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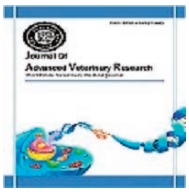

Mitigation of Air Gas Emission, and Litter Microbial Quality in Muscovy Duck Pens: The Effectiveness of adding Clinoptilolite Zeolite as a Litter Amendment

Asmaa N. Mohammed*, Mohamed A. ElBably

Department of Hygiene, Zoonoses and Epidemiology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt

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ABSTRACT

The current study aimed to investigate the effectiveness of adding clinoptilolite zeolite to litter on a mitigating of air gas emission, litter moisture content and microbial load in Muscovy duck pens. Eighty litter samples were collected in polyethylene bags to detect pH, moisture content and for isolation of different pathogenic bacteria during the entire length of study. In addition, air gas emission; ammonia (NH_3), hydrogen sulfide (H_2S), and carbon dioxide (CO_2 as a greenhouse gas); besides micro-climatic factors (in-door temperature, relative humidity, and air velocity) in duck pens were measured twice a week using measurement instruments. Litter microbial quality, litter temperature, pH and moisture content were evaluated pre- and post-litter amendment. For duck's litter amendment, the effectiveness of adding clinoptilolite zeolite at different concentrations of 10 and 20 g/kg of litter on alleviation of air gas emission, litter moisture content and the microbial load was evaluated. The average values of air gas emission (NH_3, CO_2, and H_2S) were recorded at higher concentration levels during the sixth week of age (16.44, 2671, 4.21 ppm, respectively). Total viable count (TVC) of litter was significantly increased during the fourth, fifth and sixth weeks of age ($6.6x10^6 \pm 3.5x10^4$, $5.73x10^6 \pm 3.1x10^4$ and $5.14x10^5 \pm 5.1x10^3$ CFU/g, respectively) at $P<0.05$. Clinoptilolite zeolite exhibited a significantly lower in the average values of air gas emission (NH_3, CO_2, and H_2S) besides alleviation of Litter pH, moisture content, TVC, and microbial load. In conclusion, Microclimatic factors are an essential element for improving the healthy environment for ducks. Adding of clinoptilolite zeolite at concentrations of 10 and 20 g/kg of litter is highly effective in lowering concentration levels of NH_3 and CO_2. The absence of H_2S reduce litter moisture and microbial load.

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Introduction

Controlling duck's environment is crucial to their flocks, particularly microclimatic factors; temperature, relative humidity, litter moisture content and gas emission such as ammonia (NH_3). The key element for duck welfare is using the good quality litter as straw (Jones and Dawkins, 2010). Using litter amendments has numerous benefits such as reducing the moisture content, absorb undesirable odors (Lee *et al*., 2015), in addition, inhibit microbial growth, lowering litter pH, and prevent the transformation of nitrogen into ammonia (Ataptattu *et al*., 2017).

In poultry farms, litter quality may act as a potential source and vehicle for pathogenic microorganism's transmission depending on the prevailing temperature and relative humidity besides litter pH, and moisture content (Ritz *et al*., 2014). Litter material types can exert an impact on the colonization of microbes such as wood shaving, sawdust, hay, and rice hull (Dunlop *et al*., 2016). Furthermore, one of the main environmental factors affecting the survival of fecal coliform is moisture content and temperature. Fecal coliforms such as *E. coli* (O157: H7) can survive in Poultry's litter for 42-49; 49-56 and 63-70 days at 37°C, 22°C, and 5°C, respectively (Blaustein *et al*., 2015). A litter with increased pH and moisture content in poultry farms can be considered as a favorable medium for increasing the survival and transmission of *Salmonella* Typhimurium (Lawal *et al*., 2016).

The airborne pathogens in combination with high gas concentrations and dust exert adverse effects on the respiratory system and the conjunctiva of birds that leading to inflammations, allergic reactions, and increasing their susceptibility to infectious diseases. Whereas excessive ammonia levels in poultry buildings lower productivity (Homidan *et al*., 2003; Miles *et al*., 2004; Davis and Morishita, 2005; Miles *et al*., 2006).

