Submission Confirmation

From: Veterinary World (vetworld@ejmanager.com)

To: rma_fispro@yahoo.com

Date: Sunday, November 26, 2017 at 10:18 PM GMT+7

Dear Erma Safitri,

Your submission entitled **Fermented Soy Husk from Tempe Industry Waste Using Cellulolytic Bacteria of Ulat Grayak (Spodoptera litura) to Improve the Quality of Mojosari Duck (Anas javanica) Eggs** (Manuscript Number: VETWORLD-2017-11-432) has been received by **Veterinary World**.

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From: Veterinary World (vetworld@ejmanager.com)

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Date: Sunday, January 21, 2018 at 12:28 AM GMT+7

Dear Erma Safitri,

Your submission entitled **Soybean Husk was Fermented Using Cellulolytic Bacteria of Spodoptera litura to Improve the Eggs Quality of Mojosari Duck (Anas javanica)** (Manuscript Number: VETWORLD-2018-01-039) has been received by **Veterinary World**.

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Article Revision Letter for Authors - (VETWORLD-2018-01-039)

From: Veterinary World (vetworld@ejmanager.com)

To: rma_fispro@yahoo.com

Date: Thursday, April 5, 2018 at 12:47 PM GMT+7

Dear Erma Safitri,

Your manuscript entitled \"Soybean Husk was Fermented Using Cellulolytic Bacteria of Spodoptera litura to Improve the Eggs Quality of Mojosari Duck (Anas javanica)\" (Ms.Nr. VETWORLD-2018-01-039) was reviewed by reviewers of the Veterinary World. As initial decision, your manuscript was found interesting but some revisions have to be made before it can reach a publishable value. Please refer comments given at bottom.

You should send your revised manuscript via the online system of ScopeMed on my.ejmanager.com.

Sincerely yours,

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COMMENTS for Authors:

EDITORIAL COMMENTS:

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- Please answer all the comments below point-by-point in an accompanying response letter to your revised submission and include your responses at appropriate paragraphs in the revised word file.
- Include all authors name, affiliation and email address in revised word file. Please check latest article from www.veterinaryworld.org for format of this section.
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- All reference no. in the text must be in continuous no. as per style of Veterinary World and amend the reference section accordingly if you have not done it.
- If you will not revise strictly as per suggestion then there will be chance of rejection. So, revise carefully. If you have any query then please mention it in revision letter or email to Editor-in-Chief.

=> Reviewer # 1

The methods are not appropriate.
Method of egg yolk color and shell thickness measurement is not specified.
The diets should be balanced in terms of energy and protein. The amount of protein in the T3 and T4 diets is higher than the others. This leads to the effect of the protein factor.
There are incompatible sentences in the article. Line 66 “design with 5 treatments and 4 replicates” and Line 91 “randomized design (5 × 5 replicates”
Please use the Standard error in the tables.

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Revised Article Submission

From: Veterinary World (vetworld@ejmanager.com)

To: rma_fispro@yahoo.com

Date: Thursday, April 12, 2018 at 01:19 AM GMT+7

Dear Erma Safitri,

Your REVISED ARTICLE entitled **Soybean Husk was Fermented Using Cellulolytic Bacteria of Spodoptera litura to Improve the Eggs Quality of Mojosari Duck (Anas javanica)** has been received by **Veterinary World**.

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Article Revision Letter for Authors - (VETWORLD-2018-01-039)

From: Veterinary World (vetworld@ejmanager.com)

To: rma_fispro@yahoo.com

Date: Friday, April 20, 2018 at 07:29 PM GMT+7

Dear Erma Safitri,

The revisions for your manuscript titled -Soybean Husk was Fermented Using Cellulolytic Bacteria of Spodoptera litura to Improve the Eggs Quality of Mojosari Duck (Anas javanica)- and manuscript number (VETWORLD-2018-01-039) was reviewed by Editorial Board of Veterinary World and decided that the following revisions should be done. Please answer all the comments below, in your answer letter.

You should send your revised manuscript by journal Submit Article page.

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Dr. Anjum Sherasiya
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COMMENTS for Authors:

EDITORIAL COMMENTS:

- Highlight all corrections/additions in red color font in revised manuscript.
- Please answer all the comments below point-by-point in an accompanying response letter to your revised submission.
- Include all authors name, affiliation and email address in word file. Please check latest article for format of name and affiliation, email etec.
- All journal names in references must be as per standard journal abbreviation. We do not allow full name or non-standardised abbreviation. You can check the abbreviation at http://images.webofknowledge.com/WOK46/help/WOS/T_abrvjt.html, www.journalseek.net etc.
- Include authors\' contributions if you have not added.
- Include Acknowledgements along with source of fund for this study if you have not included.
- If you will not revise strictly as per suggestion then there will be chance of rejection. So, revise carefully. If you have any query then please mention it in revision letter or email to Editor-in-Chief.

=> Reviewer # 1

Reviewer - 1 --- 2. Review

There are still incompatible sentences in the article. Line 108 and 113 “design with 5 treatments and 4 replicates” and Line 133 “randomized design (5 × 5 replicates”

Change the abbreviation to \"SE\" for the standard error values you add.

Line 143-147: This sentence has been copied from the following article.
Ultrasonic eggshell thickness measurement for selection of layers. Kibala L, Rozempolska-Rucinska I, Kasperek K, Zieba G, Lukaszewicz M. Poult Sci. 2015 Oct; 94(10):2360-3

Lines 72-99: This sentence has been copied from the following article.
Investigating internal bacteria of Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) larvae and some Bacillus

strains as biocontrol agents Filiz ÖZKAN ÇAKICI1 , Ali SEVÝM2 , Zihni DEMÝRBAÐ1 , Ýsmail DEMÝR1,* Turk J Agric For (2014) 38: 99-110

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Revised Article Submission

From: Veterinary World (vetworld@ejmanager.com)

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Date: Saturday, April 21, 2018 at 02:12 PM GMT+7

Dear Erma Safitri,

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Re: Final Revision

From: Veterinary World (editorveterinaryworld@gmail.com)

To: rma_fispro@yahoo.com

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> **Key words** : *Spodoptera litura*, cellulolytic bacteria, soybean husk fermentation, eggs quality of duck
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Dr. Sri Hidanah

Departmen of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Campus C UNAIR, Jl. Mulyorejo Surabaya 60115 Indonesia
E-mail: s_hidanah@yahoo.com / sri-h@_fkh.unair.ac.id

Best Regard,

Corresponding Author,

Dr. Erma Safitri, M. Si., DVM
Reproduction Veterinary Departement, Veterinary Medicine Faculty and
Stem Cells Research Division of Institute Tropical Disease (ITD)
of Universitas Airlangga
Surabaya-Indonesia
Email : rma_fispro@yahoo.com / erma-s@fkh.unair.ac.id

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From: Veterinary World (editorveterinaryworld@gmail.com)

To: rma_fispro@yahoo.com

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Best Regard,

Dr. Sri Hidanah

Departmen of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Campus C UNAIR, Jl. Mulyorejo Surabaya 60115 Indonesia
E-mail: s_hidanah@yahoo.com / sri-h@_fkh.unair.ac.id

Best Regard,

Corresponding Author,

Dr. Erma Safitri, M. Si., DVM
Reproduction Veterinary Departement, Veterinary Medicine Faculty and
Stem Cells Research Division of Institute Tropical Disease (ITD)
of Universitas Airlangga
Surabaya-Indonesia
Email : rma_fispro@yahoo.com / erma-s@fkh.unair.ac.id

Erma Safitri and co-authors: Acceptance letter

From: Veterinary World - Publisher (veterinaryworldpublisher@gmail.com)

To: rma_fispro@yahoo.com; sri-h@fkh.unair.ac.id; dady_sn_drh@yahoo.com

Cc: editorveterinaryworld@gmail.com

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NAAS (National Academy of Agricultural Sciences -INDIA) - 5.71
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By E-mail

Ref No. VW/Accept/83/2018 25-04-2018

To,
Erma Safitri
Departement of Veterinary Reproduction,
Faculty of Veterinary Medicine,
Universitas Airlangga,
Surabaya,
Indonesia.
E-mail: rma_fispro@yahoo.com

Acceptance of article for publication in Veterinary World

Dear Dr.

I am pleased to inform you that your manuscript titled as -

Soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura* to improve the eggs quality of *Mojosari duck* (*Anas javanica*) - Sri Hidanah**,** Dady Soegianto Nazar and Erma Safitri

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Yours Sincerely,

Dr. Anjum V. Sherasiya
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Erma Safitri and co-authors: Proof for corrections

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To: rma_fispro@yahoo.com; sri-h@fkh.unair.ac.id; dady_sn_drh@yahoo.com

Cc: editorveterinaryworld@gmail.com

Date: Thursday, May 17, 2018 at 06:51 PM GMT+7

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51 RESEARCH ARTICLE

Soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura* to improve the eggs quality of Mojosari duck (*Anas javanica*)

Sri Hidanah[1], Dady Soegianto Nazar[1] and Erma Safitri[2,3]

1. Department of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia; 2. Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia; 3. Stem Cells Research Division of Institute Tropical Disease (ITD), Universitas Airlangga, Surabaya, Indonesia.

