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In Vivo Assessment of Dietary Supplementation of Magnetic Bentonite Nanocomposites in Baluchi Male Lambs Diets: Effects on Ruminal Fermentation, Nutrients Digestibility and Lamb Performance

Effect of Magnetic Bentonite Nanocomposites on Baluchi Male Lambs Performance

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Abstract: **This study aimed to evaluate in vivo dietary supplementation of magnetic bentonite nanocomposites on ruminal fermentation, nutrients digestibility and performance in Baluchi male lambs. Twelve Baluchi male lambs [30.95 ± 2.41 kg body weight (BW), 5-6 months] were randomly divided into four experimental diets (n = 3 per group). The dietary treatments were: (1) control (basal diet without supplementation); (2) natural bentonite (NB) (20 g/kg DM); (3) bentonite plus (BP) (0.5 g/kg DM) and (4) magnetic bentonite nanocomposites (MBNC) (0.5 g/kg DM). There was significant difference for final BW ($P< 0.001$), average daily gain ($P< 0.007$), dry matter intake ($P< 0.001$) and nutrients digestibility ($P< 0.001$) between diets. The pH ($P< 0.001$), NH_3-N ($P< 0.001$), TVFA ($P< 0.001$), acetate ($P< 0.001$), propionate ($P< 0.001$) and acetate to propionate ratio ($P= 0.001$) differed between treatments. These results indicated that modifying bentonite structure to nanocomposite improves chemical stability, physicochemical properties, and their efficiency as feed additives.**

Keywords-component; Baluchi, bentonite, fermentation, nanocomposites

1. Introduction

Recently, the application of clay-based feed additives to improve animal performance has been developed [1]. Bentonite is a natural clay ((Na,Ca)(Al,Mg)(Si4O10)3(OH)6nH2O) containing montmorillonite as a major constituent and characterized by colloidal properties due to its aluminosilicate structure [2,3]. By altering bentonite's structure via different methods, its performance can be optimized. Removing of phosphate species by a new type of bentonite-alum adsorbent suggested by [4]. Huang et al. (2017) could synthesize the organobentonite via using cetyl trimethyl ammonium bromide (CTAB) in the bentonite's structure [5]. The ability of the modified bentonite-alum polymer to coat ceramic substrates was demonstrated by [6]. Recent advances in the synthesis and applications of bentonite nanocomposite to remove inorganic materials from water were reviewed by [7]. The synthesized of nano bentonite and its effects on the Salmonella typhimurium mutation process were studied [8]. Bama and Sundrarajan (2017) synthesized an antibacterial Ag/TiO2/bentonite nanocomposite against Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) [9].

According to recent studies, modifying the structure of bentonite to nano and nanocomposite improved its chemical stability and physicochemical properties [10]. As a result of the synthesis of bentonite nanocomposites and the emergence of new physicochemical properties, we anticipate that ruminal fermentation will be more effectively managed. Therefore, the objective of this study was to investigate the in vitro and in vivo dose–response effects of magnetic bentonite nanocomposites supplementations on ruminal fermentation parameters, nutrient degradation and lamb performance.

2. Materials and Methods

2.1. Experimental feed additives

Natural aluminosilicate structured bentonite (Zarin Binder™) and bentonite plus (Zarin Binderplus ™) were supplied from Vivan trading company, Qaen, Iran. in which they are mentioned, and separated from the main text by a short line.

In a typical synthesis of $Fe3O4^{-}$ bentonite nanocomposite, 2 g of $FeCl_2 \cdot 4H_2O$ and 5.2 g of $FeCl_3 \cdot 6H_2O$ were dissolved in 50 mL of distilled water under vigorous stirring at 80 °C. Thereafter, 200 mL of 25 % ammonium hydroxide solution was slowly added to the solution. 3 g of previously prepared bentonite was added to the solution under constant stirring and the reaction was continued for 3 h. The coated bentonite particles were separated by a magnet and washed several times using ultrapure water and dried at 50 °C for 24 h [11].

To detect the surface morphology of adsorbent the scanning electron microscopy (SEM) images (SEM, TESCAN Mira3) and Energy Dispersive X-Ray (EDX) spectrum were used. The accelerating voltage of the microscope was kept in the range of 15 kV [11]. Fourier transformed infrared (FTIR) spectrum of $Fe3O4^{-}$ bentonite sample within the range of 400–4000 cm^{-1} was recorded on Perkin Elmer 1750 FTIR Spectrophotometer [11,12].

