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Advances in Animal and Veterinary Sciences Research Article Effect of Sorghum and Indigofera Leaf Flour on Egg Quality, Daily Protein Intake, Phosphorus Availability and Total Colonies of Bacillus sp in Small Intestines of Laying Hens Ade Djulardi, Riesi Sriagtula, Ahadiyah Yuniza, Wizna*, Zurmiati Department of Animal Feed and Technology, Faculty of Animal Science, Andalas University, Padang 25163, Indonesia. Abstract | An experiment was performed to investigate the effects of sorghum and indigofera leaf flour as substitutes for corn in laying hen rations on egg quality, daily protein intake, phosphorus availability, and total colonies of Bacillus sp. in the small intestines of laying hens. A completely randomized study design was used in this research, with 4 treat- ments, and each treatment was repeated 5 times: A (0% sorghum + 0% indigofera); B (20% sorghum + 4% indigofera); C (30% sorghum + 5% indigofera); and D (40% sorghum + 6% indigofera). The phosphorus availability, daily protein intake, eggshell thickness, eggshell strength, egg yolk color, egg yolk fat, egg yolk cholesterol, and total colony count of Bacillus sp. in the small intestines were measured. The results showed that the use of sorghum and indigofera leaf flour did not significantly ($P>0.05$) affect the daily protein intake, phosphorus availability, eggshell thickness, eggshell strength, egg yolk color, egg yolk fat, and total colonies of Bacillus sp. in the small intestine. However, there was a highly significant ($P<0.01$) effect on egg yolk cholesterol. In conclusion, 40% sorghum and 6% indigofera leaf flour can be used instead of 100% corn in laying hens ration and can reduce egg yolk cholesterol up to 26.29%. Keywords | Egg yolk cholesterol, Egg quality, Indigofera leaf flour, Laying hens, Sorghum Received | March 30, 2021; Accepted | April 10, 2021; Published | June 01, 2021 *Correspondence | Wizna, Department of Animal Feed and Technology, Faculty of Animal Science, Andalas University, Padang 25163, Indonesia; Email: wizna@ansci.unand.ac.id Citation | Djulardi A, Sriagtula R, Yuniza A, Wizna, Zurmiati (2021). Effect of sorghum and indigofera leaf flour on egg quality, daily protein intake, phosphorus availability and total colonies of bacillus sp in small intestines of laying hens.. Adv. Anim. Vet.

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INTRODUCTION

T he availability of feed ingredients in the poultry farming business has been affected by imports of cereal feed ingredients such as corn, as sources of protein and al., 2019). Giving sorghum to poultry diet can harm egg yolk color as poultry that consumes sorghum may produc- es a paler egg yolk color. According to Freitas et al. (2014), quails fed containing sorghum without pigment added in a paler egg yolk color compared to quails fed containing sor- energy. Thus, the price of corn on the world market large- ghum that pigment added. In this study, the addition of in- ly determines the price of corn on the domestic market. digofera leaf flour, up to 6% in the ration can maintain egg One way to reduce the use of corn is to look for alter- yolk color, caused by β-carotene content in indigofera leaf native feed ingredients, such as sorghum [Sorghum bicol- flour. Indigofera leaves contain 507.6 mg/kg β-carotene, or (L.) Moench]. Sorghum has been used as a feed in- which functions as a source of antioxidants and egg yolk gredient in poultry rations (Ochieng et al., 2018; Selle et pigmentation (Palupi et al., 2014a). The use of carotenoids July 2021 | Volume 9 | Issue 7 | Page 956 NE US Advances in Animal and Veterinary Sciences in rations can improve the egg yolk color of laying hens 15% moisture. after drying, the Sorghum was used as a feed (Kotrbacek et al., 2013). Carotenoid content in egg yolk is ingredient for laying hens ration. b). Indigofera leaf flour: influenced by the consumed