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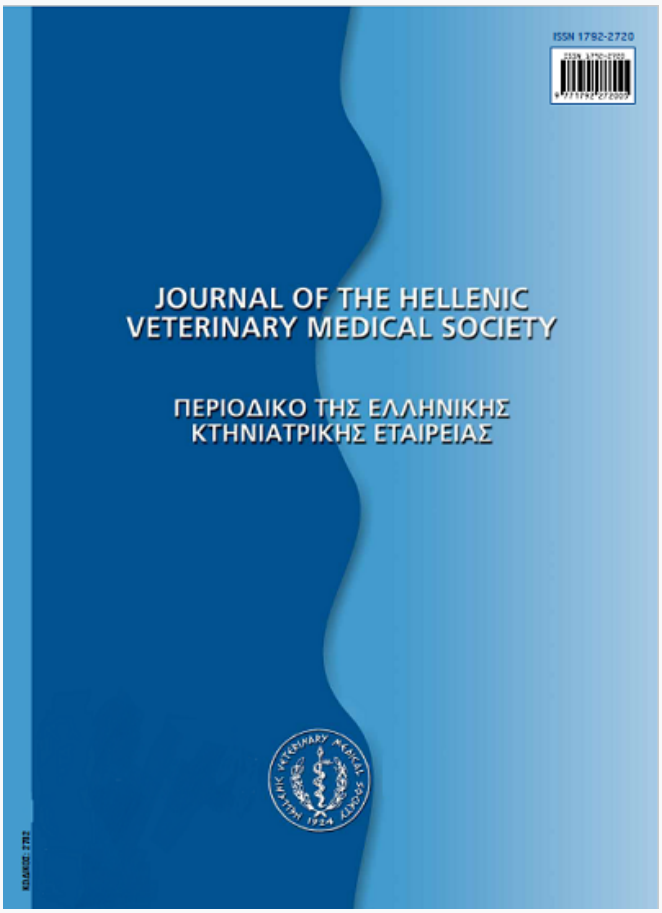

Intestinal morphograhy of broilers fed diets supplemented with perlite

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Research article
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Intestinal morphograhy of broilers fed diets supplemented with perlite

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ABSTRACT. Aim of the study was to investigate effects of different levels of perlite on intestinal morphometry in broilers (Ross 308). A hundred and eighty broiler cockerels were randomly allocated into three experimental groups (3 replications and 20 broilers per pen) and fed experimental diets supplemented with different levels of perlite (0%, 2%, 4%). At 21, 28, 35 and 42 days of the study, 2 broilers were randomly selected from each replication, slaughtered and various sections of small intestine (1, 10, 30, 50, 70 and 90% of small intestine length) sampled for morphometry characteristics. Villi height, crypts depth and villus height / crypt depth ratio were measured microscopically. According to the results, a significant difference was observed on small intestine morphology post-perlite supplementation in experimental groups compared to control group. Supplementation of diet with perlite (2%) significantly increased average villi height in various sections of small intestine (1, 70 and 90%) in experimental birds on days 28 and 35 ($P < 0.05$). In addition, similar findings were observed after addition of perlite (4%) on villi height on day 42 ($P < 0.05$). Furthermore, on day 28, average villi height and depth of liberkuhn crypts in small intestine (10%) differed significantly in cockerels fed diets containing 2% perlite in comparison to controls ($P < 0.05$). These results suggest that supplementation of perlite in broilers' diet can improve intestinal morphometry.

Keywords: broilers, depth of liberkuhn crypt, perlite, villus height

INTRODUCTION

Perlite is one of the volcanic, aluminum-silicate minerals characterized by crystalline and 3-dimensional structure (Incharoen et al., 2009). Raw perlite is transparent and greyish or gloss black, hydrated alumino-silicates of alkali (i.e., Na^+, K^+) and alkaline-earth cations (i.e., Mg^{2+}, Ca^{2+}). Concerning the chemical constituency, it contains aluminum and silicate components (Eleroğlu et al., 2011). Several researches have focused on the role of perlite and zeolite in ani-

