

## Correlation of Heavy Metals Cd, Cr, Cu, Hg, Pb, and Zn with Intestinal Bacteria in *Anas platyrhynchos* L. Duck

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### ABSTRACT

Intestinal bacterial species play an essential role in the duck body metabolism and productivity. They are affected by various factors, such as heavy metal contamination. This research aims to study metal emission levels and their correlation with the composition of intestinal bacteria. A total of twenty-five duck samples (*Anas platyrhynchos* L.) were obtained from Semarang, Temanggung, Magelang, Pati, and Salatiga. Five g of intestinal substances were employed in the isolation 16S rRNA gene V3-V4 region. Data sequence in fastq format was then analyzed using QIIME. Also, the heavy metals in water, feed, and meat samples were analyzed using inductively coupled plasma-optical emission spectroscopy (ICP-OES). The highest heavy metal contaminant was Hg, with a total concentration reaching  $5.55 \pm 1.79$  ppm of input and  $4.42 \pm 0.80$  ppm in meat. The bacterial composition comprises the four highest phyla, sequentially including Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes. The relationship between heavy metals and intestinal bacteria shows a significant value with a coefficient of  $>0.90$ . Therefore, there was a correlation between heavy metals and intestinal bacteria composition, suggesting that bacteria are likely to protect ducks from the adverse effects of heavy metals in water and feed.

## 1. Introduction

Gut microbiota is bacterial communities found in the digestive tracts of living things. The intestinal tract is a habitat for various microbial species that live in symbiosis with the host, including duck species. They play a vital role in body metabolism by secreting short-chain fatty acid (Even *et al.* 2018; Wu *et al.* 2018) and affecting the immune system by increasing cytokine production (Elson and Alexander 2015). The duck intestinal bacteria determine physiological conditions (Clavijo and Flórez 2018), and the level of its health relates with diversity, composition, and density of the gut microbiome (Goubet *et al.* 2018). Notably, some essential nutrients needed by the duck as a host are synthesized by intestinal bacteria, such as vitamins and fatty acids that influence homeostasis (Bedford and Gong 2018; Even *et al.* 2018).

Several studies have proven that the composition and activity of duck intestinal bacteria are determined by various factors such as feed (Liu *et al.* 2018), antibiotic use (Konstantinidis *et al.* 2020), maintenance patterns (Susanti *et al.* 2020a), and genetics according to its host (Elson and Alexander 2015). Recent research in the gut microbiome of ducks has revealed that husbandry techniques and feed compounds (organic or pellet) influence the gut microbiome composition and play a role in protecting the host from parasites and pathogens. Moreover, previous research also found that intestinal bacteria are also affected by heavy metal contamination (Yausheva *et al.* 2018; Duan *et al.* 2020).

Accordingly, the heavy metal contamination and the sources need to be investigated and considered in anticipation of rapid growth in the industrial sectors. Many industrial wastes have been involved as the primary source of heavy metals such as Hg, Cu, Cd, and Zn to the environment pollutes water sources from the riverbank to the animal husbandry. Therefore, it is necessary to have a deeper study

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of metal emissions levels in the composition of intestinal bacteria related to poultry productivity. This study intended to investigate the correlation of heavy metals, including Cd, Cr, Cu, Hg, Pb, and Zn, with intestinal bacteria in *Anas platyrhynchos* L. duck.

## 2. Materials and Methods

The analyses of composition, abundance and the relationship between intestinal bacteria and heavy metal Cd, Cr, Cu, Hg, Pb, and Zn input from feed and water were performed. The sampling step was conducted to get representative samples from April to May 2019 during the dry monsoon season. Five duck samples were obtained from each intensive duck farm in five regencies in Central Java, i.e., Semarang, Temanggung, Magelang, Pati, and Salatiga. Samples were obtained purposively with criteria (inclusion), including 1) female duck, 2) three months old, 3) no feed or drugs containing antibiotics taken within two weeks. Laying eggs samples obtained were excluded from the sampling method. A total of 5 ducks were taken from each farm to be sacrificed, then the intestinal content and meat were taken separately.

