Effect of Copper Bearing Egyptian Bentonite on the Growth Performance and Intestinal Microflora of Rabbits

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Abstract: This work was carried out to evaluate the effect of copper bearing Egyptian bentonite (Cu-BNT) on the growth of *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (*in vivo*). Also, the effect of Cu-BNT on growing rabbit performance and intestinal microflora (*in vivo*). Sixty weanling New Zealand White (NZW) male rabbits with average body weight of 625 ± 20g were randomly assigned to 5 groups (12 rabbits in each). The 1st group was fed basal diet as control, the 2nd – 5th test groups were fed basal diet supplemented with 0.003% Cu, 0.15% bentonite (BNT), 0.003% Cu + 0.15% BNT and 0.15% Cu-BNT (the copper concentration in Cu-BNT compound was 2%, the copper in Cu-BNT diet was 2 x 0.15/100 = 0.003%), respectively for 7 weeks. Obtained results revealed that all additives inhibit significantly the growth of pathogenic bacteria (*E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) and the best results were obtained with addition of Cu-BNT and Cu + BNT. In addition, Cu-BNT supplementation increased significantly daily body weight gain, feed conversion, digestability of nutrients & nutritive values and economical efficiency. Also, the intestinal pathogenic bacteria (*E. coli*) decreased significantly and the beneficial bacteria spore former and lactic acid bacteria increased significantly by Cu-BNT addition compared with control. The other additives (Cu, BNT, BNT+Cu) insignificantly improved rabbit performance. In conclusion, indicated that Cu-BNT can be used as practicale, safe and cheap feed additive for improving rabbit performance, microflora of intestine and economical efficiency.
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1. Introduction

Many efforts were carried out to discover new additives to improve animal production. Copper is an essential trace element that plays a vital role in feotal growth & early postnatal development, bone development and inflammatory process **(McDowell, 1992)**. The copper presents in certain other plasma proteins such as ceruloplasmin which is concerned with the release of iron from the cell into the plasma **(McDonald *et al.*, 2002)**. Also, addition of low levels of copper more than normal requirements has a growth promoting effect **(Adu and Egbunike, 2010)**.

The bentonite (BNT) present in Alamein area and south of El-Hammam city in Egypt. It was the most important bentonitic clays due to the high proportion of montmorillonite content (60-90%) **(Egyptian General Peteroleum Corporation, EGPC, 1992)**. Egyptian BNT can be used to improve the animal performance and reduces toxicity of aflatoxin **(Shehata, 2002)**. Also, it can be used in many medical applications **(Cara *et al.*, 2000 and Veniale *et al.*, 2007)**. Montmorillonite (MMT) is aluminosilicate clay. It has specific physical-chemical properties such as high surface area, strong adsorptive capacity, high structural stabitlity, and chemical inertia (Borchardt, 1989). Animal feed containing MMT promote growth performance, reduce both bacterial colonization of the gut and the detrimental effects of mycotoxin-contaminated diets **(Tauqir and Nawaz, 2001)**. Moreover, MMT is a protector of intestinal mucosa **(Droy-Lefain *et al.*, 1985)**. It can adhere to enteric pathogens selectively and exert them, reinforce intestinal mucosal barrier and help in the regeneration of the epithelium **(Girardeau 1987)**.

The Cu-bearing MMT (Cu-MMT) have many advantages as compared to copper or MMT alone as it has a highly antibacterial effect on *E. coli*, *Clostridium* and *Salmonella* (adsorbed *E. coli* and reduced its bacterial number by more than 97 %, while MMT only reduce it by 20% only **(Ye *et al.*, 2003 and Xia *et al.*, 2005)**. Using low levels of Cu-MMT (1.5 g MMT and 36 mg copper / kg diet) increased significantly daily body weight gain, improved microflora and morphology of pigs **(Xia *et al.*, 2005)** and fish intestine **(Hu *et al.*, 2007)**. Also, Cu-MMT contain low level of copper (2%), therefore there is no copper residue in animal organs after supplementation of 0.15% of Cu-MMT to diet **(Ye *et al.*, 2003)**.