Corresponding author: Erma Safitri, e-mail: rma_fispro@yahoo.com/erma-s@fkh.unair.ac.id

Co-authors: SH: sri-h@fkh.unair.ac.id/s_hidanah@yahoo.com, DSN: dady_sn_drh@yahoo.com

Received: 06-03-2018, **Accepted:** 25-04-2018**, Published online:** ***

doi: *** **How to cite this article:** Hidanah S, Nazar DS, Safitri E (2018) Soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura* to improve the eggs quality of Mojosari duck (*Anas javanica*), *Veterinary World*, 11(5): 0-0.

Abstract

Aim : This study was aimed to improve the eggs quality of Mojosari duck (*Anas javanica*) through complete feeding containing soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura*.

Materials and Methods: This study was consisted of three stages: The first stages, isolation and identification of cellulolytic bacteria from *S. litura*; the second stage, the fermentation of soybean husk through the application of bacterial cellulolytic isolate from the first stage; and the third stage, the application of the best complete feed formulation from the second stage to Mojosari duck.

Results: There are four dominant bacteria: *Bacillus*, *Cellulomonas* spp., *Pseudomonas*, and *Cytophaga*. Furthermore, the best reduction of the crude fiber of soybean husks is the use of *Cellulomonas* sp. bacteria. The final of the study, the eggs quality of Mojosari duck, was improved, as indicated by cholesterol decrease from the yolk without the decrease of egg weight and eggshell thickness, although the decrease in egg yolk color was inevitable.

Conclusion: Soy husk fermentation using cellulolytic bacteria of *S. litura* was added to complete feeding can be performed to improve the eggs quality of Mojosari duck.

Keywords: cellulolytic bacteria, eggs quality of duck, soybean husk fermentation, *Spodoptera litura.*

<H1>Introduction

Soybean is an agricultural product that has been utilized to meet the needs of industry and food, such as Tempe, tofu, soy sauce, and soy milk. In general, the use and utilization of soybean are limited to seeds only, while the waste, such as soybean husk, is still discarded and has not been widely utilized. Analysis of dry matter (DM)=91.11, crude protein (CP)=5.04, ether extract=1.65, nitrogen-free extract, calcium=21, phosphorus=0.06, and gross energy=(kcal/g. DM) 3.98 according to the methods described in AOAC. The analysis of neutral detergent fiber=60.15 and acid detergent fiber=42.08 was carried out according to detergent method [1]. In other research, the chemical composition of soybean husk comprises 47.01% crude fiber, 14.45% CP, 3.04% crude fat, 3.15% ash, and 3.060, 48 kcal kg of metabolic energy. Soybean husk contains 42-49% dry weight of cellulose, 29-34% hemicellulose, and 1-3% lignin and has anti-nutritional antitrypsin substances [2].

On the other hand, *Spodoptera litura* is a pest of soybean crop that has a very high ability in damaging the plant. The leaves and pods attacked by *S. litura* become holes even then torn

99 [3]. Based on its ability to damage the leaves and pods, allegedly the digestive tract of *S.*
100 *litura* contains cellulolytic bacteria capable of digesting crude fibers well [4].

101 In general, cellulolytic bacteria have three cellulose enzymes called endoglucanase or
102 carboxymethylcellulose (CMC-ase), exoglucanase or cellobiohydrolase, and beta-glucosidase.
103 The enzymes can degrade cellulose into glucose [5]. CMC-ase breaks the hydrogen bonds
104 present in the cellulose crystalline structure, forming single cellulose chains. Exoglucanase
105 cuts off the ends of single chains cellulose, producing disaccharides and tetrasaccharides,
106 cellobiose, beta-glucosidase hydrolyzes disaccharides, and tetrasaccharides into glucose [6].
107 Therefore, the utilization of cellulolytic microbes in the fermentation process of the feed
108 material from the waste can allegedly improve the quality of complete feed formulation with
109 the indication of the decrease of crude fiber and the increase of CP.

110 Based on another study, the soybean husk waste fermented with *Aspergillus niger* and
111 Lactobacillus was only able to decrease crude fiber from 44% to 40%. The decrease in crude
112 fiber content is still relatively small. In addition to the decrease in crude fiber, the
113 fermentation process is also expected to increase CP from processed waste material [7].
114 Therefore, we need an alternative bacterium that has higher capability in breaking down

115 crude fiber along with an increase in CP content of the soybean husk.

116 This study aims to determine the potential of cellulolytic bacteria was contained in *S. litura* as
117 a source of probiotics that can reduce the soybean crude fiber derived from the Tempe
118 industry through the fermentation process, but followed by increased CP. If this is realized,
119 then the quality of complete feed formulation on feed given to Mojosari duck (*Anas javanica*)
120 will be improved. Furthermore, improving the quality of complete feed formulation on feed
121 was given to Mojosari duck (Anas javanica) is expected to affect the quality of the eggs
122 produced, such as low cholesterol levels with maintaining eggs' weight, yolk color, and
123 thickness of the shell.

124 **<H1>Materials and Methods**

125 **<H2>Ethical approval**

126 ???

127 **<H2>Stage of study**

128 This study was consisted of three stages: The first stage, isolation and identification of
129 cellulolytic bacteria from *S. litura* digestive tract [4,8]; in total, 4 bacteria, i.e., Bacillus,

130 *Cellulomonas* sp., Pseudomonas, and *Cytophaga* were characterized based on their colony
131 color, morphological, biochemical, and molecular characteristics of bacteria.

132 We explored the culturable bacterial community in the digestive tract of *S. litura* using
133 culture-dependent technique based on 16S rRNA gene sequencing and screening of these four
134 isolates. Bacterial isolation was performed on living larvae separately. The larva was
135 homogenized in nutrient extract using a glass pounder, and the homogenate is filtered 2 times
136 to remove larvae debris than input into sterile tubes. The larvae extract a number of 50 µL
137 were placed on nutrient agar and incubated at 37°C in a humidified atmosphere containing at
138 5% CO_2 moisture and allowed to increase the number of bacteria for 3 days. Isolates were
139 distinguished based on colony color and morphology. After that, the pure cultures of bacterial
140 colonies were added into 20% glycerol and prepared at the Laboratory of Microbiology of the
141 Department of Microbiology, Faculty of Veterinary Medicine, Airlangga University.

142 Identification of bacterial isolates was identified by various tests, such as the utilization of
143 organic compounds, spore formation, Gram staining, NaCl tolerance, optimum temperature,
144 optimum pH, and catalase [4].

145 The isolate identification of four bacteria was confirmed using 16S rRNA gene sequencing.

146 The standard protocol was used for confirm of total genomic DNA extraction. The isolated
147 DNAs of each bacteria, i.e., Bacillus, *Cellulomonas* sp., Pseudomonas, and *Cytophaga* were
148 stored at −20°C until use. Furthermore, the polymerase chain reaction (PCR) amplification of
149 the 16S rRNA genes was performed using the universal primers UNI16S-L (5'-
150 ATTCTAGAGTTTGATCATGGCTCA-3') as the forward primer and UNI16S-R (5'-
151 ATGGTACCGTGTGA CGGGCGGTGTGTA-3') as the reverse primer and then
152 Amplification process in a thermocycler (Eppendorf, Mastercycler Gradient, Hamburg,
153 Germany) for 36 reaction cycles. Reactions were routinely performed in 50 µL including 1.5
154 µL of 10 mM dNTP mix, 1.5 µL of 10 pmol each of the opposing amplification primers, 1 µL
155 of 5 U/µL Taq DNA polymerase (Fermentas), 3 µL of MgCl2, 5 µL of Taq DNA polymerase
156 reaction buffer, 1 µL of genomic DNA, and 35.5 µL of dH2 O. PCR conditions were 5 min at
157 95°C for the initial denaturation of template DNA, 36 amplification cycles (1 min at 94°C, 1
158 min at 56°C, and 2 min at 72°C), and 10 min at 72°C for the final extension. PCR products
159 were separated on 1.0% agarose gels, stained with ethidium bromide, and viewed under
160 ultraviolet light. After checking the PCR products, they were sent to Macrogen (the
161 Netherlands) for sequencing. The obtained sequences were used to perform BLAST searches
162 using the NCBI GenBank database. In addition, sequences were used for phylogenetic

163 analysis for further characterization [9].