Clinoptilolite (CPL) is used in various applications as a useful natural salt such as absorber, a chemical sieve, environmental protection, a feed additive, an odor control agent, and a water filter. Clinoptilolite can easily absorb toxic gases from the air such as ammonia (Tzia and Zorpas, 2012; Bintaş *et al*., 2014). Literature studies assessed the toxicology of natural

*Corresponding author: Asmaa N. Mohammed
E-mail address: asmaa.mohamed2@vet.bsu.edu.eg

salts and proved that certain natural zeolites, e. g. CPL is non-toxic and completely safe for use in both human and veterinary medicine (Pavelić *et al*., 2001). Currently, in the poultry industry, there is little information available on domestic ducks reared for meat production (Rodenburg *et al*., 2005). This study was designed for measurement of in-door microclimatic factors, air gas emission (NH_3, H_2S, and CO_2) in duck pens and assess the microbial quality of litter in relation to litter temperature, pH, and moisture content then evaluate the influence of adding of clinoptilolite zeolite to duck's litter on a mitigating of air gas emission, litter moisture content and microbial load.

Materials and methods

Ethical approval

The protocol of the study was carried out in accordance with the ethical guidelines of Institutional Animal Care and Use Committee (IACUC), Beni-Suef University, Egypt.

Study location and duck pens description

The current study was conducted on a private poultry farm located in Beni-Suef province, Egypt that involved five duck pens. Muscovy duck flocks were reared in a deep litter system with a semi-controlled environment and kept on wood shaving litter. There were six exhaust fans in the duck pen, each having 435 m^3/min of the air exchange rate. The thickness of a duck litter was 6 cm. Hygienic measures inside pens were fair to some extent. In the current study, microclimatic factors (in-door temperature, relative humidity, and air velocity) and air gas emission (NH_3, H_2S, and CO_2) in all duck pens were measured in addition to the microbial quality of litter (TVC, *E. coli*, *Klebsiella* spp., *Listeria monocytogenes*, and *Salmonella* spp.) in relation to its moisture content, temperature, and pH was evaluated pre and post using clinoptilolite zeolite for litter amendment that assessed in an alleviation of air gas emission, litter moisture content, and microbial loads.

Microclimatic factors

In-door air temperature and relative humidity (RH%) were recorded twice a week using Clock Thermo-Hygrometer (Boeco Germany, BOE Model 325) and located about 1.0-1.5 meters above the ground level. Whilst air velocity was measured by digital anemometer [(Van E probe microprocessor digital meter) n 233569, accuracy ± 2.0 %+1.0 d, resolution 0%] during the entire length of the study period.

Measurement of air gas emission

The concentration level of each gas (NH_3, CO_2, and H_2S) was measured twice a week using measurement instruments. The level of NH_3 was measured at different heights from the duck litter surface (30, 90, and 150 cm). Measurements were taken in a zig-zag arrangement, using a portable gas detector (Crowcon Detection Instruments, Gas-Pro, Oxford shire, UK). Meanwhile, the CO_2 level was measured using a CO_2 measuring instrument (Testo535, Germany) with a securely attached probe. Hydrogen sulfide gas (H_2S) was measured using a single gas detector (Gasman, H_2S).

Ducks' litter measurement

Litter pH was recorded two times a week during this work. For detecting the litter pH, 10 g of litter samples were taken from five different areas of each duck pen (four from corners and one from the center) and added to 100 mL of distilled water then stirred in a magnetic stirrer for 15 min and filtered through filter paper. The filtered liquid was used to measure litter pH using a pH meter (JENWAY, 061 663/REV A/08-08). To measure the moisture content of duck litter, five samples were collected from different areas of duck pen, and then thoroughly mixed before analysis. The pooled litter were weighted (W1) using an electronic analytical balance (HOCHOICE, HC, Shanghai, China) and dried in a hot air oven at 105°C for 24-48 h and the dry weight (W2, two equal successive weights) was subtracted from the ini¬tial weight (W1) to determine the duck litter moisture content (%). Whilst Ordinary Thermometer were used to measure litter temperature in-situ.