This study aimed to study effect of copper

bearing Egyptian bentonite (Cu-BNT) on *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa in vitro* and rabbit growth performance, digestibility of nutrients and intestinal microflora were investigated.

2. Materials and methods

This experiment was carried out at the Rabbit Research Unit, Faculty of Agriculture and Microbiological Lab., Faculty of Science, Zagazig University, Egypt.

2.1. Preparing of copper bearing Egyptian bentonite (Cu-BNT):

Egyptian BNT was provided by Misr Company for bentonites from Alamein area, it contained (%): 51.08 SiO_2; 16.37 Al_2O_3; 0.97 CaO; 2.68 MgO; 1.07 K_2O; 0.84 Na_2O; 9.27 Fe_2O_3, 0.03 MnO, 1.26 TiO_2, 0.13 P_2O_5, 0.017 Cr_2O_3, 0.09 TOT/C, 0.01 TOT/S, 15.80 LOl (Abdel-Motelib *et al*., 2011). The row BNT was dried in the oven at 80°C over night and then milled to less than 300 mesh. The milled material was dispersed in water to form a 10% suspension and kept for about 10 min. with stirring. Particles larger than 1 µm were separated out by sedimentation, while the suspension was centrifuged at 3000 r.p.m. for 15 min. to get refined BNT. The refined BNT was dried at 80°C followed by another milling to less than 300 mesh for use. Cu-BNT was prepared by using the method of Hu *et al*., (2007). Ten grams of the refined BNT were mixed with 100 ml of 0.2 mol/L NaCl solution. The dispersion was kept for 5 hrs. with stirring. The Na-BNT was then separated by centrifugation at 3000 r.p.m. for 15 min. and washed with deionized water for three times. The washed Na-BNT was then dispersed in 200 ml of 0.05 mol/L $CuSO_4$ solution and pH value was adjusted to pH 5 by NaOH. The dispension was kept at 60°C with stirring for 6 hrs. After centrifugation, the sediment was washed with deionized water for three times, dried at 80°C over night, then ground to a size less than 300 mesh. The Cu content in Cu-BNT was found to be 2% on the basis of atomic absorption spectral analysis (Central *Lab*., Faculty of Veternary Medicine, Zagazig Univ., Egypt).

2.2. Experimental design and sampling procedure

Sixty weanling New Zealand White (NZW) male rabbits with average body weight of 625 ± 20 g were randomly assigned to 5 groups (12 rabbits in each). The copper concentration in CU-BNT was 2%. The copper in Cu-BNT diet was 0.003% (2 x 0.15/100= 0.003%). The control group fed basal diet (Table 1), the 2^{nd} – 5^{th} groups fed the basal diet supplemented with 0.00744% $CuSO_4$ which represent 0.003% Cu, 0.15% BNT, 0.15% BNT + 0.003% Cu and 0.15% Cu-BNT, respectively for 7 weeks. All additives were added to diet components before pelleting.

The formulation and chemical composition of the basal diet and bentonite are shown in Table 1. Daily fresh water was available all time. At the last week, feed intake and feces excreted of 6 rabbits from each treatment were recorded daily for digestibility trials. Proximate chemical analysis of feed and feces were determined according to A.O.A.C. (1990). Animals were housed in individual cages under the same managerial, hygienic and environmental conditions all over the experimental period. The initial and finial live body weight were recorded for each rabbit to calculate the daily body weight gain. At the end of the experimental period, six male rabbits from each treatment group were randomly taken and fastened for 12 hrs. with free water supply then slaughtered. The carcass traits were determined. Samples of the contents from the small intestine (from the distal end of the duodenum to the ileo-caecal junction) and proximal colon were collected into glass container, sealed and put on ice until enumeration of microbial populations.