ration (Nys and Guyot, 2011). Indigofera leaf was collected and dried to obtain 10-15% Some researchers have previously reported the ability of moisture. Then the milling was carried out. After grinding carotenoids to improve the egg yolk color. Hammershoj et the Indigofera leaf flour, it was ready to be used as a feed al. (2010), stated carotenoid contain in rations can increase ingredient to prepare the ration for laying hens. the brightness of egg yolk color. Furthermore, Sangeetha and Baskara (2010) also report that laying hens can not Ration: HASH(0x7f842fd804d8) turn all carotenoids into vitamin A, however, the remaining concentrate for laying hens, corn, sorghum seeds, is used to color the yolk. Indigofera leaf flour, coconut oil, vitamin B12, and rice bran (composition of the ration is showed in Table 1). Furthermore, sorghum contains a phytic acid which range This study was conducted for six weeks with an adaptation from 0.2 to 2.4 mg/g (Abdelhalim et al., 2019). Phytic acid period of 10 days. Treatment rations are given twice a day cannot be digested by monogastric and has anti-nutritional i.e., at 08.00 PM and 15.00 AM as much as 125 g/bird/ properties that inhibit the absorption of various minerals, day, and drinking water was given ad-libitum. Probiotic including calcium and phosphorus. Phosphorus is one of waretha contains Bacillus amyloliquefaciens) as much as the essential nutrients for livestock, most of the phosphorus (43x1012 CFU/mL) in all treatments. Administration of in plant seeds is in the form of phytic acid (Shanmugam, probiotic waretha through drinking water. Before giving 2018). Phosphorus utilization can be increased by generally the probiotic chicken was satisfied for two hours. During adding phytase enzymes in poultry diets (Dersjan et al., the research, waretha probiotic was given once. 2015). In the present study, a probiotic waretha (probiotic waretha contains Bacillus amyloliquefaciens bacteria) is added. Bacillus amyloliquefaciens can produce the phytase Experimental Design and Treatment enzyme (Idriss et al., 2020). Phytase enzymes can degrade HASH(0x7f842fe2da68) phytate in the digestive tract of laying hens. Thus the use of study. There were four treatments A (0% sorghum + 0% calcium and phosphorus in the digestive tract of laying hens indigofera); B (20% sorghum + 4% indigofera); C (30% sorghum + 5% indigofera); and D (40% sorghum +

6% is more optimal. Calcium and phosphorus significantly indigofera), and all treatment was repeated five times. 10 affect eggshell quality, such as egg strength and thickness. laying hens were placed in battery cages for each treatment According to Ahmed et al. (2013), eggshell quality is influenced by calcium content in the diet. Furthermore, and replication. Jiang et al. (2013) reported that chickens fed with calcium levels of 2.62% in ration have weaker eggshells than Parameters Measured those fed with calcium levels ranging from 3.70 to 4.40%. Phosphorus availability: Phosphorus availability calcu- Additionally, Bacillus amyloliquefaciens also produces lated according to Sibbald (982). various types of enzymes that help digest food substances in the small intestine of laying hens, including cellulase, P= Phosphorus protease, and amylase enzymes (Ye et al., 2017; Nassar et al., 2015; Abd-Elhalem et al., 2015). Furthermore, Bacillus Daily protein intake: Daily protein intake is the amount amyloliquefaciens has been reported as a bacterium that can of protein consumed by the chickens. Protein consumption be used as a probiotic and can increase ration efficiency in is expressed in grams, calculated according to Tillman et al. pitalah ducks (Zurmiati et al., 2017). Based on the above (1998). Daily protein intake (g) = feed consumption (g) x findings, a study was conducted to investigate the egg crude protein of the rations (%). quality, daily protein intake, phosphorus availability, and total colonies of