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The interaction of intestinal bacteria and feed: a case study of intensive-duck husbandry in Central Java

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ABSTRAK

Penelitian ini bertujuan untuk membandingkan kandungan gula dan asam amino dalam pakan itik dan usus serta pengaruhnya terhadap bakteri usus, guna meningkatkan kualitas itik. Penelitian ini merupakan penelitian observasional eksplorasi dengan melibatkan lima peternakan itik dari Kabupaten Semarang, Temanggung, Magelang, Pati, dan Salatiga, Jawa Tengah. Sebanyak 5 g isi usus dikumpulkan dari masing-masing lima itik, yang dipilih secara acak dari setiap peternakan. Pakan dan isi usus kemudian dianalisis menggunakan *high-performance liquid chromatography* (HPLC). Jenis asam amino tertinggi adalah glutamat, aspartat, lisin, valin dan isoleusin sedangkan yang terendah adalah asparagin yang terdapat di semua peternakan sampel. Isi usus segar yang baru diekstraksi kemudian ditaruh dalam botol gelap steril untuk analisis microbiome dengan primer 6S rRNA genome V3-V4 identifikasi. Konsentrasi glukosa, fruktosa, dan galaktosa di dalam usus mengalami peningkatan yang signifikan disebabkan oleh polisakarida yang tercerna. Komposisi bakteri berperan penting dalam mencerna polisakarida, sehingga mudah diserap oleh sel usus itik. Kelimpahan bakteri di semua lokasi sampel didominasi oleh filum Firmicutes, terutama ordo Lactobacilalles, Bacilalles, dan Clostridialles. Pemberian pakan yang berlebihan mungkin tidak efektif dalam penyerapan gula dan asam amino esensial, namun memainkan peran penting dalam perubahan keanekaragaman bakteri usus dalam menghasilkan komponen yang diperlukan untuk fisiologi bebek.

Kata kunci: itik, pakan, metagenomik, metabolisme, Jawa Tengah.

ABSTRACT

This study was aimed to compare sugars and amino acid content in duck's feed and the intestinal bacteria's effects to improve ducks' quality. This research was an observational exploration involving five duck husbandries from Semarang, Temanggung, Magelang, Pati, and Salatiga District, Central Java. A total of 5 g of intestinal contents were collected from each of the five ducks randomly selected from each husbandry. The feed and intestinal contents were then analyzed using high-performance liquid chromatography (HPLC). The highest amino acid types were glutamate, aspartate, lysine, valine, and isoleucine, while the lowest was asparagine found in all sample farms. Intestinal contents freshly extracted then placed in dark bottles sterile for microbiome analysis with primers 6S rRNA V3-V4 genome identification. The concentration of glucose, fructose, and galactose in the intestine has increased significantly caused by digested polysaccharides. The composition of bacteria plays a vital role in digesting polysaccharides, makes them quickly absorbed by duck's intestine cells. The abundance of bacteria in all sample locations was dominated by the phylum Firmicutes, especially Lactobacilalles, Bacilalles, and Clostridialles. Over feeding may not effective in sugar and essential amino acid absorption, however, it may play an essential role in the diversity of gut bacteria to produce necessary component for duck's physiology.

*Keywords***:** duck, feed, metagenomic, metabolomic, Central Java.

INTRODUCTION

In the current day, Statistics Indonesia or *Badan Pusat Statistik* (BPS) compiles a positive increase in the demand for duck's meat, reached 4.50% per year (BPS, 2020a). The market demand is supported by an increased duck population of more than 61 million in 2019, which dominated Java Island. Central Java Province contributes at least 11.14% of the national ducks demand and becomes the third largest duck producer in Java (BPS, 2020b). Interestingly, duck husbandry is located in different geographical conditions and may help the researcher develop a practical new approach to duck productivity.

Duck productivity is influenced by several factors, such as environment and maintenance patterns (Susanti *et al*., 2020), genetics, and mainly feed (Abouelezz *et al*., 2019). The component and feed compound on each farm is strongly influenced by local resources and financial conditions (Fouad *et al*., 2018). The duck feed must be composed by considering duck's sugar needs as energy sources and protein to gain muscle mass (improving meat quality) (Best *et al*., 2017). The composition of feed not only affects the productivity of duck meat but affects host-intestinal bacteria interactions (Clavijo and Flórez, 2018). Intestinal bacteria have an essential role in improving the quality of ducks' life. Therefore, enhancing intestinal bacteria diversity can be used as a treatment for increasing duck livestock quality. Intestinal bacteria have physiological support on providing essential vitamins, lipid acid, and sugar for ducks' growth. Understanding the effect of nutrition on the intestinal bacteria condition, and *vice versa*, the researcher and breeder, can formulate appropriate material for making duck feed. It leads poultry farming to reduce cost production but increase productivity, effectively at the same time.

