# EFFECTS OF DIETARY MONTMORILLONITE NANOCOMPOSITE ON THE DIGESTIVE ENZYME ACTIVITIES IN THE PANCREAS AND INTESTINAL VILLI OF BROILER CHICKENS

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**Abstract**
*A total of 200 one-day-old Marshal broiler chickens were used to investigate the effects of montmorillonite nanocomposite (MMT-NC) in the diets on pancreas digestive enzyme activities and villus height of the birds. The birds were allotted to 5 dietary treatments for 56 days; each of the treatment group was replicated 4 times with 10 chicks per replicate. MMT-NC was added to the starter and finisher basal diets at 0, 1, 2, 3 and 4 g/kg. Data on villus height for duodenum, jejunum and ileum indicated that addition of MMT-NC to broilers diet improved (P<0.05) the villus height of the small intestine. Supplementation of MMT-NC in the diet of broilers significantly (P<0.05) increased the activities of amylase, trypsin and lipase in the pancreas. The present study showed that MMT-NC could be supplemented at 3 g/kg in the diet of broiler chickens for improved pancreas digestive enzyme activities and villus height.*

**Keywords:** montmorillonite, enzyme activity, morphology of what?, broiler chickens

## Introduction

Montmorillonite (MMT) is used as carrier for modulating drug delivery because of its high surface area, strong adsorptive capacity, high cation exchange capacity and standout adhesive ability (Park *et al.*, 2008). MMT intercalated by drug molecules exhibit novel physical and chemical properties. Many researchers have studied the drug- MMT interactions and found that drug--MMT hybrid can target drug release, improve drug dissolution, increase drug stability, and modify drug delivery patterns (Kollar *et al.*, 2003; Zheng *et al.*, 2007; Joshi *et al.*, 2009; Meng *et al.*, 2009). Monmorillonite can absorb organic materials either on its surface or within its inter-laminar spaces through an exchange of cations present in these spaces, Due to these physical-chemical properties, montmorillonite has been extensively used in animal diets to promote production, reduce the detrimental effects induced by mycotoxin-contaminated feeds and protect against enteric diseases (Duan *et al.*, 2013).

Animal feed containing montmorillonite has been shown to promote weight gain and feed efficiency, reduce bacterial contamination of the gut, reduce the detrimental effects of mycotoxin-contaminated diets (Tauqir and Nawaz, 2001). MMT have been reported as a protector for intestinal mucosa (Droy-Lefain *et al.*, 1985), also known to reinforce the intestinal mucosal barrier and helps regeneration of epithelium (Girardeau, 1987).

Clays reduce the speed of passage of feed along the digestive tract which allows more time for digestion. Feeding clays also causes morphological changes in the intestinal mucosa such as an increase in villus height and an increase in the villus height to crypt depth ratio. These changes increase the surface area of the gastrointestinal tract thus increasing nutrient digestibility (Almeida, 2013). Montmorillonite nanocomposite (MMT-NC) is an innovative technology to create materials and change structure, enhanced quality and texture of montmorillonite at the molecular level by synthesis at the atomic level.

The aim of this study was to determine the effects of montmorillonite nanocomposite in the diets of broiler chickens on pancreatic amylase, trypsin, lipase and the villus height of the small intestine.

## Materials and methods

A total of two hundred (200) unsexed day old Marshal Strain broiler chickens were randomly allotted into five treatments in a completely randomized design. Each treatment group had forty birds with four replicates. A commercial montmorillonite nanocomposite which contained sea lettuce, montmorillonite, diatomaceous earth, yeast extracts, seaweed extracts and essential oils was used. MMT-NC was added to basal diets (Table1) at 0, 1, 2, 3 and 4 g/kg. At the end of 56 days, data were collected on the pancreatic enzyme activities and villus height.

**Digestive enzyme activities of the pancreas**

The pancreas sample from one bird per replicate was harvested, frozen, thawed and homogenized in 4 volumes of ice-cold 0.9% NaCl. The homogenate was centrifuged (13,800 × *g* for 20 min at 4°C), and the supernatant were analyzed for digestive enzymes activities. Amylase (a-1, 4-glucan 4-glucanohydrolase, EC 3.2.1.1) activity was determined using the method of Somogyi (1960). Amylase activity unit was defined as the amount of amylase that will cause formation of reducing power equivalent to 1 mg of glucose in 1 h. The substrate used in the analysis was cornstarch. Lipase (triacylglycerol lipase EC 3.1.1.3.) activity was assayed using the method described by Tietz and Fiereck (1966). Lipase activity unit was equal to the volume (ml) of 0.05 *M* NaOH required to neutralize the fatty acid liberated during 2 hr incubation. Olive oil was used as the substrate in this assay. The substrate used for trypsin determination was Nα-Benzoyl-dl-arginine 4-nitroanilide hydrochloride (B4875, Sigma, St. Louis, MO). One unit of trypsin activity was defined as the hydrolysis of 1 μmol of substrate per minute.

**Villus height**

The segments from the duodenum, jejunum, and ileum were flushed with physiological saline and fixed in 10% formalin. Three cross-sections for each intestinal sample were stained with hematoxylin and eosin using standard paraffin embedding procedures. A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. Villus height was measured from the tip of the villi to the villus crypt junction.

***Statistical Analysis***

The data obtained were analyzed using the General Linear Model procedure of Statistical Analysis System 9.1 version (2000) and Polynomial contrast analysis. Significant means were compared using Duncan's multiple range test of same package.

