

Effect of clay minerals supplementation on nutrient digestibility, blood parameters, and performance of buffalo calves

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Abstract

This study was conducted to investigate the effect of adding clay minerals such as bentonite and choline on the digestion of nutrients, blood parameters, and growth performance of buffalo calves. Twelve clinically healthy male buffalo calves aged approximately 11–12 months with average body weight (336.50 ± 20.55 kg) were randomly distributed into three different groups (four animals each). The 1st group was a control or basal diet without supplements, while the 2nd (T1) and 3rd (T2) groups were fed the same basal diet with a supplement of 1% bentonite and choline, respectively. The results revealed that the average daily gain and total dry matter intake (DMI) did not affected by dietary supplementation. However, the feed conversion ratio was improved ($P < 0.05$) in the clay minerals groups in comparison to the control group. The treatment groups were higher ($P < 0.05$) in serum glucose, and calcium and lower ($P < 0.05$) in triglycerides, and urea-N concentrations than the control. Digestibility of crude protein (CP), crude fiber (CF), and ether extract (EE) was increased ($P < 0.05$) with the supplementation of clay minerals. Also, the supplement bentonite increased ($P < 0.05$) dry matter (DM), organic matter (OM), and nitrogen-free extract (NFE) digestibility as compared with other groups. The feeding value in terms of starch value (SV) and digestible crude protein (DCP) was improved ($P < 0.05$) with clay minerals groups. It can be concluded that clay minerals such as bentonite and choline are considered one of the effective ways to improve the average daily gain, blood biochemical parameters, nutrient digestibility, and feed conversion ratio of buffalos calves while the carcass characteristics were not significantly affected.

Keywords: buffalo calves, bentonite, choline, supplementation, nutrient digestibility.

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1. Introduction

The buffalo is a great agricultural component in Egypt and is the primary source for the production of meat and milk. One of the more popular feed additives used by commercial dairies in ruminant diets is clay minerals. This additive decreases the passage rate of the digesta through the digestive tracts, enhancing digestion efficiency and, as a result, increasing feed utilization (Salem *et al.*, 2001). Clay minerals mainly hydrate include aluminum silicate (brucite), kaolinite, montmorillonite, illite, chlorite, sepiolite, bentonite, zeolite, and hydrated sodium calcium aluminosilicate (Eng *et al.*, 2003). By adding clays to the ration, toxic substances can be bound and immobilized in animals' gastrointestinal tracts, reducing their toxicity (Subramaniam and Kim, 2015). Clay has the ability to absorb ammonia produced in the rumen, and because of its great capacity for adsorption, it can protect animals from hazardous ammonia buildup by preventing excessive ammonia from building up in the rumen (Diaz *et al.*, 2004). Bentonite mostly consists of silicon dioxide (SiO₂), magnesium oxide (MgO), aluminum oxide (Al₂O₃), and sodium oxide (Na₂O) (European food safety authority (EFSA, 2013)) and it is a proven safe feed additive for animals (EFSA, 2016), where the safe level for all animal species was 20 mg/kg of complete feed (EFSA, 2013). Bentonite is one of the frequent natural clays in Egypt used in

animal diets to increase feed intake, nutrient digestibility, and growth rate (Saleh *et al.*, 1999; Salem *et al.*, 2001), with no changes to mineral metabolism, bentonites minimized the harmful effects of aflatoxins on efficiency and liver function (Marroquin-Cardona *et al.*, 2009). In nature, choline is found in large quantities as free choline, acetylcholine, more complex phospholipids, and their metabolic intermediates. It is a beta-hydroxyethyltrimethylammonium hydroxide, it has a complicated function within the body. It is required for the creation of the neurotransmitter acetylcholine, the cell membrane signaling phospholipids, the transport of lipids via lipoproteins, and the metabolism of methyl groups (homocysteine reduction) (Zeisel and Da Costa, 2009). There is little information has been published showing the effects of bentonite and choline on buffaloes calves performances. Therefore, the main objective of this study was to investigate the effect of adding bentonite and choline on performance, blood constituents, nutrient digestibility, feeding value, and carcass characteristics of buffaloes calves.