2.3. Effect of additives on bacteria

2.3.1. *In vitro*

The effect of Cu, BNT, Cu + BNT and Cu-BNT on some pathogenic bacteria (*Staphylous aureus*, *E. coli* and *Pseudomonas aeruginosa*) were studied. These pathogenic bacteria were obtained from Faculty of Medicine, Zagazig University, Egypt. The pathogenic bacteria were inoculated (1% v/v) in 100 ml nutrient broth containing 0.00744% $CuSO_4$ which represent 0.003% Cu, 0.15% BNT, 0.003% Cu + 0.15% BNT and 0.15% $CuSO_4$-BNT. Incubate at 37 °C for 48 hrs. then measure the optical density (O.D.) at 600 nm and calculate % of inhibition (% of inhibition = control-treatment/control x 100). Each treatment has its own blank.

2.3.2. Intestinal microbial populations (*in vivo*)

Under sterile conditions, 10 g of samples from the rabbit intestine and proximal colon were collected into sterile glass containers containing 90 ml sterile saline solution. The initial dilution was shaked for 30 min. and was used as a source for serial dilutions in saline solution for enumeration of bacterial populations. Triplicate plates were then inoculated with 1 ml samples and incubated at 37 °C for 48 hrs. & 72 hrs., bacteria were enumerated on nutrient agar (for spore former) (Difco Manual, 1994), MRS agar plus sodium thioglucolate (for anaerobic *Lactobacillus*) and MacConkey's agar (for *E. coli*) (Xia *et al*., 2005). To obtain the number of spore former, the tubes containing the different

dilutions kept in water bath at 80 °C for 15 min. then cooled to room temperature before delivering the 1 ml inoculum.

2.4. Statistical analysis:

Data were statistically analyzed by the General Linear Model using SAS® Software Statistical Analysis (SAS, 1996). Differences among means were tested by Duncan's multiple range test (Duncan, 1955).

3. Results and discussion

3.1. Effect of additives on pathogenic bacteria *(in vitro)*

As shown tn Table (2) addition of Cu-BNT or Cu + BNT inhibited significantly (P<0.05) the growth of *E. coli*, *Staphylococcus aureus* and *Pseudomonas auegionosa* compared to untreated control. The % of growth inhibition were 94.16, 96.50 and 93.94% for Cu-BNT; 92.34, 95.25 and 59.38% for Cu+BNT; 58.57, 77.5 and 51.8% for BNT; 49.0, 46.13 and 21.39% for Cu, respectively. These results are consistent with those of Ye *et al*., (2003) and Xia *et al*., (2005) who reported that Cu-MMT has highly effect as antibacterial activity on *E. coli*, *Clostridium* and *Salmonella* (adsorbed *E. coli* and reduced its bacterial number by more than 97 % but the inhibition by MMT only was 20%). The higher inhibition by Cu-BNT and Cu+BNT may be due to the synergistic effect where BNT adsorbed the bacteria and Cu killed the bacteria (Hu *et al*., 2002 and Adu and Egbunike, 2010).

3.2. Digestibility and nutritive values

Addition of Cu-BNT significantly (P <0.05) increased dry matter, organic matter, crude protein, crude fiber, nitrogen free extract digestibilities, total digestible nutrients (TDN%) and digestible crude protein (DCP%) in comparison with untreated control, Cu, BNT and Cu + BNT (Table 3). The improvement in digestibility of nutrients as a result of Cu-BNT addition may be attributed to improvement microflora in digestive tract by inhibition the pathogenic bacteria and enhancing the growth of desirable gastrointestinal microbes of the rabbits, also due to improvement morphology of intestine (Cu-MMT had significantly higher villus height and the villus height to crypt depth ratio at the small intestinal mucosa then increase the absorption of nutrient) (Xu *et al*., 2003 and Xia *et al*., 2005).

3.3. Growth performance

Addition of Cu-BNT significantly (P<0.05) increase the daily body weight gain as compared to the control and other additives, which represent 115.09 % of control group (Table 4). These results agree with those obtained by Xia *et al*., (2005) and Hu *et al*., (2007) who reported that Cu-MMT significantly (P<0.05) increased body weight gain of pigs and Nile tilapia fish by 120.42% and 111.61%, respectively in comparison with control (100%).