164 The second stage, the process of soybean fermentation from Tempe industry waste (Usaha
165 Tempe Rakyat, Surabaya, Indonesia), with the addition of Epidopt (Sugar Factory Candi,
166 Sidoarjo, Indonesia), urea (Petrokimia Gresik, Gresik, Indonesia), and various bacterial
167 isolates obtained from Stage 1 studies compared with control (without addition of bacterial
168 isolate). Fermentation is one of the major processes used in the production of food from
169 soybeans. This fermentation changes the physicochemical and organoleptic properties of soy
170 products such as color, flavor, and active components [10].

171 The second stage used complete randomized design with 5 treatments and 4 replicates [11].
172 The treatment was: T0: Soybean husk + 1% molasses + 1% urea + without bacterial isolate;
173 T1: Soybean husk + 1% molasses + 1% urea + 5% bacillus bacterial isolate; T2: Soybean
174 husk + 1% molasses + 1% urea + 5% bacteria *Cellulomonas* sp. isolate ; T3: Soybean husk +
175 1% molasses + 1% urea + 5% pseudomonas bacterial isolate; and T4: Soybean husk+ 1%
176 molasses + 1% urea + 5% *Cytophaga* bacterial isolate.

177 A total of 20 samples of soybean husk, each weighing 200 g, were randomly divided into 5
178 treatments with 4 replicates, 1% urea and epidopt and 5% of cellulolytic bacteria (108/cc)

dissolved in a diluent solution of sterile water as much as 30% of the sample weight. Subsequently, the solution was sprayed on the husk of the soybeans and inserted into a plastic bag (clear, hollow in some places, and tied at the top), and fermented for 7 days. After the fermentation process ended, organoleptic examination was done, including color, odor, texture, and pH measurement. Then, the fermented husk was aerated. Furthermore, to determine the content of DM, crude fiber, and CP, the proximate analysis was performed according to the method recommended by Wendee [1]. The best results of this second stage were T2: Soybean husk + *Cellulomonas* sp. suspension (1% Molasses + 1% urea + 5% isolate *Cellulomonas* sp. as fermenter).

The third stage of this study was the application of a complete feed formulation by adding fermentation of the best result of second stage: Various percentage of soybean husk + *Cellulomonas* sp. suspension, compared with control (without *Cellulomonas* sp. suspension). Furthermore, prepared complete feed formulation was given as feed on the Mojosari duck (Anas javanica). The complete feed formulation is shown in Table-1.

The third stage of this study was giving complete feed formulation to Mojosari duck (Anas javanica) in improving the quality of Mojosari duck (Anas javanica) egg. This study used 100

laying Mojosari duck*s* (Anas javanica), aged about 20 weeks, divided into 5 treatments in the form of 5 types of formula feed which were T0: Complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension; T1: Complete feed + 15% soybean husk without *Cellulomonas* sp. bacteria suspension; T2: Complete feed + 15% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension; T3: Complete feed + 30% soybean husk without *Cellulomonas* sp. bacteria suspension; and T4: Complete feed + 30% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension (Table-1). The experimental design was complete randomized design (5×5 replicates). Parameters to improve the quality of Mojosari duck (Anas javanica) eggs included egg cholesterol levels, egg weight, egg yolk, and eggshell thickness. Egg cholesterol (mg/100 g) levels were measured on day 7 before the end of the study. Cholesterol levels were tested using the Liebermann–Burchard's method [12,13]. Egg weight is measured by weighing using digital scales. Evaluation of egg yolk color estimated by the usual method applying La Roche scale (DSM Yolk Color Fan) with spectrophotometric determination of β-carotene by AOAC method, and by new rapid analyzer iCheckTM Egg photometer (BioAnalyt). The yolk color varied between the values of 4 and 13 of La Roche scale. The carotenoid content expressed as β-carotene measured by AOAC method varied between 11 and 87 mg/kg. The carotenoid content expressed as β-

carotene measured with the analyzer Check TM Egg photometer was lower and varied between 7.5 and 68.5 mg/kg [14].

The measurements of eggshell thickness were done using ultrasonography technology. The measurements beginning from the large end of the egg and repeated at each parallel on 3 meridians. The measurements were taken with an electronic micrometer measurement predominantly at the wider end of eggs [15].

<H2>Statistical analysis

Cholesterol, egg weight, and eggshell thickness were statistically analyzed using SPSS 13 for Windows XP with the confidence level of 99% (α=0.01) and the level of significance 0.05 (p=0.05). Hypothesis tests were as follows: Normality test of the data with Kolmogorov–Smirnov test, homogeneity of variance test, analysis of variance, and *post hoc* test using Tukey test with very significant difference 5% [16].

<H1>Results

<H2>Isolation and Identification of cellulolytic bacteria of *S. litura*

The results of isolation and identification of the digestive tract of *S. litura*, which was the first

stage of this study, it's found of 4 isolates of cellulolytic bacteria, they are bacillus, *Cellulomonas* sp., Pseudomonas, and *Cytophaga*. Furthermore, the four isolates were, respectively, used as fermenters on the soybean husk from Tempe industry wastes derived from Usaha Tempe Rakyat Surabaya, Indonesia, at the next stage of the study.

<H2>Improving the quality of soybean husk waste

Improving the quality of soybean husk waste, which is the second stage of this research, is done through fermentation process with the addition of epidopt (Sugar Factory of Candi, Sidoarjo, Indonesia), urea (Petrokimia Gresik, Gresik, Indonesia), and various bacterial isolates which was obtained from first stage of the study (*Bacillus*, *Cellulomonas* sp., *Pseudomonas*, and *Cytophaga*) and compared with control (without addition of bacterial isolates). The results of this second stage study can be seen in Table-2.

<H2>The eggs quality of Mojosari duck (Anas javanica)

The egg's quality of Mojosari duck (Anas javanica) after feeding with a wide variety of complete feeds (both with the addition of soybean husk and the suspension of cellulolytic bacteria) compared with no addition was observed through cholesterol levels from egg yolks,

egg weight, eggshell thickness, and egg yolk color.

<H2>The cholesterol eggs level of duck

The cholesterol eggs level (mg/100 g) based on Liebermann–Burchard's method [12,13] was measured on day 7 before the end of the study. The mean and standard deviation of cholesterol eggs levels of duck which is given with various feeding complete either by addition of soybean husk fermented using suspension of *Cellulomonas* sp. bacteria from *S. litura* was compared with not addition. The cholesterol eggs level can be seen in Table-3.

<H2>Egg weight, eggshell thickness, and egg yolk color

Mean and standard deviation of duck egg quality, including egg weight, eggshell thickness, and egg yolk color which is given with various feeding complete either by addition of soybean husk waste fermentation using suspension of Cellulomonas sp. cellulose from *S. litura* was compared with not addition. Egg weight, eggshell thickness, and egg yolk color can be seen in Table-4.

<H1>Discussion

Based on the results of variance analysis, it was found that the content of crude fiber and CP

of soybean husk fermentation using 4 bacterium: Bacillus, *Cellulomonas* sp., Pseudomonas, and *Cytophaga* have shown significantly different results ($p<0.05$), while content of dry material was not showed significant difference ($p>0.05$). Based on Duncan's distance test for crude fiber content, the best result, the highest decrease of the crude fiber, was in T2 treatment, which was treated with a suspension of *Cellulomonas* sp.

According to Holt [17], the bacteria *Cellulomonas* sp. is Gram-positive, rod-shaped, and non-motile. The characteristic of this bacterium is as follows: Respiratory metabolism using oxygen as electron acceptor, catalase positive, lives at optimum temperature 300°C, and neutral pH, with growth rate 0.15-0.23/h. These bacteria have been known to digest cellulose, xylene, and starch. According to Gupta *et al*. [18], *Cellulomonas* sp., possesses extracellular enzymes that play a greater role in the breakdown of amorphous cellulose.

Observations on cholesterol levels were showed that feeding complete in T0, which is produce the highest cholesterol levels and significantly different ($p<0.05\%$) than T1, T2, and T3. The feeding complete in T4 was yielded the lowest cholesterol level compared with T3 treatment but significantly different ($p<0.05\%$) with T1 and T2 treatment, where between T1 and T2 treatment were not significantly different ($p>0.05\%$).

This result provides an opportunity to the utilization of complete feed with the addition of fermented soybean husk using *Cellulomonas* sp. bacteria suspension from which gives the best result as an the lowest cholesterol level.

Several other studies, such as the provision of katuk leaf flour which also contains high crude fiber as well as soybeans husk, showed that katuk leaf flour at level ≥5% was also able to decrease cholesterol levels of eggs Mojosari duck without decreasing percentage of egg yolk weight [19]. However, since the use of katuk leaf flour must compete with food consumed by humans, the utilization of the soybean husk waste can be an alternative to consider. Furthermore, in many other studies on the use of various foliage powders with a high content of crude fiber, egg cholesterol levels of duck cannot be reduced. A study conducted by Palupi *et al*. [20], eggs cholesterol level of duck with an additional meal of beluntas leaves up to 2%, the level had not effect on egg cholesterol level of duck, where cholesterol levels at the treatment were still at 27.79 mg/g egg yolks.