Bacillus sp in small intestines of laying Eggshell thickness: The eggs were cleaned and then bro- hens fed sorghum and Indigofera leaf flour. ken on a glass plate. The shells were separated from the egg contents. The thickness of the shell was measured by using MATERIALS AND METHODS a shell thickness micrometer on the pointed end, the blunt end, and the middle part. The measurement was recorded in mm (Aydin et al., 2008). The number of eggs used to Birds measure the eggshell thickness was 20, with four eggs for Two hundred ISA Brown laying hens aged 63 weeks were each treatment. used, with egg production at 80%. Eggshell strength: The measurement of eggshell strength Preparation of sorghum and Indigofera leaf flour: a). was performed by weighing the eggs, then placing them in Sorghum: sorghum was harvested and dried to obtain 10- a vertical position on an Instron plate. The eggs were com July 2021 | Volume 9 | Issue 7 | Page 957 NE US Advances in Animal and Veterinary Sciences HASH(0x7f842fd80748) experimental ration Feedstuff Experimental diets Sorghum and indigofera leaf flour in rations (%) A B C D Concentrate for laying hens 26.00 23.00 22.00 21.00 HASH(0x7f842fd809e8).00 100.00 100.00 Calculated analysis HASH(0x7f842fe401c8),63 Metabolism energy (kcal/kg) 2653.1 2692.6 2678.1 2659.3 Probiotic waretha (contain the Bacillus amyloliquefaciens bac- 43x1012 terium) (CFU/mL) 43x1012 43x1012 43x1012 Table 2: Average phosphorus availability, daily protein intake, eggshell thickness, and eggshell strength of laying hens fed sorghum supplemented with Indigofera leaf flour Sorgum and Indigofera leaf flour in rations (%) Phosphorus availability (%) Daily protein intake Eggshell thickness Eggshell strength (g/bird/d) (mm) (kg/mm2) A (0% sorghum + 0% Indigofera) 77.40 18.72 0.47 4.86 B (20% sorghum + 4% Indigofera) 60.30 18.71 0.46 4.42 C (30% sorghum + 5% Indigofera) 86.78 18.71 0.43 4.04 D (40% sorghum + 6% Indigofera) 72.67 18.70 0.45 4.33 SE 5.09 0.15 0.004 0.11 SE: Standard Erorr HASH(0x7f842fe42020)). pressed until they broke. Reference graphs can be used to calculate the strength of the eggshells (kg/mm); the higher the value in kg/mm2 for each measured egg, the higher the eggshell strength (Ranggana, 1986). The number of eggs used to measure the eggshell strength was 20, with four HASH(0x7f842fe41e28) eggs were HASH(0x7f842fd84280) quality of the egg yolk color was deter- mined visually HASH(0x7f842fe455a0) colors on the Roche Yolk Fan stand- ard score on a scale of 1-15 from pale to dark orange (con- centrated) (Vuilletjmier, 1968). The number of eggs used to measure the egg yolk color was 20, with four eggs for each treatment. HASH(0x7f842fd84610) test sample: 1 g sample of HASH(0x7f842fe45d50) in oil pa- per, and

dried in an oven at 105-110 oC. After that, HASH(0x7f842fd80688) 105-110 oC. HASH(0x7f842fd857d8) fat content was determined by using the Sox- hlet method. Fat content = a – b/c × 100%. Description: a = sample weight after the extraction process; b = sam- ple weight before the extraction process; and c = sample weight (AOAC, 2005). Egg yolk cholesterol: preparation of egg yolk cholesterol test sample: egg yolk cholesterol was measured using spec- trophotometry. 1 g of egg yolk sample is weighed and put into a centrifuge tube containing 10 ml of acetone al July 2021 | Volume 9 | Issue 7 | Page 958 NE US Advances in Animal and Veterinary Sciences Table 3: Average egg yolk color, egg yolk fat, egg yolk cholesterol, and total colony of Bacillus sp. in the small intestines of laying hens fed sorghum supplemented with Indigofera leaf flour Sorgum and Indigofera leaf flour in ration Egg yolk Egg yolk fat Egg yolk cholesterol Total colony of Bacillus (%) color (%) (mg/100g) sp (log CFU/ml) A (0% sorghum + 0% Indigofera) 8.50 26.66 903.96a 8.22 B (20% sorghum + 4% Indigofera) 8.75 26.37 981.49a 8.49 C (30% sorghum + 5% Indigofera) 8.50 26.10 881.66a 7.98 D (40% sorghum + 6% Indigofera) 8.25 25.84 509.79b 8.11 SE 0.26 26.08 726.70 0.12 SE: Standard Erorr Different superscript lowercase letters in the same column indicate a significant difference (P<0.01). cohol solution (1: 1), then boiled until the solution boils. After chilling, centrifuged at 3000 rpm for 15 minutes, the supernatant was taken and evaporated in a boiling water bath until the residue remained after that dilution was car- ried out with chloroform readings at 680 nm after turning green (± 5 minutes). Cholesterol level is calculated by com- paring the absorbance with a standard cholesterol curve. The analysis of egg yolk cholesterol was performed by the method of Salkowski and Liebermann-Burchard (Schunack et al., 1990). The equation was Y = 2.354X + 0.005 Y = Absorbance of cholesterol sample X = level of cholesterol (mg / 100ml) Total colony of Bacillus sp. in the small intestines: The population of Bacillus sp bacteria was observed after six weeks of treatment by taking one chicken per unit. The small intestine fluid is taken as much as 1 gram, then dilut- ed from 10-1 to 10-7. 1 ml of each 10-7 dilution of the sam- ple was inserted into the Petri dish, which had been filled with selective media of Bacillus sp then incubated at room temperature for 24 hours. The colonies grown in the Petri dish were then counted using a colony counter (Cappucino and Sherman, 1987; Hadioetomo, 1991). HASH(0x7f842fd85688) (ANO- VA), and HASH(0x7f842fe47350) (Steel and Tor- rie, 1995). RESULTS AND DISCUSSION The use of sorghum and indigofera leaf flour in the rations of laying hens did not affect (P>0.05) the daily protein intake (Table 2). Over time, no difference in phosphorus availability, thickness, and the eggshell strength of laying hens from the five treatments was observed (P>0.05; Table 2). The use of sorghum and indigofera leaf flour in the ra- tions of laying hens did not affect (P>0.05) egg yolk color, egg yolk fat, and Bacillus sp. total or colony count in the small intestines (Table 3); however, it had a highly signifi- cant effect (P<0.01) on the egg yolk cholesterol of the lay- ing hens (Table 3). The sorghum and indigofera leaf flour are as palatable as the corn in the rations. The uniformity of the values of dai- ly protein intake and phosphorus availability indicate that there were no negative effects from using 40% sorghum and 6% indigofera leaf flour in the rations of the laying hens. This was due to the nutritional quality, especially the ration protein content in all treatment rations that were equal in amount so that the inclusion of each level of sor- ghum and Indigofera leaf flour in rations had the same ration quality. This results in the same amount of protein consumed and utilized by layer chickens so that the re- sulting daily protein consumption is the same. Leke et al (2018), stated that protein intake affects egg production and that a decrease in protein consumption can cause a de- crease in egg production. The HASH(0x7f842fe47878) et al., 2019). The average daily protein intake in this study ranged from 18.70 to 18.72 g/bird/day. These averages HASH(0x7f842fd849d0) aver-

age daily protein intake HASH(0x7f842fe1fee8) 17.20 g/bird/day. The use of up to 40% sorghum and 6% indigofera leaf flour also does not affect phosphorus availability. Sorghum con- tains a phytic acid ranging from 0.2 to 2.4 mg/g (Abdel- halim et al., 2019). The utilization of phytic acid as a source of phosphorus in chicken rations through the hydrolysis of phytate-bound phosphate can increase the efficiency of the use of phosphorus in rations. The use of feed ingredi- ents that contain high phytic acid levels hurts poultry be- cause they cannot break down phytic acid in their digestive tract. In this study, the use of waretha probiotic (containing Bacillus amyloliquefaciens) can produce phytase enzymes, which will help degrade phytate in sorghum in the small July 2021 | Volume 9 | Issue 7 | Page 959 NE US Advances in Animal and Veterinary Sciences intestine of poultry. Bacillus amyloliquefaciens can produce the phytase enzyme (Idriss et al., 2020), which can degrade the phytate in the digestive tract of laying hens. Thus the use of calcium, phosphorus, and protein HASH(0x7f8430c8d670) more optimal. The addition of the phytase enzyme to poultry rations increases the hydrolysis of phytic acid, and the availability of minerals, amino acids, and energy for the poultry and therefore increasing their growth (Khan et al., 2013). Besides that, Bacillus amyloliq- uefaciens also produces various types of enzymes that help digest food substances in the small intestine, including cel- lulase, protease, and amylase enzymes (Ye et al., 2017; Nas- sar et al., 2015; Abd-Elhalem et al., 2015). Furthermore, Bacillus amyloliquefaciens has been reported as a bacterium that can be used as a probiotic and can increase ration effi- ciency on pitalah ducks (Zurmiati et al., 2017). Furthermore, the use of sorghum and indigofera leaf flour in the rations HASH(0x7f842fdec5e0) of eggshells. Sorghum contains a phytic acid range from 0.2 to 2.4 mg/g (Abdelhalim et al., 2019). Phyt- ic acid cannot be digested by monogastric and has anti-nu- tritional properties that inhibit the absorption of various minerals, including calcium and phosphorus. Phosphorus utilization can be increased by generally adding phytase enzymes in poultry diets (Dersjan et al., 2015). In this study, a probiotic waretha was added (probiotic waretha is containing Bacillus amyloliquefaciens bacteria) Bacillus am- yloliquefaciens can produce the phytase enzyme (Idriss et al., 2020), which can degrade phytate in the digestive tract of laying hens. Thus the use of calcium and phosphorus HASH(0x7f842fdce0f0) more optimal. Calcium and phosphorus significantly affect eggshell quality, such as egg strength and thickness. The primary nutrients that affect eggshell quality are calcium, phosphorus, and vitamin D3 (Neijat et al., 2011; Xiao et al., 2014). According to Ahmed et al. (2013), eggshell quality is influenced by calcium con- tent in the diet. Furthermore, Jiang et al. (2013) reported that chickens fed with calcium levels of 2.62% in the ration had weaker eggshells than those fed with calcium levels of 3.70 to 4.40%. Besides that, the inclusion of sorghum and HASH(0x7f842fddc288) eggshell due to the almost equal presence of calcium (ranging from 2.47 to 3.01%) and phosphorus (ranging from 0.41 to 0.43%) in each treatment. The use of sorghum and HASH(0x7f842fe01690) egg yolk color. It is feared that the replacement of corn with sorghum up to 40% in the ration will harm the egg yolk color because sorghum does not contain carotenoids as does corn. It is believed that giving sorghum to poultry may harm egg yolk color. Chicken consuming sorghum produce a paler egg yolk color. According to Freitas et al. (2014), quails fed sorghum without added pigment have in a paler egg yolk color compared to those fed with pigment added sorghum. In this study, the addition of Indigofera leaf flour, up to 6% in the ration can maintain egg yolk color due to the β-car- otene contained in Indigofera leaf flour. Indigofera leaves contain 507.6 mg/kg of β-carotene, which functions as a source of antioxidants and egg yolk pigmentation (Palupi et al., 2014a). The use of carotenoids in rations can improve the egg yolk color HASH(0x7f840b199a18)HASH(0x7f842fdd34e0) ration consumed (Nys and Guyot, 2011). Some research- ers have previously suggested

