

## The use of zeolite in low protein diet added with critical amino acids to reduce pollution

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**ABSTRACT:** The purpose of the experiment was to investigate the effects of zeolite addition to 17%- and low (14%) protein diets added with critical amino acids on fecal ammonia emission and house flies (*Musca domestica*) larvae populations. Ninety layers of Lohmann brown strain at 50 weeks of age were randomly divided into 6 dietary treatment groups of 5 birds with 3 replications. Six dietary treatments were as follows: R1 (17% crude protein or CP, without zeolite addition), R2 (17% CP with 2% zeolite addition), R3 (17% CP with 4% zeolite addition), R4 (14% CP + Lys, Met, Thr, Trp without zeolite addition), R5 (14% CP + Lys, Met, Thr, Trp with 2% zeolite addition), R6 (14% CP + Lys, Met, Thr, Trp with 4% zeolite addition). Parameters observed were moisture content, fecal ammonia emission, populations of house flies larvae, and dynamic population of house flies. Data were analyzed by 2 x 3 factorial (2 dietary protein x 3 zeolite levels) followed by Duncan's multiple range test. The result of the experiment showed that 17% CP dietary and 14% CP dietary with critical amino acids addition had effects ( $P < 0,01$ ) on moisture content (73,46% and 73,12%), fecal ammonia emission (4,241 mg/100ml and 3,749 mg/100ml), and populations of house flies larvae (398 and 281 birds). It is concluded that the use of zeolite at the rate of 2 and 4% in 14% CP diet with critical amino acids addition can reduce moisture content (72,89% and 70,53%), fecal ammonia emission (3,469 mg/100ml and 3,312 mg/100ml), and population of house fly larvae (258 and 183 birds).

**Key words:** low dietary crude protein, zeolite, fecal ammonia emission, house flies larvae population

### INTRODUCTION

A major environmental issue faced by poultry industry in the future is the management of animal manure and odour that are associated with intensive livestock production. Livestock manure consists basically of faeces and urine, sometimes mixed with bedding, spilled feed and water spillage. In poultry production, manure may also be mixed with litter, feathers and broken eggs. Odour and flies are produced in the manure and other waste products, and will become a nuisance and a source of environmental pollution if not handled judiciously, hence causing environmental pollution and public protest.

Poultry houses of laying birds have particular problem with toxic gases especially ammonia, because the birds dropping are rich in nitrogen (uric acid). Koerkamp (1994) found that the nitrogen components of uric acid, urea and undigested proteins were potential sources for ammonia volatilization. Furthermore, uric acid and undigested protein were the two main nitrogen components in faeces, representing about 70 and 30% of total nitrogen, respectively.

Ammonia is one of the chemical pollutants from the poultry industry, which is the result of microbial activities in the faeces in which uric acid and undigested proteins are degraded. The rate of breakdown and the production of ammonia are influenced by moisture and temperature (Huton, 1987). Carbon dioxide is also produced in this process (Middelkoop, 1995).

Fresh layers manure contains 75-80% moisture (Poel, 1994; Wihandoyo, 2005). Trung reported that composition of layer manure was 1.50 to 8.55% nitrogen, 1 to 4% ether extract, 8.8% calcium and 2.5% phosphorous and poultry litter were 1.1 to 6.7% nitrogen, 1-6% ether extract, 2.4% calcium and 1.8% phosphorous. Manure accumulated in pits beneath the cages when not frequently removed, serves as an excellent medium for fly breeding (Quiswberry and Foster, 1984). Flies develop very fast in poultry manure, and often abundantly, causing a nuisance to farmers, their neighborhoods and the birds. Removing the fly breeding materials and disposing of it properly would break the life cycle of the fly (Nolan, 1981).

It has been observed that layer manure is highly suitable for the development of fly eggs to pupae. Many species of *Diptera* grow and develop naturally in animal waste particularly the house fly

(*Musca domestica*). Bowman and Lynn (1995) reported that house flies lay their eggs on animal manure or almost any kind of decaying organic matter. A female house fly may deposit about 2,000 eggs in average lifetime of 6 to 8 weeks. Moon and Meyer (1985) stated that house flies frequently deposited their eggs in batches with individual females producing approximately 100 eggs. The eggs took from 8 to 40 hours to hatch depending on environmental temperature, and the larvae usually feed on aggregations below the surface of the medium, requiring 3-8 d to complete the larvae period.