### 2.1. DNA Isolation and Making Gene Library for Next Gene Sequencing (NGS) Analysis

Five g of intestinal contents were used in isolation of the region V3-V4 16S rRNA gene (Dennis *et al.* 2013; Yarla *et al.* 2014), using QIAamp DNA Stool Mini Kit Qiagen (San Diego, California, US) according to the manufacturer's protocol for DNA isolation and SYBR™ Green PCR Master Mix ThermoFisher Scientific. The amplification program with PCR is denaturation at 94°C for 3 min, followed by 35 cycles (denaturation was run at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 45 sec), and final extension at 72°C for 10 min. The primers used were 338F (5'-GGACTACHVGGGTWTCTAAT-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Dennis *et al.* 2013). The PCR reaction was carried out with a total volume of 20 ml containing 0.5 µl (5 U/µl) of EasyTaq DNA polymerase, 2 µl of 10× EasyTaq buffer, 2 µl of 0.25 mmol/L dNTPs, 0.2 µmol/L primers, 10 ng of DNA template, and to 20 µl of ddH<sub>2</sub>O.

The microbiome diversity was identified using next-generation sequencing (NGS) using Illumina HiSeq 2500 System Platform (ThermoFisher Scientific; Singapore) following the manufacturer's protocols. The read length was set at Paired-End 250 bp, and the objective was amplicon sequencing-application:

metagenomics/microbiomics. Data output from the NGS process was approximately 100,000 raw tags (200,000 reads), then analyzed using metagenomic software analysis.

### 2.2. Metagenomic Analysis

Data from the 16S rRNA gene sequence region V3-V4 was obtained in the form of fastqc. Quality control was performed using (Callahan 2018). Fastqc that has passed quality control was then analyzed using QIIME. Taxonomic preparation was carried out using Greengenes data 1313\_8 99% OTU (McDonald *et al.* 2012). Phylogenetic trees were built using MAFFT (Katoh and Standley 2013) and FastTree (Price *et al.* 2010).

### 2.3. Heavy Metal Analysis

Water samples were collected from local water resources used for drinking water of the duck. As much as 1.5 L of water samples was placed in black plastic-jerry cans at each farm location. Preparation was done by taking as much as 100 ml of water and adding four drops of 65% HNO<sub>3</sub> or until the pH becomes 2 (Junianto *et al.* 2017). Then the water was added with sterile distilled water up to 200 ml, and the prepared sample was stored at room temperature before analysis.

As much as 1.5 kg of feed consisting of the bran, coconut pulp, dried rice, concentrate, shrimp waste (head and exoskeleton), and wasted-fish taken from the farm and then dried for 48 h at 70°C, after drying the feed was mashed with a blender, weighed as much as 6 g and added 2 ml of 65% HNO<sub>3</sub> to be furnace for 2 h at 500°C (Demirel *et al.* 2013). After becoming perfectly white ash, it was then dissolved with distilled water until it reached a volume of 50 mL and stored at room temperature.

The thigh and breast meats were taken. Each thigh and breast meat were cut 2 × 2 × 3 cm in size, put into a petri dish, and weighed. The meat was dried in the oven for more than 72 hours at 70°C. After drying, the meat was taken as much as 6 g, added with 2 ml of 65% HNO<sub>3</sub>, then furnace at 500°C for 2 h (Demirel *et al.* 2008). Meat ash is dissolved with distilled water up to 50 ml volume for analysis. All samples (water, feed, and meat) that have been through the preparation stage were analyzed using the inductively coupled plasma-optical emission spectroscopy (ICP-OES) method using The PerkinElmer® Optima™ 8300 (Massachusetts: USA) followed manufacturer-specific procedures. All samples were analyzed with

Scott's spray chamber and a gem cone nebulizer and replied for three replication per sample.

### 2.4. Data Analysis

All data obtained were analyzed statistically using SPSS 23 for Mac. Data were tested for normality and homogeneity using the Kolmogorov-Smirnov test, correlation analysis was performed using the Pearson two-tail correlation test with the following assessment criteria: if the correlation coefficient value of 0.00-0.20 shows the weak relationship, 0.21-0.40 correlates weakly, 0.41-0.60 is moderately correlated, 0.61-0.80 correlates strongly, and 0.81-1.00 correlates very strongly; significantly correlation determined if  $p < 0.05$ .

### 3. Results

Measurements of heavy metals in water and feed were carried out on all samples from each farm. The results showed that the highest content of contamination was Hydragyrum (Hg) or mercury in both animal feed and drinking water, followed by copper (Cu) (Figure 1). The highest Hg content in water was found in most farms located in

Temanggung Regency, precisely in the highland area and the freshwater supplies from the mountain. Based on field observations, no factories or industries were found as sources of pollution in the area around the livestock water source. The water comes from mountain water resources channeled directly from community waterways.