2. Materials and methods

2.1 Animals, diets, and management

This study was performed at the research farm of the Faculty of Agriculture, Al-Azhar University, Assiut, Egypt. Twelve clinically healthy male buffalo calves, aged 11-12 months and weighing 235.50

± 20.55 kg on average, were used in this study. The animals were divided into three groups, each four animals. The experimental period was divided into two parts, the first was for 15-days as adaptation period and second was for 135-days trial phase. The animals of each group were housed separately in pens, and throughout the experiment were fed individually. The basal diet was prepared to meet the nutritional needs of calves according to the recommendations of NRC (2001). A concentrate mixture covered 70% of the nutrient requirements, while wheat straw was given *ad libitum* as roughage to cover the rest of the requirements (30%). The control group received the basal diet without supplements. The animals in the second and third groups were fed the same basal

diet supplemented with 1% choline and bentonite to CFM, respectively. The CFM is composed of 20% wheat bran, 20% undecorticated cotton seed cake, 50% com, 8% soya bean meal, 1% limestone, 0.5% salt, and 0.5% premix. The chemical analysis of the diets and wheat straw is presented in Table (1). Rations were offered to animals daily and the refusals were collected and weighed daily to calculate the daily feed intake. The animals were weighed at the beginning and biweekly throughout the experiment. Feed conversion ratio was calculated by dividing the feed intake by the weight gain. Fresh water was available *ad libitum*. The calves were dewormed with an anthelmintic injection before the start of the trial, and clinically examined to be sure that they are healthy.

Table (1): Chemical analysis of the diets and wheat straw (% on DM basis).

Item	Treatments			Wheat straw
	Control	Choline	Bentonite	
Dry matter (DM)	90.77	90.95	91.20	91.20
Organic matter (OM)	82.55	82.50	81.18	83.90
Crude protein (CP)	16.60	16.00	15.10	3.55
Crude fibre (CF)	8.30	9.40	8.80	36.40
Ether extract (EE)	3.01	3.08	3.40	1.76
Nitrogen free extract (NFE)	54.64	54.02	53.88	42.19
Ash	8.22	8.45	10.02	7.30

2.2 Blood sampling

Every month, blood samples were collected from each calf jugular vein at 6 hrs. after the morning feeding. Blood samples were then centrifuged at 3000 rpm for 20 min for harvesting of serum and then stored at -20°C until analyzed for

blood parameters. Blood biochemical parameters (urea, calcium, glucose, total protein, albumin, and globulin) were analyzed by spectrophotometer (Hitachi 911 automated analyzer) (Spinreact, Spain), and the analyses were performed according to the manufacturers company.

2.3 Digestibility trials

The nutrient digestibility of the experimental diets was determined at the end of the feeding trial using chromic oxide as an external marker in three digestibility trials. Each digestibility trial for each diet lasted for 14 days, the first 7 days serving as an adaptation period, followed by 7 days of data collection. Each calf got exactly 10 g of powdered Cr₂O₃ on the first day of the preparatory period, which was manually mixed with the concentrate mixture. For chemical analysis, daily feed samples were collected, mixed, dried, and ground through a 1 mm sieve screen. In supplementation, from day 8 to day 14, about 200 g of fresh feces were gathered twice daily by fecal grabbing and kept in a refrigerator. The fecal samples from each animal were collected at the end of the digestibility trial, dried at 60 °C, and ground through a 1 mm mesh screen for chemical analysis using the procedures of the Association of the Official Analytical Digestibility of nutrients was estimated according to Maynard and Loosli (1969). Samples of feed and feces were analyzed for DM, CP, CF, EE, and ash contents according to AOAC (1990). Nitrogen free extract (NFE) of feed and feces was obtained by difference.