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mals (Ghiasi Ghalehkandi et al., 2009; Abaş et al., 2011). Based on its beneficial properties, use of perlite as feed additive has been adapted by domestic animal feed industries (Alkan and Do¢gan, 2001; Talebali and Farzinpour, 2006). Perlite contributes to breakdown of faeces, absorption of moisture and quality of litter in birds (Alkan and Do¢gan, 2001; Karamanlis et al., 2008). It has been reported that supplementation of diet with zeolite promotes binding of nitrogen cations such as NH^{4+} (Karamanlis et al., 2008), decreases ammonia toxicity (Gupta et al., 1997) and reduces aflatoxine toxicity in corn, wheat and soybean (Miazzo et al., 2000). As a feed additive for livestock it has several benefits, e.g. promoting growth rate (Abaş et al., 2011). In previous studies, it has been found that supplementation of diets with zeolite increased bodyweight gain and improved food conversion ratio in broiler chickens (Zhang and Hung, 1992; Ghiasi Ghalehkandi et al., 2009). Khambualai et al. (2009) reported that supplementation of vermiculite herbal extract diet using zeolite caused to hypertrophy of intestinal villi, villous area, cell mitoses, absorptive epithelium cells and results to improved bodyweight gain in Aigamo ducks.

The gastrointestinal tract, especially small intestine, is the main organ for digestion and absorption accomplished by secretory enzymes of small intestine epithelium and pancreas. The mucous layer of intestine wall is composed of a zigzag form structure named villi. A variety of factors, such as age, food deprivation, luminal microflora, chemicals present in feeds, affects small intestine villi height and growth rate (Bayer, 1975; Antheony et al., 1999). According to Obst and Diamond (1992), in broilers duodenum villi growth is completed on day 7 after hatch, but jejunum and ileum villi growth continues until day 14. In agreement, it is suggested that villi growth and depth of liberkuhn crypts in duodenum, jejunum and ileum is extended by two days. By contrast, intestinal morphology changes form days 4 to 21 of age, which indicates increase in villi volume and liberkuhn crypts depth (Geyra et al., 2001). Absorptive epithelial cells originate form liberkuhn bases as immature reproductive cells and then they are differentiated, migrate across villi apex, drop form villi apex and finally they are excreted (Cheng and Leblond, 1974).

It has been reported that intestine villi morphology and epithelial cell morphology are related to intestine function and growth rate of intestine (Ruttanavut et al., 2009). In cockerels, numerous epithelial cells are observed on the villous apex surface, suggesting a particular role in improved feed conversion ratio and increased growth rate (Yamauchi et al., 2006). The largest size of villi is related to the intestines, with dynamic cell proliferation (Antheony et al., 1999). Narrow and long villi indicate rapid proliferation of crypts, which migrate to villus apex rapidly and turnover of this type of epithelial cell is shorter (Nordstrom and Dahlqvist, 1973). Huge villi height and excessive mitoses in intestine indicate active intestine villi function (Langhout et al., 1999). On the basis of previous findings and considering the participation of zeolite on intestine epithelial cells, the hypothesis of recent study was to clarify possible effects of different levels of perlite on average villi height, depth of liberkuhn crypts and comparison of average villi height and depth of liberkuhn crypts in various sections of small intestine (1, 10, 30, 50, 70 and 90 percent of small intestine length) in broiler cockerels in days 21, 28, 35 and 42, respectively.

MATERIALS AND METHODS

Birds and diet

One hundred and eighty day-old broiler cockerels (Ross 308) (Eshragh Co. Iran) were divided into three experimental groups (3 replications and 20 broilers per pen). Chickens were kept at a temperature of 22±1 ^{o}C with 50% humidity, continuously lighted and raised in litter condition (Olanrewaju et al., 2006). Birds were fed mesh diet [starter: 20.50% protein and 2850 kcal/kg of metabolisable energy - grower: 18.17% protein and 2920 kcal/kg of metabolisable energy] formulated using User Friendly Feed Formulation Done Again (UFFDA) (Pesti et al., 1992). Composition of

experimental diet is in Table 1. The control group received basal diet (no perlite supplement) throughout the experimental period. Treatment groups were fed diets containing 2% and 4% perlite, respectively. During the study, all experimental birds had free access to fresh water. Experimental perlite was purchased from Kan Azar Tabriz Mining Co. (Kat Co., Tabriz, Iran). All doses of perlite were calculated on the basis of previous and pilot studies (Ingram et al., 1988; Khademi, 2008; Shariatmadari, 2008).

