Original Article

Effects of Effective Microorganisms on Growth Performances, Ammonia Reduction, Hematological Changes and Shedding of *Salmonella enterica* and *Campylobacter* spp. in Broilers

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Abstract

The aim of this study was to determine the effects of effective microorganisms (EM) product on ammonia reduction, growth performances, *Salmonella* and *Campylobacter* reduction and hematological changes in broilers. Five hundred and ninety four day-old female Arbor Acres were divided into 3 groups with 6 replicates in each group. Group 1 was the control group, while groups 2 and 3 received EM product in forms of daily spraying at 1:50 EM solution/m^2 on litter and in drinking water at 1:800 dilution, respectively. Ammonia concentrations were measured weekly, while blood samples were collected at 21 and 42 days of age for hematological evaluation. Growth performances were determined at the end of the trial. In addition, fecal samples were collected weekly and cultured for *Salmonella enterica* and *Campylobacter*. It was found that the ammonia concentrations were significantly decreased (p<0.05) between the EM spraying group and the control group at 42 days of age. No significant differences in the growth performances and most of the hematological parameters were found at the end of the trial. However, stress indicator determined by heterophil and lymphocyte (H/L) ratio was significantly decreased (p<0.05) by the end of the trial in the EM spraying group. Although the EM product tended to have a beneficial effect in reducing ammonia in poultry houses and broilers' stress, the effect of this product on the reduction of *Salmonella enterica* and *Campylobacter* spp. at the farm level was not clearly noticed.

Keywords: ammonia, broilers, *Campylobacter*, effective microorganisms, growth performances, *Salmonella*

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Introduction

Malodor, which is mainly caused by ammonia, is one of the major pollutants on poultry farms. Ammonia is usually formed through the microbial breakdown of uric acid, unused feed nitrogen excreted by birds. There have been several reports showing how ammonia affects birds' health and performance (Miles et al., 2002). Suppressing ammonia production can be beneficial to animal health and, as a result, can enhance the growth of animals (Chang and Chen, 2003).

Effective microorganisms (EM) are mixed cultures of beneficial microorganisms that are not genetically modified or harmful to humans and animals. Generally, EM is composed of a diverse group of bacteria, yeasts and fungi including *Lactobacilli*, *Bifidobacteria*, *Pediococcus*, *Bacillus*, moulds, yeasts, *Actinomyces, Bacteroides* and other undefined organisms (Patterson and Burkholder, 2003). After antibiotics as a growth promoter are prohibited in several countries, EM products are used as the alternatives in animal husbandry and organic livestock farming to improve growth performance of animals and control odor and waste produced on farms (Kalavathy et al., 2003). It has been shown that some EM products can reduce ammonia concentrations in chicken houses by 42.12% and 54.25% when used in drinking water and fermented feed, respectively (Yongzhen and Weijiong, 1994). Additionally, Chiang and Hsieh (1995) have also reported that feeding an EM supplemented diet containing *L. acidophilus*, *S. faecium* and *B. subtilis* can noticeably reduce the concentration of ammonia in the excreta of broilers. Although the effects of EM on the growth performance of broilers and the ammonia reduction in poultry houses have been reported, none of these studies has revealed the effects of EM on the changes of hematological profiles of broilers, which can indicate stress level of the birds. Gross and Seigel (1983) proposed that the heterophil and lymphocyte ratio (H/L ratio) could be used as an indicator of the stress level of chickens specifically stress due to surrounding environment such as an increased level of ammonia in poultry house.

