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Research Article

Decrease Air Biological Contamination by Improving the Litter Characteristics in Broiler Barns During Winter

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Abstract

The study was conducted in the Lattakia Governorate, Syria. The data was collected during the winter months from December 2022 to January 2023. In this experiment, 3,000 birds of the Roos hybrid were randomly assigned to four groups, each comprising 750 birds. The experiment was conducted using a completed randomized design. The objective of this experiment was to ascertain the impact of three distinct ratios of Syrian natural zeolite (Tz_1, Tz_2, Tz_3) on the bacterial contamination of the air in broiler barns. The results demonstrated that at the sixth week of the fattening period, the bacterial concentrations in the Tz_3 treatment were 7.9 x 105 and 9.5 x 10^3 CFU/m^3 for Staphylococcus spp. and Escherichia coli, respectively. The results demonstrated that incorporating Syrian natural zeolite into broiler litter resulted in a reduction of airborne bacteria for both Staphylococcus spp. and Escherichia coli, with a more pronounced effect observed in the Tz_3 treatment within the interior airspace. This study presents a novel approach for the mitigation of airborne microorganisms within broiler barns.

Keywords: *Air Bacteria; Broiler; Gram-Positive Bacteria; Gram-Negative Bacteria; Natural Zeolite.*

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1. Introduction

The atmosphere within broiler barns contains a considerable quantity of diverse pollutants, exceeding the permitted limits and resulting in unnatural conditions. These pollutants are collectively referred to as air-polluting compounds. Furthermore, they pose a significant risk of contamination with microorganisms, a phenomenon known as bio-pollution. The prevalence of detrimental illnesses is heightened when broiler farms are subjected to semi-closed systems. The microorganisms in question represent a significant hazard, with the potential to cause harm to both birds and humans. The dissemination of these microorganisms can have a detrimental impact on both health and production, particularly when coupled with the presence of volatile organic dust and the pathogens it carries (Saleh, 2018). Furthermore, the arrival of these elements in the surrounding environment air, via the ventilation system, presents an additional risk of transmission to the external environment (Plewa & Lonc, 2011; Hartung & Schulz, 2008).

Broiler litter is regarded as a significant source of biological contamination. This is due to its role as a container for birds' manure, as well as the impact of various factors, including the quality of the litter used, its management, temperature, acidity and humidity. These factors have been identified as key contributors to the increased concentration of bacteria in the air of broiler barns (Witkowska & Sowińska, 2017; Mihina et al., 2012). Furthermore, bio-pollution is influenced by the relative moisture and temperature of the air, as well as the season of the year (Xin et al., 2011). In addition to the density of the birds, the age of the birds, the ventilation rates and the amount of dust, feed and feces emitted by the birds, there is the issue of the combined effect of these factors on the health risks associated with the release of such materials into the external environment (Redding, 2013; Calvet et al., 2011).

In recent years, there has been a notable increase in interest in utilising the natural zeolite mineral by incorporating it into the broiler litter. This is with the aim of identifying the ammonia (NH_3) gas concentrations (Jilenkerian et al., 2023) and the humidity of the broiler litter (Jilenkerian et al., 2021a). The aforementioned studies have proposed methodologies for the regulation of air and litter quality in broiler farms, employing alternative natural materials for the safe incorporation into broiler litter. The term 'zeolite' was first coined by the Swedish mineralogist Axel Fredrik Cronstedt in 1756 (Mumpton, 1999). Natural zeolite has the potential to act as an effective acidifier and mitigator of ammonia (NH3) gas pollution in broiler farms. Furthermore, it is economically viable and environmentally benign, in addition to exhibiting favorable litter moisture absorption characteristics. These findings attracted the interest of researchers, who sought to utilise zeolite in broiler litter (Baerlocher et al., 2007). Furthermore, there is a paucity of experimental data on airborne biological contamination in broiler barns. Of the studies that have been conducted, none have included the addition of natural zeolite (Sanz et al., 2021; Saleh, 2018; Plewa & Lonc, 2011; Abdelaziz & Nisafi, 2009). A study was recently conducted in the Lattakia Governorate, Syria, during the spring and autumn seasons (Jilenkerian et al., 2022) to assess the efficacy of using Syrian natural zeolite in the litter as a means of reducing air bio-pollution. Given the dearth of studies examining the impact of natural zeolite on airborne bioaerosols in broiler barns during the winter season, this investigation represents a pioneering effort on a global scale. It aims to identify novel strategies for mitigating elevated microbial concentrations in the air through the utilization of a safe alternative material capable of absorbing moisture from broiler litter.