The improvement in rabbit growth performance as a result of Cu-BNT addition may be attributed to reduction the total viable counts of pathogenic bacteria and increasing the benficial bacteria in the small intestine which reflected on improvement the rate of passage, thickness of intestinal mucosa, nutrient digestibility and absorption (Hu *et al*., 2002., Ye *et al.*, 2003 and Xia *et al*., 2005). Also Cu-BNT improves the digestibility and utilization of nutrients in diets, influencing activities of the hormones such as growth hormones and thyroid hormones by copper (Underwood and Suttle, 1999 Adu and Egbunike, 2010).

Addition of Cu or BNT alone insignificantly affect daily growth rate compared to control. However, Adu and Egbunike, (2010) reported that copper inclusion in rabbit diet (0.01, 0.02 and 0.03%) enhance growth performance. This disagree may be due to using different concentration. Also, may be due to the, sanitary condition, level performance, diet composition, animal species and so on (Xia *et al*., 2005). Addition of Cu + BNT increased significantly daily body weight gain in comparison with the control. However, Cu + BNT still significantly lower than Cu-BNT. These results may be due to that the interaction between Cu and BNT in the digestive tract (Cu + BNT) was lower than in under control conditions (*In vitro*) where the aqueous solutions, pH, temperature and time help the interaction between Cu-BNT.

The average daily feed intake did not significantly changed due to all additions. These results are in accordance with those obtained by Xia *et al*., (2005) on pigs. The best feed conversion (P<0.05) was obtained in rabbits fed diet supplemented with Cu-BNT compared to the other treatments, these results may be due to an improvement the digestibility of nutrients (Table 3) and absorption.

3.4. Intestinal microbial populations *(in vivo)*:

As shown in Table (5) supplementation with Cu-BNT reduced significantly (P<0.05) pathogenic bacteria (*E. coli*) and increased (P<0.05) the benificial bacteria (spore former and lactaic acid bacteria) in small intestine and colon. These results agree with those obtained by Xia *et al*., (2005) who reported that Cu-MMT reduced significantly (P<0.05) *Clostridium* and *E. coli* in the small intestine and proximal colon in pigs as compared to the control. Cu + BNT addition improved significantly (P<0.05)

intestinal microflora compared to control, but the values were still lower significantly (P<0.05) than Cu-BNT. Most results of 0.003% Cu or 0.15% BNT alone had not significant effect. However, Shurson *et al*., (1990) reported that the postive effect of copper on gut flora of animals occurred with level 0.03% copper in diet.

The mode of action of Cu-BNT was reported by Stadler and Schindler (1993) who found that Cu^{+2} in aqueous solution with pH>4.5 tended to enter the interlayer position of MMT and form $[Cu(AlO)n(H_2O)4\text{-}n]^{x+}$. When Na^{+} or Ca^{+} was replaced by $[Cu(AlO)n(H_2O)4\text{-}n]^{x+}$, or Cu^{2+} entered the tetrahedron and octahedron, MMT lost its electrical balance. This made the mineral has surplus postive charge. On the other hand, the bacterial cell wall is negatively charged due to functional groups such as carboxylates present in lipoproteins at the surface (Breeen *et al*., 1995), so that Cu-MMT particles would attract bacteria, due to the postive static charge. The released Cu^{+2} would act directely on the attracted bacteria, instead of into the medium and indirectly on the bacteria. In summary, electrostatic attraction and the antibacterial effect of Cu^{2+} ion on bacteria are two ways of the antimicrobial action of Cu-MMT.

3.5. Carcass traits:

Addition of additives insignificantly affect carcass traits (Table 6).