Among the variety of species of beneficial microorganisms, a combination of *L. acidophilus* and *S. faecium* showed the ability to reduce colonization and fecal shedding of foodborne pathogens in broiler chickens (Morishita et al., 1997). *Salmonella* and *Campylobacter* are the two most important causes of foodborne disease in many countries worldwide. These organisms are commonly found in the intestinal tract of poultry and usually contaminate carcasses during processing (Hue et al., 2011). The control and prevention of *Salmonella* and *Campylobacter* species at the farm level is considered to be a main factor of the reduction of *Salmonella* and *Campylobacter* in poultry carcasses. Since it is unlikely that the control and prevention of flock exposure to *Salmonella enterica* and *Campylobacter* spp. can be accomplished by using strict biosecurity measures alone (Newell and Davison, 2003), other supportive measures such as vaccination or the use of competitive exclusion, probiotics, prebiotics or EM seem to be necessary. over the years, a number of studies have shown that competitive exclusion, probiotics and prebiotics can reduce the colonization and shedding of *Salmonella* and *Campylobacter* (Patterson and Burkholder, 2003; Zhang et al., 2007; Willis and Reid, 2008). However, no studies revealed the efficacy of EM on the reduction of these foodborne pathogens. Therefore, the purpose of this study was to determine the effects of EM product on the reduction of fecal shedding of *Salmonella enterica* and *Campylobacter* spp. Moreover, the effects of EM on ammonia reduction, growth performance and hematological changes in broilers were also investigated.

Materials and Methods

***Birds and environment*:** The experiment was performed at the University research farm located in Nakhon Pathom province. Five hundred and ninety four day-old female Arbor Acres were divided into 3 groups in a random manner. Each group consisted of 6 replicates with 33 birds per replicate. Group 1 was the control group, while groups 2 and 3 received a commercial EM product from 22 to 42 days of age in different forms. The EM product used in this study contained 4 types of viable organisms including lactic acid bacteria ($5.0x10^8$ cfu/ml), yeasts ($5.3x10^5$ cfu/ml), *Bacillus* spp. ($4.3x10^5$ cfu/ml) and *Actinomycetes* ($2.8x10^2$ cfu/ml). Group 2 was sprayed daily (11:00 to 12:00) with 1:50 EM solution/m^2 at a particle size of 150-200 μm on the top of the litter. Chicks in group 3 received EM in drinking water at 1:800 dilution. All groups were raised under the same conditions with a density of 7.5 birds/m^2. Clean water was given to the chicks throughout the rearing period. All chicks were fed ad-libitum with commercial broiler feed and vaccinated with Newcastle disease plus Infectious Bronchitis disease vaccine (sprayed at the hatchery and eye dropped at 9 days of age) and Infectious Bursal disease vaccine (dissolved in drinking water at 14 days of age). Temperature and relative humidity were recorded daily until the end of the experiment. Guidelines and legislative regulations on the use of animals for scientific purpose were certified by the Chulalongkorn University Animal Care and Use Committee (permission No. 0731039).

Determination of efficacy of EM on growth performances and hematological parameters of broilers: The growth performances, i.e. body weight (BW) gain, average daily gain (ADG), feed conversion ratio (FCR), mortality rate and the European Broiler Index (EBI) of the control and the two treatment groups were measured and compared at the end of the trial. EBI was calculated by ADG (g) x survival (%) /10 x FCR (Lückstädt et al., 2004). At 21 and 42 days of age, blood from 2 birds per replicate was collected by venipuncture and sent to the laboratory to determine total red blood cell count (RBC count), total white blood cell count (WBC count) and percentage of hemoglobin (Hb) and hematocrit (Hct). In addition, the ratio of absolute numbers of heterophil and lymphocyte (H/L ratio), which indicates stress factor, was also determined.

Determination of the efficacy of EM on ammonia reduction: Ammonia was measured using an ammonia detector (BW Technologies, Canada). The measurement was performed in the center spot of each replicate at a level of 8 to 10 cm over the litter surface. Ammonia levels were determined in the morning (08:00 to 09:00), afternoon (13:00 to 14:00) and evening (16:00 to 17:00) of each day for two weeks to check for any fluctuation. After that, the concentrations of ammonia were measured twice a day (08:00 to 09:00 and 15:00 to 16:00) when the birds were 14, 21, 28, 35 and 42 days of age.