Animal handling and slaughter procedures were performed according to the Guide for the Care and Use of Laboratory animals by the National Institutes of Health (USA) and the current laws of the Iranian government.

Sample collection

At 21, 28, 35 and 42 days of age, three hours before the start of experimental procedures, animals were deprived from food (FD3). Two broilers from each group were randomly selected and slaughtered (totally 18 chickens in each experiment). A post-mortem examination was performed, during which the entire gastrointestinal tract was quickly removed for further studies. Various sections of small intestine (1, 10, 30, 50, 70 and 90% of small intestine length) were sampled, rinsed with phosphate to measure villi length and depth of liberkuhn crypts (Talebali and Farzinpour, 2006). A 5 cm length of each intestinal

Table 1. Ingredient and nutrient compositions of experimental diets.

	1-21 days			21- 42 days		
Perlite levels	0%	2%	4%	0%	2%	4%
Ingredient						
Corn	54.50	54.00	45.00	62.64	39.00	59.00
Soybean meal	34.14	34.19	35.81	27.00	27.70	27.70
Oil	2.5	2.50	2.50	2.50	2.50	2.50
Methionine	0.60	0.60	0.80	0.60	0.60	0.60
Lysine	0.00	0.00	0.00	0.20	0.20	0.20
Vit-permix	0.25	0.25	0.25	0.25	0.25	0.25
Min-permix	0.25	0.25	0.25	0.25	0.25	0.25
DCP	1.60	1.60	1.62	1.13	1.13	1.13
Oyster	1.44	1.40	1.33	1.48	1.44	1.39
Salt	0.28	0.28	0.28	0.28	0.28	0.28
Perlite	0.00	2.00	4.00	0.00	2.00	4.00
Starch	1.06	1.41	7.37	0.00	2.60	2.60
Fine sand	3.38	1.46	0.07	3.67	2.05	0.10
Nutrients composition calculated						
ME, Kcal/kg	2850.21	2850.11	28050.14	2920.54	2920.03	2920.03
Crude protein [a]	20.50	20.51	20.50	18.17	18.18	18.17
Calcium	0.99	0.99	0.99	0.89	0.89	0.89
Phosphorus	0.44	0.44	0.44	0.34	0.34	0.34
ME / Protein	139.00	138.96	139.03	160.69	160.64	160.64
Calcium / Phosphorus	2.23	2.23	2.23	2.56	2.54	2.58

DCP: dicalcium phosphate, ME: Metabolisable energy.

Per 2.5 kg mineral supplement containing 99200 mg magnesium, 84700 mg zinc, 50000 mg iron, 10000 mg copper, 990 mg iodine, 200 mg selenium, 250000 ml g choline chloride.

Per 2.5 kilogram vitamin supplement containing 900000 IU of vitamin A, 200000 IU of vitamin D_3, 19000 IU of vitamin E, 200 mg vitamin K_3, 18050 mg vitamin B1, 49000 mg vitamin B_2, 9800 mg vitamin B_3, 29650 mg vitamin B_5, 2940 mg vitamin B_6, 1000 mg vitamin B_9, 15 mg vitamin B_{12}, 100 mg Biotin, 190000 mg Choline Chloride, 1000 mg antioxidant.

[a] Accorded to AOAC (1984).

sample of intestine was immediately rinsed by sodium phosphate buffer and stabilized by Clark stabilizer solution (Clarke, 1977). Each sample was divided into two parts; one part for measuring villus dimensions and the remaining for determination of depth of liberkuhn crypts. Each sample was prepared for microscopically study after staining with Periodic Acid schiff Solution, separating muscular layer and preparing lamella. In each sample, villi height was measured from the tip to the bottom of villus. Mean villi heights from two birds (80 villi from different sections in each sample per bird) were attributed as a mean villi height in each group. Depth of liberkuhn crypts were measured eighty cases, using the second sample per birds. Measurement was carried out in an image analyser (Nikon Cosmozone 1S; Nikon Co., Tokyo, Japan).

Statistical analysis

The data were analyzed using one-way analysis of variance using SAS software (2001) and are presented as mean±standard deviation. Differences among means were compared by Duncan's multiple range tests. $P < 0.05$ was considered as significant differences between treatments.