2.2. In vivo experiment

Twelve Baluchi male lambs [30.95 ± 2.41 kg body weight (BW), 5-6 months] were randomly divided into four experimental diets (n = 3 per group). The dietary treatments were: (1) control (basal diet without supplementation); (2) natural bentonite (NB) (20 g/kg DM); (3) bentonite plus (BP) (0.5 g/kg DM) and (4) magnetic bentonite nanocomposites (MBNC) (0.5 g/kg DM). The experimental diets were formulated according to NRC (2007) [13], with a forage to concentrate ratio of 50:50 (Table 1). Animals were adapted to the experimental diets for 15 days, followed by a 60-days experimental period. The animals were housed according to guidelines suggested by the Iranian Council on Animal Care (1995) [14] in 2 m × 2 m individual pens with a concrete floor. Lambs were fed ad libitum (5% orts) twice a day at 7am and 7pm. Lambs allowed free access to fresh water. Dry matter intake (DMI) and refusals were recorded daily for each lamb throughout the experiment period. The diet samples (offered and orts) were dried in a forced-air oven at 65 °C for 48 h and stored in plastic containers for further study. Lambs were weighed immediately before the morning feeding at the start of the experiment and every 15 days thereafter, and these weights were used to calculate BW changes. Fresh fecal samples were obtained daily from each goat at 9am and 5pm, about 1 h post-feeding during a 7-days period (from day 53–60). These samples were pooled per lamb and stored at -20 °C for later analysis. Feces samples were dried for 48 h at 65 °C in a forced-air oven (Behdad Co., Iran), and then ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) before analysis. The nutrient digestibility was measured based on the amount of nutrient consumed and excreted. The collected rumen fluid was strained through four layers of cheesecloth and pH was immediately measured with a portable pH meter (Hana, Model HI 2210− 01, USA), and then preserved at −18 °C for the future analysis. Ruminal NH_3-N and VFA were prepared according to that described in the in vitro section. The ether extract (EE, method no. 991.36), ash (method no. 942.05), and crude protein (CP, Kjeldahl, N × 6.25, method no. 954.01) concentrations were measured according to AOAC (2005). An Ankom fiber analyzer (ANKOM, model A2001, New York, USA) was used to determine neutral detergent fiber (NDF) using reagents described by Van Soest et al. (1991) [15] and protocol of Ankom Technology (2006b) [16]. The samples of treatments were placed in an air-forced oven (Behdad Co., Iran) (method no. 930.15) to constant weight (AOAC, 2005) [17] for dry matter determination.

Table 1. Major ingredients and chemical composition of the experimental basal feed substrate based on dry matter (DM).

Corn silage	38.8
Alfalfa Hay	7.19
Wheat Straw	3.60
Barley grain	42.0
Corn grain	25.0
Wheat bran	14.0
Soybean meal	13.5
Vitamins and minerals mixture[1]	5.00
Urea	0.50
Chemical composition (%DM)	
Dry matter	64.75
Metabolizable energy (Mcal/Kg DM)	02.67
Crude protein	15.27
Neutral detergent fiber	31.00
Non-fiber carbohydrates	43.36
Ether extract	02.95
Ash	07.42

[1] Mineral and vitamin mixture (mg/Kg): Vit E, 100 mg; Vit B, 10 mg; Vit B2, 20 mg; Vit A, 400,000 IU; Vit D, 100,000 IU; Ca, 30 g; P, 12 g; Na, 40 g; Cu, 1000 mg; I, 60 mg; Co, 60 mg; mg, 11,000; Mn, 2000 mg; Zn, 2000 mg; Fe, 3000 mg.

2.3. Statistical analysis

The *in vivo* data were analyzed in a completely randomized design using the procedure of SAS (9.4) with the following model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij}= the value of each observation, μ= overall mean, T_i= treatment effect, and e_{ij}= experimental error. The statistical difference between the treatments was determined based on the Tukey’s procedure at $P< 0.05$.

3. Results and Discussion

The effect of experimental diets on body weight (BW), average daily gain (ADG), feed conversion ratio (FCR), DMI and nutrient digestibility of Baluchi male lambs are presented in Table 2. There was significant difference for final BW ($P< 0.001$), ADG ($P< 0.007$), DMI ($P< 0.001$) and nutrients digestibility ($P< 0.001$) between diets (Table 2). feed conversion ratio ($P= 0.13$) did not differ between treatments. (Table 2).

Consistent with our findings, Al-Dbaisi (2019) [18] reported that bentonite (2 and 4%) increased total BWG compared to the control group. Similarly, Mohammad et al. (2018) [19] observed enhancements in total BWG and nutrients digestibility with supplementation of 20 g bentonite per head per day for the Karadi lambs. This can be due to improving the ruminal pH via adding bentonite maintaining the amount of water inside the gut and reducing ammonia toxicity [20]. Additionally, stimulating and growing cellulolytic bacteria via eliminating or reducing the side effects of mycotoxins and improving the rumen environment can increase organic matter degradation and nutrient utilization [21].

Large effective surfaces in nano clays might increase chemical catalysis due to a larger area of mass compared to volume. This is why supplemented MBNC treatment is superior compared to the other groups. Moreover, Nanomaterials display distinct size-dependent mechanical properties compared to bulk materials. These new properties are believed to arise due to the diverse interaction forces between Nanomaterials or between them and a surface [22,23].

Table 2. Effects of natural bentonite (NB), bentonite plus (BP) and magnetic bentonite nanocomposites (MBNC) supplementation on growth performance, dry matter intake and nutrients apparent digestibility.

