

Water filter application with zeolit composite material in laying hens farm towards the egg quality

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ABSTRACT

This study aimed to analyze the physical, chemical, and microbiological properties of applying an antibacterial water filter made from zeolite composites in laying hens. This study used a completely randomized design with a factorial pattern with 2 factors and 3 replications for egg quality. Factor A is the zeolite treatment, and factor B is the day of egg collection after being filtered. If the measures show a significant difference, it is continued with the least significant difference test, and the chemical quality of the eggs and the microbiology of the eggs are tested using the t-test analysis (paired samples). The results showed that there was a significant interaction between the zeolite treatment and the day after the filter was installed after the eggs were collected. This increases the value of egg length, egg width, shell weight, shell thickness, and haugh units. Furthermore, the chemical quality of eggs treated with zeolite alone on day 3 had a significant effect ($P < 0.05$) on the dry matter and egg fat content. The microbiological quality of the eggs also showed that the zeolite treatment reduced the TPC value and effectively killed *Escherichia coli* and *Salmonella sp*. It was concluded that the application of zeolite filters to the drinking water of laying hens could improve physical quality, maintain chemical quality, and is effective as an antibacterial against bacterial populations, *Escherichia coli* and *Salmonella sp* bacteria in eggs.

Keywords: Laying hens, Microbigical quality, Physicochemical quality, Zeolite filter

INTRODUCTION

Laying hens are a potential poultry livestock in various parts of the world. This typerof chickens is cultivated in producing eggs for daily consumption in large commercial production across the globe (Duman *et al.*, 2016). Purebred

chicken eggs are highly nutritious livestock, so oftenly they become a highly comestibles need (Tolimir *et al.*, 2017). Laying hens obtain water from 3 sources: drinking water, water from food, and water from the oxidation of carbohydrates, fats, and proteins. Water consumption in laying hens is generally influenced by age, ambient

temperature, production, ration consumption, and the chickens' health. The water used to feed chickens must be sufficient and in good quality. Water quality is affected by *Escherichia coli* bacteria, water pH, magnesium levels, nitrate and nitrite levels, sodium/chloride levels, and another minerals. (Swick *et al.*, 1999).

Water quality has an essential role in the maintenance of laying hens. The growth of bacteria, fungi, minerals, and water additives interact in water sources and piping and drinking places for laying hens to cause an optimal performance impact on laying hens (Oviedo *et al.*, 2006). Although 1000 bacteria per millimeter are acceptable for poultry drinking water, up to 1 million bacteria per millimeter have been found in contaminated water (Watkins *et al.*, 2002). Based on the results of the drinking water test before zeolite treatment on drinking water of laying hens on farms, the total number of bacteria was 8×10^4 CFU/mL, *Escherichia coli* 1.4×10^1 CFU/mL and *Salmonella sp.* 1.5×10^1 CFU/mL and water quality standard values for livestock were grouped as classes II with microbiological requirements of 5000 MPN/100 mL of *Coliform* and 2000 MPN/100 mL of *Escherichia coli*. Ideally, bacteria should not be present in drinking water; the presence of coliform bacteria contamination in drinking water is related to fecal contamination and other contaminants produced from the livestock environment, which contaminate water sources and can have an impact on the productivity of laying hens and the quality of the eggs produced (Brake and Hess 2001).

Boetius *et al.* (2015) stated that several factors, such as sufficient nutrients, the appropriate pH, and sufficient temperature around the chicken drinking bowl, caused microorganisms to grow correctly. Using chlorine to suppress bacterial populations in drinking water hurts the health of chickens and negatively impacts humans who consume chicken eggs (Polder *et al.*, 2016). Due to the adverse effects of chlorination on drinking water for chickens, in several developed countries, the use of chlorination for drinking water is prohibited, and chlorination as a disinfection process is no longer used (Shi *et al.*, 2017).

