THE EFFECT OF NONI (*Morinda citrifolia*) FRUIT MEAL AS FEED ADDITIVE ON INTESTINAL MICROFLORAS AND VILLI CHARACTERISTICS OF HYBRID DUCK

PENGARUH TEPUNG BUAH MENGKUDU (Morinda citrifolia) SEBAGAI ADITIF PAKAN TERHADAP MIKROFLORA DAN KARAKTERISTIK USUS ITIK HIBRIDA

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ABSTRACT

Noni fruit is a medicanal plant with biological activity such as antimicrobial and antioxidant that could potentially be used as a feed additive in poultry. This research investigated the effect of noni fruit powder as feed additive on intestinal microflora and villi characteristics of hybrid duck. The subjects for this study were one hundred fifty of 2-week-old hybrid ducks were used in this study, divided into 5 dietary groups, namely P0: basal feed serving as negative control, P1: basal feed + 1% noni fruit powder, P2: basal feed + 2% noni fruit powder, P3: basal feed + 3% noni fruit powder, P4: basal feed supplemented with tetracycline antibiotic (300 mg/kg feed) as positive control. Data were analyzed by one-way of Completely Randomized Design and if there was significant effect followed by Duncan's Multiple Range Test. The result show that levels of noni fruit powder significantly influence (P<0.01) intestinal microflora. Total Escherichia coli of intestinal microflora was decreasing, however total Lactic acid bacteria was equal to negative control. Intestinal villi characteristics show that significantly different (P<0.01) with used noni fruit powder. Treatment noni fruit powder 1% can increase villus length and villus surface area.

(Key words: Duck, Intestinal changes, Noni fruit, Phytobiotic)

INTISARI

Mengkudu merupakan salah satu jenis tanaman obat yang mempunyai senyawa bioaktif antimikroba dan antioksidan yang berpotensi digunakan sebagai feed additive pada ternak unggas. Penelitian ini bertujuan mengetahui pengaruh penggunaan tepung buah mengkudu sebagai aditif pakan terhadap mikroflora usus dan karakteristik villi itik hibrida. Materi penelitian adalah 150 ekor itik hibrida yang berumur 2 minggu terbagi menjadi 5 kelompok perlakuan, yaitu P0: pakan basal disebut kontrol negatif, P1: pakan basal + 1% tepung buah mengkudu, P2: pakan basal + 2% tepung buah mengkudu, P3: pakan basal + 3% tepung buah mengkudu, P4: pakan basal dengan suplementasi antibiotik tetraciklin (300mg/kg pakan) sebagai kontrol positif. Data yang diperoleh dianalisa Rancangan Acak Lengkap satu arah dan jika terdapat perbedaan yang nyata dilanjutkan dengan uji lanjut Duncan's. Hasil penelitian menunjukkan bahwa level tepung buah mengkudu memberikan pengaruh yang sangat nyata (P<0,01) terhadap mikroflora usus. Jumlah Escherichia coli pada mikroflora usus mengalami penurunan, meskipun jumlah bakteri asam laktat sama dengan kontrol negatif. Karakteristik villi menunjukkan perbedaan yang nyata (P<0,01) dengan penggunaan tepung buah mengkudu. Perlakuan tepung buah mengkudu 1% dapat meningkatkan panjang villi dan luas permukaan villi.

(Kata kunci: Buah mengkudu, Fitobiotik, Itik, Perubahan usus)

Introduction

A variety of chemical feed additives are used in poultry feeds to maximize the production efficiency, product quality and control diseases. However, the negative effect of feed additives on the consumers is due mainly to the remaining residue in the poultry products. Because of that, it is necessarily important to explore more about natural phytobiotic to replace the chemical ones. Phytobiotic is widely used in traditional medicine in Asia and Africa.

Feeding diets containing phytobiotic may result in inhibition of the growth and colonization of enterophatogenic microbes in the digestive tract, thus contributing to

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improve balance of gut microflora (Harris *et al*., 2001). Improvement of balance digestive microflora could enhance performances and health of poultry. Changes structure and morphology of villi might be increase performances production of poultry which play a substantial role in the digestion and absorption of nutrients in the gastrointestinal tract. An increases in the size and height of intestinal villi contributes to the nutrients absorption which leads to improvement on the productivity (Jamroz and Kamel, 2002; Zulkifli *et al.,* 2009). The health effects may be partly due to the development and responses of the host toward immune system against pathogenic and nonpathogenic antigens (Jang *et al.,* 2007).