**Results and discussion**

Supplementation with MMT-NC ($P<0.05$) increased the amylase, trypsin and lipase activities in the pancreas. As the level of MMT-NC increased in the diets, unit of digestive enzymatic activities increased. The exchange properties of clay could alter the pH and ionic composition of gastrointestinal fluids, thereby changing the enzymatic activity of gastrointestinal secretions (Martin-Kleiner et al., 2001). Xia *et al.* (2004) showed that Cu-MMT significantly suppressed intestinal *Escherichia coli* and *Clostridium*, such changes might contribute to the increased in digestive enzyme activities.

Compared with the control, supplemental MMT-NC increased($P<0.05$) villus height at the small intestinal mucosa, duodenum, jejunum and ileum. The increase in the villus height in different segments of the small intestine may be attributed to the fact that MMT-NC reduces the growth of many pathogenic or non-pathogenic intestinal bacteria, thereby decreasing the intestinal colonization and infectious processes, ultimately reducing the inflammatory reactions at the intestinal mucosa, resulting in the improvement of villus height in the intestines of broiler chicken (Pelicano *et al.*, 2005). Stressors that are present in the digesta can lead to quick changes in the intestinal mucosa (Choct, 2009). The increased length of the mucosal villi of the intestine and the increased gut length due to the dietary inclusion of humic substance or in the drinking water of broiler chickens (Ozturk and Coskun, 2006; Taklimi *et al.*, 2012) has been associated with the increased digestibility of nutrients due to a reduction of the passage rate of the intestinal content. The increased villus height in the small intestines has been related to a higher absorptive intestinal surface (Loddi *et al.*, 2002) which might have facilitated nutrient absorption and boosted growth promoting effect.

The results indicated that supplementing MMT-NC in broiler chickens improved digestive enzyme activities and villus height. However, supplementation at 3 g/kg could be added for economic advantage.

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**Table1:** Percentage composition of broiler chickens basal diets

| Ingredient | Starter (0-4 weeks) | Finisher (4-8 weeks) |
|---|---|---|
| Maize | 53.90 | 60.00 |
| Fish meal | 2.50 | 0.00 |
| Soya bean meal | 36.60 | 28.00 |
| Wheat offal | 4.00 | 7.70 |
| Bone meal | 1.30 | 1.80 |
| Oyster shell | 1.80 | 1.50 |
| Premix | 0.30 | 0.30 |
| Salt (NaCl) | 0.30 | 0.30 |
| L-lysine | 0.10 | 0.10 |
| L-methionine | 0.20 | 0.30 |
| **Analyzed values** | | |
| Crude protein (%) | 23.64 | 19.91 |
| Ether extract (%) | 3.61 | 3.51 |
| Crude fibre (%) | 3.22 | 3.27 |
| **Calculated values** | | |
| Calcium (%) | 1.08 | 1.09 |
| Phosphorus (%) | 0.43 | 0.39 |
| Metabolizable energy (MJ/kg) | 11.98 | 12.00 |

**Starter premix provid ed:** Vitamin A -10,000,000iu, Vitamin D $_3$-2,000iu, Vitamin E -40,000mg, Vitamin K -2,000mg, Vitamin B $_1$-1,500mg, Vitamin B $_2$-4,000mg, Vitamin B $_6$-40,000mg, Vitamin B $_{12}$-20mg, Niacin -40,00mg, Pantothenic -10,000mg, Folic -1,000mg, Biotin -100mg, Choline -300,000mg, Mang anese-80,000mg, Zinc-60,000mg, Iron -40,000mg, Copper -80,000mg, Iodine -800mg, Selenium -200mg, Cobalt -300mg, Antioxidant-100,000mg.

**Finisher premix provided:** Vitamin A -12,000,000iu, Vitamin D $_3$-2,500,000iu, Vitamin E -30,000mg, Vitamin K-2,000mg, Vitamin B $_1$-2,250mg, Vitamin B $_2$-6,000mg, Vitamin B $_6$-4,500mg, Vitamin B $_{12}$-15mg, Niacin -40,00mg, Pantothenic -15,000mg, Folic -1,500mg, Biotin -50mg, Choline -300,000mg, Manganese -80,000mg, Zinc-50,000mg, Iron -20,000mg, Copper -5,000mg, Iodine -1,000mg, Selenium -200mg, Cobalt -500mg, Antioxidant-125,000mg.

**Table 2:** Digestive enzyme activities and intestinal villus height of broiler chickens supplemented with montmorillonite nanocomposite (MMT-NC)

| | MMT-NC inclusion (g/kg) | | | | | | P-values | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameter | 0 | 1 | 2 | 3 | 4 | SEM | L | Q | C |
| **Enzyme** | | | | | | | | | |
| Amylase | 37.00 | 38.00 | 40.47 | 43.00 | 44.33 | 0.83 | 0.000 | 0.000 | 0.000 |
| Trypsin | 10.70 | 12.30 | 12.47 | 14.10 | 15.73 | 0.54 | 0.000 | 0.001 | 0.003 |
| Lipase | 37.00 | 40.00 | 40.67 | 42.00 | 43.53 | 0.65 | 0.000 | 0.000 | 0.000 |
| **Villus height** | | | | | | | | | |
| Duodenum | 757.67 | 768.67 | 799.00 | 792.67 | 805.00 | 6.29 | 0.003 | 0.010 | 0.031 |
| Jejunum | 891.67 | 897.00 | 898.67 | 901.67 | 905.00 | 2.19 | 0.037 | 0.123 | 0.253 |
| Ileum | 636.67 | 649.67 | 662.00 | 680.67 | 692.00 | 5.62 | 0.000 | 0.000 | 0.000 |

SEM = standard error of the mean. L= Linear, Q = Quadratic, C = Cubic