2. Methods

2.1. Experimental Site:

The experiment was conducted at a private broiler farm situated in the northern region of Lattakia during the winter seasons (December and January) of the 2022-2023 academic years, as illustrated in **Figure 1**. Atmospheric air temperatures ranged between +15°C and +18°C, with internal temperatures in the broiler house varying from 22°C to 33°C. The indoor relative humidity was observed to be within the range of 72 to 80%, while the outdoor relative humidity exhibited a variation between 65 to 78%.

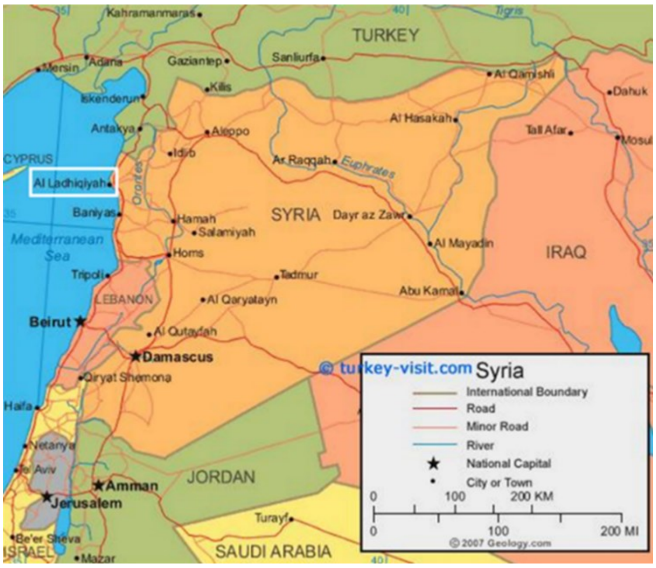

Figure 1. The Location of Broiler Farm Located in The North of Lattakia Syria.

2.2. Design of the Experiment:

In this study, the Syrian zeolite (**Figure 2**) was incorporated into the litter for the three treatments, with the following ratios: The following ratios were employed: Tz_1 (25%) (75:25), Tz_2 (50%) (50:50), and Tz_3 (75%) (25:75). In addition to the control treatment, Tz_0 (0%) was included. The source of the Syrian zeolite is the Al-Sis area of Tell Mkehelat, situated 170 km to the southeast of Damascus. The mineralogical composition of the zeolite is as follows: quartz, calcite, olivine, and two types of zeolites, namely Phillip sites $(Ca, Na2, K2)_3Al_6Si_{10}O_{32}\cdot 12H_2O$ and Analcime NaAlSi2O6·H2O (Hatem et al., 2017). The Syrian natural zeolite has a surface area of 76.5 m^2/g and a granule size ranging from 3 to 4 mm (Salameh et al., 2022). The powder X-ray diffraction (XRD) pattern is presented in **Figure 3** (Hatem et al., 2018). The general chemical composition (Al-Sis site) is presented in **Table 1** as a weight percentage. (Al-Safarjalani et al., 2010).

Table 1. The General Chemical Composition of Syrian Natural Zeolite, which was Added in The Broiler Litter.

Components	Si_2O	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	TiO	P_2O_5	Na_2O	K_2O	H_2O+CO_2
% wt.	38.26	10.2	10.86	0.14	9.90	11.94	1.78	0.56	2.44	1.03	12.8

Figure 2. Syrian Natural Zeolite.

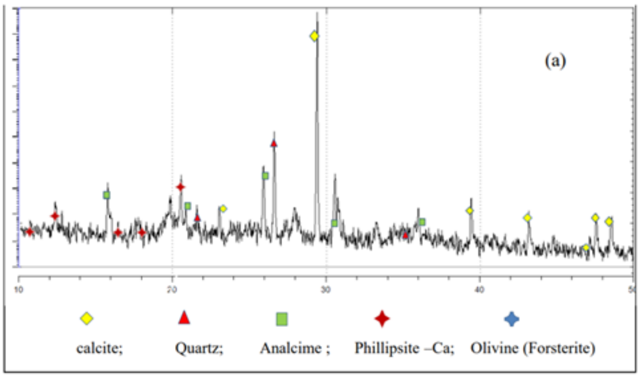

Figure 3. XRD Pattern of Syrian Natural Zeolite Ore.