Based on the problems above, it is necessary to carry out proper handling to overcome the problem of bacterial contamination in laying hens' drinking water with modified natural zeolite as an antibacterial. It does not hurt the health of laying hens and humans who consume chicken eggs. Therefore an antibacterial water filter is made with the addition of Copper (Cu) modified zeolite. Zeolite is a porous alumina-silica material found in nature or synthesized and is often applied as an adsorbent, ion exchanger, and catalyst (Nik Malek *et al.*, 2018). Zeolite has a structure composed of an alumina tetrahedron which acts as a negative charge, and a silica tetrahedron which acts as a positive charge (Drakhshankhah *et al.*, 2020). As an ion exchanger, zeolite can contain metal ions or nanoparticles. Metal ions which have the potential as antibacterial and are contained in inorganic materials, can make these composite materials antibacterial (Ferreira *et al.*, 2012). Natural zeolite + 0.5 M CuSO_4 can inhibit bacteria because natural zeolite has been activated with the heavy metal CuSO_4 0.5 M by soaking the zeolite so that the zeolite and CuSO_4 combine perfectly to form Cu^{2+} and then burning at 700°C changes to $\text{CuO} + \text{Cu}_2\text{O}$ so that the zeolite CuSO_4 0.5 M has inhibitory power against *Escherichia coli* bacteria. This study aims to analyze the physical, chemical, and microbiological properties of water filter applications made from zeolite composites in laying hens farms on egg quality.

MATERIALS AND METHODS

Material

The material used was laying hens of the Lohman Brown strain, with the age between 80-83 weeks. The laying hens used have a population of 2000 laying hens. The cages used for the research used a battery cage system made of wood and wire equipped with feeders, drinking water containers, and lights. One battery box contains one laying hen. The battery floor is designed to be inclined, with a slope angle of between $20\text{-}25^\circ$. The goal is that the eggs removed from the chickens can easily roll out.

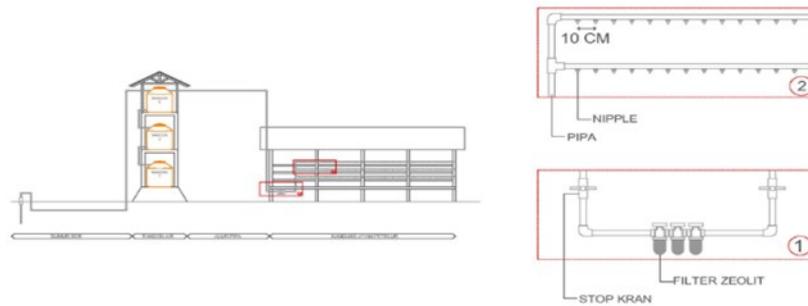


Figure 1. Zeolite water filter installation design

Chickens are fed once a day at 15.00 WIB in the afternoon. The feed given was 120 grams/head, and the total feed given to one cage was 240 kg. The feed used in this study was self-mixed with the feed ingredients used consisting of yellow corn, rice bran, soybean meal, meat, bone meal, and vegetable oil with a nutritional content consisting of 13.00% moisture, 14.00% ash, and crude protein. 17.00%, crude fat 3.00%, crude fiber 7.00%, Calcium (Ca) 3.25-4.25%, Phosphorus (P) 0.45%, total aflatoxin (max) 50 μ g/kg and amino acids consisting of lysine 0.80%, methionine 0.40%, methionine + cystine 0.67%, tryptophan 0.18% and threonine 0.55%.

Drinking water is given ad libitum; before installing the zeolite filter, drinking water from the nipple is filtered using an ordinary water filter, while after installing the drinking water filter from the nipple that has been filtered using a composite zeolite filter. Avian Influenza (AI) vaccine is given five times in 1 period, Newcastle Disease (ND) and Infectious Bronchitis (IB) vaccines are given every 12 weeks by injection, and vitamins are given anti-stress vitamins (vita stress) 2 times a month. Environmental conditions on the farm have temperatures of 26 - 33 $^{\circ}$ C during the day and 18 $^{\circ}$ C at night, with rainfall ranging from 1.200 to 2.200 mm/year with an altitude of 300 meters above sea level and environmental conditions of Relative Humidity (RH) ranging from 70-90%. The lighting conditions in

the cage during the day use sunlight, and at night use yellow light.

Procedure

Installing a Zeolite-based filter installation on a Laying Chicken Farm using a water filter made from zeolite containing copper oxide is put in a filter container suitable for chicken farming (standard water filter container) and then installed in a chicken farm cage containing 2000 laying hens. The water filter is a filter from healthy water filtered directly in the laying hen house. Egg sampling was carried out before and after filter installation. Before installation, eggs were selected randomly with replicates on different collection days. After installing the filter for 3 days, egg samples were taken on different days. The number of samples is 20 eggs once taken to test egg quality and 20 samples for replication.