Meanwhile, the highest Hg content in duck feed was found in Pati, a coastal area. It is likely because the fish as a food source for duck feed may be contaminated with heavy metals from industries in the upstream area. The analysis also showed that the Cd, Cr, Pb, and Zn levels in water and feed did not exceed 1 ppm, despite exceeding normal limits.

The metal content was also found in duck meat used as a sample. Metals analysis shows that there was a reduction of more than 20% from metals input (water and feed) that enters the duck's body to the metal content in meat for all metals except zinc (Zn) (Table 1). Cd, The Cr, Cu, Hg, and Pb metals concentrations had decreased from input to meat. There may be a mechanism possessed by duck physiology or bioremediation to reduce metal concentration inside their body. However, the concentration of Zn in meat is much higher than the metal input entering a duck's

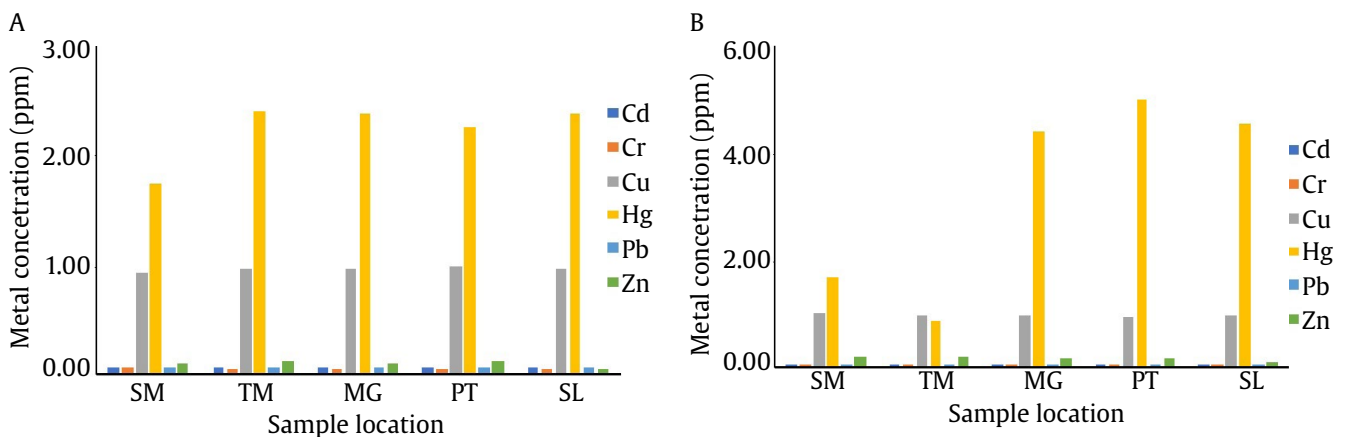


Figure 1. Metal concentration from water (A) and feed (B) from sample location of duck husbandry in five subdistricts in Central Java Province. SM: Semarang, TM: Temanggung, MG: Magelang, PT: Pati, SL: Salatiga

Table 1. Comparison metals input (in water and feed) to duck's meat

Metals	Concentration (ppm)			
	Input	Meat	Balance	%
Cd	0.15±0.00	0.07±0.00	-0.08±0.00	-50.93±0.13
Cr	0.11±0.00	0.06±0.00	-0.06±0.00	-50.96±1.97
Cu	1.95±0.02	0.86±0.24	-1.09±0.24	-55.75±12.41
Hg	5.55±1.79	4.42±0.80	-1.12±1.88	-20.25±37.88
Pb	0.14±0.01	0.06±0.01	-0.08±0.01	-55.82±6.59
Zn	0.27±0.07	1.41±0.85	1.14±0.83	417.48±263.30

Negative mark shows total residue that possibly eliminated from duck's body, positive mark indicates existed deposit or possibly contaminated from the environment, percentage (%) is calculated from metals concentration in duck's meat divided by metals input

body. Zn probably causes this is needed as a cofactor of various types of metabolism enzymes, resulting in accumulation rather than elimination. The highest concentration reduction was found in the Hg metal, a toxic metal for living things. Therefore, the possibility of the metal deposit period is also shorter and be immediately eliminated by the body.

