA	35.05	34.17	34.76	34.72	33.83	1.54	p<0.05	NS
ΣMUFA	50.02	49.72	48.99	50.09	48.89	1.62	NS	NS
ΣPUFA	14.93	16.09	16.25	15.19	17.27	1.66	p<0.05	NS
Σ n-6	14.04	15.11	15.24	14.26	14.23	1.58	p<0.05	NS
Σ n-3	0.79	0.87	0.89	0.82	0.91	0.11	NS	NS

For each group n = 10, TL: Total lipid, results expressed as Mean and Standard Error of the mean (SEM), p<0.05: Significant effect, NS: Not significant

Bentonite appeared in comparable proportions to the control, if not higher (p<0.05) to the control, especially when bentonite is added to 5%. Overall, except CaBR2 group, the level of unsaturated fat of meat seems increased with the addition percentage of bentonite regardless of the applied treatment.

Growth performance, digesta and carcass characteristics: Overall, results indicate that the final BW and WG increased respectively about 13 and 18% with CaBR supplementation, compared to the C group, although the CaBT generates a low performance. This improvement in growth performance can be attributed to bentonite effect on slowing intestinal transit and leading so to an increased retention time in the intestine[10]. Our measurements of dry matters digesta and intestinal viscosity confirmed this observation. The findings of Lala *et al.*[23] and Chen *et al.*[24] showed that the use of modified clay and palygorskite (atapulgite) respectively in turkey and broiler improved weight gain, feed efficiency and final weight. In the same way, Damiri *et al.*[16] reported that the best results were observed when the bentonite was added at a low dose (3% or less) which might be due to an increase in the digesta retention time in the lumen and thus a better utilization of nutrients. The decreasing trend of body weight in broiler is in accordance with the results of Pappas *et al.*[25]. Petkova and Ivanov[26] and Khanedar *et al.*[27] who observed a decrease about 4%. This might be due to the highly adhesive nature of the bentonite which absorbs more moisture and resist the flow of digesta through gastrointestinal tract, which can affect the feed intake negatively as described by Zhang *et al.*[28] for use of palygorskite at 300 mg kg^{-1} in weaned piglets instead at 200 mg kg^{-1}. In this fact, our finding, in agreement with Damiri *et al.*[16] showed that the inclusion of treated bentonite at 5% had strongly affected the digesta moisture and intestinal viscosity that switched from 1.76-2.12, which may be had depressed the growth performance. It is assumed that increased intestinal viscosity reduces nutrient digestibility by interference with the diffusion of digestive enzymes to their substrates and with the movement of digesta across the intestine[28]. The same observation was reported with viscous polysaccharides that may also complex directly with digestive enzymes and decrease their activity[29,30]. In the contrast, Qiao *et al.*[31] recently found that neither dietary 2.0% natural Palygorskite nor 2.0% heat-modified palygorskite affected negatively laying hens performance. In this study, 0.5 and 1.0% mass palygorskite inclusion did not affect the growth performance of broilers. The eviscerated and thigh weights in CaBR2 group were the highest among experimental groups. This result can be explained by good muscular development attributed to better weight gain. The finding of Chen *et al*.[24] showed carcass yield of broilers fed on palygorskite supplementation did not differ between treatments being about 71% for all treatments, but the relative weights of heart, liver and spleen were increased. In the contrast with these results but in agreement with our finding, Katouli *et al*.[10], which showed respectively that use of 1, 2 and 3% of sodium bentonite improved carcass weight and yield. In agreement with the results of Khanedar *et al*.[27] and Bailey *et al*.[32]. Our finding demonstrated that liver, abdominal fat and breast weights were not affected by bentonite inclusion. In this study, our findings contradict those of Chen *et al*.[24] that used diets with added palygorskite but with contamination by aflatoxin. On other side, Miazzo *et al*.[33] have found a significant decrease in relative weight of liver, kidney and heart of broilers feed by diets supplemented by bentonite and contaminated with aflatoxins.

Blood biochemical parameters: In our trials we have observed that the glucose, protein and albumin concentrations were not affected by bentonite supplementation in all treatments. However, Che *et al*.[34] and Yang *et al*.[35] indicated a decrease in serum levels of these compounds whatever animals received a diet supplemented with clay but contaminated with *Fusarium poae*. The chickens receiving respectively treated and raw bentonite have increased serum concentration of triglycerides ($p<0.05$) about 2.3-2.5 fold compared to the control group. Lv *et al*.[36] observed a decrease in serum triglyceride in weaned piglets fed on palygorskite supplementation. The high triglycerides concentration observed in our trials may be attributed to the nature of the calcium bentonite that has slowed intestinal transit and therefore induced a higher intestinal absorption of starch and there by contributing to an increase in the hepatic synthesis of lipid. The LDL cholesterol was decreased in both groups of chickens fed on CaB compared to control group. However, the tendency is reversed with HDL cholesterol. These observations indicate the positive effect of treated bentonite addition in chicken diet on HDL Cholesterol. The similar concentrations were recorded by Hermier and Chapman[37] in broilers selected for a high adiposity. Wahl *et al*.[38] indicated that, in many instances, the positive relationship between LDL cholesterol and other lipoprotein lipids becomes inverse in the presence of high proportions of triglycerides.