Variables

Egg samples were taken for analysis of egg quality. The length and width of the eggs were measured using a caliper by measuring the length and width of the eggs. The egg index was converted from the length and width of the eggs using a caliper. Egg weight was obtained by weighing each egg using a digital scale and recording the results based on (Mota *et al.*, 2017). The white and yolk weights were weighed using

a digital balance based on egg whites and yolks. White height and yellow height were measured using a caliper. The weight of the eggshell that has been broken is removed from the egg white residue attached to the inside of the shell, and then the shell weight is measured using a digital scale. The thickness of the eggshell, the egg which has been cracked and the inner membrane removed, is measured by measuring the thickness of the shell using a screw micrometer. Haugh unit is a parameter of egg interior quality calculated based on albumin height and shell weight (Keener *et al.*, 2006).

Variable chemical quality of dry matter eggs is obtained by reducing the water content of the material based on (Relling, 2011). Ash, protein, and fat content were calculated according to

the formula (AOAC, 2005) and carbohydrates (by difference). Egg microbiological quality test, Total Plate Count test were analyzed using the method (BAM, 2001) and using Plate Count Agar (PCA) media with a dilution of 10^{-1} to 10^{-3} , total *Escherichia coli* was analyzed using the method (BAM, 2001) with Eosin media Methylene Blue Agar (EMBA) at a dilution of 10^{-1} to 10^{-3} and *Salmonella sp.* were analyzed by the method (BAM, 2001) with Xylose Lysine Deoxycholate Agar (XLDA) medium at a dilution of 10^{-1} to 10^{-3} .

Data Collection

Egg quality data were analyzed using a factorial completely randomized design (CRD) with 2 factors and 3 replications. Factor A is the zeo-

Table 1. Average Value of Egg Quality in Egg Length, Egg Width, Egg Index, Egg Weight, White Weight, Yellow Weight Before and After Zeolite Treatment.

Zeolite Treatment	Days After Filter			Average	Significance and P-Value		
	3 days	6 days	9 days		TZ	DAF	INT
Egg Length (mm)							
Before	57.71±0.55 ^b	58.24±1.57 ^b	62.28±2.59 ^a	59.41±1.02	NS	NS	*
After	59.76±0.62 ^{ab}	59.70±0.23 ^{ab}	58.53±1.75 ^b	59.33±0.79	0.90	0.14	0.01
Average	58.73±0.05	58.97±0.94	60.41±0.59				
Egg Width (mm)							
Before	44.58±0.43 ^b	44.54±0.50 ^b	49.18±3.54 ^a	46.10±1.78	NS	*	*
After	46.02±0.27 ^b	46.66±0.53 ^{ab}	45.98±0.57 ^b	46.22±0.16	0.87	0.04	0.02
Average	45.30±0.11 ^b	45.60±0.02 ^b	47.58±2.10 ^a				
Egg Index (%)							
Before	76.79±0.59	77.79±3.37	78.24±1.21	77.60±0.60	NS	NS	NS
After	78.78±0.80	78.10±1.21	76.97±0.36	77.95±0.74	0.71	0.95	0.37
Average	77.78±0.99	77.94±0.15	77.60±0.63				
Egg Weight (g)							
Before	65.31±0.49 ^c	65.21±0.51 ^c	69.16±2.70 ^a	66.56±1.27 ^b	**	**	**
After	70.10±0.90 ^b	71.86±4.39 ^b	68.87±3.48 ^b	70.27±1.81 ^a	0.00	0.00	0.00
Average	67.71±0.29 ^b	68.53±2.74 ^b	69.01±0.55 ^a				
White Weight (g)							
Before	40.71±2.66	40.78±1.11	43.13±1.16	41.54±0.88 ^b	**	NS	NS
After	44.62±2.57	46.23±0.78	43.64±2.77	44.83±1.10 ^b	0.00	0.74	0.13
Average	42.67±0.06	43.51±0.23	43.39±1.14				
Yellow Weight (g)							
Before	18.20±0.34	17.77±1.17	18.68±1.33	18.21±0.53	NS	NS	NS
After	18.83±1.06	19.49±0.20	17.91±0.73	18.74±0.43	0.24	0.81	0.09
Average	18.52±0.51	18.63±0.68	18.29±0.42				

^{a,b,c} Value with superscript that is significantly different ($P < 0.05$); TZ = Treatment zeolite; DAF = Day after filter ; INT = Interaction between treatment zeolite and days after filter; ** = highly significant ($P < 0.01$); * = Significant ($P < 0.05$); ns = no significant ($P > 0.05$).

lite treatment, and factor B is the day of egg collection after filtering. If the treatment showed a significant difference, it was continued with the Least Significant Difference (LSD) test, and the chemical quality of the egg, the microbiology of the egg was tested using the t-test analysis (Paired Sample). Data analysis was carried out using the SPSS version 22 application.