Further, giving an incorrect feed composition results in dysbiosis that leads to duck obesity or low meat quality (Ran *et al*., 2020). This condition increases the risk of digestive system diseases and contributes to food insecurity. Hence, this study was aimed to compare the types of nutrition sugars and amino acids from feed and intestinal contents and their effect on the diversity of intestinal bacteria composition, as an effort to improve the quality of ducks as livestock.

MATERIALS AND METHODS

This study used an observational exploration design involving five duck-husbandries in Central Java. The sample location was selected by purposive sampling following inclusion criteria: 1) largest meat duck producer in Central Java; 2) intensively caged duck; representing the area of coastline, lowland, and highland regions; 3) not in an area affected by industrial contaminants and chemical pesticides, and 4) managed well in nurture. Based on the criteria, the research sites were determined, presented in Table 1.

The duck samples were selected based on the following criteria: 1) female and or male ducks aged more than three-months-old; 2) in health condition without any disease symptoms during the sampling process; 3) not being exposed to antibiotics within two weeks before sampling from either feed or drugs. The exclusion criteria used were any endoparasite found in duck's internal organs, possibly affecting the host's anatomical and physiological condition. Twenty-five ducks were randomly selected, and sacrificed, then 5 g of intestinal contents were taken aseptically. The intestinal contents of ducks were collected in a 25 mL container and 3 mL microtube as many as six tubes and frozen at -20°C until further analysis. Then, 1 kg of duck feed samples was taken from each farm. Besides, the ingredients of the feed were documented by interviewing with husbandry owners. The feeds per location were homogenized and dried in the oven, setting up at 40-50°C for three days.

High-performance liquid chromatography (HPLC) analyzes sugars and amino acids

The feed and intestinal contents were re-evaporated to leave 20% of humidity, then as much as 0.5 mL of solid intestinal contents were grinded and put in a threaded tube. Then, the sample was added with 10 mL of 6 N HCl to hydrolysis the organic material. The solution was incubated in the water-bath at 100ºC for 24 hours,

Table 1. Coordinate of research loci

Sites	City or Regency	Code	Degree Decimal (DD) Coordinate	Poultry farm characteristic
Tanjung Mas sub-district, North Semarang district	Semarang	A	-6.947028, 110.439056	Coastal area near to fish auction center
Gilingsari sub-district, Temanggung district	Temanggung	B	-7.315402, 110.151809	In high-land rice field area and far from resident
Plosogede sub-district, Ngluwar district	Magelang	C	-7.642265, 110.264626	In low-land rice field area, but closes to resident
Rejoagung sub-district, Trangkil district	Pati	D	-6.658436, 111.088812	In coastal area but near to rice field
Gemenggeng village, Ngrapah sub-district, Banyubiru district	Salatiga	E	-7.288893, 110.395703	In low-land rice field area, but closes to from resident

cooled at room temperature, and carefully evaporated, leaving 1 mL of solution. The sample was added with 0.01 N HCl until the volume up to 10 mL, the solution was filtered. Five standard solutions of sugar and amino acids were made by mixing 0.1; 0.5; 1; 2.5; 5 µg / mL of the measured component with distilled water using the ratio (v:v) for 5 mL total volume. The ortho-phthalaldehyde (OPA) reagent and buffer citrate pH 3.25 was used to stabilize the solution.

Before injecting into the HPLC, the OPA reagent and mobile phase solution (eluent) were filtered with a PTFE membrane and degassing. The standard solution and the sample solution were also filtered using cellulose nitrate membranes in the HPLC injector. The sample or standard solution was mixed with OPA reagent in a ratio of 1: 1, homogenized using a vortex, degassed and put into a syringe. Analyzes were performed using a Shimadzu LC-20AT HPLC with an SPD-20A (RF 20 A Fluorescence Detector) detector (Shimadzu U.S.A. Manufacturing, Inc: Oregon. USA) with buffered Na citrate pH 3.25, Wavelength detector at 450 nm, and a run time of 30 minutes.

Intestinal Bacteria Identification

The microbial genome was extracted from intestinal contents using the QIAamp DNA Stool Mini Kit (Qiagen, San Diego, California, US) and identified based on the 16S rRNA genome V3-V4 region for accurate and precise results (Yarza *et al*., 2014). The next-generation sequencing analysis then followed by bioinformatic analysis, and all required procedures, including diversity analysis, were described before in Susanti *et al*. (2020). The analyzed data were arranged and displayed using Microsoft Excel 2019.