2.4. Carcass characteristics

At the end of the experiment period, all animals in all groups were slaughtered after eight hrs. of fasting with free access

to water. Body weight was measured immediately before slaughter. Then, directly after slaughtering, the hot carcass weight was recorded. The animals were slaughtered according to the Islamic method (Alhidary *et al.*, 2019). The weights of blood, head, skin, feet, liver, whole gastrointestinal tract, spleen, kidney, heart, and lung were also recorded. The carcass right and left sides were cut into four pieces, the right posterior quadrant, right anterior quadrant, left hind quadrant and left anterior quadrant, then all of them were weighed. The dressing percentage to fasting body weight was calculated. Also, the recovery percentage for hot carcasses was calculated.

2.5 Statistical analysis

Statistical analysis was done according to the general linear model in SAS (2001) version 8.2. Differences among groups for feed intake, daily gain, blood parameters, nutrient digestibility, and carcass characteristics were evaluated by one-way analysis of variance. The Duncan Multiple Range Test (Steel and Torrie 1980) was used to test the effect of treatments. The data are presented as mean ± S.E. Level of significance was set at $p < 0.05$. The statistical model was as follows:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where, Y_{ij} = experimental observation, μ = general mean, T_i = the effect of treatment, i = control, choline and

bentonite, Eij = the errors related to individual observation.

3. Results and Discussion

3.1 Growth performance

Total body gain and average daily gain were not significantly affected by dietary supplementation (Table 2). However, the average daily gain tended to be increased with the supplement choline and bentonite to calves' diets by about 27.9 and 19.8% compared with the control group, respectively. The improve performance may be attributed to choline or bentonite supplementation with diets decreased the breakdown of feed protein contents, thereby increasing the flow of dietary N from the stomach to the small intestine (Ivan *et al.*, 1992). Young-Jik *et al.* (2017) reported that bentonite activity may be related to the improvement in growth performance. This activity caused a delay

in food particles passed through the gut, enhancing nutrient digestion. Similarly, Fenn and Leng (1990) stated that the increased growth rates could be caused by the impact of bentonite in diets, which may boost the flow rate of protozoa from the rumen to the intestine rather than feeds. These results are in agreement with those reported by Lee *et al.* (2010) who found no differences in body weight gain of steers fed concentrate mix supplemented with 1% bentonite during the feeding trials. However, Saleh (1994), they found that the supplementation of bentonite at the levels of 2, 2.5 and 5% increased the growth rate of lambs. Also, Cho *et al.* (2000) found that the mineral-supplemented groups (illite-, bentonite-, and kaolinite) to calves ration improved average daily gain and feed intake in comparison with the control. Feed intake from concentrate and roughages as total DMI were not significantly ($P>0.05$) different between groups.

Table (2): Effect of dietary choline and bentonite supplementation on growth performance of buffalo calves.

Items	Treatments			P-value
	Control	Choline	Bentonite	
Number of Animals	4	4	4	-
Duration (day)	135	135	135	-
Initial weight (kg)	235.00± 2.52	236.33± 5.81	234.66± 3.7	0.957
Final weight (kg)	322.33± 12.20	348.00± 1.53	339.33± 8.08	0.175
Total gain (kg)	87.33± 10.09	111.67± 7.13	104.67± 4.91	0.148
Average daily gain (g/h/d)	646.88± 74.72	827.18± 52.78	775.33± 36.37	0.148
Daily feed intake (kg /h/ d)				
DMI of concentrate	5.813 ± 0.28	5.710 ± 0.36	5.720± 0.18	0.960
DMI of roughages	4.965 ± 0.18	5.189± 0.23	5.150± 0.21	0.715
Total DM intake	10.77 ± 0.11	10.89± 0.59	10.87± 0.38	0.976
Feed conversion (Kg DM /K gain)	16.64 ^a ± 0.167	13.16 ^b ± 0.71	14.01 ^b ± 0.49	0.000

^{a,b} Means within the same row carrying different superscripts are significantly different at ($P<0.05$).