RESULTS AND DISCUSSION

Egg Quality

Egg quality is an indicator that relates to the quality standard of the egg's exterior (Kraus *et al.*, 2021). Table 1 shows the physical quality of eggs before and after zeolite treatment.

Variations in Egg Length, Egg Width, and Egg Index

The egg length values in Table 1 show an interaction ($P < 0.05$) between the zeolite treatment and the days after the filter in the egg length values. Further tests on the interaction of the zeolite treatment and the day after the filter showed that the highest egg length value was found in the treatment before the 9th day zeolite treatment with a value of 62.28 mm, while the lowest egg length value was in the treatment before the 3rd day with a value of 57.71 mm. The variation in egg length produced in this study is thought to be influenced by the application of zeolite treatment in drinking water affecting the absorption of nutrients in laying hen feed, especially calcium, and egg length in this study was categorized into oval, oval, and round shapes.

The results in Table 1 show a significant interaction ($P < 0.05$) between the zeolite treatment and the day-after filter in increasing the egg width value. Further tests on the interaction of the zeolite treatment and the day of egg collection after the filter showed that the highest egg width value was in the treatment before the zeolite treatment on day 9 with a value of 49.18 mm, and the lowest value was in the treatment before the 6th day with a value of 44.54 mm. The longer the day of egg collection, the higher the egg width; this is due to variations in egg

width which are affected during the egg formation process.

The research results in Table 1 show no interaction ($P > 0.05$) between the zeolite treatment and the day of egg collection on the egg index value. In this study, the zeolite treatment and egg collection day it has maintained the egg index value. This is presumably because the zeolite contains minerals that affect the index value of the eggs produced. The index value of the eggs produced in this study ranged from 76.79-78.78% the index value of the eggs produced was still in the ideal category. Overall, the results of this study suggest that zeolite treatment can affect the value of egg length, egg width, and egg index. This can be assumed because there has yet to be any previous research similar to and related to this research.

Variations in Egg Weight, Albumen Weight, and Yolk Weight

The research results in Table 1 show a significant interaction ($P < 0.05$) between the zeolite treatment and the day of egg collection after filtering, increasing the egg weight value. Further tests on the interaction of the zeolite treatment and the day of egg collection after the filter showed that the highest egg weight value was found in the treatment after the 6th day zeolite treatment with a value of 71.86 g while the lowest value was in the treatment before the zeolite treatment on the 6th day with a value of 65.21 g. Additional days of zeolite treatment affected increasing egg weight, this was due to the increasing age of the chickens, the egg weight increased. This was in accordance with John-Jaja *et al.* (2016) stated that egg weight is affected by age, the older the laying hens, the heavier the eggs produced. Based on the SNI standard (2008), the egg weight in this study was included in the large group. Egg weight values before and after zeolite treatment ranged from 65.21-71.86 g. Egg weight is affected by ovarian development. The ovary is where the yolk is formed. Egg weight will be low if the formation of the yolk is less than perfect. In addition, according to Tugiyanti (2012), low nutrient absorption inhibits

ovarian development, making egg weight less than optimal.

The research results in Table 1 show that the zeolite treatment before and after had a significant effect ($P < 0.05$) on the egg white weight value. The resulting white weight test further shows that the average value of zeolite treatment after zeolite treatment is higher than before on egg white weight. The higher the white weight value in the treatment after the zeolite treatment of 44.83 g is influenced by the density of the denser the egg white, the heavier the weight, which is supported by the intake of nutrients needed for egg formation, both protein, minerals,

and vitamins. The lowest white weight value was in the pre-treatment zeolite treatment of 41.54 g, which was thought to be low in egg white due to insufficient nutrient intake during egg formation and the influence of the length and width of the eggs produced. The white weight results of this study were higher than that of Fendri *et al.* (2012), who used zeolite on the egg quality of Tunisian laying hens around 34.15-36.02 g. Yields of egg white weight in several countries; according to Zhu *et al.* (2020), egg weight in China is 63.98 g, and Guo *et al.* (2020) egg weight in Japan is 71.80 g.