Microbial quality of ducks' litter

A total of eighty litter samples was collected from different location of two duck pens for isolation of different bacterial pathogens. The total viable count (TVC) of bacteria was enumerated using the Pour Plate Technique according to APHA (2012). Five grams of each litter samples were suspended in phosphate-buffered saline (30 mL) and mixed well for 5 min then serial dilutions were performed from 10-1:10-6 thereafter, 0.1 µL from each serial dilution was spread onto two sterilized Petri dishes of plate count agar (CM 0325; Oxoid Ltd., Basingstoke, UK). The number of colonies on all plates (CFU/g) was enumerated after 24 h of the incubation period (Kirk *et al*., 2004). For isolation of *E. coli* and *Klebsiella* spp., a loopful of diluted samples were streaked on MacConkey agar (Oxoid; CM 0115) and incubated at 37°C for 24 h. Whilst for *L. monocytogenes*, diluted samples were taken on Listeria enrichment broth, then spread on Palcam agar (PA, Biokar Diagnostics, France). *Salmonella* spp. was streaked on buffer peptone water (BPW) as peri enrichment broth, then enrichment on Rapaport broth was done and incubated at 42.5°C for 24 h. Thereafter, a loopful of broth spread on selective agar media Xylose Lysine Deoxycholate (XLD) and incubated at 37°C for 24 h. All suspected colonies of bacterial isolates were preliminarily identified by Gram's staining and colony morphology in addition to API 20E (Biomerieux, Crappone, France) used for the identification of suspected colonies of *E. coli* and *Klebsiella* spp. Whilst, different biochemical tests were used to identify both *Salmonella* spp. and *L. monocytogenes* colonies (Collee *et al*., 1996; Hitchins 2001).

Assessment of adding clinoptilolite zeolite for litter amendment in-vivo trial

The clinoptilolite zeolite (Aluminum Silicate, Al2(SiO2)3) effectiveness on mitigation of air gas emission (NH_3, CO_2, and H_2S) and microbial load of ducks' litter (TVC, *E. coli*, *Klebsiella* spp., *Listeria monocytogenes*, and *Salmonella* spp.) was evaluated at a concentration of 10 and 20 g/Kg of ducks' litter and compared with a control group (without litter amendment). The in-vivo trial was applied in replicate.

Experimental study design

A total of 1500 one-day-old Muscovy duck obtained from a commercial hatchery were transported to control environmental duck pens. Ducks housed into five-floor deep litter pens with 300 ducks each. Each pen provided with six exhausted fans. The temperature in the first week was relatively kept at 32°C then progressively reduced to reach between 21°C to 24.6°C in the third and fourth weeks of age. In the 1st two groups, clinoptilolite zeolite was added at a concentration of 10 g/kg of wood sheaving litter. In the 2nd two groups,

clinoptilolite zeolite was added at a concentration of 20 g/kg of duck litter, while the 5th group was kept as a control group without any litter amendment. Air-gas emission: NH_3, CO_2, and H_2S was measured two times per week as mentioned before. Duck litter samples were collected twice a week for each study group besides control one for the determination of its pH and moisture content (Mcgrath *et al*., 2005) and investigated for microbial quality. The trial was conducted in replicate for each testing concentration. Thereafter, the mean values of all tested parameters at the same treatment groups (1st two groups) were calculated and called as the 1st group, meanwhile, the 2nd two groups were called as the 2nd group in the results section then, each 1st and 2nd study group was compared with the control group.

Data analysis

All data were collected and prepared in the Excel spreadsheet to analyze statistically. The mean values of microclimatic factors and average values of air gas emission in-door of duck pens besides litter measurements were analyzed using a One-way analysis of variance "ANOVA test". Whilst the clinoptilolite zeolite effectiveness on microbial quality of Muscovy ducks' litter were analyzed using non-parametric test "Chi-square test" using SPSS (statistical package for social sciences, version 22.0, Chicago, Illinois, USA).

Results

Environmental conditions in-door of Muscovy duck pens

Micro-climatic factors in-door of duck pens clarified that the mean values of ambient temperature during the first week of age were higher (35.81±0.31°C) followed by the fourth, sixth and fifth week of age (34.25±0.23, 33.95±1.08 and 31.70±2.15°C) compared with the second and third week (19.54±0.16 and 26.74±0.04°C, respectively). Whilst, the relative humidity (%) inside duck pens recorded the highest percentage during the sixth week (73.25±10.05 %) followed by the first three weeks of age (53.22±12.03, 50.06±9.24 and 65.33±11.61%) then gradually decreased during the fourth week (33.01±6.22%). Oppositely, air velocity rate in-door of duck pens exhibited the highest rate during the fourth week of age (2.11±0.33 Knots/h) followed by the fifth week (1.41±3.02 Knots/h) meanwhile the lower rate recorded on the first and third week of age (0.21±2.1 and 0.75±3.41 Knots/h, respectively) as shown in Table 1.