RESULTS

Effects of different levels of perlite on average villi height, depth of liberkuhn crypts and comparison of average villi height and depth of liberkuhn crypts in various sections of small intestine (1, 10, 30, 50, 70 and 90% of small intestine length) in broiler cockerels on day 21, 28, 35 and 42 are in Tables 2-5.

On day 21, no difference was detected in average villi height and depth of liberkuhn crypts in various

Table 2. Effect of different levels of perlite on intestinal morphology of broiler cockerels in day 21 (mean±sd)

Groups	Intestinal part					
	1%	10%	30%	50%	70%	90%
Effect on average villi height						
0 % perlite	4236±359	4179±197	2615±124	2128±299	1721±145	1456±129
2 % perlite	4737±131	4428±276	2807±331	2237±285	1825±283	1823±283
4 % perlite	4176±359	4200±467	3046±169	2469±232	1885±335	1487±119
Effect on average depth of liberkuhn crypts						
0 % perlite	626±108	575±44	509±51	504±131	507±63	404 ±55
2 % perlite	526±39	615±115	541±46	510±433	503±414	478 ±76
4 % perlite	569±51	582±581	515±18	497±39	492±37	427 ±9
Comparison of average villi height and depth of liberkuhn crypts						
0 % perlite	6.8±0.9	7.2±0.7	5.1±0.3	4.3±0.7	3.7±0.2	3.9 ±0.3
2 % perlite	7.5±0.8	7.3±1.4	5.2±0.70	4.4±0.4	3.6±0.2	3.9 ±0.1
4 % perlite	7.7±0.6	7.3±1.8	5.9±0.5	4.9±0.4	3.8±0.6	3.0 ±1.2

sections of small intestine length after supplementation of perlite (2% and 4%) in broiler cockerels ($P > 0.05$). In addition, no difference was detected in average villi height and depth of liberkuhn crypts in any sections of small intestine in day 21 ($P > 0.05$) (Table 2).

Effects of different levels of perlite on small intestine morphometry in broiler chickens on day 28 are in Table 3. Supplementation of different levels of perlite significantly increased average villi height in 1% sections of small intestine length in experimental groups compared to control group ($P < 0.05$). Furthermore, no difference was observed post-supplementation of perlite on average depth of liberkuhn crypts in various sections of small intestine ($P > 0.05$) (Table 3). Significant difference was detected on of average villi height and depth of liberkuhn crypts in section of 10 percent of small intestine using perlite (2%) compared to control group ($P < 0.05$).

On day 35 supplementation with 2% of perlite increased average villi height on various sections of small intestine (70 and 90%) ($P < 0.05$) (Table 4)

Effect of different levels of perlite on average villi height in various sections of small intestine in broiler cockerels in day 42 are in Table 5. There was a significant decrease of average villi height in section of 90% of small intestine length in broilers received diet containing 4% of perlite ($P < 0.05$).

DISCUSSION

While many studies have been carried out on effects of perlite on performance and carcass characteristics, very few studies have been designed to study its effects on intestinal morphometry in poultry. According to the results obtained from this study, we have found

Table 3. Effect of different levels of perlite on intestinal morphology of broiler cockerels in day 28 (mean±sd)

Groups	Intestinal part					
	1%	10%	30%	50%	70%	90%
Effect on average villi height						
0 % perlite	3937±2431^{b}	3612^{b}±408	2977±810	2466±626	1983±346	1611±212^{b}
2 % perlite	4830±100^{a}	4855^{a} ±174.2	3522±144	2557±187	2117±254	1992±149^{a}
4 % perlite	4660±208^{a}	4186^{ab}±161	3396±134	2828±307	2102±137	1738±27^{ab}
Effect on average depth of liberkuhn crypts						
0 % perlite	562±101	526±147	522±30	4835±45	491±30	439 ±7
2 % perlite	561±11	594±20	537±27	565±26	518±40	480 ±58
4 % perlite	548±48	5559±17	520±11	517±25	444±253	453 ±21
Comparison of average villi height and depth of liberkuhn crypts						
0 % perlite	7.2±1.7	6.8±0.9^{b}	5.6±1.4	5.0±0.8	4.0±0.2	3.6 ±0.4
2 % perlite	8.6±0.0	8.2±0.3^{a}	5.5±0.4	4.5±0.5	4.0±0.3	3.6 ±0.5
4 % perlite	8.5±1.0	7.5±0.4^{ab}	6.5±0.2	5.4±0.3	4.7±0.6	4.4 ±0.3

There were significant differences between groups with different letters in a column (a and b; P < 0.05).