A total of three thousand one-day-old hybrid birds (Ross) were randomly assigned to one of four groups, with each group comprising 750 birds. The concentrations of bacteria in the air were measured at six-week intervals until the conclusion of the experiment in each treatment group. The airborne bacteria were precipitated on Mannitol Salt Agar (MSA) and MacConkey Agar (McC). The data was recorded on the Petri dishes and subsequently stored in a portable thermos before being transferred to the poultry laboratory for the requisite analyses. Subsequently, the Petri dishes were incubated for a period of 24 to 48 hours, after which the bacterial colonies were counted using a mechanical optical counter. Subsequently, a Gram staining procedure was conducted in order to facilitate the differentiation of the bacterial groups. The number of airborne bacteria was calculated using the Omeliansky equation (Polyakov, 1986).

2.3. Statistical Analysis:

The experiment was conducted using a completely randomized design (CRD) with two factors. Each treatment was replicated three times. The data were subjected to a two-way analysis of variance (ANOVA) using the GenStat 12 statistical program. The mean values were compared according to Duncan's test, with a p-value of 0.05.

3. Results and Discussion

3.1. Classification of the Air Bacteria in Broiler Barn According to the Color Characteristics of the Colonies:

A total of 144 Petri dishes were utilized in the present study for the precipitation of airborne bacteria. The classification results demonstrated that, based on the color characteristics of the bacterial colonies' growth on the MSA medium, a bacterial group was isolated, namely, Staphylococcus spp. (yellow colonies). Conversely, on the McC medium, a single bacterial group, Escherichia coli, was isolated, characterized by pink colonies (see **Figures 4 and 5**).

Figure 4. Syrian Natural Zeolite.

Figure 5. Gram-Negative Bacteria on (McC).

3.2. Classification of the Air Bacteria in Broiler Barn According to Gram Staining and Microscopy:

The results demonstrated that the Gram staining process, employed for colony preparation on both MSA and McC media, was effective when examined under a microscope. It was observed that there were distinguishable, purple-colored Gram-positive cocci bacteria with a minute and round shape (*Monococcus, Diplococcus, Streptococcus, Staphylococcus*), and red-coloured Gram-negative bacilli bacteria (Escherichia coli), which have rounded ends and are observed to occur singly or in the form of strings (**Figures 6 and 7**).

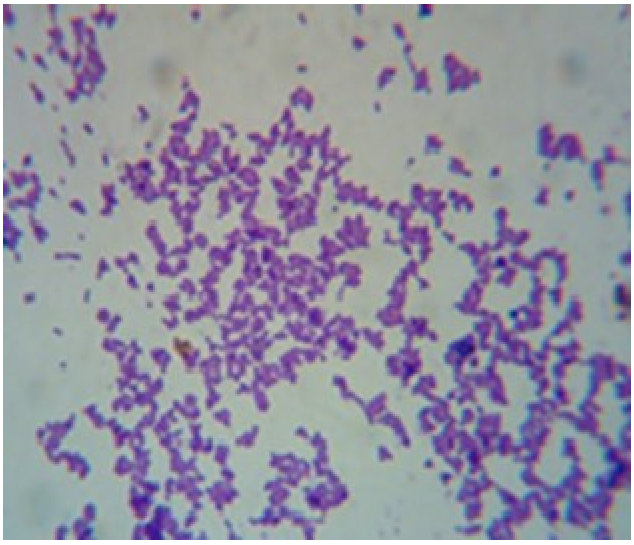

Figure 6. Gram-Positive Cocci Bacteria.

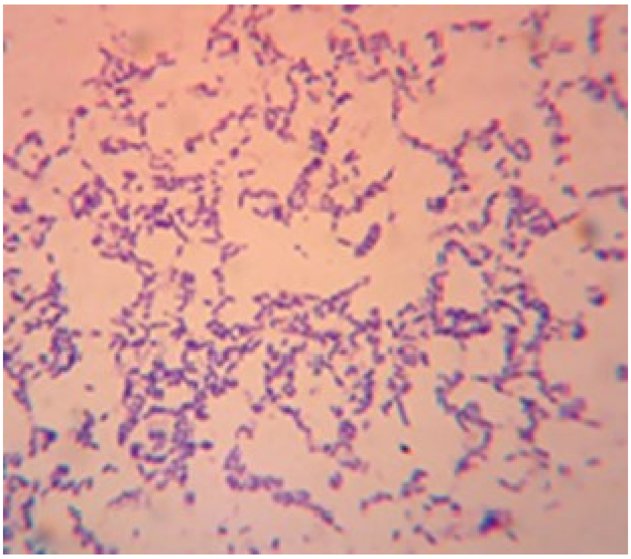

Figure 7. Gram-Negative Bacilli Bacteria.