Air gases emission in-door of duck pens

Interestingly, the average values of air gas emission (ppm) in Muscovy duck pens that included NH_3, CO_2, and H_2S were recorded at higher concentration levels during the sixth week of age 16.44, 2671, 4.21 ppm, respectively, compared with their minimum, maximum and average values during the first three weeks of age whereas average values of NH_3 emission in-door of duck pen was 9.32, 6.69 and 10.52 ppm, respectively. Whilst CO_2 average values were 2277, 2219, and 2248 ppm, respectively. Oppositely, average values of H_2S were 2.28, 2.63 and 3.02 ppm, respectively as recorded in Table 2.

Ducks' litter measurements and microbial quality

As shown in Table 3, pH of ducks' litter was relatively within the normal value that ranged from 6.2±0.2 to 8.3±0.6 during the study period. The indoor temperature of wood shaving litter was recorded 24.5±1.9, 21.3±2.2 and 26.2±4.3°C, respectively during the first three weeks. Meanwhile, it recorded the highest degree in the fourth and fifth weeks of age (32.3±1.5 and 29.6±3.6°C). Furthermore, moisture content (%) was recorded the highest percentage during the fourth, fifth and sixth weeks of age (59.3±3.6, 62.8±1.3 and 71.7±2.5%, respectively).

Microbial load of ducks' litter clarified that total viable count (TVC) was significantly increased during the fourth, fifth and sixth week of age, which was $6.6x10^6$±$3.5x10^4$, $5.73x10^6$± $3.1x10^4$, and $5.14x10^5$± $5.1x10^3$ CFU/g, respectively at P<0.05. Besides *E. coli*, and *L. monocytogenes* were the most bacterial isolates from ducks' litter followed by *Klebsiella* spp. and *Salmonella* spp. Moreover, *E. coli* isolated in the highest percent-

Table 1. Mean values of micro-climatic conditions (mean ± SE) in-door of Muscovy duck pens during the study period

	Microclimatic factors		
Age (week)	Temperature (°C)	Relative humidity (RH%)	Air velocity (Knots/h)
1st	35.81±0.31[a]	53.22± 12.03[ab]	0.21±2.1[c]
2nd	19.54±0.16[c]	50.06±9.24	1.0±1.65[b]
3rd	26.74±0.04[b]	65.33±11.61[b]	0.75±3.41
4th	34.25±0.23[a]	33.01±6.22	2.11±0.33[a]
5th	31.70±2.15[a]	36.42±4.57[c]	1.41±3.02
6th	33.95±1.08	73.25±10.05[a]	1.06±1.63[b]

Superscript letter [a-c] within the same column significantly differ at P<0.05.

Table 2. Air gas emission (ppm) levels in-door of Muscovy duck pens during the study period.

	Air gas emission (ppm) in Muscovy duck pens											
	NH_3				CO_2				H_2S			
Age (week)	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
1st	8.64	10	9.32[b]	2.1	2249	2305	2277[b]	45	1.5	3.07	2.28	1.95
2nd	5.51	7.67	6.69[ab]	1.84	2190	2248	2219[ab]	121	2.56	2.71	2.63[b]	1.02
3rd	9.72	11.32	10.52[b]	1.95	2246	2250	2248[b]	59	2.21	3.83	3.02	2.23
4th	8.7	13.52	11.11	3.4	2142	2398	2270	64	1.54	2.72	2.13[ab]	3.41
5th	10.45	16.64	13.54[a]	1.76	2086	2228	2157[c]	35	1.8	2.5	2.15	1.53
6th	13.07	19.81	16.44[a]	2.8	2182	3160	2671[a]	49	4.02	4.4	4.21[a]	1.27

- Min: Minimum, Max: Maximum, Avg: Average, SD: Standard deviation.
- Column with different superscripts [a,b&c] significantly differ at P<0.05.

ages during the first, third and fourth weeks of age (53.3;8/68, 46.7;7/68 and 60.0%;9/68, respectively). Whilst, *Klebsiella* spp. isolated at the highest percentage in the fifth and sixth week of age (26.7%;4/68 and 13.3%;2/68) compared with both of *L. monocytogenes* and *Salmonella* spp. that recorded their highest percentages in the sixth week of age (20.0;3/68 and 26.7%;4/68, respectively).