Items[1]	Treatments[2]				SEM	*P*-value
	Control	NB	BP	MBNC		
Initial BW (kg)	30.97	30.65	31.12	31.06	0.43	0.87
Final BW (kg)	42.90^a	45.42^b	46.36^b	47.68^c	0.28	< 0.001
Average daily gain (Kg/d)						
Day 15	0.180	0.238	0.248	0.282	0.040	0.44
Day 30	0.215^a	0.249^b	0.254^b	0.257^b	0.004	< 0.001
Day 45	0.212^a	0.249^b	0.251^b	0.250^b	0.003	< 0.001
Day 60	0.186^a	0.228^a	0.261^{ab}	0.318^b	0.019	< 0.007
Total BW gain	11.93^a	14.76^b	15.42^b	16.62^b	0.460	< 0.007
% FCR	6.67	6.34	7.03	6.63	0.190	0.13
DMI (kg/day)	1.357	1.562	1.658	1.851	1.06	< 0.001
Nutrient digestibility (%)						
DM	0.78^a	0.82^b	0.83^c	0.85^d	0.006	< 0.001
CP	0.80^a	0.83^b	0.84^{bc}	0.86^c	0.005	< 0.001
NDF	0.45^a	0.54^b	0.56^{bc}	0.61^c	0.015	< 0.001
OM	0.80^a	0.83^b	0.84^{bc}	0.86^c	0.005	< 0.001

Different letters along the same row are significantly different according to *P*-value indicated.

[1] BW: body weight, FCR: feed conversion ratio, DMI; dry matter intake, CP: crude protein, NDF: Neutral detergent fiber, OM: Organic Matter, SEM: Standard error of the mean.

[2] NB: natural bentonite supplemented at 20 g/kg DM feed substrate, BP: bentonite plus and MBNC: magnetic bentonite nanocomposites supplemented at 0.5 g/kg DM feed substrate, respectively.

Some ruminal fermentation parameters of lambs fed on different experimental diets are shown in Table 3. The pH (P< 0.001), NH_3-N (P< 0.001), TVFA (P< 0.001), acetate (P< 0.001), propionate (P< 0.001) and acetate to propionate ratio (P= 0.001) differed between treatments (Table 3). However, Butyrate (P= 0.52) was unchanged among treatments (Table 3).

Table 3. Effects of natural bentonite (NB), bentonite plus (BP) and magnetic bentonite nanocomposites (MBNC) supplementation on some ruminal fermentation parameters.

Items[1]	Treatments[2]				SEM	*P*-value
	Control	NB	BP	MBNC		
pH	6.30^a	6.37^b	6.46^{cd}	6.52^d	0.15	< 0.001
NH_3-N (mg/dl)	15.54^a	14.33^b	13.68^c	12.72^d	0.11	< 0.001
TVFA (mM)	63.51^a	62.15^a	70.50^b	66.35^b	0.59	< 0.001
Acetate (mol/100 mol)	40.92^a	39.88^b	42.77^c	42.95^c	0.20	< 0.001
Propionate (mol/100 mol)	12.55^a	13.86$_b$	15.12^c	15.66^c	0.24	< 0.001
Butyrate (mol/100 mol)	10.37	10.95	10.16	10.00	0.45	0.52
Acetate/Propionate	3.26^a	2.87^b	2.82^b	2.74^b	0.04	0.001

Different letters along the same row are significantly different according to *P*-value indicated.

[1] NH_3-N: ammonia nitrogen, TVFA: total volatile fatty acids, SEM: Standard error of the mean.

[2] NB: natural bentonite supplemented at 20 g/kg DM feed substrate, BP: bentonite plus and MBNC: magnetic bentonite nanocomposites supplemented at 0.5 g/kg DM feed substrate, respectively.

The pH in vivo enhanced for both natural or nano zeolite forms [10], which is in agreement with our study. Compared to other 1:1 phyllosilicate clays, natural bentonite is a 2:1 with a high CEC and surface area [24]. Increases in pH values caused by bentonite addition may be due to the high CEC of bentonite forms to improve ruminal fermentation. However, interlayer's collapse due to manipulation of the natural bentonite structure can impact on the swelling capacity and surface charge [24]. The higher CEC of MBNC type could provide favorable conditions for the incorporation of metal hydrolysates and ions into its interlayer space [25]. So, MBNC was the most effective treatment for increasing pH. In the current study, lower NH_3-N concentration in bentonite supplemented groups was in agreement with El-Nile et al. (2021) [10] who found significantly lower

NH_3-N concentration in goats fed on both zeolite forms. It can be speculated that the ability of bentonite forms as cation exchangers to retain and exchange the saliva ammonium ion might be the reason for the decreased NH_3-N concentration in the rumen [10]. However, compared to the control group, the nano bentonite forms show greater CEC. The concentration of VFAs depends on DMI, nutrients degradability and availability of substrate for ruminal fermentation [26, 27]. The VFAs pattern indicates that nano bentonite has a different effect on rumen fermentation than natural form. These findings support our initial hypothesis that modifying bentonite structure to nano and nanocomposite forms improves chemical stability, physicochemical properties, and their efficiency as feed additives.

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