Noni fruit (*Morinda citrifolia*) is a plant with bioactives substances like phenolic compounds, organic acids and alkanoids. The phenolic compounds are antraquinones, aucubin, asperuloside and scopoletin. The main organic acids are caproic and caprylic acid. The alkanoid is xeronine (Wang and Su, 2001; Dittmar, 1993; Chan-Blanco *et al.,* 2006). Singh (2012) has reviewed biological activity of noni including antibacterial, antifungal, antiviral, antihelmintic, antioxidant, hepatoprotective, antiobesity and hypoglycaemic, analgesic, anxiolytic, anti-imflamatory, hypotensive, cardiovascular activity, estrogenic, immunological and anticancer.

The aim of this study is to investigate to response of the hybrid duck toward the gradual-supplemented diet of the noni fruit powder in their intestinal microflora and villi characteristics.

Materials and Methods

Material

One hundred and fifty of 2-weeks-old hybrid ducks unsexing from local hatchery were used. They were randomly allotted to 30 experimental units. Each experimental unit was 70x80x40 cm in size and it was used for 5 duck up to they reach 56 days of age. Each unit was equipped with waterer and feeder. The ducks were raised on litter-type floor.

The basal experimental diet was formulated according to the standards of National Research Council (1994). The composition of basal diet used shown in Table 1 was formulated as an antibiotic-free diet. Feed and water were provided *ad libitum* throughout the experimental period.

Methods

The method used for this study is experimental with 5 different treatments and 6 replications. The treatments were as follow: P0: basal feed without supplementation of either noni fruit or tetracycline antibiotic serving as negative control; P1: basal feed + 1% noni fruit powder; P2: basal feed + 2% noni fruit powder; P3: basal feed + 3% noni fruit powder; P4: basal feed supplemented with tetracycline antibiotic (300mg/kg feed) as positive control.

Feeds were given twice a day in the morning (07.00 am) and afternoon (15.00 pm). Noni fruit powder was prepared by selecting ripe yellowish fruit, cutting into small slice, dried in the 50^{o}C oven for 48 h and then ground into powder .

Intestinal microflora. Approximately 1 g intestinal digesta samples were taken gently from 15 cm end of small intestine of 156-days old duck per experimental unit. The digesta samples were then placed in a sterile test tube, kept in an ice containing stereoform box, then brought to the laboratory. In the laboratory, the samples were then immediately diluted with distilled water at a ratio of 1:1. The diluted samples were then plated onto selective agar, of which Mac-Conkey agar and de Man Rogosa Sharpe agar were used for growing *Escherichia coli* and lactic acid bacteria, respectively. All plates were incubated at 37^{o}C for 24 h, before calculating the number of colony (Benson, 2002).

Intestinal characteristics. Parameters recorded included villus height (from trip of villus to the crypt opening), villi surface area (μm^2/villi) calculated as [(villi basal width+villi apical width)/apical width] x villi height, and crypt depth (from the base of the crypt to the level of crypt opening) (Iji *et al.*, 2001). The ileum, defined as region Mackel's diverticulum to a point 10 cm proximal to the ileo-caecal junction, was dissected and the contents were collected. The collected samples were put into bottles immersed with 10% formalin solution. Then the samples were cut perpendicular to the longitudinal axis and embedded in paraffin wax. Transversal sections were cut (2-3μm), stained by Hematoxylin-Eosin and analyzed under a light microscope (Sugito *et al*., 2007).

Data analysis

Data were analyzed using one-way of Completely Randomized Design ANOVA and

Table 1. Composition and nutrition content of basal diet

Feedstuffs	-- (%) --
Yellow corn	56.52
Soybean meal	11.68
Polished rice	20
Fish meal	10
Coconut oil	1.5
Premix*	0.3
Analyzed composition, DM (%)	
Metabolizable energy (kcal/kg)**	3150
Crude protein (%)	18.28
Crude fat (%)	5.93
Crude fiber (%)	4.08

(*) One kg Premix contained: Vit A 12,000 IU, Vit D3 2,000 IU, Vit E 8 IU, Vit K3 2 mg, Vit B1 2 mg, Vit B2 5 mg, Vit B6 0.5 mg, Vit B12 0.012 mg, Vit C 25 mg, Ca-D-pantothenate 6 mg, Niacin 40 mg, Cholin Chloride 10 mg, Methionine 30 mg, Lysine 30 mg, Manganese 120 mg, Iron 20 mg, Iodine 0.2 mg, Zinc 100 mg, Cobalt 0.2 mg.
(**) calculated as 70% of Gross Energy.

if there was significant effect followed by Duncan's Multiple Ranges Test (Gomez and Gomez, 1995).