The variance results also showed no interac-

Table 2. Average Value of Egg Quality in White Height, Yellow Height, Eggshell Weight, Eggshell Thickness, Haugh unit Before and After Zeolite Treatment

Zeolite Treatment	Days After Filter Installation			Average	Significance and P Value		
	3 days	6 days	9 days		TZ	DAF	INT
White Height (mm)							
Before	7.67±0.27	7.67±0.40	8.87±0.44	8.07±0.09			
After	8.03±0.11	7.25±0.99	7.93±0.57	7.74±0.44	NS	*	NS
Average	7.85±0.11 ^{ab}	7.46±0.42 ^b	8.40±0.09 ^a		0.21	0.03	0.15
Yellow Height (mm)							
Before	17.66±0.17	17.74±0.31	18.8±1.06	18.07±0.48			
After	18.47±0.40	17.98±0.67	18±0.170	18.15±0.25	NS	NS	NS
Average	18.07±0.16	17.86±0.26	18.4±0.63		0.75	0.28	0.07
Eggshell Weight (g)							
Before	7.07±0.2 ^{ab}	6.67±0.24 ^b	7.34±0.4 ^a	7.03±0.11			
After	7.1±0.27 ^{ab}	7.2±0.44 ^{ab}	6.82±0.27 ^{ab}	7.06±0.10	NS	NS	*
Average	7.12±0.05 ^b	6.93±0.14 ^b	7.08±0.10 ^a		0.79	0.56	0.04
Eggshell Thickness (mm)							
Before	0.24±0.04 ^b	0.26±0.01 ^b	0.38±0.02 ^a	0.29±0.01 ^b			
After	0.36±0.02 ^a	0.36±0.03 ^a	0.36±0.02 ^a	0.36±0.01 ^a	**	**	**
Average	0.30±0.01 ^b	0.31±0.02 ^b	0.37±0.00 ^b		0.00	0.00	0.00
Haugh Unit (HU)							
Before	85.97±1.56 ^b	71.97±3.47 ^c	91.59±2.14 ^a	83.17±0.98			
After	86.74±0.90 ^{ab}	82.47±4.39 ^b	86.13±2.81 ^b	85.11±1.75	NS	**	**
Average	86.35±0.47 ^a	77.22±0.65 ^b	88.86±0.47 ^a		0.16	0.00	0.00

^{a,b,c} Value with superscript that is significantly different ($P < 0.05$); TZ = Treatment zeolite; DAF = Day after filter ; INT = Interaction between treatment zeolite and days after filter; ** = highly significant ($P < 0.01$); * = Significant ($P < 0.05$); ns = non significant ($P > 0.05$).

tion ($P>0.05$) between the zeolite treatment and the day of egg collection after filtering on egg yolk weight. The average value of yolk weight before and after treatment zeolite ranged from 18.21 -18.74 g. The importance of the yolk is influenced by the weight of the eggs produced in the study. The higher the weight of the eggs obtained, the higher the weight percentage of the yolk. Tugiyanti and Iriyanti (2012) stated that yolk weight is influenced by ovarian development, chicken body weight, age at reaching sexual maturity, quality and quantity of feed, disease, environment, and feed consumption.

Variation in Albumen Height, Yolk Height

The study's results in Table 2 show no interaction ($P>0.05$) between the zeolite treatment and the day of egg collection after filtering on white height. The white height test showed that the average result before the zeolite treatment was higher than the white height value after the zeolite treatment. The high value of white height before zeolite treatment of 8.07 mm was influenced by optimal nutrient absorption, so the egg white content increased, and white height correlated with egg weight gain. The lowest value of white height after zeolite treatment was 7.74 mm which was affected by the increasing age of the chickens, so nutrient absorption was not optimal. in Table 2 shows that there was no interaction ($P> 0.05$) between the zeolite treatment and the day of egg collection after filtering, on egg yolk height. The average value of yellow height before and after zeolite treatment ranged from 18.07 – 18.15 mm. This is because the high value of the yolk also correlates with the weight of the yolk; the weight of the yolk in this study did not significantly affect the weight, so the height of the yolk also did not have a significant effect after zeolite treatment in the drinking water of laying hens.

Variations in Shell Weight, Shell Thickness and Haugh Units

The results in Table 2 show a significant interaction ($P<0.05$) between the zeolite treatment and the day of egg collection after filtering

on shell weight. Further test results on the interaction of the zeolite treatment and the day of egg collection after the filter showed that the highest value in shell weight was in the treatment before the 9th day zeolite treatment with a value of 7.34 g and the lowest value was in the 6th day with a value of 6.67 g while the value after the weight treatment shell shows a value of 6.93 – 7.12 g. The value of shell weight before and after zeolite treatment dramatically influences the absorption of feed nutrients and the age of laying hens; this is in line with John-Jaja *et al.* (2016) that shell weight is related to genetic factors, age factors, and physiological characteristics of laying hens. The age factor gives the progress of the variation of the shell weight coefficient in various ages of laying hens under 25 weeks, namely 8.39%, 10.05% at 25, 10.18% at 51 weeks, and 13.18% at 72 weeks, and Yuwanta (2010) states that eggshell quality can be affected by the age of the broodstock. The older the parent, the quality of the chicken shell decreases.