RESULT AND DISCUSSION

The ducks' feed was arranged from various components from vegetable to animal-based product. Besides, most farmers in the sampling areas applied concentrate-feed to increase weight and antibiotics supplementation to boost duck health (Table 2).

The feed component composites varied both in proteins and sugars, which is the highest protein found in fish and shrimps contained feed, then the sugars were mostly found in dried-rice and bran. However, the analysis of amino acids

Table 2. The feed composition in each husbandry-based location

Feed composition	Husbandry Location				
	Semarang	Temanggung	Magelang	Pati	Salatiga
Shredded coconut	+				
Dried-rice	+		+	+	
Bran			+	+	
Chopped-fish	+			+	
Shrimp head-carapace	+				
Mussel		$+^{**}$			
Water spinach	$+^{*}$				
Concentrate-feed		$+^{(A)}$	+	$+^{(B)}$	$+^{(C-D)}$
Palm sugar		+	+		
Molasses			+		

Note: asterisk mark (*) indicates feeding period: *) for once a week; **) for once a month. The alphabet letter (A-D) indicate concentrate type with protein composition of 17-19% (A); maximum 37% (B); 37-39 (C and D). the maximum fiber content is 6% (A); 5% (B); 6% (C and D). Brand name and complete nutritional content are not presented for research purposes and avoiding conflicts of interest.

(Table 3) and glucose (Table 4) indicating the various feed components affects the concentration of digested amino acids and sugars in duck's intestine. Interestingly is, the feed mixed with chopped-fish and shrimp in Semarang has lower amino acids and sugar than Temanggung. Whereas the Temanggung duck breeder only use concentrate-feed, but it provides enough nutrient for the ducks. The high amount of amino acid absorption (more than 70%) was observed in several types of essential amino acids except methionine (Met), while the highest absorption occurs in the amino acid Tryptophan (Trp), which reaches 71-72% in Semarang and Temanggung; and 78-80% from the other loci (Figure 1).

The average sugar content in the gut fluctuated uniformly across duck in all husbandry. Polysaccharides found in duck's feed were not measured in the ducks' intestinal contents, but monosaccharides concentration has increased significantly (Figure 2). This situation confirms that the polysaccharide has digested and produced glucose and other monosaccharides.

The duck's feed was made from shrimp carapace containing much trehalose (Figueroa and Lunn, 2016), whereas seeds and plants have lots of raffinose and stachyose (Xu *et al*., 2016). The trehalose is a non-reducing sugar formed from two glucose units joined by C1α and C1 bonds. This bonding model keeps the non-reducing sugar in a closed ring. It does not experience glycation (binding to lysine or arginine in the group of aldehydes or ketones) (Figueroa and Lunn, 2016). The raffinose molecules are composed of glucose, galactose, and fructose, which bond to each other at C1 and C6 atoms (Gangl and Tenhaken, 2016). Simultaneously, the tetrasaccharide group stachyose comprises two α-D-galactose molecules and one α-D-glucose β-D-fructose molecule each (Gangl and Tenhaken, 2016; Tian *et al*., 2019). Both types of oligosaccharides cannot be digested by monogastric animals such as ducks but can be fermented into short-chain fatty acids by intestinal bacteria using the α-galactosidase enzyme (Dong *et al*., 2020).

In contrast to carbohydrates, a small proportion of amino acids may not experience absorption, especially non-essential amino acids (Figure 1). The remaining amino acids may be the residue from absorption by both ducks and bacteria. The absorption of amino acids and sugars was consistently at the same rate. Hence, the provision of excess amino acids may unnecessarily improve duck's metabolism and productivity. Moreover, extra feeding may instead result in lipid buildup inside cutaneous and liver tissue (Wei *et al*., 2020). Besides, intestinal bacteria may also offer several types of amino acids, both essential and non-essential (Apajalahti and Vienola, 2016). This is probably due to the retrograde peristaltic ability, which causes urine to have an influx from the cloaca to the cecum. This event provides uric acid for cecum bacteria, which is then catabolized into ammonia to compose