Determination of the efficacy of EM on fecal shedding of Salmonella enterica and Campylobacter spp.: To determine the efficiency of EM on fecal shedding of *Salmonella* and *Campylobacter*, 9 replicates of broiler chickens (3 replicates from each group) were challenged with *Salmonella* Typhimurium and *Campylobacter jejuni* at a final concentration of 5 x 10^6 CFU per bird when the birds were 3 days old. The other 9 replicates that were not challenged with *Salmonella* or *Campylobacter* represented naturally infected birds. Three fecal samples from each replicate were collected weekly and cultured for *Salmonella enterica* and *Campylobacter* spp. In addition, fecal samples from 6 replicates of the challenged birds (2 replicates per group) and 6 replicates of the naturally infected birds (2 replicates per group) were obtained when the birds were 28, 35 and 42 days of age to compare the number of *Campylobacter* spp. shed in the feces between the control and the EM-treated groups.

Salmonella isolation and identification: One gram of fecal sample was added to 9 ml of buffered peptone water (BPW) and then incubated at 37°C for 24 h. After incubation, 0.1 ml of BPW was inoculated onto modified semi-solid Rappaport-Vassiliadis (MSRV) medium and incubated at 42°C for another 24 h. After the enrichment process, a loopful of culture from the MSRV medium was streaked onto xylose lysine deoxycholate (XLD) agar and triple sugar iron (TSI) agar slant and then incubated at 37°C for 24 h. The identification of *Salmonella* was based on colony morphology on the XLD agar as well as the biochemical characteristics in TSI agar slant and lysine indole motility (LIM) medium.

Campylobacter isolation, identification, and enumeration: One gram of fecal sample was added to 9 ml of 0.85% normal saline. For isolation, the suspension was directly streaked onto modified charcoal-cefoperazone-deoxycholate (mCCD) agar. Additionally, this suspension was also serially diluted and 0.1 ml of each diluted suspension was spread onto mCCD agar in duplicate for enumeration purpose. The inoculated plates were incubated at 42°C for 48 h under microaerophilic conditions. Suspect *Campylobacter* colonies were identified by colony morphology and biochemical characteristics. In addition, the polymerase chain reaction (PCR) method was performed to confirm the biochemical results.

Statistical analyses: The data were statistically analyzed using One-Way Analysis of Variance (ANOVA). When the means of each group were different, the determination of the difference was made using the Duncan's new multiple range test (DMRT). In addition, the hematological changes before and after the trial were compared using a student paired t-test, whereas the significant differences in the fecal shedding of *Salmonella enterica* and *Campylobacter* spp. between the control and treatment groups were determined by a Fisher's exact test at a significance level of $p<0.05$.

Results

Table 1 shows the environmental conditions and ammonia concentrations at different ages. Ammonia concentrations in every group tended to slightly increase from 14 to 35 days of age. At the end of the experiment (42 days of age), the control group had the highest ammonia concentration, followed by group 3 (EM in drinking water) and group 2 (EM in spray form), respectively. Group 2 showed a significant ammonia reduction ($p<0.05$) compared to the control group, while no significant differences in the ammonia reduction were observed between group 3 and the other groups. The temperature and relative humidity were not significantly different among the groups during the experimental period. The average ranges of temperature and relative humidity were 26.57-28.50°C and 59.46-78.93%, respectively.

At the end of experiment, there were no significant differences among the groups for all growth performances (*i.e.* body weight gain, mortality rate, feed consumption, ADG, FCR and EBI) (Table 2).

The hematological parameters of broilers before and after the experiment (21 and 42 days of age, respectively) are demonstrated in Table 3. There are no significant differences in hematological parameters among the groups at both ages except the RBC count between group 1 and group 2 ($p<0.05$) at 42 days of age. However, when the hematological parameters of each individual group at 21 days of age were compared to those at 42 days of age, it was found that the hemoglobin and hematocrit increased significantly ($p<0.05$) in all groups, whereas a significant difference ($p<0.05$) in the H/L ratio between both periods was only observed in group 2.

No statistically significant difference in the fecal shedding of *Salmonella enterica* and *Campylobacter* spp. was observed between the control and treatment groups (Table 4). Likewise, the number of *Campylobacter* spp. shed in the feces of broiler chickens receiving EM product in both forms was not significantly different from those of the control group (Table 5).