The results in Table 2 show a significant interaction ($P<0.05$) between the zeolite treatment and the day of egg collection after filtering on the shell thickness value. The test results on the interaction of the zeolite treatment and the day of egg collection after the filter showed that the highest values were found on days 3, 6, and 9 after 0.36 mm zeolite treatment, and the lowest values were found in the treatment before 0.24 mm treatment. This study showed that the thickness of the shell after the zeolite treatment showed an increase in the thickness of the eggshell, and the presence of antibacterial substances in the zeolite in the chicken drinking water filter could also optimize the absorption of nutrients, especially in calcium. This is in line with Ketta and Tumova (2016) that the eggshell thickness of the Lohman Brown strain at 20-24 weeks of age is 0.354 mm, and when it is 56-60 weeks, the thickness of the shell can reach 0.372 mm. The shell thickness in this study was higher than the eggshell thickness based on Aguillón-Páez *et al.* (2020) from Colombia with a shell thickness of 0.20 mm, Saudi Arabia 0.04 mm (Attia *et al.*, 2014) and Zhu *et al.* (2020) shell thickness in

China is 0.31 mm.

The research results in Table 2 shows a significant interaction ($P < 0.05$) between the zeolite treatment and egg retrieval results after filtering on the high value of egg units. Further tests on the interaction of the zeolite treatment and the day of egg collection after the filter showed that the highest value was in the treatment before zeolite treatment, 91.59, and the lowest value was in the treatment before zeolite treatment, 71.97. This is because the measurement time for each repetition is carried out at the same hour so that the haugh unit values produced are almost the same. The high value of the research unit is still balanced with the high unit value in I, Attia *et al.* (2014) state that in Saudi Arabia, the high unit value is 60.37, which is lower than the results of the study. Overseas, TW *et al.* (2019) state that the high unit value in Brazil is 87.36.

The high haugh value of egg units obtained was because after taking the eggs, the eggs were immediately measured. The haugh unit value decreases with the increasing age of the egg because the egg albumen will melt more due to the evaporation of CO_2 and the entry of microorganisms into the albumen so that it is damaged. Based on this, the eggs produced during the study were included in the quality category AA, namely eggs of the highest quality with a value of haugh unit > 72 or still fresh eggs.

Egg Chemical Quality

The results of the chemical analysis of eggs which include dry ingredients, ash, protein, and carbohydrates before and after zeolite treatment, show the results presented in Table 3.

Dry Ingredients

Organic matter is a nutrient component comprising carbon, hydrogen, oxygen, and nitrogen (McDonald *et al.*, 2010). The study results in Table 3 show that the dry matter on day 3 before and after the zeolite treatment showed significantly different values ($P < 0.05$) before the zeolite treatment. Another in the egg. Kocatepe *et al.* (2011) stated that an increase in dry matter value was correlated with an increase in other nutrient

content, such as carbohydrates, fats, and protein. Meanwhile, days 6 and 9 did not show different values for dry matter before and after zeolite treatment. The dry matter value of the zeolite treatment is high compared to that after the treatment experienced shrinkage. This is because before and after the zeolite treatment in the treated drinking water did not affect the nutritional value of the eggs, especially the dry matter. The material's ash, protein, and fat content influences the high dry matter content. The high ash, protein, and fat content before and after zeolite treatment is the cause of the high dry matter. The dry matter value in this study was higher than that of Kabir *et al.* (2015), namely local chicken 25.58, exotic chicken 38.52, Guinea fowl 32.90, and quail 30.67 dry matter value.

Ash Content

The research results in Table 3 show that the ash content on days 3, 6, and 9 before and after the zeolite treatment showed no significant difference ($P > 0.05$) in the egg ash content. Before treatment on days 3, 6, and 9, the value of high ash content after treatment was experienced. This shrinkage is due to the absence of a treatment effect on the value of ash content both before and after zeolite treatment. The ash content of eggs is related to the levels of minerals contained in eggs, such as Ca and Phosphorus in eggs. The value of the ash content before and after treatment was more excellent, ranging from 3.32-3.77%. This proves that zeolite treatment in chicken drinking water can increase the ash content, resulting from burning organic matter in the form of minerals. The organic matter content is inversely proportional to the ash content. The higher the ash content, the lower the organic matter content. \