The increase of serum minerals can be attributed to the addition of calcium bentonite to high doses 2 and 5%, especially the calcium content which was higher in group that was fed by CaB supplemented diet. However, Khanedar *et al*.[27], reported that the addition of bentonite at 1 and 1.5% did not increase the mineral concentrations in the serum of broiler. The improved bioavailability of mineral might be due to the high swelling capacity of clay minerals[39,40], resulting in a slowing in the rate of digesta passage through the gastro-intestinal tract or might be related to the high cation exchange capacity of the clay mineral[41].

It is clear from our findings that the use of calcium bentonite increases the mineral fraction in serum and probably affects the skeleton and muscle mineral proportion. In this context, Yalcin *et al*.[42] reported that supplementation of the diet chicken by sodium zeolite increased the sodium content of tibia.

Intramuscular fat and fatty acids composition: Higher intramuscular fat in the muscle of broilers fed raw CaB was consistent with body weight and carcass composition. Results indicate greater lipid deposition. In our experiment, the increase in intra muscular lipid concentration with Ca bentonite supplementation can be attributed to the slowdown of intestinal transit and highest concentrations of serum triglycerides caused by bentonite as previously explained for the body weight gain. However, Safaei *et al*.[14] shown that supplementation of clay minerals decreased the intramuscular fat and abdominal fat.

The FA composition was greatly modified by diets supplemented with bentonite compared to control diet, although both CaBT diets have generated a similar FA composition to the control group. The bentonite supplementation effect on meat FA composition is most likely due to differences in the amount and composition of FA intake and lipid metabolism. In this study, meat fat quality in chickens is affected by various potential intrinsic and extrinsic factors. Among factors, phosphorus and calcium (as provided by calcium bentonite) were critical and expensive minerals in poultry nutrition. Research on effects of dietary P levels on animal growth and bone development showed that high P intake negatively impacts calcium metabolism and bone properties, whereas low P diets will limit the growth of the animals and it is obvious that excess of calcium with a low phosphorus induces opposite effects[43,44]. Therefore, we think that low dietary phosphorus and high calcium provided by Ca bentonite may change the enzyme activities in broiler chickens, thus affecting meat quality through lipid metabolism, since fatty acid composition and biosynthesis play a role during the regulation for intra muscular fat contents[45].

The MUFA were not affected with bentonite treatment. However, SFA increased in bentonite groups. The increase in SFA is due to an increase in serum triglycerides, as it has been shown in this results and membrany polar lipid remains constant. In bird liver, SFA are synthesized from dietary starch and then stearic acid was desaturated into oleic acid[46]. In fact, our findings could be explained by an increase the lipogenesis in birds fed CaB diet. Because of a long intestinal transit, chickens fed CaB digested starch and thus have a very high lipogenic activity in theirs livers.

However, n-3 PUFA were decreased in group that received CaBR2 diet. This difference can be explained by a high lipogenic activity of chickens liver which directs their metabolism to the production of more SFA rather than PUFA. But, when the calcium bentonite is added at 5%, chickens probably reduce their hepatic lipogenesis of SFA due to excess calcium and thus incorporate more PUFA in muscle. Govers and van der Meet[47] showed that dietary calcium supplementation reduced drastically the solubility of fatty acids in the ileum and this effect, which was maintained in the colon and faeces, reflects the inhibition of the intestinal absorption. Additionally some FA, such as n-6 C18:2, n-3C18:3, n-6 and n-3 long chain-PUFA are selectively incorporated into polar lipids, where astriacylglycerols incorporate more SFA and cis-9 18:1[48].

CONCLUSION

In conclusion, results from this experiment suggest that the addition of 2% calcium bentonite (CaB) has the best beneficial effects on the growth performance, carcass parameters, compared to the supplementation at 5%. On the other hand, the best form of bentonite incorporation was the raw form without previous humidification of feed but. The intra muscular n-3 PUFA was slightly improved by the bentonite addition at 5%. The meat produced may be considered acceptable nutritionally as a component of a healthy consumer diet, which compared favorably with nutritional recommendations for reducing the risk of coronary heart disease. The further researches must be conducted in order to explore the hepatic metabolism through the measurement of the activity of key lipogenesis enzymes and the peroxidation of fatty acids.

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