that carotenoids can improve the egg yolk color. Hammershoj et al. (2010) suggest that carotenoid contained in rations can increase the brightness of egg yolk color. Furthermore, Sangeetha and Baskara (2010) also suggest that although laying hens cannot turn all carotenoids into vitamin A, the remaining is used as colorant to their yolk. Furthermore, the use of sorghum and HASH(0x7f842fdd1bf8) egg yolk fat content because the fat contents of sorghum and corn are almost the same i.e., 2.71 and 2.66%, respectively (Non-Ruminant Nutrition Laboratory, Universitas Anda- lals, 2013). The fat content of rations affects the egg yolk fat content (Yamamoto et al., 2007). According to Schreiber et al. (2013), β-carotene is a potential natural antioxidant. In this study, the use of sorghum and indigofera leaf flour in the rations of laying hens HASH(0x7f8430c98ce8) 30% sorghum and 5% indigofera leaf flour, and 40% sorghum and 6% indigofera leaf flour in rations of laying hens significantly reduced the egg yolk cholesterol content. Increased carotenoid content in the rations of laying hens can reduce egg yolk cholesterol (Ak- demir et al., 2012). Researchers have previously reported the effect of using indigofera on cholesterol contain. Palupi et al. (2014b), reported the use of indigofera sp., top leaf meal in rations of laying hens, reduces egg yolk cholesterol as much as 54.1%. Furthermore, Palupi et al. (2018), re- ported that the duck fed indigofera zollinggeriana top leaf meal have lower cholesterol content compared to duck that not fed indigofera zollinggeriana top leaf meal. The use of sorghum and indigofera leaf flour in the ration does not affect the total colony count of Bacillus sp in the small intestines of the laying hens due to the inclusion of probiotic waretha that contain the bacterium Bacillus am- yloliquefaciens at concentrations as high as 43x1012 CFU/ mL. In this study, the total colony count of Bacillus sp in the small intestines of laying hens found ranged from 7.98 to 8.49 log CFU mL-1. Bacillus amyloliquefaciens increase the total colony count of nonpathogenic bacteria and de- crease the total colony count of pathogenic bacteria such July 2021 | Volume 9 | Issue 7 | Page 960 NE US Advances in Animal and Veterinary Sciences as Escherichia coli and Salmonella spp. (Tang et al., 2017). REFERENCES Bacillus amyloliquefaciens increase the efficiency of Pitalah duck rations by reducing feed conversion by as much as • Abd-Elhalem BT, El-Sawy M, Gamal RF, Abou-Taleb KA 15%, and it was found that the total colony count of Bacil- (2015).Production of amylases from Bacillus amyloliquefaciens lus sp. in the small intestine of Pitalah ducks is as high as under submerged fermentation using some agro-industrial 14.84 log CF U/mL (Zurmiati et al., 2017a). The inclusion by-products Ann. Agric. Sci. 60 (2): 193-202. http://dx.doi. of up to 2000 ppm of the probiotic Bacillus amyloliquefa- org/10.1016/j.aoas.2015.06.001. ciens increase the efficiency of Pitalah duck rations by re- • Abdelhalim TS, Kamal NM, Hassan AB (2019). Nutritional potential of wild sorghum: Grain quality of Sudanese wild ducing the energy requirements of the rations by as much sorghum genotypes (Sorghum bicolor L. Moench). Food Sci. as 3.57% and reduce the protein requirements by as much Nutr. 7:1529–1539. http://dx.doi.org/10.1002/fsn3.1002. as 5.56% (Zurmiati et al., 2017b). • Afify AEMR, Beltagi HSE, Salam SMAE, Omran AA (2012). Biochemical changes in phenols, flavonoids, tannins, vitamin CONCLUSION E, β- karoten and antioxidant activity during soaking of three white sorghum varieties. Asian Pacific J. Trop. Biomed. 203- 209. www.elsevier.com/locate/apjtb In conclusion, the use of 40% sorghum with the addition • Ahmed NM, Atti KAA, Elamin KM, Dafalla KY, Malik of 6% indigofera leaf flour can replace the use of 100% HEE and Dousa BM (2013). Effect of dietary calcium corn in laying hen rations without disrupting phosphorus HASH(0x7f8430c93910). J. availability, daily protein intake, eggshell thickness, egg- Anim. Prod. Adv. 3(7): 226-231. https://doi.org/ 10.5455/ shell strength, egg yolk color, egg yolk fat, and total colony japa.20130718034818 • Akdemir F, Orhan C, Sahin N, Sahin DK, Hayirli A (2012). count of Bacillus sp in the small intestines of laying

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