Table 1 Environmental conditions and ammonia concentrations at different ages[1]

Age (days)	Temperature (°C)	Relative Humidity (%)	Ammonia Concentrations (ppm)		
			Group 1	Group 2	Group 3
14	26.57 ± 1.21	78.93 ± 5.03	6.58 ± 0.42	6.42 ± 0.55	6.00 ± 0.37
21	28.50 ± 0.76	62.46 ± 2.28	9.25 ± 0.34	8.75 ± 0.60	8.33 ± 0.42
28	28.25 ± 1.07	64.14 ± 6.31	10.94 ± 1.31	8.17 ± 1.44	9.61 ± 1.47
35	26.64 ± 1.44	67.14 ± 8.94	11.83 ± 2.64	9.17 ± 2.14	9.42 ± 2.29
42	26.86 ± 0.50	59.46 ± 7.62	18.67 ± 1.63[a]	15.33 ± 1.37[b]	16.83 ± 1.94[ab]

[1] Each data entry represents mean ± standard error.
[a-b] Means within a row with different superscripts are significantly different (p<0.05).

Table 2 Growth performances of broilers at the end of the experiment (42 days of age)[1]

Performance[2]	Group 1	Group 2	Group 3
Mortality rate (%)	6.06 ± 1.92	6.06 ± 2.59	10.10 ± 2.43
Body weight gain (kg/bird)	2.32 ± 0.05	2.32 ± 0.06	2.49 ± 0.10
Feed consumption (kg/bird)	4.02 ± 0.08	4.00 ± 0.08	4.19 ± 0.11
ADG (g/bird/day)	55.33 ± 1.22	55.25 ± 1.49	59.28 ± 2.44
FCR	1.73 ± 0.02	1.73 ± 0.01	1.69 ± 0.03
EBI	300.05 ± 5.76	299.62 ± 2.60	314.84 ± 10.90

[1] Each data entry represents mean ± standard error. The number of broilers at the beginning of the experiment was 198 birds/group, whereas the number of broilers at the end of the experiment was different among groups (186 birds in groups 1 and 2 and 178 birds in group 3).
[2] ADG, average daily gain; FCR, feed conversion ratio; EBI, European broiler index

Table 3 Hematological parameters of broilers at 21 and 42 days of age[1]

Parameter[2]	21 days of age			42 days of age		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
RBC counts(x 10^6 cell/µl)	2.48 ± 0.10	2.50 ± 0.09	2.57 ± 0.09	2.25 ± 0.08	2.51 ± 0.06	2.39 ± 0.06
Hemoglobin(g/dl)	8.51 ± 0.22[a]	8.84 ± 0.19[a]	8.28 ± 0.17[a]	9.74 ± 0.31[b]	10.12 ± 0.21[b]	9.87 ± 0.24[b]
Hematocrit (%)	26.94 ± 0.66[a]	26.82 ± 0.50[a]	27.23 ± 0.60[a]	28.88 ± 0.89[b]	31.70 ± 0.68[b]	30.18 ± 0.81[b]
WBC counts(x 10^3 cell/µl)	2.27 ± 0.20	2.18 ± 0.19	2.15 ± 0.21	2.36 ± 0.43	2.09 ± 0.30	2.15 ± 0.41
Differential counts						
Heterophil (H)(x 10^3cell/µl)	0.96 ± 0.09	0.93 ± 0.10	0.90 ± 0.12	1.06 ± 0.29	0.68 ± 0.14	0.70 ± 0.13
Lymphocyte (L)(x10^3cell/µl)	1.11 ± 0.12	1.01 ± 0.10	1.02 ± 0.09	1.17 ± 0.24	1.14 ± 0.15	1.24 ± 0.31
H/L ratio	0.94 ± 0.07	0.98 ± 0.10[a]	0.90 ± 0.09	0.99 ± 0.13	0.64 ± 0.09[b]	0.80 ± 0.13

[1] Each data entry represents mean ±standard error.
[2] RBC, red blood cell; WBC, white blood cell; H/L ratio, heterophil and lymphocyte ratio
[a-b] Means within a row with different superscripts are significantly different (p<0.05).