Protein

The research results in Table 3 show that the protein values on days 3, 6, and 9 before and after zeolite treatment did not appear to be significantly different ($P > 0.05$). The protein value remained stable both before and after treatment, and this proved that the zeolite treatment did not

Table 3. Chemical Value of Eggs in Dry Material, Ash Level, Protein, Fat, and Carbohydrate Before and After Zeolite Treatment

Variable	DAF	Zeolite Treatment		P Value	Significance
		Before	After		
Dry Material (%)	3	97.31 ± 0.11	91.44 ± 0.17	0.00	**
	6	96.10 ± 0.33	89.48 ± 0.17	0.04	*
	9	91.62 ± 0.19	89.85 ± 0.35	0.41	NS
Ash Level (%)	3	3.77 ± 0.54	3.58 ± 0.12	0.62	NS
	6	3.63 ± 0.08	3.56 ± 0.27	0.06	NS
	9	3.32 ± 0.04	3.40 ± 0.16	0.5	NS
Protein (%)	3	46.83 ± 0.76	48.38 ± 0.63	0.36	NS
	6	48.63 ± 0.75	48.09 ± 0.42	0.63	NS
	9	47.93 ± 0.12	48.24 ± 0.59	0.5	NS
Fat (%)	3	30.97 ± 0.80	32.26 ± 0.77	0.01	**
	6	32.97 ± 2.75	32.26 ± 0.04	0.16	NS
	9	34.43 ± 0.69	34.92 ± 0.00	0.5	NS
Carbohydrate (%)	3	13.29 ± 1.21	7.24 ± 0.32	0.12	NS
	6	11.38 ± 0.68	5.46 ± 0.05	0.76	NS
	9	5.92 ± 0.66	5.23 ± 0.32	0.50	NS

Score of P Value P<0.05 showing real difference; DAF = Day after filter; ** = highly significant (P <0.01); * = Significant (P<0.05); ns = non significant (P>0.05).

reduce the protein value in eggs. The value after zeolite treatment showed a constant range in egg protein. Chicken egg protein levels are generally influenced by the nutrients consumed by livestock. According to Argo *et al.* (2013) high protein content in feed contributed to high protein in eggs. The protein content in chicken eggs without the effect of zeolite treatment a content in the range of 46.83- 48.38 %. At the same time, the fat content in chicken eggs with the influence of zeolite treatment found 48.24 – 48.38% results. The results of Bakhtra *et al.* (2016) stated that the protein content of purebred chicken eggs through nitrogen analysis using the Kjeldahl method obtained an average egg protein content value of purebred chicken eggs of 6.45%, native chicken eggs 6.91%, duck eggs 6.59% and quail eggs 6.55%.

Fat

Based on the results of the study in Table 3, shows that the fat value on day 3 after zeolite

treatment showed an increase in the fat value significantly different (P> 0.05) in eggs; this was allegedly due to the increase in egg fat content caused by high egg yolk and white egg values. Almost no fat content. Considering that the highest content in egg yolk was fat, while on days 6 and 9, the fat value was not significantly different (P> 0.05) before and after zeolite treatment. The fat content in chicken eggs without the effect of zeolite treatment content in the range of 30.56% - 34.43%. While the fat content in chicken eggs with the effect of adding zeolite found results of 32.26% for observations on the 3rd and 6th days and 34.92% on the 9th day of observation. Iman research (2003) stated that the fat in the eggs of Merawang chickens, by feeding them with omega-3 supplements, obtained a fat value of 21.69% lower than the study's results.

Carbohydrate

Carbohydrate levels in eggs generally have a small amount. The research results in Table 3

Table 4. The Score of Egg Microbiology Before and After Zeolite Treatment

Egg Microbiology Test	Unit	DAF	Zeolite Treatment	
			Before	After
Total Plate Count (TPC)	Log CFU /mL	3	1.73 ± 0.96	1.41 ± 0.21
		6	1.63 ± 0.09	1.38 ± 0.18
		9	1.26 ± 0.17	1.28 ± 0.00
<i>Escherichia coli</i>	Log CFU/mL	3	Negative	Negative
		6	1.43 ± 0.34	Negative
		9	Negative	Negative
<i>Salmonella sp</i>	Log CFU /mL	3	Negative	Negative
		6	Negative	Negative
		9	1.32 ± 0.74	Negative

DAF = Day after filter; there is real difference of CFU mL log between before and after zeolite treatment. The data of colony numbers were transformed into the form of log.

show that the carbohydrate values on days 3, 6, and 9 before and after the zeolite treatment were not significantly different ($P > 0.05$). The hydrolysis process in the zeolite treatment in drinking water decreased the carbohydrate value in eggs. Carbohydrate content in chicken eggs before zeolite treatment has content in the range of 5.92% - 13.29%. Meanwhile, the fat content in chicken eggs after the zeolite treatment resulted in 5.23% - 7.24%.