Table-4 shows that egg yolk color parameters on T0, T1, T2, and T3 treatments result in a significantly different color of egg yolk ($p<0.05$), whereas T4 yields a lower yolk color than the other four treatments. This is shows that the provision of soybean husk fermentation from

289 Tempe industry waste as much as 30% as a substitute for corn can affect the color of egg
290 yolks. Based on research by Subhan [21] was reported that the egg yolk colour of the Tegal
291 duck only 7.120, while Beardsworth and Hernandez [22] stated that the good egg yolk color
292 was in the range of 8-12. The good egg yolk color in the range of 8-12 was obtained with the
293 addition of corn to the feed. Corn is one of the agricultural commodities very important for
294 livestock. Corn is a high-energy feed ingredient, with a protein content of about 8.6-9.0%, but
295 corn protein cannot fermented or degraded by rumen microorganisms [23].

296 The parameter observation of egg weight and eggshell thickness was not showed significant
297 difference between treatments ($p>0.05$). This is shows that the utilization of soybean husk
298 waste fermented with *Cellulomonas* sp. bacteria up to 30% dose does not affect on egg
299 weight or eggshell thickness. Silversides and Villeneuve [24] reported that with increasing
300 age, the egg size will increase as a result of increased yolk weight.

301 In poultry, including ducks, the process of egg formation known as folliculogenesis, in
302 addition to affecting the development of the oocyte (egg cell), also affects the weight of the
303 egg yolk. The number of follicles during one cycle is influenced by factors such as animal
304 species, reproductive phase, circumstances, age, mother, genetic [25], and feed [26-28].

<H1>Conclusion

The fermentation of soybean husk from Tempe industry waste through the utilization of cellulolytic bacteria of *S. litura* added to complete feed can be done as an effort to improve the quality of Mojosari duck (Anas javanica) eggs in the form decrease of egg yolks cholesterol level without decreasing egg weight and eggshell thickness, although the decrease in yolk color is unavoidable statistically does not show significant differences ($p>0.05$).

<H1>Authors' Contributions

All the authors conceptualized the manuscript. Both SH and ES drafted the manuscript. SH: Research project leader and coordinating research, collected and processed samples. Carried out the data collection and gathering assay samples. DSN has done the statistical analysis part and critically reviewed the manuscript. ES: Assisted in manuscript preparation and corresponding author. All the authors have read and approved the final version of the manuscript.

<H1>Acknowledgments

The study was supported by funding from the Directorate General of Higher Education

(DIKTI) 2015. The National Education Ministry, Republic of Indonesia.

<H1>Competing Interests

The authors declare that they have no competing interests.

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403 **Tables**

Table-1: Complete feed formulation was given to Mojosari duck (Anas javanica) using soybean husk waste fermented with *Cellulomonas* sp. bacteria suspension.					
Materials (%)	T0 (Control) complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension.	T1 (Treatment 1) complete feed + 15% soybean husk without *Cellulomonas* sp. bacteria suspension	T2 (Treatment 2) complete feed + 15% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension	T3 (Treatment 3) complete feed + 30% soybean husk without *Cellulomonas* sp. bacteria suspension.	T4 (Treatment 4) complete feed + 30% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension.
Yellow corn	61.00	46.00	46.00	31.00	31.00
Fish meal	13.80	13.75	13.80	13.80	13.75
Soy meal	5.60	5.60	5.60	5.60	5.60
Rice bran	14.70	14.70	14.70	14.70	14.70

Soybean	4.30	4.30	4.30	4.30	4.30
Coconut oil	0.30	0.30	0.30	0.30	0.30
Premix	0.30	0.30	0.30	0.30	0.30
Soybean husk	-	15	15	30	30
Cellulomonas sp. bacteria suspension	-	-	0.05	-	0.05
Total	100	100	100	100	100

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Table-2: The content of dry material (%), crude fiber (%), and CP (%) of fermented soybean husk using various cellulolytic bacteria isolates from *S. litura.*			
Treatment	Dry material (%)±SE	Crude fiber (%)±SE	CP (%)±SE

T0 (soybean husk + 1% molasses + 1% urea + without bacteria isolate)	78.15[a]±0.05	48.60[a]±0.14	15.63[a]±0.26
T1 (soybean husk + 1% molasses + 1% urea + 5% bacillus bacteria isolate)	78.79[a]±0.82	48.73[a]±0.53	15.85[a]±0.73
T2 (Soybean husk + 1% molasses + 1% urea + 5% *Cellulomonas* sp. bacteria isolate)	78.18[a]±0.20	43.81[b]±0.78	15.90[a]±0.55
T3 (soybean husk + 1% molasses + 1% urea + 5% *Pseudomonas* bacteria isolate)	78.67[a]±0.16	48.07[a]±0.50	17.10[b]±0.90
T4 (soybean husk + 1% molasses + 1% urea + 5% *Cytophaga* bacteria isolate)	78.40[a]±0.19	48.58[a]±1.38	17.57[b]±0.68
[a,b,c]Values in the same column with different superscripts indicate significant difference $p<0.05$ (n=4), CP=Crude protein, *S. litura*=*Spodoptera litura*,			

SE=Standard error

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Table-3: Mean and standard deviation of egg yolk cholesterol levels of Mojosari ducl (Anas javanica).	
Treatments	Mean±SE
T0 (complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension)	18.74^{a}±2.19
T1 (complete feed + soybean husk 15% without *Cellulomonas* sp. bacteria suspension)	15.61^{b}±2.12
T2 (complete feed + soybean husk 15% + 0.05% *Cellulomonas* sp. bacteria suspension)	16.53^{b}±2.52
T3 (complete feed + soybean husk 30% without *Cellulomonas* sp. bacteria suspension)	13.35^{c}±1.92
T4 (complete feed + soybean husk 30% + 0.05% *Cellulomonas* sp. bacteria suspension.)	12.69^{c}±2.23

[a,b,c]Values in the same column with different superscripts indicate significant difference p<0.05 (n=5), SE=Standard error

407

408

Table-4: Mean and standard deviation of egg weight, egg yolk color, and egg shell thickness of duck.

Variable	Treatment				
	T0 (complete feed without soybean husk and *Cellulomonas* sp.	T1 (complete feed + 15% soybean husk without *Cellulomonas* sp.	T2 (complete feed + 15% soybean husk + 0.05% *Cellulomonas* sp.	T3 (complete feed + 30% soybean husk without *Cellulomonas* sp.	T4 (complete feed + soybean husk 30% + 0.05% *Cellulomonas* sp.

	bacteria suspension.)	bacteria suspension)	bacteria suspension)	bacteria suspension)	bacteria suspension.)
Egg weight (g)±SE	47.60[a]±4.07	50.96[a]±3.38	52.26[a]±2.48	42.17[a]±20.12	47.94[a]±9.37
Egg yolk color±SE	10.20[b]±1.79	8.20[ab]±1.92	9.40[b]±2.30	8.00[ab]±1.41	6.40[a]±2.30
Eggshell thickness (mm)±SE	0.55[a]±0.08	0.52[a]±0.08	0.53[a]±0.03	0.53[a]±0.07	0.53[a]±0.08
[a,b,c]Values in the same line with different superscripts indicate significant difference p<0.05 (n=5), SE=Standard error					

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RESEARCH ARTICLE
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The improvement of eggs quality of Mojosari duck (*Anas javanica*) with soybean husk fermentation using cellulolytic bacteria of *Spodoptera litura*

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Received: 06-03-2018, **Accepted:** 25-04-2018, **Published online:** ***

doi: 10.14202/vetworld.2018.720-725 **How to cite this article:** Hidanah S, Nazar DS, Safitri E (2018) The improvement of eggs quality of Mojosari duck (*Anas javanica*) with soybean husk fermentation using cellulolytic bacteria of *Spodoptera litura*, *Veterinary World*, 11(5): 720-725.

Abstract

Aim: This study was aimed to improve the quality of the eggs of Mojosari duck (*Anas javanica*) through complete feeding containing soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura*.

Materials and Methods: This study consisted of three stages: The first stages, isolation and identification of cellulolytic bacteria from *S. litura*; the second stage, the fermentation of soybean husk through the application of bacterial cellulolytic isolate from the first stage; and the third stage, the application of the best complete feed formulation from the second stage to Mojosari duck.

Results: There are four dominant bacteria: *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp. Furthermore, the best reduction of the crude fiber of soybean husks is the use of *Cellulomonas* sp. bacteria. The final of the study, the quality of the eggs of *Anas javanica*, was improved, as indicated by cholesterol decrease from the yolk without the decrease of egg weight and eggshell thickness, although the decrease in egg yolk color was inevitable.