The results of feed intake in this study were similar to that obtained by Saleh (1994) reported that feeding bentonite to goats and sheep had no effect on their daily dry matter intake. Also, Aiad (1990) reported that supplements of bentonite, kaolin, and tafla clays with 3% urea in sheep rations had no effect on feed intake. Also, Norouzian *et al.* (2010) found no effect for clay minerals on daily dry matter intake of lamb. In this context, Mohsen and Tawfik (2005) reported that the variation in dry matter intake may be due to the type of diet and bentonite levels. Feed conversion was considerably ($P < 0.05$) improved in treatment groups in comparison to the control group (13.16 and 14.01 *vs.* 16.64 Kg DM/Kg gain, respectively). In this context, Ibrahim (2012) reported that tafla and bentofarm-supplemented lamb rations had higher feed efficiency than non-supplemented rations. Similar to that, adding bentonite to the rations at levels of 0, 2.5, and 5% improved ($P < 0.05$) feed conversion efficiency (Mohsen and Tawfik, 2005).

3. 2 Blood metabolites.

The results of blood metabolites are summarized in Table (3). Blood metabolites usually are taken as a tool to assess the general health and vitality of animals. The dietary supplementation of choline and bentonite increased ($P < 0.05$) the serum glucose, and calcium and decreased ($P < 0.05$) triglycerides and urea-N concentrations as compared with the control group. The calves that received

bentonite were higher ($P < 0.05$) in globulin and lower ($P < 0.05$) in the A/G ratio compared with other groups. However, the total protein and albumin did not differ among the groups. These results confirm the finding of Ghanem (1995), who observed that the globulin fraction was improved ($P < 0.05$) by bentonite. Albumin fraction in the urea-treated rice straw rations was unaffected by the supplementation of bentonite at levels of 2.5 and 5% when compared to the control (Mohsen and Tawfik, 2005). Moreover, Aydin *et al.* (2020) reported that the supplementation of sodium bentonite at rates of 1% and 2% to lambs' diets had no significant impact on the albumin, total protein, or globulin values of the lambs. Similarly, EI-Gendy (1985) reported that the calves fed urea-treated diets containing 0% or 4% bentonite did not experience any changes in blood protein content. Also, Saleh (1994) stated that bentonite had no effect on the total plasma protein of sheep. Animals fed on bentonite or choline had the chance to release ammonia very steadily into the rumen environment, allowing the rumen pool to have a chance to balance microbial protein production through better utilization of glucose and ammonia and thereby improving the blood total protein concentrations. This is indicated by the decreased urea N concentration of serum with dietary supplements of the animals (Khadem *et al.*, 2007). Similarly, Saleh (1994) indicated that sheep's blood urea nitrogen decreased significantly ($P < 0.05$) when bentonite was added to the urea-

containing diet. Increasing the level of glucose in blood serum with clay minerals supplement may be refer to release significant physiological doses of thyroid hormones as T3 that oxidizes glycogen to glucose (Mehany and Shams, 2019). Roussel *et al.* (1992) found that adding sodium zeolite supplements of 1.0% and 1.5% to a mid-lactation Holstein diet based on corn silage significantly increased serum glucose and alkaline

phosphate. The decrease in serum total triglycerides in clay minerals groups was consistent with the finding of Mehany and Hegazy (2020) who reported that the concentration of cholesterol significantly ($P<0.05$) decreased by toxic binders (bentonite and zeolite) under heat stress conditions. However, Moheesn and Tawfik (2002) noted that toxic binder bentonite did not affect the serum cholesterol of Angora goats.

Table (3): Effect of dietary choline and bentonite supplementation on blood serum constituents of buffalo calves.