Table 4 Fecal shedding of *Salmonella enterica* and *Campylobacter* spp.at different ages

Age (days)	Infection status[1]	No. of positive samples/no. of samples tested (%)					
		Group 1		Group 2		Group 3	
		Salmonella	*Campylobacter*	*Salmonella*	*Campylobacter*	*Salmonella*	*Campylobacter*
28	NI	2/9 (22.22)	9/9 (100.00)	4/9 (44.44)	9/9 (100.00)	2/9 (22.22)	9/9 (100.00)
	C	9/9 (100.00)	9/9 (100.00)	8/9 (88.89)	9/9 (100.00)	9/9 (100.00)	9/9 (100.00)
35	NI	3/9 (33.33)	9/9 (100.00)	4/9 (44.44)	9/9 (100.00)	3/9 (33.33)	9/9 (100.00)
	C	6/9 (66.67)	9/9 (100.00)	8/9 (88.89)	9/9 (100.00)	9/9 (100.00)	9/9 (100.00)
42	NI	9/9 (100.00)	8/9 (88.89)	6/9 (66.67)	7/9 (77.78)	8/9 (88.89)	8/9 (88.89)
	C	9/9 (100.00)	7/9 (77.78)	9/9 (100.00)	9/9 (100.00)	8/9 (88.89)	8/9 (88.89)
Total		38/54 (70.37)	51/54 (94.44)	39/54 (72.22)	52/54 (96.30)	39/54 (72.22)	52/54 (96.30)

[1] NI: naturally infected birds, C: challenged birds

Table 5 Number of *Campylobacter* spp. shed by broilers at different ages

Age (days)	Infection status	Average number of *Campylobacter* spp. in feces (log CFU/g)		
		Group 1	Group 2	Group 3
28	Naturally infected	3.93	3.91	4.09
	Challenged	4.41	4.03	4.45
35	Naturally infected	3.08	3.41	3.02
	Challenged	4.05	3.99	3.62
42	Naturally infected	3.78	3.62	3.70
	Challenged	4.16	4.72	4.12

Discussion

The results show that ammonia concentrations in all groups gradually increased from 14 to 35 days of age. After that, the ammonia volatilization (*i.e.* ammonium nitrogen being converted to dissolve ammonia gas) increased substantially especially in the final week of the experiment. This finding is likely the result of the considerable growth of broilers after 35 days old, which led to correspondingly increased excreta. The ammonia concentration at the end of the experiment (42 days of age) in group 2 (EM spraying group) and group 3 (EM in drinking water) was lower than that of the control group. This result is in accordance with the study in Aichi Prefecture, which revealed that the addition of EM in drinking water or feed or use in spraying form had positive effects on foul odor (mainly caused by ammonia) reduction in laying houses (SCD probiotics, 2011). EM could eliminate ammonia emission by dominating the microbial ecology with organisms that manipulate a fermentative pathway. It is possible that *Lactobacillus* spp., one of the key components of EM used in this study, might help improve nitrogen retention (Angel et al., 2005) and nitrogen utilization (Yongzhen and Weijiong, 1994).

The ammonia concentrations found in this study (less than 20 ppm) were lower than the recommended limit for chronic exposure in poultry houses (25 ppm) (MAFF, 1987). The low level of ammonia in our study was likely due to the low density of broilers and good ventilation in the house. In addition, the temperatures during the study period were not so high as to influence ammonia volatilization. Furthermore, the great thickness of litter used in this study (approximately 4 inches) well absorbed the moisture and ammonia emission from the litter content.