Pearson analysis results also show the heavy metals that enter the body with metal concentrations in duck meat (Table 2). A positive correlation was shown between the concentration of Hg input and Cr in meat. In addition, another correlation was also shown from the interaction between Pb with Cr ( $p = 0.04$ ) and Zn with Cu ( $p = 0.03$ ).

The presence of heavy metals contamination in water and feed affects body physiology and may also affect the composition of intestinal bacteria. Based on metagenome analysis, the composition of the intestinal bacteria also shows the low diversity in some phyla. The main bacterial content found is phylum Firmicutes, followed by Actinobacteria and Bacteroidetes. Almost all samples of duck intestinal contents did not show any striking variation in bacteria. Phylum Proteobacteria was found in all groups even though the density was low (Figure 2).

In addition, bacteria can absorb, accumulate, and remediate heavy metals and transform them into harmless, including intestinal bacteria that may also have a role in eliminating metals that enter the duck's body.

This study emphasized the four bacteria phyla with the highest abundance and diversity in the intestinal

gut, and there were sequentially Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Based on the metagenomic analysis, the four phylum bacteria consisted of 33 families, with the highest density being Streptococaceae (Figure 2b).

Correlation analysis of the abundance of bacteria with the concentration of metals in meat shows some family of bacteria family correlated with the concentration of the metals of meat. Bacteria in the family Bacillaceae, Family XI (unidentified), Aerococcaceae, and Actinomycetaceae have a robust positive correlation with Zn concentrations in meat, whereas Streptococaceae is negatively correlated. It suggests that an increase in the concentration of heavy metals in duck meat will increase along with an increase in the density of the bacterial family. This study also showed a robust positive correlation between Clostridiaceae with Hg and Zn metals, whereas Corynebacteriaceae had a robust negative correlation with Cr concentration and positively correlated with Zn. Besides Zn. A robust negative correlation was also shown between the Dietziaceae, Intrasporangiaceae, Promicromonosporaceae, Nocardiodaceae, and Ruaniaceae families correlated with copper (Cu), and the Peptococcaceae family with Pb metal (Table 3). The negative correlation shows that the decrease in metal content is associated with increased bacterial density. In other words, the decrease in heavy metal content in duck meat is closely related to the rise in the bacterial number in certain families, possibly because of the role of bacteria as heavy metal bioremediation.

Table 2. Pearson's coefficient correlation and significant value among metals input and duck's meat

Metals	Input						Meat						
	Cd	Cr	Cu	Hg	Pb	Zn	Cd	Cr	Cu	Hg	Pb	Zn	
Input	Cd	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
	Cr	NA		0.451	-0.494	-0.533	-0.790	0.640	-0.535	0.523	0.322	0.899*	0.299
	Cu	NA	0.45		0.364	-0.545	0.161	0.173	0.485	0.216	-0.381	0.723	0.924*
	Hg	NA	0.40	0.55		0.475	0.850	-0.220	0.878*	0.211	-0.906*	-0.308	0.617
	Pb	NA	0.36	0.34	0.42		0.346	0.026	0.134	0.383	-0.409	-0.754	-0.231
	Zn	NA	0.11	0.80	0.07	0.57		-0.437	0.940*	-0.306	-0.628	-0.554	0.299
Meat	Cd	NA	0.25	0.78	0.72	0.97	0.46		-0.281	0.622	0.348	0.344	0.074
	Cr	NA	0.35	0.41	0.05	0.83	0.02	0.65		-0.178	-0.670	-0.249	0.576
	Cu	NA	0.37	0.73	0.73	0.52	0.62	0.26	0.78		-0.347	0.312	0.409
	Hg	NA	0.60	0.53	0.03	0.49	0.26	0.57	0.22	0.57		0.115	-0.689
	Pb	NA	0.04	0.17	0.61	0.14	0.33	0.57	0.69	0.61	0.85		0.558
	Zn	NA	0.63	0.03	0.27	0.71	0.63	0.91	0.31	0.49	0.20	0.33	

\*Correlation is significant at the 0.05 level (2-tailed)

\*\*Correlation is significant at the 0.01 level (2-tailed). NA: Cannot be computed because at least one of the variables is constant