Effectiveness of natural zeolite on mitigation of air gases emission, and litter microbial quality (%)

Evaluating the clinoptilolite zeolite effectiveness on mitigation of air gas emission in Muscovy duck pens (Table 4) exhibited a significantly decreased in the average values of air gas emission in both of 1st and 2nd groups compared with the control group at P<0.01. Furthermore, the values of NH_3 gas emission was significantly decreased during the third and fourth week (3.62±1.6 and 4.35±0.8 ppm, respectively) in the 1st group and its value in the 2nd group was 3.53±0.5 and 3.01±1.0 ppm compared with a control group, which was 10.32±1.4 and 15.41±2.3 ppm. Interestingly, the average values of CO2 were recorded in the least values in the last two weeks of in-vivo trial, where its value was 1241±23.5 and 1457±19.2 ppm, respectively, in the 1st group, whilst 1352±12.5 and 1572±11.7 ppm, respectively in the 2nd group compared with their average values in the control group that was 2130±13.7 and 2289±10.1 ppm, respectively. Meanwhile, H2S has recorded 0.0±0.0 ppm in each examined group.

On the other hand, the influence of clinoptilolite zeolite on microbial quality (%) of Muscovy duck's litter in Table 5, showed that TVC of bacteria in both of 1st and 2nd group were gradually decreased compared with the control group. TVC of bacteria in both study groups was $2.45x10^3±3.5\ x10^2$ and $3.1x10^3±3.5x10^2$ CFU/g, respectively, during the third and fourth week of duck age compared with their level in the control group ($4.33x10^5±1.3\ x10^4$ and $4.6x10^4±3.5x10^2$ CFU/g). In addition, after adding of clinoptilolite zeolite, the microbial quality of litter showed significantly decreased in the level of *E. coli* and *Salmonella* spp. during the third and fourth week of age in the 1st and 2nd study group compared with control group. Meanwhile, the percentage of *Klebsiella* spp. and *L. monocytogenes* were not achieved any significant difference compared with a control group.

Effectiveness of natural zeolite on litter pH and moisture content (%)

Adding of clinoptilolite zeolite to duck's litter has influenced by litter moisture content (%) during the in-vivo trial (Fig. 1). It has been found that the moisture content (%) during the third and fourth weeks of age recorded the least percentage (29.8, 35.7; 32.6 and 28.7 %, respectively) compared with a control group (46.5 and 39.3%, respectively). Litter pH was significantly reduced during the in-vivo study (Fig. 2). Litter pH in both study groups showed an obvious reduction during the fourth week of age to become more acidic (4.5 and 4.8, respectively) compared to the control group.

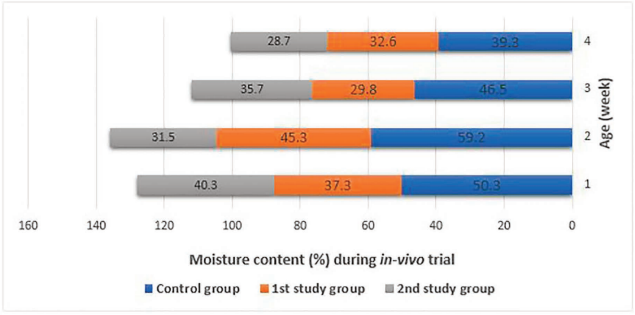

Fig. 1. Effectiveness of clinoptilolite zeolite on moisture content (%) of ducks' litter during the in-vivo trial.

Table 3. Microbial quality of ducks' litter in relation to temperature, pH, and moisture content of wood shaving litter.