3.6. Economical efficiency:

The economical efficiency results indicated that all additions improved the economical efficiency (Table 7). The relative profit was 139.33, 114.65, 107.04, 109.78% for Cu-BNT, Cu+BNT, Cu and BNT, respectively compared with control group. These results may be due to improvement feed utilization and growth performance.

Table 1. Formulation and chemical composition (%) of basal diet

Ingredients	%
Yellow corn	17.00
Clover hay	35.00
Wheat bran	20.00
Barley	10.00
Soybean meal	13.00
Molasses	3.00
Sodium chloride	0.20
Methionine	0.20
Vitamin and minerals	0.30
Bone meal	1.00
Limestone	0.30
Chemical composition	
(DM)	89.50
OM	16.80
CP	16.00
CF	2.50
EE	54.20
NFE	10.50
Ash	

Table 2. Effect of feed additives on growth of some pathogenic bacteria (*in vitro*)

Items	Control		0.003% Cu		0.15% Bentonite (BNT)		0.003% Cu + 0.15% bentonite (Cu+ BNT)		0.15% Cu bearing bentonite (Cu-BNT)	
	O.D. 600	% of inhibition	O.D. 600	% of inhibition	O.D. 600	% of inhibition	O.D. 600	% of inhibition	O.D. 600	% of inhibition
E. coli:	1.371	0.0^{c}	0.699	49.00^{b}	0.568	58.57^{b}	0.105	92.34^{a}	0.080	94.16^{a}
Staphylococcus aurius:	0.800	0.0^{d}	0.431	46.13^{c}	0.180	77.5^{b}	0.038	95.25^{a}	0.028	96.50^{a}
Pseudomonasauegionosa:	0.832	0.0^{d}	0.654	21.39^{c}	0.401	51.80^{b}	0.338	59.38^{a}	0.300	63.94^{a}

% of inhibition = control – treatment / control x 100.

a, b,… Means in the same row bearing different letters differ significantly (P<0.05).

Table 3. Effect of feed additives on digestibility and nutritive values

Items	Control	0.003% Cu	0.15% Bentonite (BNT)	0.003% Cu + 0.15% bentonite (Cu+ BNT)	0.15% Cu bearing bentonite (Cu-BNT)
Digestibility (%):					
DM	$60.94^{b} \pm 1.18$	$62.25^{b} \pm 0.96$	$62.50^{b} \pm 1.21$	$63.58^{b} \pm 1.16$	$71.58^{a} \pm 1.12$
OM	$60.99^{b} \pm 1.17$	$63.52^{b} \pm 0.63$	$63.20^{b} \pm 0.90$	$64.11^{b} \pm 1.11$	$71.93^{a} \pm 1.09$
CP	$71.44^{b} \pm 1.15$	$71.50^{b} \pm 1.07$	$71.69^{b} \pm 0.77$	$72.10^{b} \pm 1.13$	$76.16^{a} \pm 1.10$
CF	$29.91^{b} \pm 0.67$	$29.93^{b} \pm 0.53$	$30.40^{b} \pm 0.83$	$32.50^{b} \pm 1.07$	$34.93^{a} \pm 1.47$
EE	70.86 ± 1.97	71.48 ± 1.28	71.00 ± 1.67	73.15 ± 1.33	75.19 ± 1.13
NFE	$77.50^{b} \pm 1.96$	$76.33^{b} \pm 0.55$	$76.37^{b} \pm 0.81$	$79.15^{b} \pm 1.52$	$87.10^{a} \pm 1.72$
Nutritive values (%):					
TDN	$62.78^{b} \pm 1.02$	$62.20^{b} \pm 0.54$	$62.30^{b} \pm 0.56$	$64.32^{b} \pm 1.06$	$69.83^{a} \pm 1.06$
DCP	$12.00^{b} \pm 0.19$	$12.01^{b} \pm 0.18$	$12.05^{b} \pm 0.13$	$12.11^{b} \pm 0.18$	$12.79^{a} \pm 0.18$

a, b,… Means in the same row bearing different letters differ significantly (P<0.05).