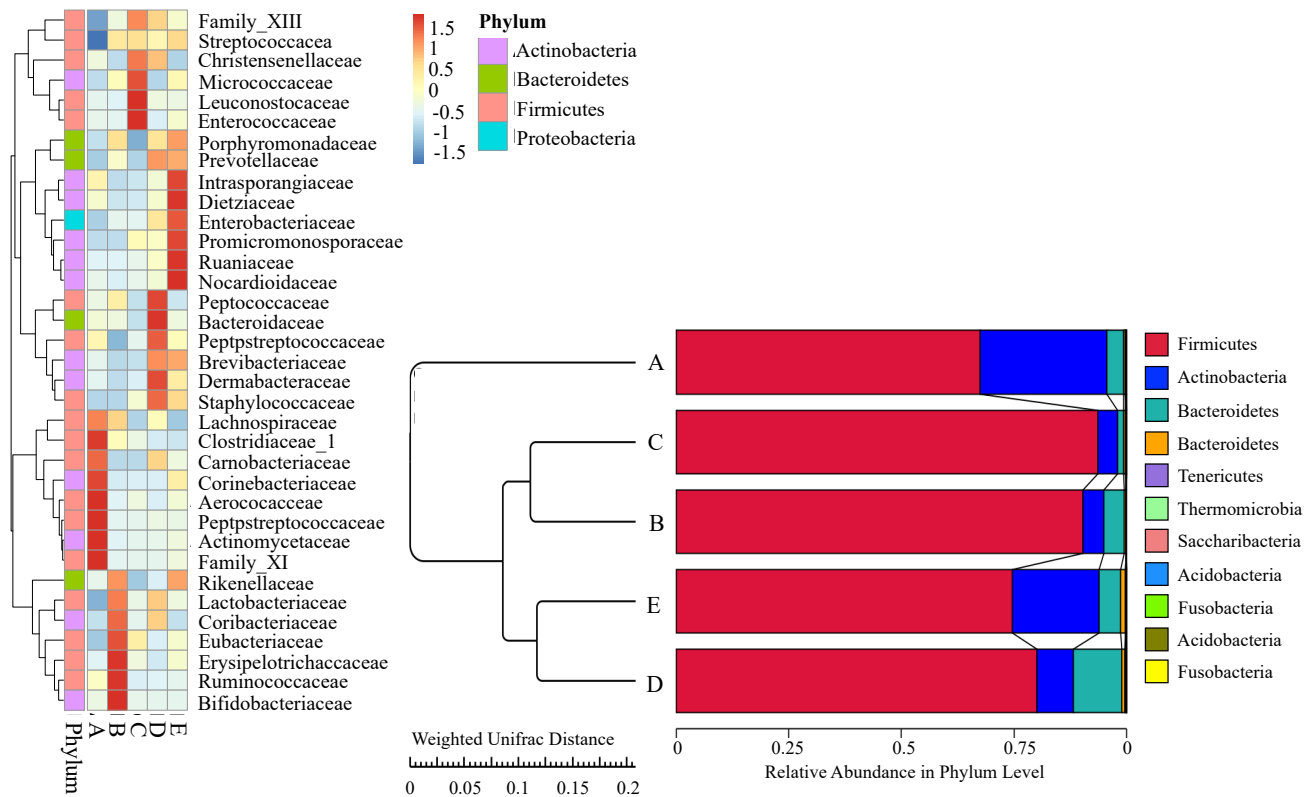


Figure 2. The diversity of bacteria at the Phylum level (a: bar chart) and abundance at the family level (b: heatmap chart). Alphabetic code representing location sample groups A: Semarang, B: Temanggung, C: Magelang, D: Pati, E: Salatiga

Table 3. The coefficient correlation score of heavy metals in duck' meat and bacteria family