	Wood shaving litter in Muscovy duck pens							
	Measurements			Microbial load of ducks' litter (%) Total No. of litter samples (*n* =80); Total positive= 68/80;85.0%.				
Age (week)	Temperature (°C)	pH	Moisture content (%)	TVC (CFU/g)	*E. coli*	*Klebsiella* spp.	*L. monocytogenes*	*Salmonella* spp.
1st	24.5±1.9^{ab}	6.2±0.2^{b}	17.8±3.1^{c}	$7.63\ x\ 10^4± 5.1\ x10^{4ab}$	53.3	13.3	20	6.7
2nd	21.3±2.2	7.1±1.4^{a}	19.2±1.2	$8.32\ x\ 10^5± 3.1\ x10^{4b}$	33.3	6.7	0	13.3
3rd	28.2±4.3^{b}	6.5±0.05	40.5±5.4^{ab}	$5.23x10^5±1.5\ x10^{3b}$	46.7	6.7	13.3	0
4th	32.3±1.5^{a}	8.3±0.6^{a}	59.3±3.6^{b}	$6.6x10^6±3.5x10^{4a}$	60	0	6.7	6.7
5th	29.6±3.6	6.7±2.1^{b}	62.8±1.3^{b}	$5.73\ x\ 10^6± 3.1\ x10^{4a}$	33.3	26.7	13.3	13.3
6th	27.6±0.8	7.5±1.3	71.7±2.5^{a}	$5.14\ x\ 10^5± 5.1\ x10^{3b}$	20	13.3	20	26.7

- TVC: Total viable count; CFU: Colony forming unit.
- In litter measurements, column with different superscripts $^{a,b\&c}$ is significantly differ at P<0.05.
- Distribution of microbial load of ducks' litter (%) is statistically significant at $\chi 2 = 18.6$, P<0.05.

Table 4. Influence of clinoptilolite zeolite on mitigation of air gas emission (mean ± SE) in Muscovy duck pens during the in-vivo trial.

	Influence of clinoptilolite zeolite on mitigation of air gases emission (ppm) in-door								
				Study groups					
Age (week)	Control group			1st group			2nd group		
	NH_3	CO_2	H_2S	NH_3	CO_2	H_2S	NH_3	CO_2	H_2S
1st	12.26±2.6^{a}	1958±11.2^{a}	2.34±0.03	5.21±2.1^{b}	1653±12.8^{ab}	0.0±0.0	4.31±0.01^{ab}	1730±9.6^{b}	0.0±0.0
2nd	9.53±1.1^{a}	1792±14.6^{a}	1.50±0.05	7.52±1.3^{a}	1592±16.6^{b}	0.0±0.0	3.66±1.2^{b}	1684±20.1^{c}	0.0±0.0
3rd	10.32±1.4^{a}	2130±13.7^{a}	2.11±0.23	3.62±1.6^{b}	1241±23.5^{ab}	0.0±0.0	3.53±0.5^{b}	1352±12.5^{b}	0.0±0.0
4th	15.41±2.3^{a}	2289±10.1^{a}	1.22±0.42	4.35±0.8^{b}	1457±19.2^{ab}	0.0±0.0	3.01±1.0^{ab}	1572±11.7^{b}	0.0±0.0

-Different superscript letters $^{a,b\&c}$ within a row of each gas are significantly differ at P<0.01.

Table 5. Effectiveness of clinoptilolite zeolite on microbial quality of Muscovy ducks' litter during the in-vivo trial.

Antimicrobial effect of clinoptilolite zeolite on microbial quality (%) in Muscovy ducks' litter													
Age (week)	Control group					Study groups							
						1st group				2nd group			
	TVC (CFU/g)	*E.*	*KL.*	*L.*	*S.*	TVC (CFU/g)	*E.*	*KL.*	*L.*	*S.*	TVC (CFU/g)	*E.*	*KL.*	*L.*	*S.*
1st	$10.52 \times 10^5 \pm 4.1 \times 10^3$	38.9	16.7	27.8	31.7	$6.43 \times 10^4 \pm 2.1 \times 10^2$	36.4	18.2	27.3	18.2	$4.23 \times 10^4 \pm 5.2 \times 10^2$	23.1	23.1	30.8	23.1
2nd	$5.34 \times 10^6 \pm 2.1 \times 10^{2a}$	50	25	15	10	$3.52 \times 10^4 \pm 1.2 \times 10^{2b}$	50	21.4	21.4	7.1	$4.63 \times 10^3 \pm 1.8 \times 10^2$	40	30	20	10
3rd	$4.33 \times 10^5 \pm 1.3 \times 10^{4a}$	53.3	26.7	13.3	6.7	$2.45 \times 10^3 \pm 3.5 \times 10^{2b}$	22.2	44.4	33.3	0	$3.72 \times 10^3 \pm 3.3 \times 10^2$	37.5	25	37.5	0
4th	$4.6 \times 10^4 \pm 3.5 \times 10^2$	50	12.5	25	12.5	$3.1 \times 10^3 \pm 3.5 \times 10^2$	30	30	40	0	$4.3 \times 10^3 \pm 2.8 \times 10^3$	20	50	30	10