Table 4. Effect of feed additives on rabbit growth performance

Items	Control	0.003% Cu	0.15% Bentonite (BNT)	0.003% Cu + 0.15% bentonite (Cu+ BNT)	0.15% Cu bearing bentonite (Cu-BNT)
Initial live body weight (g)	628.33 ± 24	625.83 ± 20	621.67 ± 21	625.30 ± 20	622.50 ± 25
Finial live body weight (g)	$1933.69^{c} \pm 46$	$1961.67^{bc} \pm 35$	$1956.67^{bc} \pm 61$	$2000.00^{b} \pm 56$	$2125.00^{a} \pm 50$
Total body weight gain (7 weeks)	$1305.36^{c} \pm 35$	$1335.84^{bc} \pm 28$	$1335.00^{bc} \pm 31$	$1374.7^{b} \pm 26$	$1502.50^{a} \pm 24$
Average daily body gain (g)	$26.64^{c} \pm 1.20$	$27.26^{bc} \pm 0.86$	$27.25^{bc} \pm 1.38$	$28.06^{b} \pm 1.00$	$30.66^{a} \pm 0.94$
% increase in daily body gain	100	102.33	102.29	105.33	115.09
Average daily feed intake (g)	134.67 ± 4.33	140.00 ± 4.65	143.33 ± 3.07	142.00 ± 4.68	138.33 ± 5.58
Feed conversion (feed /gain)	$5.18^{a} \pm 0.20$	$5.14^{a} \pm 0.09$	$5.14^{a} \pm 0.33$	$5.17^{a} \pm 0.08$	$4.84^{b} \pm 0.06$

a, b,… Means in the same row bearing different letters differ significantly (P<0.05).

Table 5. Effect of feed additives on intestinal microflora of rabbits

*Log $_{10}$N.	Control	0.003% Cu	0.15% Bentonite (BNT)	0.003% Cu + 0.15% bentonite (Cu+ BNT)	0.15% Cu bearing bentonite (Cu-BNT)
			After 48 hrs.		
Small intestine :					
Spore former	Uncount**	$7.0^{C} \pm 0.11$	$9.0^{b} \pm 0.13$	$9.5^{b} \pm 0.64$	$11.5^{a} \pm 0.54$
Lactic acid bacteria (anaerobic)	No growth	No growth	No growth	No growth	No growth
E. coli	$18.0^{a} \pm 1.45$	$15.0^{ab} \pm 1.73$	$14.4^{ab} \pm 0.92$	$13.4^{b} \pm 1.16$	$12.0^{b} \pm 0.87$
Colon :					
Spore former	$9.0^{c} \pm 0.58$	$9.5^{c} \pm 0.29$	$9.4^{c} \pm 0.35$	$11.5^{b} \pm 0.81$	$13.2^{a} \pm 0.08$
Lactic acid bacteria	No growth	No growth	No growth	No growth	No growth
E. coli	$30.0^{a} \pm 1.73$	$28.0^{ab} \pm 2.5$	$25.4^{b} \pm 0.92$	$22.5^{c} \pm 1.04$	$20.0^{d} \pm 1.16$
			After 72 hrs.		
Small intestine :					
Spore former	Uncount*	$9.2^{b} \pm 0.17$	$12.1^{a} \pm 0.29$	$13.2^{a} \pm 0.64$	$13.5^{a} \pm 0.40$
Lactic acid bacteria (anaerobic)	No growth	$12.0^{c} \pm 0.23$	$27.1^{b} \pm 1.73$	$32.0^{ab} \pm 1.73$	$34.0^{a} \pm 2.02$
E. coli	$26.8^{a} \pm 1.16$	$21.4^{b} \pm 0.58$	$19.5^{b} \pm 1.73$	$18.4^{b} \pm 1.16$	$17.5^{b} \pm 1.45$
Colon :					
Spore former	$10.5^{b} \pm 0.58$	$12.5^{ab} \pm 0.87$	$13.2^{ab} \pm 0.75$	$14.4^{a} \pm 1.16$	$15.2^{a} \pm 1.04$
Lactic acid bacteria	$16.0^{d} \pm 1.45$	$24.5^{c} \pm 0.87$	$33.2^{b} \pm 1.73$	$36.0^{ab} \pm 2.02$	$42.5^{a} \pm 1.45$
E. coli	$48.0^{a} \pm 1.73$	$37.1^{b} \pm 2.31$	$34.5^{bc} \pm 1.45$	$31.6^{bc} \pm 0.75$	$30.5^{c} \pm 1.73$