Table 3. The amino acid composition in feed and duck intestine

Amino Acid	Semarang (µmol/gr)			Temanggung (µmol/gr)			Magelang (µmol/gr)			Pati (µmol/gr)			Salatiga (µmol/gr)		
	Feed	IC	Abs.	Feed	IC	Abs.	Feed	IC	Abs.	Feed	IC	Abs.	Feed	IC	Abs.
Cys	2.07	0.73	1.34	2.26	0.80	1.46	1.94	0.70	1.24	1.75	0.63	1.11	1.81	0.66	1.16
Met †	5.14	1.80	3.35	5.54	1.82	3.71	4.87	1.62	3.25	4.49	1.49	3.00	4.62	1.54	3.08
Ser	7.51	2.44	5.07	8.07	2.49	5.57	7.14	2.13	5.01	6.60	1.94	4.66	6.78	2.00	4.77
Gly	8.15	2.54	5.61	8.74	2.75	5.99	7.74	2.45	5.29	7.16	2.01	5.15	7.35	2.13	5.22
Ala †	9.98	3.07	6.91	10.70	3.19	7.51	9.49	2.85	6.64	8.80	2.68	6.12	9.03	2.79	6.24
Ile †	9.78	2.97	6.81	10.48	3.09	7.39	9.30	2.69	6.61	8.63	2.47	6.15	8.85	2.54	6.31
Glu	**25.19**	7.61	**17.57**	**26.93**	**8.00**	**18.93**	**24.02**	**7.08**	**16.94**	**22.36**	**6.30**	**16.06**	**22.90**	**6.75**	**16.15**
Arg	12.32	3.72	8.61	13.20	4.03	9.17	11.74	3.37	8.37	10.90	3.07	7.83	11.17	3.17	8.00
His †	13.41	4.04	9.37	14.36	4.38	9.97	12.77	3.68	9.09	11.87	3.35	8.51	12.16	3.47	8.69
Asn	1.63	0.49	1.14	1.79	0.55	1.24	1.52	0.47	1.05	1.36	0.41	0.94	1.41	0.43	0.98
Tyr	9.40	2.82	6.59	10.08	3.06	7.02	8.94	2.72	6.22	8.29	2.49	5.80	8.51	2.57	5.93
Leu †	**14.00**	4.19	**9.81**	**14.99**	**4.52**	**10.47**	**13.34**	**3.82**	**9.51**	**12.40**	**3.49**	**8.91**	**12.70**	**3.61**	**9.09**
Asp	**20.52**	6.13	**14.40**	**21.95**	**6.64**	**15.31**	**19.57**	**5.70**	**13.87**	**18.21**	**4.99**	**13.22**	**18.65**	**5.16**	**13.49**
Pro	6.49	1.92	4.57	6.98	2.10	4.88	6.16	1.85	4.31	5.69	1.68	4.00	5.84	1.74	4.10
Thr †	8.51	2.50	6.00	9.13	2.73	6.40	8.09	2.39	5.69	7.49	2.18	5.31	7.69	2.16	5.52
Val	**17.96**	5.26	**12.69**	**19.21**	**5.62**	**13.59**	**17.11**	**5.09**	**12.03**	**15.92**	**4.52**	**11.40**	**16.31**	**4.67**	**11.63**
Phe †	7.07	2.06	5.01	7.59	2.25	5.34	6.72	1.98	4.73	6.21	1.80	4.41	6.38	1.87	4.51
Lys †	**19.02**	5.43	**13.59**	**20.34**	**5.90**	**14.44**	**18.13**	**5.01**	**13.12**	**16.87**	**4.56**	**12.31**	**17.28**	**4.95**	**12.33**
Gln	1.57	0.45	1.12	1.72	0.51	1.22	1.96	0.43	1.53	1.30	0.37	0.93	1.36	0.39	0.97
Trp †	3.12	0.87	2.26	3.38	0.96	2.42	2.95	0.61	2.34	2.70	0.52	2.18	2.78	0.55	2.23
Total AA content	202.86	61.03	141.82	217.42	65.40	152.03	193.49	56.66	136.84	178.98	50.95	128.03	183.57	53.16	130.41

Note: the bold number represent the five highest amino acid concentration in all observed subject. The dagger mark (†) indicates the essential amino acids