-E.: *E. coli*; KL.: *Klebsiella* spp.; L: *L. monocytogenes*.; S: *Salmonella* spp.; TVC: Total viable count
-No. of litter samples/control group/week = 20, Total positive samples in 1st week= 18, in 2nd week= 20, in 3rd week=15 and in 4th week= 16.
-No. of litter samples/ study group/week = 20, Total positive samples/1st group in 1st week=11, in 2nd week= 14, in 3rd week=9 and in 4th week=10.
-Total positive samples/2nd group in 1st week= 13, in 2nd week= 10, in 3rd week = 8 and in 4th week= 10.
-Different superscript letters a&b within the same row are significantly differ at P<0.001.

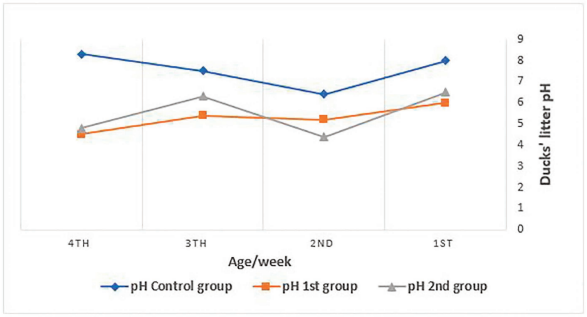

Fig. 2. Effectiveness of clinoptilolite zeolite on ducks' litter pH during the in-vivo trial.

Discussion

For improving the healthy environment for ducks during this study, there are various potential environmental factors include ambient temperature (°C), relative humidity (RH%), airflow rate (Knots/h), litter moisture (%), air gases emission (ammonia, carbon dioxide, and hydrogen sulfide), stocking density, and housing design besides biosecurity measures should be taken into consideration for an achievement of duck welfare. Interestingly, increasing of ambient temperature associated with high ducks' litter temperature and improper stocking density were very implicated in growth rate reduction, increased mortality, and worsening gait (Jones and Dawkins, 2010).

In the current study, microclimatic factors were strongly associated with air gas emission and litter moisture besides microbial loads of wood shaving litter used in duck's pen. Furthermore, the higher temperature was observed during the first, fourth and fifth week of age together with high relative humidity in the first three weeks of duck age then gradually decreased throughout the fourth and sixth week of age during the production cycle of duck. Moreover, air velocity recorded a low rate in the first and third weeks. Jones and Dawkins (2010) found that high ambient temperature affected growth rate and reduced the percentage of ducks with the best gait due to ducks ate less when rearing under higher temperatures.

The obtained results revealed that air pollutant gases indoor of duck pens (NH_3, CO_2, and H_2S) recorded at the highest concentration levels during the sixth week of age that associated with increased relative humidity (%) and lower air movement rate compared with the first week of age. Jones *et al.* (2005) found that during the production period, relative humidity increase was implicated with ammonia concentration in the occurrence of footpad dermatitis. In addition, the hygienic problem associated with a high level of ammonia concentration included depressing growth rate, feed intake, and severe irritation of mucous membrane of the eyes and upper respiratory system (Kristensen and Wathes, 2000; Homidan *et al.*, 2003; Nurzillah *et al.*, 2018).