Results and Discussion

Intestinal microflora

The effects of noni fruit powder on intestinal microflora including non-phatogenic (lactic acid bacteria) and phatogenic (*Escherichia coli*) bacteria were summarized in Table 2. The suplementation of noni fruit powder increased significantly ($P<0.05$) population of small intestinal lactic acid bacteria and *Escherichia coli* as compared with positive control (P4). However, comparative result toward negative control (P0) indicated that lactic acid bacteria was significantly decreased ($P<0.05$) as levels of noni extract increase, except for P2. This implies that the use of noni extract suppressed count of lactic acid bacteria, but not as severe as the antibiotic use. The reason is antibiotics which has wide spectrum would be able to kill not only gram positive but also gram negative bacteria. As a result, the more non-pathogenic bacteria like lactic acid bacteria were also killed by antibiotics.

On the other hand, the result for *Escherichia coli* count indicated that positive control group was significantly ($P<0.05$) able to kill more *Escherichia coli* than those of noni powder added groups. The antimicrobial compounds of noni fruit which was able to decrease *Escherichia coli* count in the intestine included phenolic compounds, organic acid and alkanoids. Singh (2012) reported that *Morinda citrifolia* has bioactive compounds which function as antimicrobial enable to suppress *Escherichia coli* count in small intestine.

In particular phenolic compound, Oliver *et al.* (2001) proposed that the mechanism by which count of bacteria decrease is the phenolic substance in high concentration destructs cell membrane and protein denaturation of bacteria cell. As result, the supplementation 3% noni fruit powder decreased *Escherichia coli* (4.882) as compared with negative control (5.444 log CFU/ml) in intestinal microflora. Decreasing number of *Escherichia coli* due to the active compound of noni fruit powder, especially flavonoid. Puspitasari *et al*. (2012) reported that flavonoid compound would be able to inhibit gram positive bacteria better than gram negative bacteria. While organic acid according to Langhout (2000) could improve morphology of intestinal wall.

The highest amount of lactic acid bacteria (6.832 log CFU/ml) indicated by supplementation 2% noni fruit powder. The compound organic acid and essential oil of noni fruit powder increase total lactic acid bacteria in the digestive tract. Kurniaagung *et al*. (2012) reported that organic acid of *Citrus aurantifolia* in duck diets significantly increased ($P<0.05$) total lactid acid bacteria in the digestive tract. The growth of *Lactobacillus* species may repress pathogenic microorganism such as aerobic bacteria, *Coliform, Escherichia coli, Enterococus* in chicken jejunum and caecum. In Addition, *Lactobacilli* bacteria can cause some changes in the villi of the small intestine (Lan *et al.* 2004). Ghazanfari *et al*. (2014) reported that *Myrtus communis* essential oil decrease the colony forming unit of *Escherichia coli* and

Table 2. Intestinal microflora of hybrid duck supplemented by noni fruit powder in diet

Variable	Treatment	
	Lactic acid bacteria (log cfu/ml)	*Escherichia coli* (log cfu/ml)
P0	5.273±0.038[a]	6.815±0.035[d]
P1	5.175±0.041[bc]	6.481±0.035[b]
P2	5.277±0.031[a]	6.591±0.014[c]
P3	5.209±0.035[b]	6.627±0.01[c]
P4	4.885±0.067[c]	5.237±0.051[a]

P0: basal feed serving as negative control, P1: basal feed + 1% noni fruit powder, P2: basal feed + 2% noni fruit powder, P3: basal feed + 3% noni fruit powder, P4: basal feed supplemented with tetracycline antibiotic (300 mg/kg feed) as positive control.
[a,b,c] Means with different in treatment are significantly different (P<0.05).

Table 3. Intestinal characteristics of hybrid duck supplemented by noni fruit powder in diet

Treatment	Variable		
	Villi height (µm)	Villi surface area (mm^2)	Crypt depth (µm)
P0	571.74±145.8[a]	52.40±27.74[a]	137.52±13.72[a]
P1	753.24±161.1[b]	72.00±23.17[b]	170.27±32.63[b]
P2	514.32±37.3[a]	29.72±12.23[a]	160.71±16.8[b]
P3	588.10±33.7[a]	33.08±11.17[a]	166.06±25.9[b]
P4	568.74±78.8[a]	36.54±20.40[a]	125.25±33.1[a]

P0: basal feed serving as negative control, P1: basal feed + 1% noni fruit powder, P2: basal feed + 2% noni fruit powder, P3: basal feed + 3% noni fruit powder, P4: basal feed supplemented with tetracycline antibiotic (300 mg/kg feed) as positive control.
[a,b,] Means with different in treatment are significantly different (P<0.05).

increase the colony forming unit of *Lactobacilli* (P<0.05). Generally, the essential oil possesses the strongest antibacterial due to their property to fight against pathogens. Parts of essential oil components which have antibacterial effect are phenolic compounds such as carvacrol, eugenol and tymol (Brene and Roura, 2010).