a, b,… Means in the same row bearing different letters differ significantly (P<0.05).
*Bacterial numbers are expressed as $\log_{10}$ colony-forming unit / g of fresh weight.
** Uncount (less than 30).

Table 6. Effect of feed additives on carcass traits

Items	Control	0.003% Cu	0.15% Bentonite (BNT)	0.003% Cu + 0.15% bentonite (Cu+ BNT)	0.15% Cu bearing bentonite (Cu-BNT)
Dressing %	57.00 ± 1.33	55.63 ± 0.74	57.62 ± 0.93	57.40 ± 0.97	57.29 ± 0.87
Liver*	2.99 ± 0.16	3.10 ± 0.08	3.07 ± 0.03	3.15 ± 0.08	3.25 ± 0.12
Kidneys*	0.90 ± 0.05	0.92 ± 0.03	0.99 ± 0.05	0.93 ± 0.06	0.97 ± 0.04
Lungs*	0.48 ± 0.03	0.48 ± 0.01	0.46 ± 0.02	0.45 ± 0.02	0.47 ± 0.01
Hearts*	0.36 ± 0.06	0.33 ± 0.01	0.35 ± 0.02	0.32 ± 0.03	0.30 ± 0.02

*= % of live body weight

Table 7. Effect of feed additives on economical efficiency

Items	Control	0.003% Cu	0.15% Bentonite (BNT)	0.003% Cu + 0.15% bentonite (Cu+ BNT)	0.15% Cu bearing bentonite (Cu-BNT)
Total gain (g)	1250.84	1335.84	1335.00	1374.7	1502.50
Total feed intake (kg)	6.598	6.860	7.023	6.96	6.778
Feed cost (LE)*	13.196	13.740	14.047	13.941	13.580
Gain price (LE)**	25.017	26.717	26.700	27.494	30.050
Profit***	11.821	12.977	12.653	13.553	16.470
Relative profit (%)****	100	109.78	107.04	114.65	139.33

a, b,… Means in the same row bearing different letters differ significantly (P<0.05).
*= total feed intake x price. The price of 1 kg control, Cu, bentonite, Cu + bentonite and Cu bearing bentonite were 2, 2.00290, 2.00015, 2.00305 and 2.00305 pound respectively (price 2010). One kg of CuSO4 and bentonite were 40 and 0.10 pound, respectively. **= total gain x 20 (one kg 20 pound). ***= gain price – feed cost
****= relative profit for treatment/ net revenue of control x 100.

Conclusions

The results of this work indicate that Cu-BNT inhibited the tested pathogenic bacteria effectively (*in vitro*). Also, addition of Cu-BNT to the growing rabbit diets improved rabbit performance, microflora of intestine and economical efficiency.

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References

Abdel-Motelib, A., Abdel-Kader, Z., Ragab, Y.A., Mosalamy, M. 2011. Suitability of a Miocene bentonite from North Western Desert of Egypt for pharmaceutical use. Applied Clay Sci., 52: 140-144.

Adu, O.A., Egbunike, G.N. 2010. Enhancing growing rabbits performance with diets supplemented with copper. Advances in Biological Research, 4 (1): 18-22.

AOAC. 1990. Official Methods of Analysis. The 15th Edition. Association of Official Analytical Chemists, Washington, D.C.