Items	Treatments			P-value
	Control	Choline	Bentonite	
Total protein (g/dl)	6.41 ± 0.06	6.42 ± 0.05	6.52 ± 0.06	0.287
Albumin (g/dl)	2.93 ± 0.02	2.95 ± 0.09	2.74 ± 0.05	0.101
Globulin (g/dl)	3.48 ^b ± 0.08	3.47 ^b ± 0.07	3.78 ^a ± 0.01	0.015
A/G ratio	0.84 ^a ± 0.02	0.85 ^a ± 0.03	0.72 ^b ± 0.01	0.011
Urea-N (mg/dl)	44.15 ^a ± 0.03	42.16 ^b ± 0.02	42.90 ^b ± 0.02	0.001
Triglycerides (mg/dl)	186.90 ^a ± 0.10	153.3 ^c ± 0.12	158.53 ^b ± 0.07	0.001
Glucose (mg/dl)	63.72 ^c ± 0.03	72.26 ^a ± 0.12	68.78 ^b ± 0.10	0.001
Calcium (mg%)	10.20 ^b ± 0.16	11.71 ^a ± 0.04	11.43 ^a ± 0.052	0.001

^{a, b, c} Means within the same row carrying different superscripts are significantly different at ($P<0.05$).

3.3 Nutrient digestibility and nutritive values

Results in Table (4) indicate that dietary supplementation of choline and bentonite to the buffalo calves' diets significantly increased ($P<0.05$) the digestibility of CP, CF, and EE compared to the control group. Also, the calves received the bentonite diet were higher in DM, OM, and NFE digestibility than the choline and control groups. However, no significant differences were found between the choline and control groups. Improvement in nutrient digestibility with the supplementation of choline or bentonite to diets may be attributed to increased reactive nutrient surface areas to the

impact of microorganism enzymes may be responsible for increasing the amount of feed accessible for absorption and during digestion (Pulatov *et al.*, 1983) and improving feed utilization by decelerates the feed rate time through the digestion tract and improving absorption consequently (Gabr *et al.* 2003). These results are in agreement with the findings of EL-Tahan *et al.* (2005) found that 2 and 4% clay supplemented to growing calves diets significantly improved nutrients digestibility and feeding values. Consequently, bentonite is regarded as one of the typical natural clays utilized in animal rations to improve nutrient digestibility (Saleh *et al.*, 1999). Similar results confirmed by Yahia *et al.*

(2018) showed that the digestibility of DM, OM, EE, and NFE were significantly ($P \leq 0.05$) higher for cows fed tafla-supplemented rations than those in the control diet. Moreover, Mehany and Shams (2019) found that supplemented toxin binder (bentonite and zeolite) increased ($P < 0.05$) all nutrient digestibility compared to those of control one.

However, the bentonite group recorded higher values of all nutrients than those of the zeolite group, so bentonite minerals could be considerably affecting protein and carbohydrate metabolism and digestion. Feeding value in terms of SV and DCP was significantly ($p < 0.05$) improved with dietary supplementation of clay minerals.

Table (4): Effect of choline and bentonite supplementation on nutrients digestibility and nutritive value of experimental rations (%).

Item (%)	Treatments			P-Value
	Control	Choline	Bentonite	
Nutrient digestibility				
Dry matter (DM)	73.63 ^b ± 0.03	74.20 ^b ± 0.15	77.87 ^a ± 0.28	0.001
Organic matter (OM)	77.06 ^b ± 0.01	76.76 ^b ± 0.13	79.26 ^b ± 0.09	0.001
Crude protein (CP)	75.23 ^c ± 0.09	77.62 ^b ± 0.17	80.54 ^a ± 0.28	0.001
Crude fibre (CF)	70.39 ^c ± 0.01	74.76 ^b ± 0.13	75.07 ^a ± 0.01	0.001
Ether extract (EE)	65.49 ^c ± 0.29	77.32 ^b ± 0.25	78.36 ^a ± 0.03	0.001
Nitrogen free extract (NFE)	84.17 ^b ± 0.07	84.33 ^b ± 0.09	85.22 ^a ± 0.091	0.001
Nutritive value				
TDN	65.41 ± 0.32	65.73 ± 0.23	66.06 ± 0.25	0.255
SV	62.57 ^b ± 0.26	64.10 ^b ± 0.16	67.45 ^a ± 0.17	0.001
DCP	10.65 ^b ± 0.06	11.80 ^a ± 0.04	12.80 ^a ± 0.04	0.050

^{a,b,c} Means within the same raw in each item with different superscripts are significantly different ($P < 0.05$).