3.3. Gram-Positive Bacteria Concentrations in the Air of Broiler Barn:

Table 2 illustrates the concentration of gram-positive bacteria in the air of a broiler barn during the winter season over a six-week period. At the outset of the experimental period, the concentration in the control treatment (Tz_0, 0%) was 3.8×10^5 CFU/m³, while the three treatments (Tz_1, 25%; Tz_2, 50%; Tz_3, 75%) exhibited concentrations of 3.5×105, 3.2×10^5 and 3.1×10^5 CFU/m³, respectively. By the sixth week, the control treatment (Tz_0) had reached 8.7×105 CFU/m³, while the other three treatments exhibited concentrations of 8.5×105 (Tz_1), 8.0×105 (Tz_2) and 7.9×105 (Tz_3) CFU/m³, as illustrated in **Figure 8**. The concentrations exhibited a notable decline from the first to the sixth week, ranging from 3.8×10^5 to 8.7×10^5 CFU/m³ in the control treatment. This decline was statistically significant ($P\leq0.05$) when compared to the three treatments. The contamination value exhibited a decline at the initial treatment stage (Tz_1, 25%), followed by a further reduction at the second treatment stage (Tz_2, 50%), and subsequently at the third treatment stage (Tz_3, 75%). These reductions were observed in comparison to the control. Therefore, it can be concluded that treatment Tz_3 demonstrated superior performance compared to the other treatments.

Table 2. Gram-Positive Bacteria Concentrations (CFU/m³) in the Air of Broiler Barn.

	Treatments			
Age (weeks)	$Tz_{0\ control}$ (0%)	Tz_1 (25%)	Tz_2 (50%)	Tz_3 (75%)
1	3.8×10^{5a}	3.5×10^{5b}	3.2×10^{5c}	3.1×10^{5d}
2	4.6×10^{5a}	4.3×10^{5b}	4.2×10^{5c}	4.0×10^{5d}
3	2.9×10^{5a}	2.8×10^{5b}	2.4×10^{5c}	2.1×10^{5d}
4	3.7×10^{5a}	3.5×10^{5b}	3.2×10^{5c}	3.0×10^{5d}
5	6.0×10^{5a}	5.9×10^{5b}	5.5×10^{5c}	5.2×10^{5d}
6	8.7×10^{5a}	8.5×10^{5a}	8.0×10^{5a}	7.9×10^{5a}
Mean	5.0×10^5	4.7×10^5	4.4×10^5	4.2×10^5
N	270	270	270	270

a,b,c,d Means in the Same Row with Significant Differences Among Averages ($P\leq0.05$).

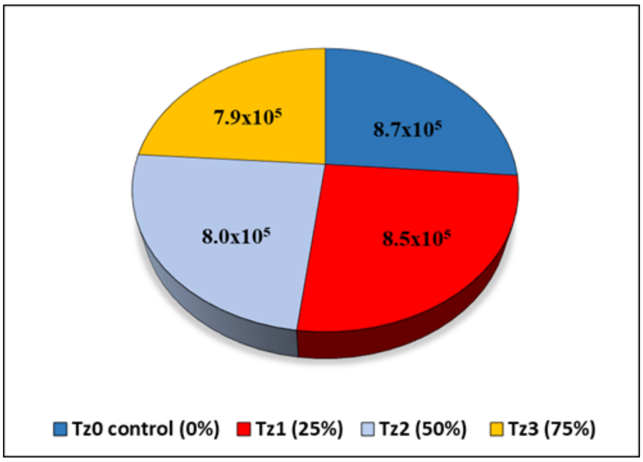

Figure 8. Gram-Positive Bacteria Concentrations (CFU/m^3) in the Air of Broiler Barn in the 6^{th} Week.