Broiler performances were not significantly different among the groups. All performances in group 2, which was sprayed with EM, were similar to those in the control group, while the feed consumption, BW, ADG, FCR and EBI in group 3 (EM in drinking water) seemed to be better than those in the other groups. The average BW gain (2.49±0.10 kg) and the FCR (1.69±0.03) of the birds in group 3 were also better than those of the Arbor Acres standard performance at 42 days of age (2.42 kg for BW gain and 1.82 for FCR). This finding is consistent with a previous study conducted in Pakistan in which EM in drinking water helped improve the BW gain of broilers (Hussain et al., 1995). Likewise, the EBI of the broilers in group 3 (314.84±10.90) was also higher than that of the control group (300.05±5.76). The above mentioned results suggest that ingestion of EM could enhance broiler performances since only the broilers in group 3 directly consumed EM (via drinking water). The result is in agreement with that of Simeamelak et al. (2012) who reported that EM supplementary administration of drinking water improved growth performance of Rhode Island Red (RIR) chicks. It is proposed that EM may act in the intestinal tract of the birds by (i) maintaining a beneficial microbial population in the alimentary tract (Fuller, 1989), (ii) increasing nutrient utilization through improved intestinal health resulting in greater intestinal enzyme activities and nutrient availability (Nahashon et al., 1994), (iii) altering bacterial metabolism (Cole et al., 1987) and (iv) competing with pathogenic microflora in the digestive tract (Wood and Abuchar, 1998). The better performance of broilers receiving EM in drinking water observed in this study is comparable to the results of earlier studies on the beneficial effects of probiotics on the growth performances of broilers (Huang et al., 2004; Murry et al., 2006; Vicente et al., 2007). Although the overall performances seemed to be better in the group of broilers receiving EM in drinking water, it should be noted that the mortality rate of this particular group at 42 days of age (10.10±2.43%) was higher than that of the other groups.

There are no significant differences in the hematological parameters among the groups at both ages except the RBC count between group 1 and group 2 at 42 days of age. This might be partly due to the fact that ammonia concentrations in the present study did not reach a harmful level at 25 ppm. Since ammonia levels in this study were in the acceptable range, suggesting that the broilers did not face any substantial stress conditions, it is not surprising that no significant changes in the H/L ratio were observed among the groups. Gross and Siegel (1983) reported that the H/L ratio appeared to increase in response to the chicken's perception of environmental stress. In this study, the H/L ratio of the control group seemed to increase, while the H/L ratio of both treatment groups tended to decrease by the end of the trial. Since the most significant reduction of the H/L ratio was observed in group 2 (EM spraying group), this result suggests that the spraying of EM on the litter could directly hinder the reaction of ammonia volatilization and thus reduce the birds' stress, which led to a significant decrease in the H/L ratio by the end of trial when compared to the value at the beginning of trial.

No effect of EM on the fecal shedding of *Salmonella enterica* and *Campylobacter* spp. was observed in this study. Generally, beneficial bacteria such as competitive exclusions, probiotics or effective microorganisms should be given to animals at an early age so that these bacteria can become established in the intestinal tract and become competitive or antagonistic to opportunistic pathogens (Doyle and Erickson, 2006). In poultry, these beneficial bacteria are usually introduced to the chicks after hatching or on the first day of life (Wagner, 2006). Since the birds in this study first received EM after they had been raised on the farm for 3 weeks, which *Salmonella* and *Campylobacter* had already colonized the intestinal tract of the birds, it is not surprising that the effect of EM on the reduction of the fecal shedding of *Salmonella* and *Campylobacter* was not observed in the present study. However, if EM product had been given to the birds at the first day of life or before they were colonized with pathogenic microorganisms, a significant reduction of the fecal shedding of *Salmonella enterica* and *Campylobacter* spp. between the control and treatment groups may have been found. This hypothesis will be investigated in a future study.

In conclusion, the use of EM product especially by direct spraying on the litter could reduce ammonia emission and decrease the stress of birds, whereas the use of EM in drinking water was able to promote broiler performances to some extent. Further experiment should be performed under intensive or more stressful conditions in order to determine whether similar results of EM on ammonia emission, growth performances and hematological changes will be observed or not. Additionally, more research should be conducted to achieve a solid conclusion on the effects of EM on the reduction of fecal shedding of foodborne pathogens in poultry production.