Table 4. The sugar contents in feed and intestinal content

Sugar	Semarang (µmol/gr)			Temanggung (µmol/gr)			Magelang (µmol/gr)			Pati (µmol/gr)			Salatiga (µmol/gr)		
	Feed	IC	Balance	Feed	IC	Balance	Feed	IC	Balance	Feed	IC	Balance	Feed	IC	Balance
Xylose	17.13	3.19	13.94	17.89	3.29	14.60	16.70	2.99	13.71	15.37	2.42	12.95	15.68	3.00	12.68
Arabinose	13.12	1.51	11.61	13.73	1.68	12.05	12.76	1.41	11.35	11.66	1.24	10.42	11.92	1.43	10.49
Rhamnose	21.98	4.72	17.26	22.92	4.99	17.93	21.44	4.29	17.15	19.81	3.77	16.04	20.19	4.59	15.60
Glucose $^{+}$	27.38	71.01	+43.63	28.62	74.23	+45.61	26.66	65.62	+38.97	24.45	55.85	+31.41	25.09	66.43	+41.34
Galactose	34.45	18.94	15.51	35.86	20.00	15.85	33.23	17.55	15.69	30.67	14.78	15.89	31.44	17.87	13.58
Mannose	20.56	20.42	0.14	21.42	21.37	0.05	19.81	18.99	0.81	18.23	15.97	2.26	18.71	19.23	-0.52
Trehalose$^{(P)}$	65.31	0.00	65.31	67.53	0.00	67.53	63.65	0.00	63.65	58.71	0.00	58.71	59.61	0.00	59.61
Raffinose$^{(P)}$	58.40	0.00	58.40	60.60	0.00	60.60	56.14	0.00	56.14	52.02	0.00	52.02	53.28	0.00	53.28
Stachyose$^{(P)}$	82.65	0.00	82.65	85.79	0.00	85.79	79.99	0.00	79.99	73.50	0.00	73.50	74.95	0.00	74.95
Fructose	0.00	36.18	+36.18	0.00	37.87	+37.87	0.00	33.39	+33.39	0.00	28.44	-28.44	0.00	33.89	+33.89
Total sugar															
Poly	206.36	0.00	206.36	213.93	0.00	213.93	199.77	0.00	199.77	184.23	0.00	184.23	187.84	0.00	187.84
Mono	134.62	155.97	+21.35	140.44	163.43	+22.99	130.59	144.24	+13.64	120.19	122.48	+2.29	123.03	146.45	+23.41

Note: The positive mark (+) indicates additional content as a result of polysaccharide digestion. The letter (P) shows the polysaccharide sugars

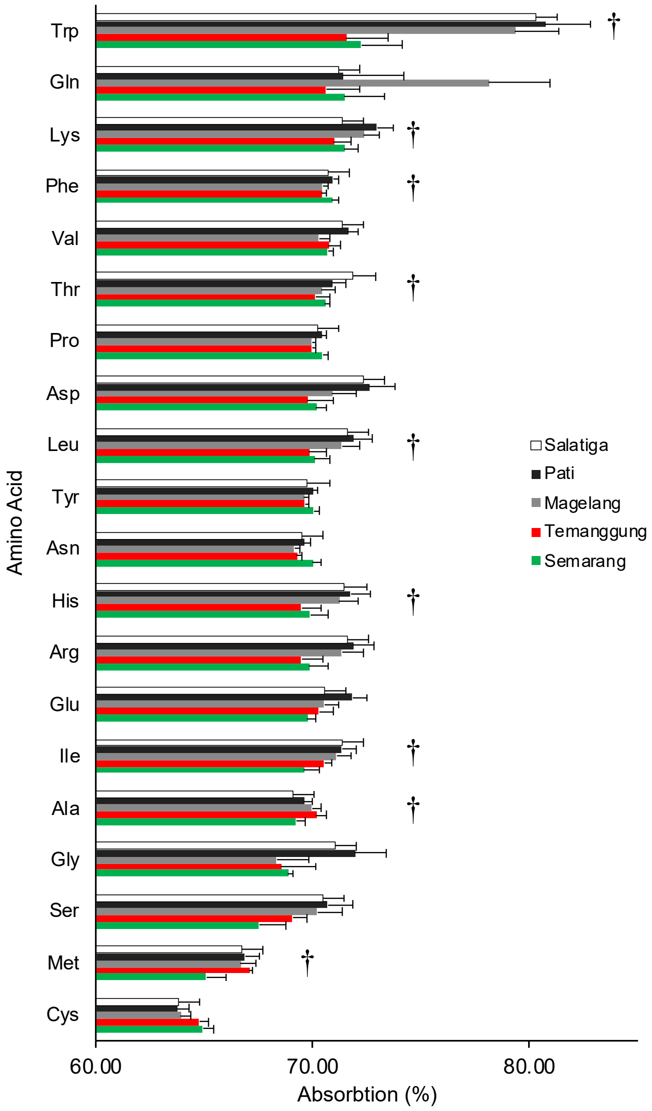

Figure 1. Percentages of amino acid absorption. The dagger mark (†) indicates the essential amino acids

amino acids (Lin *et al*., 2017).