Intestinal characteristic

The effect of noni fruit powder on intestinal characteristics including villus height, villus surface area and crypt depth were summarized in Table 3. The supplementation noni fruit powder effected significantly (P<0.05) on villus height, villus surface area and crypt depth of hybrid duck. Sunder *et al*. (2014) reported that broiler fed noni fruit juice, *Lactobacillus acidophilus* and noni fruit juice and *Lactobacillus acidophilus* mixture supplemented diets significantly improved the villi height and crypt depth. Singh (2012) reported that *Morinda citrifolia* has bioactive compounds which function as antimicrobial enable to suppress *Escherichia coli* count in small intestine. Therefore, antimicrobial activity of the noni fruit powder used in this study might be the cause of increased villus height and villus surface area, resulting in better intestinal health of the experimental birds. Garcia *et al*. (2007) reported that using medical plants in feed cause a higher villous in chickens. They suggest that medicinal plants decrease the total harmful bacteria in the intestinal wall and cause a reduction in production of toxic compounds and damage to intestinal epithelia cells such as shorter villi and deeper crypt.

Addition 1% noni fruit powder as feed additive increased villus height, villus surface area and crypt depth, while addition 2 and 3% indicated the result same as negative control. This improved intestinal characteristics of villi by the noni fruit powder justified the data on better feed efficiency and overall growth performance as reported by Singh *et al.* (2008). *Morinda citrifolia* fruit rich in the neutraceutical compounds, amino acids, vitamins, minerals and coenzymes might have played an important role in uptake of the nutrients in the gut and in turn directly or indirectly help in metabolism of the nutrient. Giannenas *et al*. (2010) reported that the counts of pathogenic bacteria increase in the gastrointestinal tract cause a shorter villus and a deeper crypt. The size and height of the villi are important for intestinal function. Diet is one of the important factors that could alter the morphology of intestinal villi.

In this study, supplementation of 1% noni fruit powder showed the increase villi height (753.24 µm), villi surface area (72,00

mm) and crypt depth (170,27 µm) if compared negative control. The increase may be indicated that the development of intestinal characteristics is a function of ratio between pathogenic and non-pathogenic population. The compound bioactive and essential oil of noni fruit powder are contributing to improve small intestinal morphology. Cross *et al*. (2007) reported that the essential oils or plant extract may act as balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry. The mucus layer in the small intestine plays an important role in protection of the small intestinal epithelial cells and in transport between the lumen and the brush border membrane and thus, the ontogeny of its development has extensive implications for intestinal function (Ghazanfari *et al*. 2014).

Conclusion

In the present study, the supplementation noni fruit powder can decrease of pathogenic bacteria and improve small intestinal morphology. The supplementation noni fruit powder 1% can increase villus length and villus surface area of hybrid duck. The result of this study suggested that noni fruit powder may be considered as an alternative to use antibiotic in poultry.

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References

Benson, H. J. 2002. Microbiological Applications 8th edn. Bacterial Population Counts. Fig. 21-1. McGraw Hill, New York.

Brenes, A. and E. Roura 2010. Essential oil composition in poultry nutrition: main effects and modes of action. Anim. Feed Sci. Technol. 158: 1-14.

Chan-Blanco, Y., F. Vaillant, A. M. Perez, M. Reynes, J. M. Brillouet and P. Brat. 2006. The noni fruit: A review of Agriculture Research, Nutritional and Teurapeutic Properties. J. Food Compos. Anal. 19: 645-654.

Cross, D. E., T. Acamovic, S. G. Deans and R. M. Cdevitt. 2007. The effect of dietary inclution of herbs and their volatile oils on the performance of growing chickens. Br. Poult. Sci. 43: 33-35.

Dittmar, A. 1993. *Morinda citrifolia* L. use in indigenous samoa medicine. J. Herbs, Spices and Medicine Plant 1: 77-79.

Garcia, V., P. Catala-Gregori, F. Hernandez, M. D. Megias and J. Madrid. 2007. Effect of formic acid and plant extracts on growth, nutrient, digestibility, intestine mucosa morphology and meat yield of broiler. J. Appl. Poult. Res. 16: 555-562.