Reducing ammonia and house fly in the manure, must reduce the nitrogen content (uric acid) and water content. Reduction in the nitrogen content of uric acid and lowering the water content decrease the activity of microorganisms and the flies will not lay eggs in the dry faeces. If the eggs hatch, the larvae will not have enough nutrients.

About 17% of protein digestibility occurs in small intestine to be absorbed in the form of amino acid by villi of small intestine wall. This will be brought in the cells for metabolism and produce waste in the form of uric acid. Uric acid is depending on feed protein. The low protein feed with critical amino acid addition causes decreasing uric acid. High uric acid content in the manure is an ideal place for house fly to grow.

The use of chemical, drug or toxins for controlling odor and flies must be addressed properly concerning with the residues that may be accumulated in the soil and water. Zeolite may be a suitable alternative material, because it has the ability for ion exchange and exhibit adsorption properties, and is not toxic to water and soil. Xia et al. (1992) showed that inclusion of zeolite in the diet consistently reduced ammonia release from faeces of broiler chickens. Zeolites are crystalline, hydrated aluminosilicates of alkaline earth cations which have the ability to lose or gain water reversibly and can, without major structural changes, exchange constituent cations (Carlile, 1984). It can be used to extract ammonia (NH<sub>4</sub><sup>+</sup>) by ion exchange (Nakaue and Koelliker, 1981; Wihandoyo, 2005). Zeolite showed the ability for adsorption of ammonia in animal litter (Nemeiya *et al.*, 2000; Wihandoyo, 2005)

This study was undertaken to evaluate utilization of addition zeolite to 17% protein dietary and 14% low dietary protein with critical amino acids addition as feed additive in the control of fecal ammonia emission and population of house fly.

## MATERIALS AND METHODS

### *Animals and Experimental Design*

Ninety Lohmann brown layers, 50 weeks of age obtained from commercial layer, were allocated in a 2 x 3 factorial design experiment consisting of two levels of dietary protein (17% and 14% + critical amino acid addition) and three levels of zeolite (0, 2, and 4%) in the layer diets, with three replicates per treatment group and five birds per group (Table 1). The layer hens were placed in battery cages placed in opened-sided layer housing.

### *Diets*

The experimental diets are shown in Table 1. There were 6 dietary treatments with protein at 17 and 14% and zeolite at 0, 2, and 4%. All the diets were formulated to be iso-caloric (2700 kcal/kg).

**Table 1.** Experimental design and dietary treatments

Treatment <sup>1</sup>	Zeolite, %	Replication	Number of hen
Protein 17% (T1)	0	3	5
Protein 17% (T2)	2,0	3	5
Protein 17% (T3)	4,0	3	5
Protein 14% (T4)	0	3	5
Protein 14% (T5)	2,0	3	5
Protein 14% (T6)	4,0	3	5

<sup>1</sup>T4, T5 and T6 plus Met, Lys, Trp, and Thr.

The experimental diets consist of corn, rice bran, soy bean meal (SBM), meat and bone meal (MBM), poultry bone meal (PBM), DL-Methionine, L-Tryptophan, L-Lysin HCl, L-Threonine, Limestone, sodium chloride, trace mineral mix, vitamin mix, sand and zeolite.

### **Data Collection and Measurements**

The experiment was conducted for 2 periods (cycles), each with duration of 28 d per cycle (a total of 56 d). The measurements taken during the study included moisture content (%), ammonia fecal emission (mg/100ml), and populations of house fly larvae (birds).

A 2 to 3 g manure sample for analysis, were collected on day 28<sup>th</sup> (period I) and 56<sup>th</sup> (period II). The moisture and Si content of manure was measured according to AOAC (1984). Ammonia content measurement was made on day 28<sup>th</sup> (period I) and 56<sup>th</sup> (period II). Manure samples (50 g) were analysed using the method of Conway. The ammonia content of manure was measured according to AOAC (1984). Fresh manure was collected on day 28<sup>th</sup> (period I) and 56<sup>th</sup> (period II). Fresh manure (200 g) were taken and sterilized at 100°C placed in polybags and exposed for three days, then were incubated at room temperature for 3 days. Calculation was made using the modified technique of Southwood (1987) and Wihandoyo (2005). The dynamic population of adult fly was identified by counting the faecal and regurgitation spots left on the white papers placed at four sides of which flies in the exposure during three d for each period.

Analysis of variance was conducted on the data based on 2 x 3 factorial design using Statistical Analysis System (SAS) software. Duncan's new multiple range test was used to determine the differences between the means of each group (Steel and Torrie, 1989).