Table 4. Effect of different levels of perlite on intestinal morphology of broiler cockerels in day 35 (mean±sd)

Groups	Intestinal part					
	1%	10%	30%	50%	70%	90%
Effect on average villi height						
0 % perlite	4340±195	4048±356	2756±349	2463±543	1792 ±48[b]	1696±103[b]
2 % perlite	4472±243	4129±316	3418.7±218	2654±73	2316 ±152[a]	2332 ±155[a]
4 % perlite	4123±696	4005±523	2893.7±208	2492±143	2247 ±298[ab]	1859±83[b]
Effect on average depth of liberkuhn crypts						
0 % perlite	529±59	511±20	537±16	543±35	477±19	513 ±41
2 % perlite	585±12	566±52	528±38	517±172	460±5	460 ±30
4 % perlite	551±22	529±23	526±5	473±62	462±6	484 ±30
Comparison of average villi height and depth of liberkuhn crypts						
0 % perlite	8.2±1.1	7.9±0.7	5.1±0.7	5.5±0.8	3.7±0.2	3.3 ±0.4
2 % perlite	7.6±0.2	7.2±0.1	6.4±0.8	7.5±4.2	5.0±0.3	5.1 ±0.6
4 % perlite	7.4±1.6	7.6±1.3	5.5±0.4	5.3±1.0	4.8±0.6	3.8 ±0.5

There were significant differences between groups with different letters in a column (a and b; $P < 0.05$).

out that supplementation of perlite (2 and 4%) was not able to cause alterations of villi height, average depth of liberkuhn crypts in various sections of small intestine in day 21. Several different action mechanisms of aluminosilicates (zeolite and perlite) remain ambiguous in animal models (Osman, 1982). These substances are sensitive to environmental conditions and their structural hydrogen ions change by condition. Furthermore, zeolite has the ability to bind with magnesium, calcium, potassium and ammonium ions and release under specific conditions. For instance, nutrients temporally attach to zeolite and passage rate through digestive tract diminishes and they are more exposed to gastrointestinal tract enzymes, e.g. amylase and peptidase (Khambualai, 2009). The life cycle of intestine absorption cells from establishment to shedding at the villous tip is 2 to 3 days (Leblond, 1981). Consequently, morphological changes at the exfoliate area of epithelial cells might be attributed to villous function. Cellular hypertrophy is by definition cell projection inward the intestine lumen (Shamoto and Yamauchi, 2000; Tarachai and Yamauchi, 2000). It is reported that charcoal administration induces hypertrophy of villi and epithelial cells in chickens, pigs and experimental animals (Coombs et al., 1997; Mumpton, 1999). These reports indicate that intestine villi and epithelial cells hypertrophy lead to increase intestinal absorptive feature (Yamauchi et al., 2006; Mekbungwan et al., 2008). It has been previously reported that bodyweight gain and performance of broilers were significantly increased after 1 to 2% perlite incorporation into diets (Talebali and Farzinpour, 2006; Ghiasi Ghalehkandi et al., 2011). In the present study, different levels of perlite significantly increased average villi height in birds in day 28. It has been reported that villi height, villus area and cell mitosis increased after administration of natural zeolite in chickens (Incharoen et al., 2009). The suggested

Table 5. Effect of different levels of perlite on intestinal morphology of broiler cockerels in day 42 (mean±sd)

Groups	Intestinal part					
	1%	10%	30%	50%	70%	90%
	Effect on average villi height					
0 % perlite	4723±135	4278±290	3833±36	3187±332	2460±194	2078 ±65 [a]
2 % perlite	4734±113	4425±141	3774±93	3235±45	2569±67	2140±69[a]
4 % perlite	4542±443	4505±71	3349±721	2839±547	24532±388	1607±265[b]
	Effect on average depth of liberkuhn crypts					
0 % perlite	562±10	526±14	522±30	483±45	491±30	439±7
2 % perlite	561±11	594±20	537±27	565±26	518±40	480±58
4 % perlite	548±48	555±17	520±11	517±25	444±253	453±21
	Comparison of average villi height and depth of liberkuhn crypts					
0 % perlite	7.2±1.7	6.8±0.9[b]	5.6±1.4	5.0±0.8	4.0±0.2	3.6 ±0.4
2 % perlite	8.6±0.0	8.2±0.3[a]	5.5±0.4	4.5±0.5	4.0±0.3	3.6 ±0.5
4 % perlite	8.5±1.0	7.5±0.4[ab]	6.5±0.2	5.4±0.3	4.7±0.6	4.4 ±0.3