A substantial body of research has been conducted to assess the impact of air contamination in broiler farms on the health and productivity of the birds and the workers. The air within broiler barns has the potential to serve as a reservoir for pathogenic microorganisms that are involved in the etiology of infectious diseases. **Table 2** reveals that, at three weeks of age, the concentration decreased in all treatments, before increasing again at the fourth week. In closed premises during the winter season, and in circumstances where the environmental conditions are unsuitable and ventilation cannot be optimally controlled, the level of relative humidity, temperature, and moisture in the litter, in the absence of daylight, increased when water spilled from the drinkers. Furthermore, elevated temperatures of the litter and the acidity number contribute to an increase in the chemical reactions of uric acid in the birds' feces. As the litter temperature rises, microbial activity in the litter is stimulated, resulting in a shift towards an alkaline pH. These factors collectively result in the emission of the highest amounts of bio aerosols. At three weeks of age, the birds were observed to be undergoing a phase of rapid body development. During this period, all windows were opened in order to facilitate the exchange of air within the barns and to reduce the concentration of ammonia (NH_3) and carbon dioxide (CO_2) gases and microorganisms. These pollutants were observed to spread with the air to the outside of the barns, and their concentration subsequently decreased. In the fourth week, bio-pollution increased once more due to the rise in airborne bacteria concentrations associated with the birds' advancing age. As the birds grow older and heavier, they produce a greater quantity of feces, which in turn leads to an increase in the number of airborne bacterial loads. In the control treatment, the concentration of colony-forming units (CFU) per cubic metre of air was recorded at 8.7×10^5. The air of a broiler barn exhibited a high concentration of gram-positive bacteria, with the highest amounts observed during the sixth week of the fattening period. Ultimately, microbial decomposition of uric acid results in the production of ammonia gas through fermentation reactions. A number of studies have been conducted on the Syrian coast with a view to determining the potential benefits of incorporating Syrian natural zeolite into the litter. Zeolite has been demonstrated to contribute to a reduction in litter moisture levels from 61.01% to 38.38% (Jilenkerian et al., 2021a), and the acidity number ranges from 7.4 to 5.2 (Jilenkerian et al., 2021b). Furthermore, the concentrations of ammonia gas have been observed to decrease from 16.6 ppm to 12 ppm in the air of broiler farms during the winter season (Jilenkerian et al., 2023). The number of bacteria belonging to the Staphylococcus genus in indoor air was recorded in Spain at a concentration of 105 colony-forming units (CFU) per cubic metre (m^3) (Sanz et al., 2021). In Wroclaw, Poland, the concentration reached 1010 CFU/m^3 (Plewa & Lonc, 2011). In the coastal region of Syria, the concentration was recorded at 9.7×10^6 CFU/m^3 (Saleh, 2018). Additionally, in a separate study conducted along the Syrian coastline, the values reached 1.1×10^5 CFU/m^3 in the absence of zeolite (Abdelaziz & Nisafi, 2009). The discrepancy in the concentration results can be attributed to the disparate climatic and geographical conditions between the continents.

3.4. Gram-Negative Bacteria Concentrations in the Air of Broiler Barn:

Table 3 illustrates the concentration of gram-negative bacteria in the air of a broiler barn during the winter season, over a period of six weeks. At the outset of the experimental period, the concentration in the control treatment (Tz_0, 0%) was 8.9×10^2 CFU/m^3, while the concentrations in the three treatments were 8.7×10^2, 8.6×10^2, and 8.2×10^2 CFU/m^3, respectively, for Tz_1 (25%), Tz_2 (50%), and Tz_3 (75%). By the sixth week, the control treatment (Tz_0) had reached 9.9×10^3 CFU/m^3, while the other three treatments (Tz_1, Tz_2, and Tz_3) had reached 9.8×10^3, 9.6×10^3, and 9.5×10^3 CFU/m^3, respectively (**Figure 9**). The concentration exhibited a range of 8.9×10^2 CFU/m^3 to 9.9×10^3 CFU/m^3 during the first six weeks of the control treatment. A notable decline was observed in the concentrations of the three treatments when compared to the control ($P\leq0.05$). The contamination value exhibited a decline at the initial treatment (Tz_1), with a reduction of 25% to 0.1 CFU/m^3, followed by a further decrease at the second treatment (Tz_2), with a reduction of 50% to 0.3 CFU/m^3, and finally at the third treatment (Tz_3), with a reduction of 75% to 0.4 CFU/m^3. These values were compared to control. Therefore, it can be concluded that treatment Tz_3 was the most effective.

Table 3. Gram-Negative Bacteria Concentrations (CFU/m^3) in the Air of Broiler Barn.