This study observed that in-door air gases emission of NH_3 and CO_2 levels were substantially affected by air movement rates inside duck's pen. In addition, the air movement rate varies with different ambient temperatures and relative humidity, this interaction proved that microclimatic factors play an essential role in controlling of air gas concentration levels. Kilic and Yasliogluu (2014) found that indoor air temperature had a significant effect on air gases concentration in the laying hen house. As well increased ambient temperature was closely associated with the high level of NH_3 and CO_2 concentrations. Witkowska and Sowińska (2017) pointed to the carbon dioxide concentration level in densely stocked poultry houses should not exceed 2000 ppm. Furthermore, comparing the magnitude of NH_3 level in ducks' pen with other poul-

try species clarified that the NH_3 concentration level in layer houses was ranged from 0.5 to 12.5 ppm (Cheng *et al*., 2011). In contrast, Ning (2008) recorded that the level of NH_3 in laying hen house reached 26 ppm that had adverse effects on the health of the birds. Besides, Prodanov *et al*., (2016) and Wang *et al*., (2010) found that the most predominant air gas pollutant is ammonia inside the poultry farms as well its concentration varies based on various factors, among which are relative humidity, temperature, ventilation rate, and manure handling inside the facility. On the other hand, H_2S as a pollutant air gas accumulated in duck pens due to decomposing of sulfur contains organic matter in ducks' manure causing objectionable odor (rotten egg). Nurzillah *et al*., (2018) reported that H_2S is one of the pollutant gases related to chicken manure besides its acute health effect, which included irritation of the respiratory and cardiovascular system.

The current study found that TVC was significantly higher during the fourth, fifth and sixth week of age besides the predominant bacterial isolates from ducks' litter were *E. coli*, *L. monocytogenes* followed by *Klebsiella* spp. and *Salmonella* spp. Sahoo *et al*., (2017) showed that litter management is an important factor that affects the rate of its emission and bird's health. As well, keeping litter dry is a key point of the poultry farms management. Soliman *et al*., (2018) clarified that litter is a favorable media for bacterial pathogens growth and transmissions such as *Salmonella* Typhimurium in the presence of increased litter pH and moisture content (%). Moreover, both litter moisture and temperature represented the most essential factors affecting the survival of Escherichia coli (Blaustein *et al*., 2015).

Ducks' litter amendment using clinoptilolite zeolite and evaluating its effect on alleviation of air gas emission, moisture content, pH, and microbial quality of litter in Muscovy duck pens is considered the first study to the best of our knowledge. The clinoptilolite zeolite effectiveness at both concentrations of 10.0 and 20.0 g/kg of litter on air gases emission exhibited a significantly lower in the average values of air gas emission (NH_3 and CO_2) in both of 1st and 2nd groups than the control group during the third and fourth week of age meanwhile, H_2S gas emission in each study group recorded 0.0 ppm. In addition, it produced an alleviation of the moisture content (%) in duck's litter. On the other hand, Schneider *et al*., (2016) concluded that at a concentration of 10%, adding zeolite to broilers litter led to reduce litter moisture and ammonia level by up to 32.0%. Meanwhile, using zeolite on the manure of laying hen at a concentration 2.5, 5.0 and 10.0% of the manure weight reduced the level of ammonia emissions by 36, 62, and 92%, respectively (Li *et al*., 2008). Furthermore, the influence of zeolites incorporated into litter occurred through its ability to adsorb ions and absorb water (Schneider *et al*., 2017). In contrast, clinoptilolite zeolite at both concentrations used 10.0 and 20.0 g/kg of wood shaving litter in this study induced a significant decrease in the total viable bacterial count and some bacterial isolates during the 3rd and 4th week of duck age in each examined group such as *E. coli* and *Salmonella* spp. Whilst, Al-Nasser *et al*., (2011) showed that addition of zeolite at 2% in the broiler diet led to reducing of more than 50 % *Salmonella* count in the study group compared to the control one. WU *et al*., (2013) showed a decrease of total viable counts of *E. coli* with clinoptilolite supplementation 2.0% from one day to 21 days. When the pH of litter and moisture content reduced, a remarkable reduction of TVC, *E. coli* and *Salmonella* spp. was observed (Line and Bailey, 2006; Sahoo *et al*., 2017).

Conclusion

Control of environmental conditions during ducks rearing is a key factor for improving and maintaining duck's health. Ducks' litter amendment using clinoptilolite zeolite at a concentration of 10.0 and 20.0 g/kg of wood shaving litter is more effective to alleviate air gas emission including NH3, CO2, and absence of H2S gas in-door of duck pens. Furthermore, litter moisture content and pH are reduced and consequently affect the TVC and microbial load of ducks' litter.

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Conflict of interest

The authors declare that they have no competing interests.

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