Ghazanfari, S., M. A. Morabi and M. M. Bardzardi. 2014. Intestinal morphology and microbiology of broiler chicken fed diets containing Myrtle (*Myrtuscommunis*) essential oil supplementation. Ir. J. Appl. Anim. Sci. 4: 549-554.

Giannenas, I., D. Tontis, E. Tsaile, E. F. Chronis, D. Doukas and I. Kyriazakis. 2010. Influence of diatery mushroom agaricus bisporus on intestinal morphology and microflora composition in broiler chickens. Res. Vet. Sci. 89: 78-84.

Gomez, K. A. and A. A. Gomez. 1995. Prosedur Statistik untuk Penelitian Pertanian. Universitas Indonesia Press, Jakarta.

Harris, J. C., S. Cottrell, S. Plummer and D. Lioyd. 2001. Antimicrobial properties of *Allium sativum* (garlic). Appl. Microb. Biotech. 57: 282-286.

Iji, P. A., R. J. Hughes, M. Choct and D. R. Tivey. 2001. Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. Asian-Aust. J. Anim. Sci. 14: 54-60.

Jamroz, D. and C. Kamel. 2002. Plant extracts enhance broiler performance. J. Anim. Sci. 80: 41-46.

Jang, I. S., Y. H. Ko, S. Y. Kahn and C. Y. Lee 2007. Effect of commercial essential oils on growth performance, digestive enzyme activity and intestinal microflora population in broiler chikens. Anim. Feed Sci. Technol. 134: 304-315.

Kurniaagung, F., V. D. B. Ismadi dan I. Estiningdriati. 2012. Pengaruh penambahan jeruk nipis (*Citrus aurantifolia*) dalam ransum terhadap total bakteri asam laktat dan bakteri colifrom pada saluran pencernaan itik Magetan jantan. Anim. Agric. 1: 405-413.

Langhout, P. 2000. New additives for broiler chicken. Feed Mix. Int. J. Feed Nutr. Technol. 9: 24- 27.

Lan, P. T., M. Sakamoto and Y. Benno. 2004. Effects of two probiotic *Lactobacillus* strains on jejunum and caecal microciota of broiler chicken under acute heat stress condition as revealed by molecular analysis of 16S rRNA genes. Microbiology and Immunology 8: 917-929.

National Research Council. 1994. Nutrien Requrement of Poultry. 9th Revision edn. National Academi Press, Washington DC.

Oliver, S. P., B. E. Gillespie, M. J. Lewis, S. J. Ivey, R. A. Almeida, D. A. Moorrehead and H. H. Dowlen. 2001. Efficiency of a New Premilking, Teat Disinfectant Containing a Phenolic Combination for The Prevention of Mastitis. J. Dairy Sci. 84: 1545-1549.

Puspitasari, G., S. Murwani, dan Herawati. 2012. Uji Daya Antibakteri Perasan Buah Mengkudu Matang (*Morinda citrifolia*) terhadap Bakteri Methicillin Resistan *Staphylococcus Aureus* (Mrsa) M.2036.T secara *In Vitro*. Program Kedokteran Hewan. Universitas Brawijaya, Malang.

Singh, D. R., J. Sunder and R. C. Srivastava 2008. Peptide and mineral profile of *Morinda citrifolia* fruits and leaves. Int. J. Noni Res. 2: 72-78.

Singh, D. R. 2012. *Morinda citrifolia* L: A review of the scientific validation for its nutritional and therapeutic properties. J. Diabetes and Endocrinology 3: 77-91.

Sugito, W. Manalu, D. A. Astuti, E. Handharyati, dan Chairul. 2007. Morfometrick usus dan performa ayam broiler yang diberi cekaman panas dan ekstrak n-Heksan kulit batang taloh (*Salix tetrasperma* Roxb). Media Peternakan 30: 198-206.

Sunder, J., S. Tamilvana, P. Natesan, A. Kundu and M. S. Kundu. 2014. Effect of *Morinda citrifolia* fruit juice and *Lactobacillus acidophilus* on broiler duodenal morphology. Adv. Anim. Vet. Sci. 2: 28-30.

Wang, M. Y. and C. Su. 2001. Cancer preventive effect of *Morinda citrifolia* (Noni). Annals of the New York Academy of Sci. 952: 161-168.

Zulkifli, I., H. S. I. Rahayu, A. R. Alimon M. K. Vidyadara and S. A. Babjee. 2009. Gut microflora and intestinal morphology of commercial broiler chickens and Red Jungle Fowl fed diets containing palm kernel meal. Arch. Geflugelkd. 73: 49-55.