Bacteria family	Coefficient correlation of metals in duck's meats					
	Cd	Cu	Cu	Hg	Pb	Zn
Streptococcaceae	NA	0.58	-0.41	-0.87	-0.20	-0.95*
Bacillaceae	NA	-0.71	0.24	0.83	0.30	0.98**
Lactobacillaceae	NA	0.55	0.19	-0.48	-0.57	-0.63
Corynebacteriaceae	NA	-0.91*	-0.21	0.68	0.54	0.92*
Ruminococcaceae	NA	0.09	0.21	0.35	0.14	-0.02
Leuconostocaceae	NA	0.63	0.12	-0.30	-0.02	-0.47
Erysipelotrichaceae	NA	0.22	0.00	0.12	0.23	-0.28
Enterococcaceae	NA	0.53	-0.01	-0.25	0.16	-0.44
Bacteroidaceae	NA	0.10	0.26	-0.42	-0.88	-0.10
Peptostreptococcaceae	NA	-0.13	0.02	-0.44	-0.69	0.04
Family_XI	NA	-0.74	0.18	0.84	0.36	0.98**
Lachnospiraceae	NA	-0.32	0.62	0.73	-0.16	0.69
Brevibacteriaceae	NA	-0.23	-0.47	-0.66	-0.42	-0.19
Staphylococcaceae	NA	0.13	-0.28	-0.83	-0.64	-0.45
Peptococcaceae	NA	0.27	0.44	-0.33	-0.91*	-0.18
Rikenellaceae	NA	-0.34	-0.62	-0.05	0.43	-0.12
Christensenellaceae	NA	0.66	0.50	-0.43	-0.71	-0.37
Bifidobacteriaceae	NA	0.24	0.26	0.29	0.12	-0.14
Dietziaceae	NA	-0.60	-0.94*	-0.36	0.35	-0.01
Clostridiaceae_1	NA	-0.54	0.41	0.97**	0.36	0.92*
Coriobacteriaceae	NA	0.53	0.48	-0.18	-0.59	-0.40
Eubacteriaceae	NA	0.53	0.01	-0.12	0.16	-0.58
Intrasporangiaceae	NA	-0.72	-0.90*	-0.22	0.41	0.17
Micrococcaceae	NA	0.56	-0.27	-0.41	0.28	-0.67

Table 3. Continued

Bacteria family	Coefficient correlation of metals in duck's meats					
	Cd	Cu	Cu	Hg	Pb	Zn
Aerococcaceae	NA	-0.74	0.12	0.83	0.44	0.96**
Prevotellaceae	NA	-0.05	-0.46	-0.72	-0.43	-0.41
Porphyromonadaceae	NA	-0.21	-0.54	-0.50	-0.17	-0.30
Promicromonosporaceae	NA	-0.19	-0.91*	-0.68	0.20	-0.40
Nocardioidaceae	NA	-0.47	-0.98**	-0.45	0.38	-0.15
Dermabacteraceae	NA	0.02	-0.11	-0.66	-0.73	-0.24
Carnobacteriaceae	NA	-0.59	0.24	0.35	-0.28	0.74
Ruaniaceae	NA	-0.42	-0.96**	-0.52	0.30	-0.22
Actinomycetaceae	NA	-0.74	0.17	0.82	0.35	0.98**

\*Correlation is significant at the 0.05 level (2-tailed)

\*\*Correlation is significant at the 0.01 level (2-tailed). NA: Cannot be computed because at least one of the variables is constant

#### 4. Discussion

The ducks in Central Java come from *Anas platyrhynchos* L. species, which are farmed intensively in cages from eggs, duckling to mature. Duck cultivation mainly aims to produce eggs harvested after 6 months of maintenance. Farmers in several locations such as Pati, Semarang, and Salatiga use concentrated feed or pellets, shrimp, and fish to increase productivity. Concentrated feed is the main feed with the main components of carbohydrates from bran and corn, while shrimp and fish in flour are used as the primary source of protein. Farmers obtain the fish and shrimp from the northern waters of Java or the coast of Semarang City.

According to the result analysis of heavy metal content, the overall feed has heavy metals concentrated inside, especially Hg, above accepted contamination level, but this research did not evaluate metals content per each feed material. Metal contamination probably originated from small fish originating from the north coast of Java exposed to heavy metals from contaminants in the ocean. It is based on research conducted by Sabdono (2009), which shows the average metal Cd:  $6.41 \pm 0.68$  ppm; Cu:  $12.62 \pm 1.88$  ppm; Cr:  $2.57 \pm 4.77$  ppm; Pb:  $58.01 \pm 6.03$  ppm; and Zn:  $14.35 \pm 4.38$  ppm in coral tissue in the northern waters of Java. Moreover, other research has proven that several metals contaminate Java seawater (Junianto *et al.* 2017; Susanti *et al.* 2020b; Herawati *et al.* 2021). The metal released into the sea waters might be absorbed by corals and plankton, which then enter the food chain to nekton (fish) and benthos (shrimp and other crustaceans), which become raw material for fish meal for duck feed. Allegedly, heavy metal is derived from anthropogenic activity (Chu *et al.* 2019; Bessa *et al.* 2021), supported by the highest heavy metal findings in the study limited to industrial disposal sites, urban

waste, and rivers, including such as agricultural activities (Pradika *et al.* 2019).