However, the feeding value expressed as a total digestible nutrient (TDN) did not differ among groups. The increasing SV may be due to improvement of digestibility of CP, CF and EE. Significant improvement in nutritive values as DCP was associated with increasing the digestibility coefficients of CP. Similar trends were obtained by the investigation of (Salem *et al.*, 2001). Also, Yahia *et al.* (2018) showed that the improvement in nutritional values as seen in TDN and DCP reflects the positive effect of dietary supplementation with 200 g tafla/h/d on the digestibility coefficients of most nutrients when compared with the control diet.

3.4 Carcass characteristics.

There were no significant ($P > 0.05$) differences in the hot carcass, dressing percentage, recovery percentage and edible and non-edible carcass components of buffalo calves fed clay minerals and control diets (Table 5). While the blood weight was lower in the choline and bentonite group than in the control group. These results agree with those reported by Young-Jik *et al.* (2017) who found that the supplementation of bentonite to the diets of growing and fattening steer had not significant effect on yield traits and quality traits between treatments. However, Khadem *et al.* (2007) found that

the dressing percentages and carcass cuts were a bit higher in sheep of bentonite-fed groups compared to those in the control group. Also, the supplementation of

bentonite to the diets of swine and steer increased productivity, meat quality, and carcass characteristics (Lee et al., 2010).

Table (5): Effect of choline and bentonite supplementation on hot carcass and edible and non-edible parts of buffalo calves.

Items	Treatments			P-value
	Control	Choline	Bentonite	
Fasting weight (kg)	339.33± 8.09	348.00± 1.53	322.33± 12.19	0.175
Hot carcass (kg)	146.66 ± 4.54	152.96 ± 5.32	140.33 ± 3.12	0.654
Anterior quadrants (kg)	73.33± 3.33	73.41± 3.564	67.00± 3.51	0.385
Hind quadrants (kg)	73.33± 3.33	79.55± 5.01	73.33± 3.33	0.486
Boon weight (kg)	26.87± 1.56	29.53± 0.62	28.33± 1.67	0.437
Dressing percentage (%)	43.22± 0.91	43.95± 0.74	47.97± 0.16	0.322
Recovery percentage (%)	81.67± 5.56	80.69± 4.81	79.81± 2.87	0.707
Blood weight (kg)	25.37 ^a ± 1.06	15.87 ^b ± 1.89	17.31 ^b ± 0.33	0.003
Total full gastrointestinal tract (kg)	101.00± 2.08	107.00 ± 1.154	98.33± 4.41	0.175
Lung weight (kg)	4.45± 0.03	4.06± 0.06	4.17± 0.09	0.056
Head weight (kg)	16.58± 0.76	19.48± 0.88	18.33± 0.89	0.125
Skin weight (kg)	27.91± 0.49	30.90± 0.35	27.67± 1.452	0.081
Feet weight (kg)	10.37± 0.31	10.67± 0.17	10.33± 0.33	0.674
heart weight (kg)	1.52± 0.02	1.50± 0.03	1.32± 0.12	0.171
Liver weight (kg)	4.07± 0.07	4.15± 0.09	3.20± 0.47	0.096
kidney weight (kg)	0.71± 0.01	0.70± 0.06	0.70± 0.01	0.555
Spleen weight (kg)	0.69± 0.06	0.70± 0.02	0.63± 0.07	0.479

^{a,b,c} Means within the same row in each item with different superscripts are significantly different (P<0.05).

4. Conclusion

It was concluded that dietary supplementation with choline and bentonite at a rate of 1% to CFM improved the average daily gain, blood biochemical parameters, nutrient digestibility, and feed conversion ratio of buffalos' calves. However, the carcass characteristics were not significantly affected.

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