Conclusion: Soy husk fermentation using cellulolytic bacteria of *S. litura* was added to complete feeding can be performed to improve the quality of the eggs of Mojosari duck.

Keywords: cellulolytic bacteria, eggs quality of duck, soybean husk fermentation, *Spodoptera litura.*

Introduction

Soybean is an agricultural product that has been utilized to meet the needs of industry and food, such as Tempe, tofu, soy sauce, and soy milk. In general, the use and utilization of soybean are limited to seeds only, while the waste, such as soybean husk, is still discarded and has not been widely utilized. Analysis of dry matter (DM)=91.11, crude protein (CP)=5.04, ether extract=1.65, nitrogen-free extract, calcium=21, phosphorus=0.06, and gross energy=(kcal/g. DM) 3.98 according to the methods described in AOAC. The analysis of neutral detergent fiber=60.15 and acid detergent fiber=42.08 was carried out according to detergent method [1]. In other research, the chemical composition of soybean husk comprises 47.01% crude fiber, 14.45% CP, 3.04% crude fat, 3.15% ash, and 3.060, 48 kcal kg of energy metabolism. Soybean husk contains 42-49% dry weight of cellulose, 29-34% hemicellulose, and 1-3% lignin and has anti-nutritional antitrypsin substances [2].

On the other hand, *Spodoptera litura* is a pest of soybean crop that has a very high ability in damaging the plant. The leaves and pods attacked by *S. litura* become holes even then torn [3]. Based on its ability to damage the leaves and pods, allegedly the digestive tract of *S. litura* contains cellulolytic bacteria capable of digesting crude fibers well [4].

In general, cellulolytic bacteria have three cellulose enzymes called endoglucanase or carboxymethylcellulose (CMC-ase), exoglucanase or cellobiohydrolase, and beta-glucosidase. The enzymes can degrade cellulose into glucose [5]. CMC-ase breaks the hydrogen bonds present in the cellulose crystalline structure, forming single cellulose chains. Exoglucanase cuts off the ends of single chains cellulose, producing disaccharides and tetrasaccharides, cellobiose, beta-glucosidase hydrolyzes disaccharides, and tetrasaccharides into glucose [6]. Therefore, the utilization of cellulolytic microbes in the fermentation process of the feed material from the waste can allegedly improve the quality of complete feed formulation with the indication of the decrease of crude fiber and the increase of CP.

Based on another study, the soybean husk waste fermented with *Aspergillus niger* and Lactobacillus was only able to decrease crude fiber from 44% to 40%.

The decrease in crude fiber content is still relatively small. In addition to the decrease in crude fiber, the fermentation process is also expected to increase CP from processed waste material [7]. Therefore, we need an alternative bacterium that has the higher capability in breaking down crude fiber along with an increase in CP content of the soybean husk.

This study aims to determine the potential of cellulolytic bacteria was contained in *S. litura* as a source of probiotics that can reduce the soybean crude fiber derived from the Tempe (Tempe is a traditional soy product originating from Indonesia. It is made by a natural culturing and controlled fermentation process that binds soybeans into a cake form) industry through the fermentation process, but followed by increased CP. If this is realized, then the quality of complete feed formulation on feed given to *Anas javanica* will be improved. Furthermore, improving the quality of complete feed formulation on feed was given to *Anas javanica* is expected to affect the quality of the eggs produced, such as low cholesterol levels with maintaining eggs' weight, yolk color, and thickness of the shell.

Materials and Methods

Ethical approval

The present study was approved by ethical committee vide Ethical Clearance KE (Komisi Etik Penelitian), Animal Care and Use Committee (ACUC). Veterinary Medicine Faculty, Universitas Airlangga, Surabaya, Indonesia.

Stage of study

This study consisted of three stages.

First stage

The first stage, isolation and identification of cellulolytic bacteria from *S. litura* digestive tract [4,8]; in total, 4 bacteria, i.e., *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp.were characterized based on their colony color, morphological, biochemical, and molecular characteristics of bacteria.

We explored the culturable bacterial community in the digestive tract of *S. litura* using a culture-dependent technique based on 16S rRNA gene sequencing and screening of these four isolates. Bacterial isolation was performed on living larvae separately. The larva was homogenized in nutrient extract using a glass pounder, and the homogenate is filtered 2 times to remove larvae debris than input into sterile tubes. The larvae extract a number of 50 µL were placed on nutrient agar and incubated at 37°C in a humidified atmosphere containing at 5% CO_2 moisture and allowed to increase the number of bacteria for 3 days. Isolates were distinguished based on colony color and morphology. After that, the pure cultures of bacterial colonies were added into 20% glycerol and prepared at the Laboratory of Microbiology of the Department of Microbiology, Faculty of Veterinary Medicine, Airlangga University.

Identification of bacterial isolates was identified by various tests, such as the utilization of organic compounds, spore formation, Gram staining, NaCl tolerance, optimum temperature, optimum pH, and catalase [4].

The isolate identification of four bacteria was confirmed using 16S rRNA gene sequencing. The standard protocol was used for confirm of total genomic DNA extraction. The isolated DNAs of each bacteria, i.e., *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp. were stored at −20°C until use. Furthermore, the polymerase chain reaction (PCR) amplification of the 16S rRNA genes was performed using the universal primers UNI16S-L (5'-ATTCTAGAGTTTGATCATGGCTCA-3') as the forward primer and UNI16S-R (5'-ATGGTACCGTGTGA CGGGCGGTGTGTA-3') as the reverse primer and then Amplification process in a thermocycler (Eppendorf, Mastercycler Gradient, Hamburg, Germany) for 36 reaction cycles. Reactions were routinely performed in 50 µL including 1.5 µL of 10 mM dNTP mix, 1.5 µL of 10 pmol each of the opposing amplification primers, 1 µL of 5 U/µL Taq DNA polymerase (Fermentas), 3 µL of MgCl2, 5 µL of Taq DNA polymerase reaction buffer, 1 µL of genomic DNA, and 35.5 µL of dH2 O. PCR conditions were 5 min at 95°C for the initial denaturation of template DNA, 36 amplification cycles (1 min at 94°C, 1 min at 56°C, and 2 min at 72°C), and 10 min at 72°C for the final extension. PCR products were separated on 1.0% agarose gels, stained with ethidium bromide, and viewed under ultraviolet light. After checking the PCR products, they were sent to Macrogen (the Netherlands) for sequencing. The obtained sequences were used to perform BLAST searches using the NCBI GenBank database. In addition, sequences were used for phylogenetic analysis for further characterization [9].

Second stage

The second stage, the process of soybean fermentation from Tempe industry waste (Usaha Tempe Rakyat, Surabaya, Indonesia), with the addition of Epidopt (Sugar Factory Candi, Sidoarjo, Indonesia), urea (Petrokimia Gresik, Gresik, Indonesia), and various bacterial isolates obtained from Stage 1 studies compared with control (without addition of bacterial isolate). Fermentation is one of the major processes used in the production of food from soybeans. This fermentation changes the physicochemical and organoleptic properties of soy products such as color, flavor, and active components [10].

The second stage used complete randomized design with 5 treatments and 4 replicates [11]. The treatment was: T0: Soybean husk + 1% molasses + 1% urea + without bacterial isolate; T1: Soybean husk + 1% molasses + 1% urea + 5% bacillus sp. bacterial isolate; T2: Soybean husk + 1% molasses + 1% urea + 5% bacteria *Cellulomonas* sp. isolate; T3: Soybean

husk + 1% molasses + 1% urea + 5% pseudomonas sp. bacterial isolate; and T4: Soybean husk+ 1% molasses + 1% urea + 5% *Cytophaga* sp. bacterial isolate.

A total of 20 samples of soybean husk, each weighing 200 g, were randomly divided into 5 treatments with 4 replicates, 1% urea + epidopt and 5% of cellulolytic bacteria (108/cc) dissolved in a diluent solution of sterile water as much as 30% of the sample weight. Subsequently, the solution was sprayed on the husk of the soybeans and inserted into a plastic bag (clear, hollow in some places, and tied at the top), and fermented for 7 days. After the fermentation process ended, the organoleptic examination was done, including color, odor, texture, and pH measurement. Then, the fermented husk was aerated. Furthermore, to determine the content of DM, crude fiber, and CP, the proximate analysis was performed according to the method recommended by Sruamsiri and Silman [1]. The best results of this second stage were T2: Soybean husk + *Cellulomonas* sp. suspension (1% Molasses + 1% urea + 5% isolate *Cellulomonas* sp. as fermenter).

Third stage

The third stage of this study was the application of a complete feed formulation by adding fermentation of the best result of the second stage: Various percentage of soybean husk + *Cellulomonas* sp. suspension, compared with control (without *Cellulomonas* sp. suspension). Furthermore, prepared complete feed formulation was given as feed on the *Anas javanica*. The complete feed formulation is shown in Table-1.