There were significant differences between groups with different letters in a column (a and b; $P < 0.05$).

mechanism is that zeolite mechanically stimulates blood perfusion to the intestinal mucosa and stomach epithelial cells leading to increase gastrointestinal tract mucosa coverage, villi height and depth of liberkuhn crypts, which subsequently amplified secretory activity of these cells (Khambualai et al., 2009). One of the impressive effects of zeolites is its ability to alter intestinal enzymes that increase enzyme activity and improve nutrients absorption (Ghiasi Ghalehkandi et al., 2011). As nutrients, electrolytes and water are absorbed in the small intestine through villi and liberkuhn crypts, any change in villi height (the first degree change) and depth of liberkuhn crypt (the second degree change) will affect digestion and absorption output (Ghiasi Ghalehkandi et al., 2011). As previously reported, there is a positive correlation between intestinal villus size and villus height (Khambualai et al., 2009). Besides their positive effects on performance and small intestine morphology, there are some reports claiming effectiveness perlite and zeolite in enhancing prevention of some diseases (Macháček et al., 2010). Our observations provide novel information for these substances on their ability to improve nutrient absorption by altering intestinal villus and liberkuhn crypts feature. Further studies are required to clarify the mode and mechanisms of actions of these feed additives.

In this experiment, in day 35, a significant increase was detected on average villi height on various sections of small intestine length (70% and 90%) after supplementation of diet with perlite in experimental chicks. In contrast, there was a significant decrease on average villi height in section of 90% of small intestine length in broilers received diet contain 4% perlite in day 42. Presumably, the reverse results can derive from different sources. Use of perlite is dose-dependent and negative effects on intestinal villi and liberkuhn crypts have been reported by administration of high levels of zeolite in

poultry. It has to be mentioned that recommended level for synthetic zeolite is 1% to 2% (Shariatmadari, 2008) and inclusion of >7% of natural zeolite has toxic effects in poultry (Khademi, 2008). Effectiveness or revers effect of perlite depends on age, sex and animal strain. In agreement with previous researches, use of perlite has been confirmed to be dose-dependent and administration of 2% has positive effects, whereas supplementation diet with 4% perlite caused negative effects in broilers. The second explanation for reversed results in day 42 is growth rate. It is reported that the rate of villi growth dramatically declines by aging (Bayer, 1975; Tarachai and Yamauchi, 2000), but there is no information regarding age and sex effects on poultry response to zeolites. However, it is reported that production of some layers strain can be effected by zeolite (Shariatmadari, 2008).

In the present study, on day 28, there was significant improvement of average villi height and depth of liberkuhn crypts in section of 10% of small intestine after 2% perlite administration. Increased intestinal villi height provides more surface area for nutrient absorption (Khambualai et al., 2009). It is reported that perlite and zeolite promote feed retention time in the gastrointestinal tract of chickens, thus nutrients are exposed to extended enzymatic action that improves digestibility of certain nutrients (Safaeikatouli et al., 2011). The results of this study are in agreement with previous findings reported by Incharoen et al. (2009). We hypothesize that increased intestinal villi height and depth of liberkuhn crypts are associated to cell mitosis amplification and activation of intestinal villi. Likewise, it has been proposed that villus growth magnitude is associated with activation of cell proliferation in the liberkuhn crypt (Khambualai et al., 2009).

CONCLUDING REMARKS

Morphological chenges were detected after administration of perlite, especially with 2% supplementation in feed, on villi height and depth of liberkuhn crypts in broiler cockerels. It seems that perlite was able to activate cell mitosis. It is important to note that supplementation of diet with perlite had no adverse effects on small intestinal morphology in experimental birds. Further research is needed to elucidate the direct effects of perlite on villi height and depth of liberkuhn crypts in poultry.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interests.

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