## **RESULTS AND DISCUSSION**

Table 2 showed that decreasing level protein in layer diets resulted in a decrease ( $P < 0.05$ ) of moisture content (73.45 and 73.12% for 17 and 14% protein, respectively), while the ammonia content also showed a significant decrease ( $P < 0.01$ ) with decreasing dietary protein (4.24 and 3.75 mg/100ml, respectively). The houses fly larvae populations also decrease with decreasing dietary protein (389 and 281 birds).

Increasing the level of zeolite in layer diets decreased ( $P < 0.01$ ) the moisture content (76.43, 72.89, and 70.53% for 0, 2 and 4% zeolite, respectively) as shown in Table 2. The ammonia content also decreased ( $P < 0.01$ ) (5.205, 3.469, and 3.312 mg/100ml, respectively), while the house fly larvae decrease ( $P < 0.01$ ) with increasing dietary zeolite (577.0, 258, and 183 birds).

There was interaction effects ( $P > 0.01$ ) between protein and zeolite dietary in fecal parameters measured. Interaction between protein and zeolite dietary showed a significant decrease ( $P < 0.01$ ) the moisture content, ammonia content and house fly larvae populations.

Utilization of low protein with amino acid addition decreased water content because metabolism of amino acid is directly absorbed so the water level is lower. Zeolite in the diet decreased fecal water content because zeolite has higher water absorbing capacity. Wihandoyo (2005) showed that fecal moisture in manure decrease significantly with 6 to 12% zeolite addition, the high water absorbing capacity of clinoptilolite may yield drier droppings, which could lead to less odor and fewer fly problems.

Low protein with amino acid addition and presence of zeolite improved protein degradation into amino acids and deamination into urine, causing a decrease in urine N, which finally lowered fecal ammonia content. According to Shurson et al. (1984), zeolite and clinoptilolite are effective in binding ammonia produced by deamination of proteins in the gastrointestinal tract during and preventing its absorption, fecal N excretion would be expected to be higher while urinary N excretion would be expected to decrease. Ferguson *et al.* (1998) reported that the higher the pH of the litter the greater potential for releases  $\text{NH}_3$ .

The higher ammonia in the manure was probably caused by the increase of moisture content. High moisture content of the faeces was probably the result of spillage and high moisture in the faeces, creating favourable conditions for the flies to breed. The moisture content is also an important factor

that can influence the activity of uric acid breakdown by microorganisms in the manure of faecal nitrogen.

**Table 2.** The Effect of level protein and level zeolite on *house fly* larvae population (birds), moisture content (%), and ammonia content (mg/100ml) in the manure of layer hens.

Treatments	Moisture, %	Ammonia, mg/100ml	Larvae population, birds
Level of Protein (%)			
17%	73.45 <sup>b</sup>	4.24 <sup>b</sup>	389.0 <sup>b</sup>
14%	73.12 <sup>a</sup>	3.75 <sup>a</sup>	281.0 <sup>a</sup>
<i>P</i>	*	**	*
Level of Zeolite (%)			
0%	76.43 <sup>c</sup>	5.205 <sup>c</sup>	577.0 <sup>c</sup>
2%	72.89 <sup>b</sup>	3.469 <sup>b</sup>	258.0 <sup>b</sup>
4%	70.53 <sup>a</sup>	3.312 <sup>a</sup>	183.0 <sup>a</sup>
<i>P</i>	**	**	**
Protein x Zeolite interaction			
<i>P</i>	**	**	*

<sup>abc</sup> Within a column in each treatment, means without a common superscript differ (\**P*<0.05, \*\**P*<0.05).

The average faecal and regurgitation spots left by adult flies on the white papers described the dynamic population of adult house fly which alight to feed and deposit their eggs on the manure during the experiment as shown in Table 3. It appeared that the faecal and vomit left by adult flies fluctuated from 219 to 576 over the different cycles. On average, there were about fly's present trough the experimental period.

**Table 3.** Dynamic of adult *house fly* population

Direction	Cycle 1	Cycle 2
North	324	219
West	489	404
South	306	576
East	475	420
Average	399	405

## CONCLUSIONS

It can be concluded that protein 14% with amino acid addition and increasing dietary levels of zeolite significantly decreased the larvae population of the flies in poultry manure, ammonia production and faecal moisture content. These results showed that supplementation of zeolite at level of 4% and protein 14% with amino acid addition was the best treatment to decrease house fly larvae population and at the time reduce ammonia production.

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