The third stage of this study was giving complete feed formulation to *Anas javanica* in improving the quality of *Anas javanica* egg. This study used 100 laying *Anas javanica*, aged about 20 weeks, divided into 5 treatments in the form of 5 types of formula feed which were T0: Complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension; T1: Complete feed + 15% soybean husk without *Cellulomonas* sp. bacteria suspension; T2: Complete feed + 15% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension; T3: Complete feed + 30% soybean husk without *Cellulomonas* sp. bacteria suspension; and T4: Complete feed + 30% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension (Table-1). The experimental design was complete randomized design (5×5 replicates). Parameters to improve the quality of *Anas javanica* eggs included egg cholesterol levels, egg weight, egg yolk, and eggshell thickness. Egg cholesterol (mg/100 g) levels were measured on day 7 before the end of the study. Cholesterol levels were tested using the Liebermann–Burchard's method [12,13]. Egg weight is measured by weighing using digital scales. Evaluation of egg yolk color estimated by the usual method applying La Roche scale (DSM Yolk Color Fan) with spectrophotometric determination of β-carotene by AOAC method, and by new rapid analyzer iCheckTM Egg photometer (BioAnalyt). The yolk color varied between the values of 4 and 13 of La Roche scale. The carotenoid content expressed as β-carotene measured by AOAC method varied between 11 and 87 mg/kg. The carotenoid content expressed as β-carotene measured with the analyzer Check TM Egg photometer was lower and varied between 7.5 and 68.5 mg/kg [14].

The measurements of eggshell thickness were done using ultrasonography technology. The measurements beginning from the large end to small end of the egg and repeated at each on 3 meridians in parallel. The measurements were taken with an electronic micrometer measurement predominantly at the wider end of eggs [15].

Statistical analysis

Cholesterol, egg weight, and eggshell thickness were statistically analyzed using SPSS 13 for Windows XP with the confidence level of 99% (α=0.01) and the level of significance 0.05 (p=0.05). Hypothesis tests were as follows: Normality test of the data with

Table-1: Complete feed formulation was given to *Anas javanica* using soybean husk waste fermented with *Cellulomonas* sp. bacteria suspension.

Materials (%)	T0 (Control) complete feed without soybean husk and *Cellulomonas* sp. bacteriasuspension	T1 (Treatment 1) complete feed+15% soybean husk without *Cellulomonas* sp. bacteria suspension	T2 (Treatment 2) complete feed+15% soybean husk+0.05% *Cellulomonas* sp. bacteria suspension	T3 (Treatment 3) complete feed+30% soybean husk without *Cellulomonas* sp. bacteria suspension	T4 (Treatment 4) complete feed+30% soybean husk+0.05% *Cellulomonas* sp. bacteria suspension
Yellow corn	61.00	46.00	46.00	31.00	31.00
Fish meal	13.80	13.75	13.80	13.80	13.75
Soy meal	5.60	5.60	5.60	5.60	5.60
Rice bran	14.70	14.70	14.70	14.70	14.70
Soybean	4.30	4.30	4.30	4.30	4.30
Coconut oil	0.30	0.30	0.30	0.30	0.30
Premix	0.30	0.30	0.30	0.30	0.30
Soybean husk	-	15	15	30	30
Cellulomonas sp. bacteria suspension	-	-	0.05	-	0.05
Total	100	100	100	100	100

Kolmogorov–Smirnov test, homogeneity of variance test, analysis of variance, and *post hoc* test using Tukey test with very significant difference 5% [16].

Results

Isolation and identification of cellulolytic bacteria of *S. litura*

The results of isolation and identification of the digestive tract of *S. litura*, which was the first stage of this study, it is found of 4 isolates of cellulolytic bacteria, they are *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp. Furthermore, the four isolates were, respectively, used as fermenters on the soybean husk from Tempe industry wastes derived from Usaha Tempe Rakyat Surabaya, Indonesia, at the next stage of the study.

Improving the quality of soybean husk waste

Improving the quality of soybean husk waste, which is the second stage of this research, is done through fermentation process with the addition of epidopt (Sugar Factory of Candi, Sidoarjo, Indonesia), urea (Petrokimia Gresik, Gresik, Indonesia), and various bacterial isolates which was obtained from first stage of the study (*Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp.) and compared with control (without addition of bacterial isolates). The results of this second stage study can be seen in Table-2.

The eggs quality of *Anas javanica*

The egg's quality of *Anas javanica* after feeding with a wide variety of complete feeds (both with the addition of soybean husk and the suspension of cellulolytic bacteria) compared with no addition was observed through cholesterol levels from egg yolks, egg weight, eggshell thickness, and egg yolk color.

The cholesterol eggs level of duck

The cholesterol eggs level (mg/100 g) based on Liebermann–Burchard's method [12,13] was measured on day 7 before the end of the study. The mean and standard deviation of cholesterol eggs levels of duck which is given with various feeding complete either by addition of soybean husk fermented using suspension of *Cellulomonas* sp. bacteria from *S. litura* was compared with not addition. The cholesterol eggs level can be seen in Table-3.

Egg weight, eggshell thickness, and egg yolk color

Mean, and standard deviation of duck egg quality, including egg weight, eggshell thickness, and egg yolk color which is given with various feeding complete either by addition of soybean husk waste fermentation using suspension of *Cellulomonas* sp. cellulose from *S. litura* was compared with not addition. Egg weight, eggshell thickness, and egg yolk color can be seen in Table-4.

Discussion

Based on the results of variance analysis, it was found that the content of crude fiber and CP of soybean husk fermentation using 4 bacterium: *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp. have shown significantly different results ($p<0.05$), while the content of dry material was not showed a significant difference ($p>0.05$). Based on Duncan's distance test for crude fiber content, the best result, the highest decrease of the crude fiber, was in T2 treatment, which was treated with a suspension of *Cellulomonas* sp.

Table-2: The content of dry material (%), crude fiber (%), and CP (%) of fermented soybean husk using various cellulolytic bacteria isolates from *S. litura*.

Treatment	Dry material (%)±SE	Crude fiber (%)±SE	CP (%)±SE
T0 (soybean husk+1% molasses+1% urea+without bacteria isolate)	78.15^{a}±0.05	48.60^{a}±0.14	15.63^{a}±0.26
T1 (soybean husk+1% molasses+1% urea+5% *Bacillus* sp. bacteria isolate)	78.79^{a}±0.82	48.73^{a}±0.53	15.85^{a}±0.73
T2 (Soybean husk+1% molasses+1% urea+5% *Cellulomonas* sp. bacteria isolate)	78.18^{a}±0.20	43.81^{b}±0.78	15.90^{a}±0.55
T3 (soybean husk+1% molasses+1% urea+5% *Pseudomonas* sp. bacteria isolate)	78.67^{a}±0.16	48.07^{a}±0.50	17.10^{b}±0.90
T4 (soybean husk+1% molasses+1% urea+5% *Cytophaga* sp. bacteria isolate)	78.40^{a}±0.19	48.58^{a}±1.38	17.57^{b}±0.68

^{a,b,c}Values in the same column with different superscripts indicate significant difference $p<0.05$ (n=4), CP=Crude protein, *S. litura*=*Spodoptera litura*, SE=Standard error

Table-3: Mean and standard deviation of egg yolk cholesterol levels of *Anas javanica*.

Treatments	Mean±SE
T0 (complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension)	18.74^{a}±2.19
T1 (complete feed+soybean husk 15% without *Cellulomonas* sp. bacteria suspension)	15.61^{b}±2.12
T2 (complete feed+soybean husk 15% + 0.05% *Cellulomonas* sp. bacteria suspension)	16.53^{b}±2.52
T3 (complete feed+soybean husk 30% without *Cellulomonas* sp. bacteria suspension)	13.35^{c}±1.92
T4 (complete feed+soybean husk 30% + 0.05% *Cellulomonas* sp. bacteria suspension.)	12.69^{c}±2.23

^{a,b,c}Values in the same column with different superscripts indicate significant difference $p<0.05$ (n=5), SE=Standard error

Table-4: Mean and standard deviation of egg weight, egg yolk color, and egg shell thickness of duck.