Diversity of Duck Intestinal Duct Metagenome

The intestinal tract of ducks provides an ideal site for bacterial colonization that can mutually influence the host's anatomy and physiology (Wang *et al*., 2018). The presence of gut bacteria positively involves intestinal development and increases the ability to absorb feed nutrients (Wei *et al*., 2020). Variations in additional feed, such as shrimp carapace and plant fiber (in this study is water spinach), affect the intestine's physiology and bacteria composition (Cao *et al*., 2020). The bacteria diversity in ducks intestinal is varied between sampling areas. In total, 62,724 operational taxonomy unite (OTUs) was the maximum number of each sampling location to simplify the diversity and abundance index (Kim *et al*., 2017). The comparison of the intestinal bacteria variability of each duck implied by richness and evenness index, which is stated by Shannon index (H') and species indicated by Simpson index (*D*) (Hill *et al*., 2003). In addition, the number of species predicted by the ACE and Chao-1 index (taking into account rare species) shows that intestinal bacteria in ducks in Temanggung were more abundant (Figure 3). The bacteria diversity in ducks' intestine in Semarang

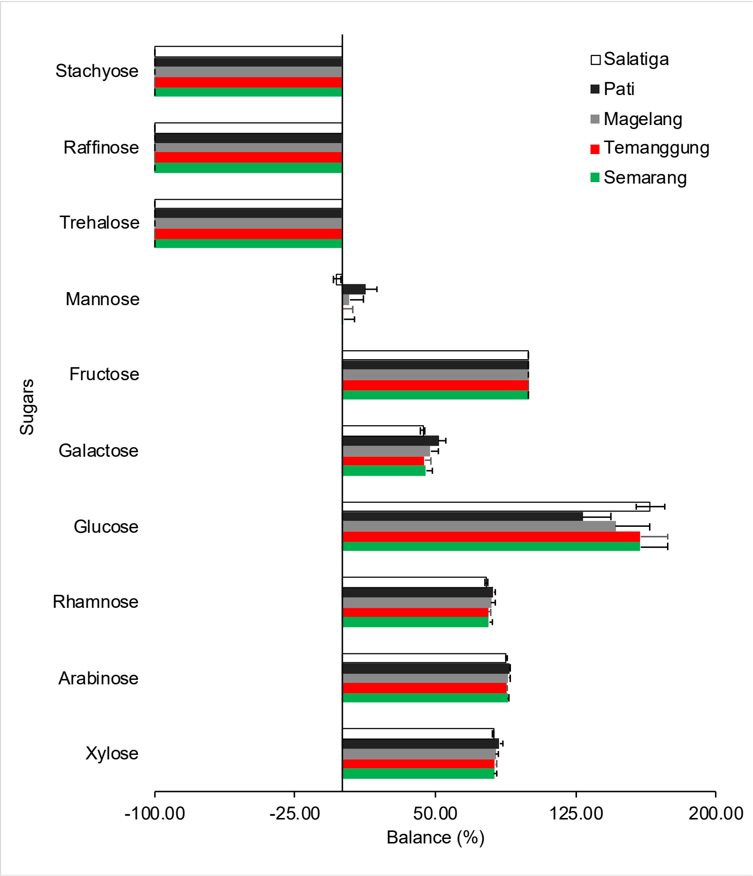

Figure 2. The sugars changing after digestion processes

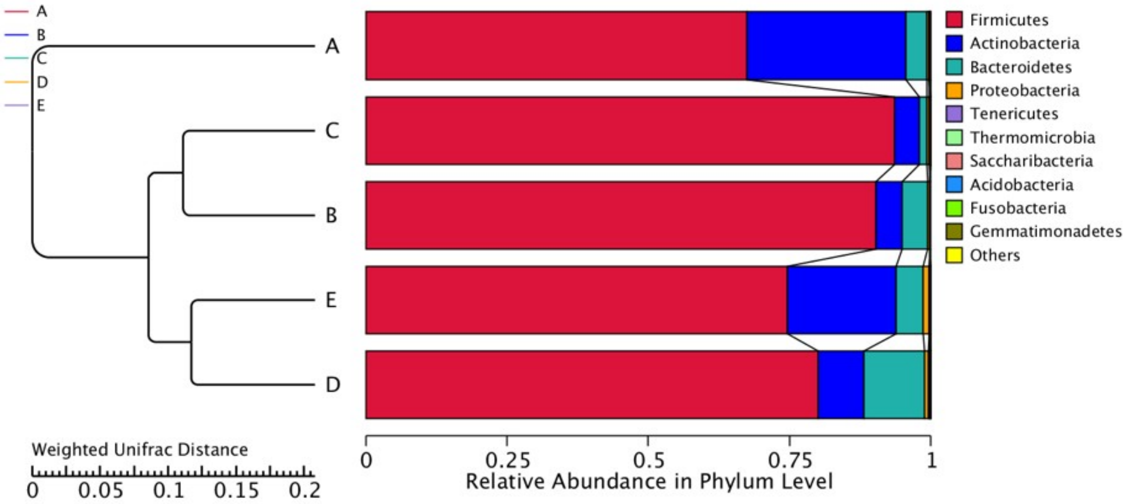

Figure 3. Abundance relative of duck's gut bacteria based on the phylum. The alphabet letter representing the research site: A) Semarang City; B) Temanggung District; C) Magelang District; D) Pati District; and E) Salatiga City.

husbandry is higher than in other places, but at the same time, maybe the highest overlapped species (dominated by particular species). Meanwhile, the highest bacteria diversity is predicted can be found in the ducks in Temanggung husbandry.