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บทคัดย่อ

ผลของจุลินทรีย์อีเอ็มต่อสมรรถภาพการเจริญเติบโต การลดก๊าซแอมโมเนีย การเปลี่ยนแปลงค่าทางโลหิตวิทยาและการขับออกของเชื้อซัลโมเนลลา เอนเทอริกา และเชื้อแคมไพโลแบคเตอร์ในไก่เนื้อ

จักรกริศน์ เนื่องจำนงค์[1*] ธราดล เหลืองทองคำ[2]

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของผลิตภัณฑ์จุลินทรีย์อีเอ็มต่อสมรรถภาพการเจริญเติบโต การลดก๊าซแอมโมเนีย การเปลี่ยนแปลงค่าทางโลหิตวิทยา และการขับออกของเชื้อซัลโมเนลลา เอนเทอริกา และเชื้อแคมไพโลแบคเตอร์ในไก่เนื้อ แบ่งไก่เนื้อเพศเมียพันธุ์อาร์เบอร์เอเคอร์ จำนวน 594 ตัว ออกเป็น 3 กลุ่ม แต่ละกลุ่มประกอบด้วย 6 ซ้ำ กลุ่มที่ 1 กลุ่มควบคุม กลุ่มที่ 2 และ 3 ได้รับผลิตภัณฑ์จุลินทรีย์อีเอ็มในรูปแบบของการพ่นเป็นละอองลงบนวัสดุรองพื้นคอก ในอัตราส่วนของสารละลาย 1:50 ต่อตารางเมตร และในรูปแบบของการละลายน้ำดื่มในอัตราส่วน 1:800 ตามลำดับ ทำการวัดความเข้มข้นของก๊าซแอมโมเนียทุกสัปดาห์ เก็บตัวอย่างเลือดเพื่อตรวจค่าทางโลหิตวิทยาที่อายุ 21 และ 42 วัน เมื่อสิ้นสุดการทดลองทำการวัดสมรรถภาพการเจริญเติบโต นอกจากนี้ทำการเก็บตัวอย่างอุจจาระแต่ละสัปดาห์เพื่อวิเคราะห์การขับออกของเชื้อซัลโมเนลลา เอนเทอริกา และเชื้อแคมไพโลแบคเตอร์ การศึกษาพบว่า มีการลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ของความเข้มข้นของก๊าซแอมโมเนียในกลุ่มที่ได้รับจุลินทรีย์อีเอ็มในรูปแบบของการพ่นเป็นละอองลงบนวัสดุรองพื้นคอกเทียบกับกลุ่มควบคุม แต่ไม่พบความแตกต่างสำหรับทุกกลุ่มการทดลองในด้านสมรรถภาพการเจริญเติบโตและส่วนใหญ่ของค่าทางโลหิตวิทยา อย่างไรก็ตามตัวบ่งชี้ความเครียดซึ่งวัดในรูปของอัตราส่วนระหว่างเฮทเทอโรฟิลและลิมโฟไซต์ (H/L) มีการลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) เมื่อสิ้นสุดการทดลองในกลุ่มที่ได้รับการพ่นจุลินทรีย์อีเอ็มลงบนวัสดุรองพื้นคอก แม้ว่าผลิตภัณฑ์จุลินทรีย์อีเอ็มมีแนวโน้มที่ดีทั้งต่อการลดระดับความเข้มข้นของก๊าซแอมโมเนียในโรงเรือนเลี้ยงไก่และความเครียดของไก่ แต่ผลิตภัณฑ์นี้ไม่มีผลอย่างเด่นชัดต่อการลดการขับออกของเชื้อซัลโมเนลลา เอนเทอริกา และเชื้อแคมไพโลแบคเตอร์ในระดับฟาร์ม

คำสำคัญ: ก๊าซแอมโมเนีย ไก่เนื้อ แคมไพโลแบคเตอร์ จุลินทรีย์อีเอ็ม สมรรถภาพการเจริญเติบโต ซัลโมเนลลา

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