Variable	Treatment				
	T0 (complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension)	T1 (complete feed+15% soybean husk without *Cellulomonas* sp. bacteria suspension)	T2 (complete feed+15% soybean husk+0.05% *Cellulomonas* sp. bacteria suspension)	T3 (complete feed+30% soybean husk without *Cellulomonas* sp. bacteria suspension)	T4 (complete feed+soybean husk 30% + 0.05% *Cellulomonas* sp. bacteria suspension)
Egg weight (g)±SE	47.60^{a}±4.07	50.96^{a}±3.38	52.26^{a}±2.48	42.17^{a}±20.12	47.94^{a}±9.37
Egg yolk color±SE	10.20^{b}±1.79	8.20a^{b}±1.92	9.40^{b}±2.30	8.00^{ab}±1.41	6.40^{a}±2.30
Eggshell thickness (mm)±SE	0.55^{a}±0.08	0.52^{a}±0.08	0.53^{a}±0.03	0.53^{a}±0.07	0.53^{a}±0.08

^{a,b,c}Values in the same line with different superscripts indicate significant difference $p<0.05$ (n=5), SE=Standard error

According to Holt [17], the bacteria *Cellulomonas* sp. is Gram-positive, rod-shaped, and non-motile. The characteristic of this bacterium is as follows: Respiratory metabolism using oxygen as electron acceptor, catalase positive, lives at optimum temperature 300°C, and neutral pH, with growth rate 0.15-0.23/h. These bacteria have been known to digest cellulose, xylene, and starch. According to Gupta *et al*. [18], *Cellulomonas* sp. possesses extracellular enzymes that play a greater role in the breakdown of amorphous cellulose.

Observations on cholesterol levels were showed that feeding complete in T0, which produces the highest cholesterol levels and significantly different (p<0.05%) than T1, T2, and T3. The feeding complete in T4 has yielded the lowest cholesterol level compared with T3 treatment but significantly different (p<0.05%) with T1 and T2 treatment, whereas between T1 and T2 treatment did not significantly different (p> 0.05%). This result provides an opportunity to the utilization of complete feed with the addition of fermented soybean husk using *Cellulomonas* sp. bacteria suspension from which gives the best result as the lowest cholesterol level.

Several other studies, such as the provision of katuk leaf flour which also contains high crude fiber as well as soybeans husk, showed that katuk leaf flour at level ≥5% was also able to decrease cholesterol levels of eggs Mojosari duck without decreasing percentage of egg yolk weight [19]. However, since the use of katuk leaf flour must compete with food consumed by humans, the utilization of the soybean husk waste can be an alternative to consider. Furthermore, in many other studies on the use of various foliage powders with a high content of crude fiber, egg cholesterol levels of duck cannot be reduced. A study conducted by Palupi *et al*. [20], eggs cholesterol level of duck with an additional meal of beluntas leaves up to 2%; the level had no effect on egg cholesterol level of duck, where cholesterol levels at the treatment were still at 27.79 mg/g egg yolks.

Table-4 shows that egg yolk color parameters on T0, T1, T2, and T3 treatments result in a significantly different color of egg yolk (p<0.05), whereas T4 yields a lower yolk color than the other four treatments. This shows that the provision of soybean husk fermentation from Tempe industry waste as much as 30% as a substitute for corn can affect the color of egg yolks. Subhan [21] reported that the score of egg yolk color of the *Anas javanicus* from Tegal region, Indonesia was < 7.5, while Beardsworth and Hernandez [22] stated that the good egg yolk color was in the range of 8-12. The good egg yolk color in the range of 8-12 was obtained with the addition of corn to the feed. Corn is one of the agricultural commodities very important for livestock. Corn is a high-energy feed ingredient, with a protein content of about 8.6-9.0%, but corn protein cannot ferment or degraded by rumen microorganisms [23].

The parameter observation of egg weight and eggshell thickness was not showed a significant difference between treatments (p>0.05). This shows that the utilization of soybean husk waste fermented with *Cellulomonas* sp. bacteria up to 30% dose does not affect egg weight or eggshell thickness. Silversides and Villeneuve [24] reported that with increasing age, the egg size would increase as a result of increased yolk weight.

In poultry, including ducks, the process of egg formation known as folliculogenesis, in addition to affecting the development of the oocyte (egg cell), also affects the weight of the egg yolk. The number of follicles during one cycle is influenced by factors such as animal species, reproductive phase, circumstances, age, mother, genetic [25], and feed [26-28].

Conclusion

The fermentation of soybean husk from Tempe industry waste through the utilization of cellulolytic bacteria of *S. litura* added to complete feed can be done as an effort to improve the quality of *Anas javanic*a eggs in the form decrease of egg yolks cholesterol level without decreasing egg weight and eggshell thickness, although the decrease in yolk color is unavoidable statistically does not show significant differences (p>0.05).

Authors' Contributions

All the authors conceptualized the manuscript. SH and ES drafted the manuscript. SH: Research

project leader and coordinating research, collected and processed samples. Carried out the data collection and gathering assay samples. DSN has done the statistical analysis part and critically reviewed the manuscript. ES: Assisted in manuscript preparation and corresponding author. All the authors have read and approved the final version of the manuscript.

Acknowledgments

The study was supported by funding from the Directorate General of Higher Education (DIKTI) 2015 with number 519/UN3/2015, Date March, 26, 2015. The National Education Ministry, Republic of Indonesia.

Competing Interests

The authors declare that they have no competing interests.

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doi: 10.14202/vetworld.2018.720-725

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51 RESEARCH ARTICLE

Soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura* to improve the eggs quality of Mojosari duck (*Anas javanica*)

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Received: 06-03-2018, **Accepted:** 25-04-2018**, Published online:** ***

doi: *** **How to cite this article:** Hidanah S, Nazar DS, Safitri E (2018) Soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura* to improve the eggs quality of Mojosari duck (*Anas javanica*), *Veterinary World*, 11(5): 0-0.

Abstract

Aim : This study was aimed to improve the eggs quality of Mojosari duck (*Anas javanica*) through complete feeding containing soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura*.

Materials and Methods: This study was consisted of three stages: The first stages, isolation and identification of cellulolytic bacteria from *S. litura*; the second stage, the fermentation of soybean husk through the application of bacterial cellulolytic isolate from the first stage; and the third stage, the application of the best complete feed formulation from the second stage to Mojosari duck.

Results: There are four dominant bacteria: *Bacillus*, *Cellulomonas* spp., *Pseudomonas*, and *Cytophaga*. Furthermore, the best reduction of the crude fiber of soybean husks is the use of *Cellulomonas* sp. bacteria. The final of the study, the eggs quality of Mojosari duck, was improved, as indicated by cholesterol decrease from the yolk without the decrease of egg weight and eggshell thickness, although the decrease in egg yolk color was inevitable.

Conclusion: Soy husk fermentation using cellulolytic bacteria of *S. litura* was added to complete feeding can be performed to improve the eggs quality of Mojosari duck.

Keywords: cellulolytic bacteria, eggs quality of duck, soybean husk fermentation, *Spodoptera litura.*

<H1>Introduction

Soybean is an agricultural product that has been utilized to meet the needs of industry and food, such as Tempe, tofu, soy sauce, and soy milk. In general, the use and utilization of soybean are limited to seeds only, while the waste, such as soybean husk, is still discarded and has not been widely utilized. Analysis of dry matter (DM)=91.11, crude protein (CP)=5.04, ether extract=1.65, nitrogen-free extract, calcium=21, phosphorus=0.06, and gross energy=(kcal/g. DM) 3.98 according to the methods described in AOAC. The analysis of neutral detergent fiber=60.15 and acid detergent fiber=42.08 was carried out according to detergent method [1]. In other research, the chemical composition of soybean husk comprises 47.01% crude fiber, 14.45% CP, 3.04% crude fat, 3.15% ash, and 3.060, 48 kcal kg of metabolic energy. Soybean husk contains 42-49% dry weight of cellulose, 29-34% hemicellulose, and 1-3% lignin and has anti-nutritional antitrypsin substances [2].

On the other hand, *Spodoptera litura* is a pest of soybean crop that has a very high ability in damaging the plant. The leaves and pods attacked by *S. litura* become holes even then torn

99 [3]. Based on its ability to damage the leaves and pods, allegedly the digestive tract of *S.*
100 *litura* contains cellulolytic bacteria capable of digesting crude fibers well [4].

101 In general, cellulolytic bacteria have three cellulose enzymes called endoglucanase or
102 carboxymethylcellulose (CMC-ase), exoglucanase or cellobiohydrolase, and beta-glucosidase.
103 The enzymes can degrade cellulose into glucose [5]. CMC-ase breaks the hydrogen bonds
104 present in the cellulose crystalline structure, forming single cellulose chains. Exoglucanase
105 cuts off the ends of single chains cellulose, producing disaccharides and tetrasaccharides,
106 cellobiose, beta-glucosidase hydrolyzes disaccharides, and tetrasaccharides into glucose [6].
107 Therefore, the utilization of cellulolytic microbes in the fermentation process of the feed
108 material from the waste can allegedly improve the quality of complete feed formulation with
109 the indication of the decrease of crude fiber and the increase of CP.

110 Based on another study, the soybean husk waste fermented with *Aspergillus niger* and
111 Lactobacillus was only able to decrease crude fiber from 44% to 40%. The decrease in crude
112 fiber content is still relatively small. In addition to the decrease in crude fiber, the
113 fermentation process is also expected to increase CP from processed waste material [7].
114 Therefore, we need an alternative bacterium that has higher capability in breaking down

115 crude fiber along with an increase in CP content of the soybean husk.