Based on the analysis, Firmicutes was the dominant bacterial families in all sample. This phylum was found in all ducks, with more than 67% of the total identified OTUs (Figure 3). The highest abundance of Firmicutes reached more than 93.65% in ducks from Magelang, followed by Temanggung, which reached 90.25%, Pati at 80.03%, and Salatiga at 74.58%. Actinobacteria dominated the second-largest abundance of bacteria with 4.37-28.19%, and Bacteriodetes in third place from 1.30-10.76%. In Pati, Bacteriodetes reached 10.76% is the second-largest bacteria phylum, above Actinomycetes, which was only 8.17%. The other seven phyla, which include Proteobacteria, Tenericutes, Thermomicronia, Saccharibacteria, Acidobacteria, and Fusobacteria Gemmatinonadetes, have an abundance of <1%.

Based on species richness and evenness, the ducks' similarity in microbiome variability was separate clade with other cities (Figure 4). This may be due to the effect of chopped-fish and shrimp head-carapace as primary protein sources and shredded coconut for fatty acids sources. Interestingly, even though Semarang and Pati's duck is cultivated in coastal areas and has the same type of feed component, they seem to have no relative similarity in intestinal bacteria composition. It is explaining the possibility of other factors besides feed may contribute to intestinal bacteria diversity. It happens is still debatable, considering the abundance of the intestinal bacteria in ducks from Pati is more similar to duck from Salatiga, whereas the environmental conditions are different.

Regarding the composition of bacteria, the top 10 abundance bacterial orders from a total of 72 orders were dominated by the phylum Firmicutes (5 orders), Actinobacteriales (3 orders), and one order Bacteroidetes and Proteobacteria, respectively. Furthermore, more than 50% of the intestinal bacteria in all duck was dominated by Lactobacillales followed by Clostridialess from Firmicutes, except in Semarang City. The abundance of the microbiome in Semarang City was dominated by Bacillales and Clostridiales from Firmicutes and Corynebacteria from the phylum Actinobacteria (Figure 4).

The distribution of bacteria by order shows that the abundance and density of bacteria are quite diverse and show unique characteristics (Figure 5). These characteristics are indicated by a dominant group of bacteria that are specifically owned only by certain husbandry. However, it needs to be realized that the microbiome's presence is strongly influenced by environmental conditions and the type of daily feed.

Intestinal bacteria digest the remaining food

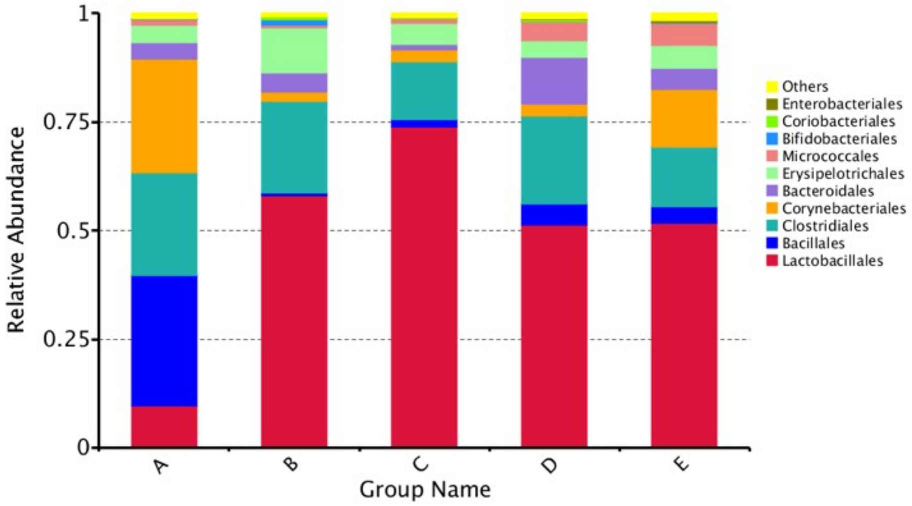

Figure 4. The intestinal bacteria abundance of ducks in five different location.