Microbiological Quality

Table 4 shows the results of evaluating the total number of microorganisms in eggs before and after zeolite treatment.

TPC (Total Plate Count)

The research results in Table 4 show that TPC contamination on days 3 and 6 significantly differed after the zeolite treatment, and there was a decrease in TPC in chicken eggs. This is because the zeolite used as a drinking water filter has antibacterial properties, which can kill bacteria by releasing Cu ions which causes bacteria to be killed. In contrast, on day 9, TPC was no different before and after zeolite treatment. The amount of TPC in this study complied with the requirements for microbiological quality (SNI 3926:2008 concerning the consumption of chicken eggs), namely that there should be no microbi-

al contamination of more than 1×10^5 or 5 logs. In contrast, in the study, the value of microbial contamination was obtained before zeolite treatment in the range of 1.73 – 1.26 log CFU/mL, whereas after treatment, the results were 1.41-1.28 log CFU/mL. The TPC contamination value from this study after zeolite treatment was lower than that of El-Kholy *et al.* (2014) in Egypt at 3.04 log CFU/g and Rizaldi *et al.* (2020) at the Tamiang Layang market, East Barito Regency at 1.90 log CFU/g.

Escherichia coli

According to Table 4, the 3 and 9 days before and after zeolite treatment had a negative value for *Escherichia coli* bacteria because the zeolite used as a water filter has been modified to have antibacterial properties capable of killing pathogenic bacteria carried by chicken drinking water. However, on the 6th day, there was *Escherichia coli* bacteria before the zeolite treatment, 1.43 log CFU/mL in chicken eggs, this was presumably due to contamination of *Escherichia coli* bacteria before the zeolite treatment, presumably because the chicken's drinking water still contained *Escherichia coli* bacteria, so it was carried into the chicken eggs. Contamination detected was still below the maximum standard for microbial egg contamination, namely 5 log CFU/g, 100 MPN/g, and 50 MPN/g for each type of microbi-

al contamination, Coliform and *Escherichia coli* according to SNI 3926:2008 microbiological quality standards for consumption chicken eggs. In the research results by Anton *et al.* (2019) on the consumption of chicken eggs from the East Jakarta city area, *Escherichia coli* bacteria were detected at 1.5 MPN/g.

Salmonella sp.

The results showed that *Salmonella sp.* bacteria in eggs on day 4 showed no different results before and after zeolite treatment. This *Salmonella sp.* bacteria were negative and, on day 6, showing the same thing. *Salmonella sp.* bacteria were negatively detected both before and after zeolite treatment. This is because the zeolite treatment used has a high concentration of Cu, which has greater anti-bacterial power. The existence of a positive charge on Cu ions and a negative charge on the bacterial cell membrane will cause a tug-of-war between the two. According to Humphrey (1994), eggs have a chemical defense in the egg white, namely lysozyme. Lysozyme is a bactericidal substance that can destroy bacteria.

The study's results on the 9th day before the zeolite treatment showed the presence of *Salmonella sp.* bacteria with a value of 1.32 CFU/mL. This was suspected because the growth of *Salmonella sp.* started to occur, which was influenced by the environment, such as temperature and humidity. This was reinforced by the statement of Momani *et al.* 2018 that bacterial contamination of eggs also occurs when microorganisms enter the eggshell through the pores on the surface of the eggshell. Chusniati *et al.* (2008) state that there are two possible ways for *Salmonella sp.* to enter the egg, namely directly vertically, through the egg yolk and egg white from the ovary the hen infected with *Salmonella sp.* *Salmonella sp.* can also enter horizontally through the pore. - pores on the eggshell. Zeolite treatment reduced the presence of *Salmonella sp.* to a negative value after treatment. This shows that the zeolite treatment of chicken drinking water is in accordance with SNI 2897 (2008) and that microbial contamination from *Salmonella*

sp. is negative.

CONCLUSION

The application of zeolite filters to the drinking water of laying hens could improve physical quality, maintain chemical quality, and is effective as an antibacterial against bacterial populations, *Escherichia coli* and *Salmonella sp.* bacteria in eggs.

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