Breen, P.J., Compadre, C.M., Fifer, E.K., Salari, H., Serbus, D.C., Lattin, D.L. 1995. Quaternary ammonium compounds inhibit and reduce the attachment of viable *Salmonells typhimurium* to poultry tissues. J. Food Sci., 60 : 1191-1196.

Cara, S., Carcangiu, G., Padalino, G., Palomba, M., Tamanini, M. 2000. The bentonites in pelotherapy: chemical mineralogical, technological and thermal properties of clay pastes from Sardinia (Italy). Applied Clay Sci., 16: 117-124.

Difco Manual. 1994. Dehydrated Culture Media and Reagents for Microbiology. 10th eddition Difco Lab., USA.

Droy-Lefain, M.T., Drouet, Y., Schatz, B. 1985. Sodium glycodeoxycholate and spinability of gastrointestinal mucus: protective effect of smectite. Gastroenterology, 88 (Suppl. 2), 1369.

Duncan, D.B. 1955. Multiple range and multiple F. test. Biometric, 11: 1-42.

Egyptian General Petroleum Corporation, EGPC. 1992. Western Desert, oil gas field (A comprehensive overview). New Maadi, Cairo, Egypt, 432 p.

Girardeau, J.P. 1987. Smectite aggregation by

Escherichia coli. Acta Gastroenterol Belg., 50: 181-192.

Hu, C.H., Xu, Y., Xia, M.S., Xiong, L., Xu, Z.R. 2007. Effect of Cu^{2+}-exchanged montmorillonite on growth performance, microbial ecology and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 270: 200-206.

Hu, X.R., Lu, G.L., Chen, L.S., Gu, J.M., Zhang, Y. 2002. Study on the mechanism of the interaction between montmorillonite and bacterium. Acta Pharmaceutica Sinica, 37: 718-720 (in Chinese).

McDonald, P., Edwards, R.A., Greenhalg, J.F.D., Morgan, C.A. 2002. Animal Nutrition, 6th ed., Pearson Education, Harlow.

McDowell, L.R. 1992. Mineral in animal and human nutrition. Academic Press in San Diego, New York, Boston, London, Sydney, Tokyo, Toronto: pp: 524.

SAS® User`s Guide. 1996. Statistics, version 6. 12 Edition. SAS inst. Inc., Cary, NC.

Shehata, S.A. 2002. Detoxification of mycotoxin contaminated animal feedstuffs. Ph.D Thesis, Zagazig Univ., Fac. of Agric., Egypt.

Shurson, G.C., Ku, P.K., Warler, G.L., Yokoyama, M.T., Miller, E.R. 1990. Physiological relationships between microbiological status and dietary copper in the pig. J. Anim. Sci., 68: 1061-1071.

Stadler, M., Schindler, P.W. 1993. Modeling of H^+ and Cu^{2+} adsorption on calcium-mintmorillonite. Clays Mineral, 41: 288-296.

Tauqir, N.A., Nawaz, H. 2001. Performance and economics of broiler chicks fed on rations supplemented with different levels of sodium bentonite. International J. of Agriculture and Biology, 3: 149-150.

Underwood, E.J., Suttle, N.F. 1999. Copper In: The mineral nutrition of livestock. Ed.3., CABI Publishing, Walling Ford, UK, : 283-342.

Veniale, F., Bettero, A., Jobstraibizer, P.G., Setti, M. 2007. Thermal muds: perspectives of innovations. Applied Clay Sci., 36: 141-147.

Xia, M.S., Hu, C.H. and Xu, Z.R. 2005. Effects of copper bearing montmorillonite on the growth performance, intestinal microflora and morphology of weanling pigs. Animal Feed Science and Technology, 118: 307-317.

Xu, Z.R., Hu, C.H., Xia, M.S., Zhan, X.A., Wang, M.Q. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry Sci., 82: 648-654.

Ye, Y., Zhou, Y.H., Xia, M.S., Hu, C.H. 2003. A new type of inorganic antibacterial material: Cu-bearing montmorillonite and discussion on its mechanism. J. Inorganic Material, 18: 569-574 (in Chinese).

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