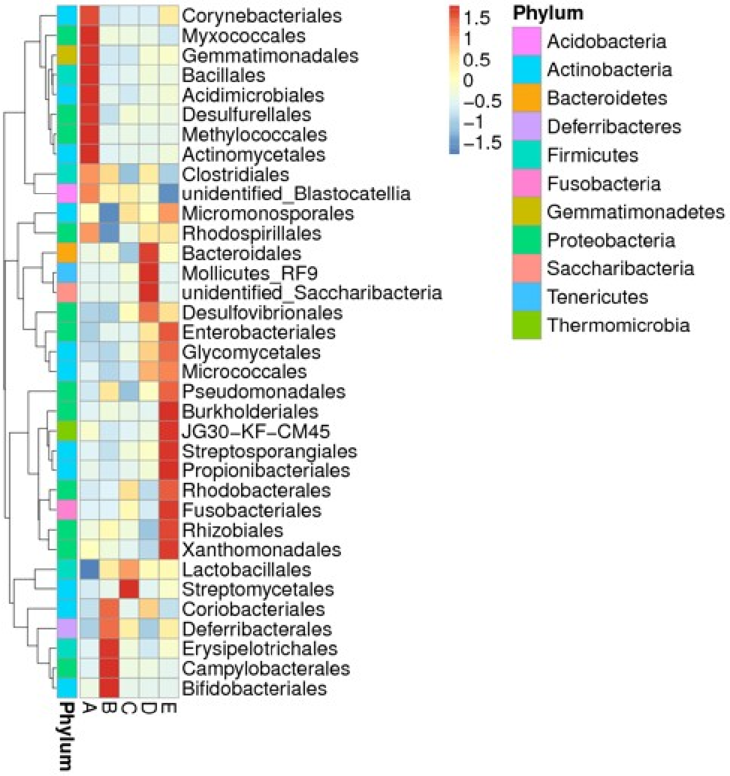

Figure 5. Order heatmap distribution of intestinal bacteria density and abundance.

substrate for an energy source. Most of the bacteria, especially Firmicutes and Bacteroidetes groups, can hydrolyze polysaccharides and oligosaccharides (Liu *et al*., 2018). The glucose or other monosaccharides then fermented by acidic and lactic bacteria producing short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate. The host can use the SCFA as a source of energy and carbon (Patterson *et al*., 2014). Previous studies have provided evidence that SCFAs, especially butyrate, can serve as an essential energy source for intestinal epithelial cells (Bedford and Gong, 2018).

Physiologically, the duck's digestive tract is not long enough (when compared to mammals) to absorb all of the nutrients. It results in fast transit molecules that make it digested unwell, then the sugars and simple peptides are abundant and not much absorbed (Khaleel and Atiea, 2017). These conditions provide a suitable environment for bacteria, especially Firmicutes, Actinobacteria, and Bacteriodetes, which can fermenting hexose, especially glucose and fructose, in limited oxygen levels (Frommeyer *et al*., 2020). The bacteria from Clostridiales and Bacillales order group play a role in hydrolyzing polysaccharides into monosaccharides. Some members of the Bacillales can ferment glucose into SCFA, which is beneficial to ducks (Even *et al*., 2018; Frommeyer *et al*., 2020; Y. Liu *et al.*, 2018). And also, many of the members of these orders have broader roles such as producing vitamins and antibiotics, which contribute to the duck's microhabitat and physiology.

This study's phylum of Actinobacteria was dominated by four orders: Corynebacteriales, Micrococales, Bifidobacteriales, and Coriobacteriales. The Corynebacteriales bacteria play a role in fixing nitrogen as an essential material for protein-making (Binda *et al*., 2018). Therefore, bacteria of this order may act as a source of amino acids for the host. Furthermore, a high protein diet stimulates an increase in Bifidobacterium, which can increase digestive enzymes' activity so that more amino acids are produced. The high level of amino acids found in the intestines is also likely to be a bacterial activity of the order Bifidobacteria (Michl *et al*., 2019).

CONCLUSION

It may unnecessary to increase duck's metabolism in order to gain the meat or eggs quality using high-sugar or protein contained feed, rather than feeding with nutritious feed in right amount to support intestinal bacteria. The abundance of duck intestinal bacteria among cities has similar characteristics, where the Firmicutes is dominant. Lactobacillus, Bacilalles, and Clostridialles' orders belong to Firmicutes was abundance likely due to high polysaccharides, including stachyose, raffinose, and trehalose from grains. Meanwhile, the majority of the remaining amino acids are non-essential amino acids that can be produced by the body. Furthermore, the presence of amino acids in the intestines may also be used by bacteria to produce digestive enzymes. Further research shall be conducted by investigating the correlation between intestinal bacteria in sugar and protein metabolism to the host's metabolism.

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