Anthropogenic activity in agriculture is also likely to contribute significantly to heavy metals released to field irrigation. It is depicted from the results of this study that show high metal Hg, Cu, and Zn concentrations in water samples used by farmers. The contamination probably originated from fertilizer and manure for paddies fields (Pradika *et al.* 2019; Bessa *et al.* 2021). Although manure as an organic fertilizer for rice fields comes from cattle, contamination has occurred in animal feed is possible (Li *et al.* 2019; Xiao *et al.* 2020). It causes heavy metals contamination in the food chain from rice to livestock back to rice fields. This assumption follows the conditions of the farms in Temanggung, Magelang, and Salatiga, which are in the rice fields.

Previous research related to the impact of heavy metals on intestinal bacteria focused on three main aspects, (1) microbial composition (Zhang *et al.* 2019), (2) changes in microbial biomass (Wang *et al.* 2009), and (3) toxicity to individual organisms (Duan *et al.* 2020; Liu *et al.* 2020). The impact caused by the increase of the heavy metal concentrations from duck nutrition intake is still needed to be studied. Scientific research on the effects of heavy metal toxicity is still contradictory because several studies show that certain metals positively affect the host and antimicrobial properties of certain bacterial families (Giambò *et al.* 2021). Some metals such as Cu show the effect of increasing the host immune system (Guo *et al.* 2011) and Zn, which has antioxidant properties (Mariani *et al.* 2008; Tarushi *et al.* 2014) and is anti-inflammatory in enteric pathogen infections (Medeiros *et al.* 2019; Xia *et al.* 2021; Zhang *et al.* 2021).

On the other hand, specific studies that discuss the effects of Cd, Cr, Cu, Hg, Pb, and Zn on intestinal bacteria do not show the antimicrobial effect (Terzi

and Civelek 2020; Brila *et al.* 2021). It is probably because the metal is only absorbed under certain conditions as a trace element. Intestinal bacteria, like environmental bacteria, may also be opportunistic or only take and store metals if needed. In addition, the nature of bioabsorption and bioaccumulation of bacteria against heavy metals is also due to habitat polluted by heavy metals in very high concentrations. Meanwhile, the number of heavy metals in the intestinal tract or intestinal bacterial habitat is unknown in this study.

Other research results have shown that the effects of each heavy metal are selective on specific bacterial populations. For example, intake of high concentrations of Fe significantly reduces the number of bacteria from the Lactobacillaceae family and increases the concentration of Enterobacteria in the human intestine, reducing the number of anaerobic bacteria, Bifidobacteria, and Lactobacilli in mice (Skrypnik and Suliburska 2018; Parmanand *et al.* 2019) and opportunistic microorganisms such as *Salmonella*, *Escherichia coli*, and *Clostridia* (Gerós *et al.* 2020; Phipps *et al.* 2020). However, in this study, the presence of specific bacterial families is thought to play a role in the concentration of metals, especially Cu and Zn, in duck meat (Table 2). Based on the analysis results, an increase in bacteria from the family Dietziaceae, Intrasporangiaceae, Promicromonosporaceae, Nocardioideae, and Ruaniaceae might influence the decrease of Cu absorption in ducks. It is caused by the ability of these bacteria to bioremediate the Cu metal before the host's body absorbs it. In addition, Corynebacteriaceae may reduce the concentration of Cr metal, and Peptococcaceae play a role in reducing Hg in duck meat. In other words, the abundance of bacteria might protect against exposure to contamination and absorption of heavy metals in ducks (Yausheva *et al.* 2018).

In conclusion, heavy metal contamination in duck meat is related to pollutants originating from water and feed. There is a negative and positive correlation between the density of certain intestinal bacterial families and the concentration of heavy metals in duck meat. Metal intakes may also be affected by various metals entering together. The highest metal input concentration is Hg, reaching  $5.55 \pm 1.79$  ppm and meat reaching  $4.42 \pm 0.80$  ppm. The bacterial composition comprises the 4 highest phyla, sequentially including *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*. The relationship between heavy metals and intestinal bacteria shows a significant value with a coefficient of more than 0.90. Bacterial abundance is likely to protect ducks from the harmful influence of heavy metals that

come along with water and feed. Further research is needed regarding the direct impact of heavy metals on intestinal bacteria to improve the feed and maintenance of normal duck intestinal bacteria.

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