	Treatments			
Age (weeks)	**$Tz_{0\ control}$ (0%)**	**Tz_1 (25%)**	**Tz_2 (50%)**	**Tz_3 (75%)**
1	8.9×10^{2a}	8.7×10^{2b}	8.6×10^{2c}	8.2×10^{2d}
2	1.5×10^{3a}	1.3×10^{3b}	1.1×10^{3c}	9.9×10^{2d}
3	1.8×10^{3a}	1.5×10^{3b}	1.4×10^{3c}	1.2×10^{3d}
4	7.3×10^{3a}	7.1×10^{3b}	6.9×10^{3c}	6.5×10^{3d}
5	8.9×10^{3a}	8.7×10^{3b}	8.5×10^{3c}	8.0×10^{3d}
6	9.9×10^{3a}	9.8×10^{3a}	9.6×10^{3a}	9.5×10^{3a}
Mean	5.0×10^{3}	4.9×10^{3}	4.7×10^{3}	4.5×10^{3}
N	270	270	270	270

a,b,c,d Means in the same row with significant differences among averages ($P\leq0.05$).

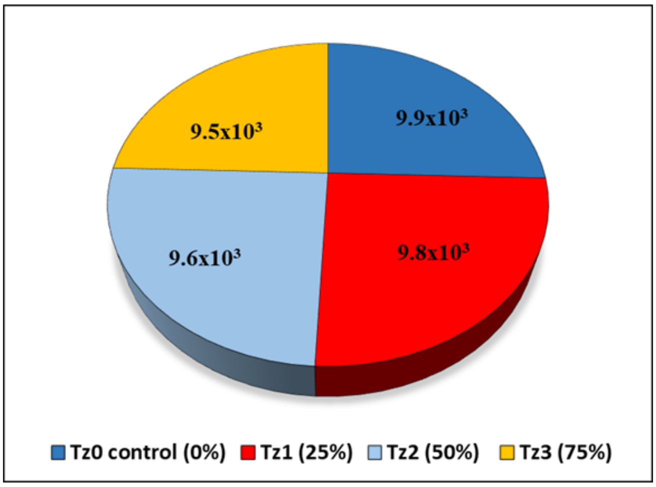

Figure 9. Gram-Negative Bacteria Concentrations (CFU/m^3) in the Air of Broiler Barn in the 6th Week.

Table 3 illustrates that at the 6th week of the study, the concentrations of gram-negative bacteria in the air of the broiler barn during the winter season for six weeks were relatively low in comparison to the gram-positive bacteria. Moreover, in a separate investigation conducted along the Syrian coastline, the absence of zeolite resulted in a recorded value of 1.5×10^3 CFU/m^3 (Abdelaziz & Nisafi, 2009). In contrast, the data from Wroclaw, Poland, indicated a considerably higher concentration of E. coli, with a value of 1.9×10^4 CFU/m^3 (Plewa & Lonc, 2011). A significant increase in air contamination has been observed in broiler houses across the globe over recent decades, as evidenced by data from scientific literature. However, the literature data typically pertains to the concentration of airborne bacteria in indoor broiler barns. There is a paucity of information concerning the dissemination of bioaerosols from these barns to the surrounding environment (Agranovski et al., 2007; Vučemilo et al., 2007). Several studies have demonstrated that the bacterial content of the indoor air of broiler barns is predominantly Gram-positive bacteria, including Streptococcus and Staphylococcus (Jilenkerian et al., 2022; Saleh, 2018; Vučemilo et al., 2006). In contrast, the concentration of gram-negative bacteria ranged from 1 to 4% of the total bacterial content (Lonc & Plewa, 2011). It was therefore concluded that a new method of controlling the high levels of airborne microorganisms should be sought, using environmentally safe alternative materials. The addition of Syrian natural zeolite to broiler litter has been found to contribute to a reduction in the bio pollutants released from broiler farms. Zeolite has the potential to be an effective method for mitigating and controlling waste produced by the broiler industry. Furthermore, it possesses distinctive physical and chemical properties, including the capacity to absorb moisture from the air and the broiler litter, as well as to act as an acidifier. These attributes have contributed to the reduction of airborne bacteria pollutants within broiler farms, thereby enhancing the overall health and productivity of the birds.

4. Conclusion

In general, this study provides a standard for the utilization of varying ratios of Syrian natural zeolite in broiler litter. Furthermore, the development of a novel methodology for the reduction of airborne bacteria is essential for the limitation of biological contamination, thereby ensuring the safety of the air from this pollution.

5. Recommendation

It is recommended that different levels of natural zeolite, at 60%, 70% and 80% respectively, be added to the broiler litter, given the absence of studies that have considered the impact of zeolite on the airborne bacteria load in broiler barns.

6. Funding

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