116 This study aims to determine the potential of cellulolytic bacteria was contained in *S. litura* as
117 a source of probiotics that can reduce the soybean crude fiber derived from the Tempe
118 industry through the fermentation process, but followed by increased CP. If this is realized,
119 then the quality of complete feed formulation on feed given to Mojosari duck (*Anas javanica*)
120 will be improved. Furthermore, improving the quality of complete feed formulation on feed
121 was given to Mojosari duck (Anas javanica) is expected to affect the quality of the eggs
122 produced, such as low cholesterol levels with maintaining eggs' weight, yolk color, and
123 thickness of the shell.

124 **<H1>Materials and Methods**

125 **<H2>Ethical approval**

126 ???

127 **<H2>Stage of study**

128 This study was consisted of three stages: The first stage, isolation and identification of
129 cellulolytic bacteria from *S. litura* digestive tract [4,8]; in total, 4 bacteria, i.e., Bacillus,

130 *Cellulomonas* sp., Pseudomonas, and *Cytophaga* were characterized based on their colony
131 color, morphological, biochemical, and molecular characteristics of bacteria.

132 We explored the culturable bacterial community in the digestive tract of *S. litura* using
133 culture-dependent technique based on 16S rRNA gene sequencing and screening of these four
134 isolates. Bacterial isolation was performed on living larvae separately. The larva was
135 homogenized in nutrient extract using a glass pounder, and the homogenate is filtered 2 times
136 to remove larvae debris than input into sterile tubes. The larvae extract a number of 50 µL
137 were placed on nutrient agar and incubated at 37°C in a humidified atmosphere containing at
138 5% CO_2 moisture and allowed to increase the number of bacteria for 3 days. Isolates were
139 distinguished based on colony color and morphology. After that, the pure cultures of bacterial
140 colonies were added into 20% glycerol and prepared at the Laboratory of Microbiology of the
141 Department of Microbiology, Faculty of Veterinary Medicine, Airlangga University.

142 Identification of bacterial isolates was identified by various tests, such as the utilization of
143 organic compounds, spore formation, Gram staining, NaCl tolerance, optimum temperature,
144 optimum pH, and catalase [4].

145 The isolate identification of four bacteria was confirmed using 16S rRNA gene sequencing.

146 The standard protocol was used for confirm of total genomic DNA extraction. The isolated
147 DNAs of each bacteria, i.e., Bacillus, *Cellulomonas* sp., Pseudomonas, and *Cytophaga* were
148 stored at −20°C until use. Furthermore, the polymerase chain reaction (PCR) amplification of
149 the 16S rRNA genes was performed using the universal primers UNI16S-L (5'-
150 ATTCTAGAGTTTGATCATGGCTCA-3') as the forward primer and UNI16S-R (5'-
151 ATGGTACCGTGTGA CGGGCGGTGTGTA-3') as the reverse primer and then
152 Amplification process in a thermocycler (Eppendorf, Mastercycler Gradient, Hamburg,
153 Germany) for 36 reaction cycles. Reactions were routinely performed in 50 µL including 1.5
154 µL of 10 mM dNTP mix, 1.5 µL of 10 pmol each of the opposing amplification primers, 1 µL
155 of 5 U/µL Taq DNA polymerase (Fermentas), 3 µL of MgCl2, 5 µL of Taq DNA polymerase
156 reaction buffer, 1 µL of genomic DNA, and 35.5 µL of dH2 O. PCR conditions were 5 min at
157 95°C for the initial denaturation of template DNA, 36 amplification cycles (1 min at 94°C, 1
158 min at 56°C, and 2 min at 72°C), and 10 min at 72°C for the final extension. PCR products
159 were separated on 1.0% agarose gels, stained with ethidium bromide, and viewed under
160 ultraviolet light. After checking the PCR products, they were sent to Macrogen (the
161 Netherlands) for sequencing. The obtained sequences were used to perform BLAST searches
162 using the NCBI GenBank database. In addition, sequences were used for phylogenetic

163 analysis for further characterization [9].

164 The second stage, the process of soybean fermentation from Tempe industry waste (Usaha
165 Tempe Rakyat, Surabaya, Indonesia), with the addition of Epidopt (Sugar Factory Candi,
166 Sidoarjo, Indonesia), urea (Petrokimia Gresik, Gresik, Indonesia), and various bacterial
167 isolates obtained from Stage 1 studies compared with control (without addition of bacterial
168 isolate). Fermentation is one of the major processes used in the production of food from
169 soybeans. This fermentation changes the physicochemical and organoleptic properties of soy
170 products such as color, flavor, and active components [10].

171 The second stage used complete randomized design with 5 treatments and 4 replicates [11].
172 The treatment was: T0: Soybean husk + 1% molasses + 1% urea + without bacterial isolate;
173 T1: Soybean husk + 1% molasses + 1% urea + 5% bacillus bacterial isolate; T2: Soybean
174 husk + 1% molasses + 1% urea + 5% bacteria *Cellulomonas* sp. isolate ; T3: Soybean husk +
175 1% molasses + 1% urea + 5% pseudomonas bacterial isolate; and T4: Soybean husk+ 1%
176 molasses + 1% urea + 5% *Cytophaga* bacterial isolate.

177 A total of 20 samples of soybean husk, each weighing 200 g, were randomly divided into 5
178 treatments with 4 replicates, 1% urea and epidopt and 5% of cellulolytic bacteria (108/cc)

dissolved in a diluent solution of sterile water as much as 30% of the sample weight. Subsequently, the solution was sprayed on the husk of the soybeans and inserted into a plastic bag (clear, hollow in some places, and tied at the top), and fermented for 7 days. After the fermentation process ended, organoleptic examination was done, including color, odor, texture, and pH measurement. Then, the fermented husk was aerated. Furthermore, to determine the content of DM, crude fiber, and CP, the proximate analysis was performed according to the method recommended by Wendee [1]. The best results of this second stage were T2: Soybean husk + *Cellulomonas* sp. suspension (1% Molasses + 1% urea + 5% isolate *Cellulomonas* sp. as fermenter).

The third stage of this study was the application of a complete feed formulation by adding fermentation of the best result of second stage: Various percentage of soybean husk + *Cellulomonas* sp. suspension, compared with control (without *Cellulomonas* sp. suspension). Furthermore, prepared complete feed formulation was given as feed on the Mojosari duck (Anas javanica). The complete feed formulation is shown in Table-1.

The third stage of this study was giving complete feed formulation to Mojosari duck (Anas javanica) in improving the quality of Mojosari duck (Anas javanica) egg. This study used 100

laying Mojosari duck*s* (Anas javanica), aged about 20 weeks, divided into 5 treatments in the form of 5 types of formula feed which were T0: Complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension; T1: Complete feed + 15% soybean husk without *Cellulomonas* sp. bacteria suspension; T2: Complete feed + 15% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension; T3: Complete feed + 30% soybean husk without *Cellulomonas* sp. bacteria suspension; and T4: Complete feed + 30% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension (Table-1). The experimental design was complete randomized design (5×5 replicates). Parameters to improve the quality of Mojosari duck (Anas javanica) eggs included egg cholesterol levels, egg weight, egg yolk, and eggshell thickness. Egg cholesterol (mg/100 g) levels were measured on day 7 before the end of the study. Cholesterol levels were tested using the Liebermann–Burchard's method [12,13]. Egg weight is measured by weighing using digital scales. Evaluation of egg yolk color estimated by the usual method applying La Roche scale (DSM Yolk Color Fan) with spectrophotometric determination of β-carotene by AOAC method, and by new rapid analyzer iCheckTM Egg photometer (BioAnalyt). The yolk color varied between the values of 4 and 13 of La Roche scale. The carotenoid content expressed as β-carotene measured by AOAC method varied between 11 and 87 mg/kg. The carotenoid content expressed as β-

carotene measured with the analyzer Check TM Egg photometer was lower and varied between 7.5 and 68.5 mg/kg [14].

The measurements of eggshell thickness were done using ultrasonography technology. The measurements beginning from the large end of the egg and repeated at each parallel on 3 meridians. The measurements were taken with an electronic micrometer measurement predominantly at the wider end of eggs [15].

<H2>Statistical analysis

Cholesterol, egg weight, and eggshell thickness were statistically analyzed using SPSS 13 for Windows XP with the confidence level of 99% (α=0.01) and the level of significance 0.05 (p=0.05). Hypothesis tests were as follows: Normality test of the data with Kolmogorov–Smirnov test, homogeneity of variance test, analysis of variance, and *post hoc* test using Tukey test with very significant difference 5% [16].

<H1>Results

<H2>Isolation and Identification of cellulolytic bacteria of *S. litura*

The results of isolation and identification of the digestive tract of *S. litura*, which was the first

stage of this study, it's found of 4 isolates of cellulolytic bacteria, they are bacillus, *Cellulomonas* sp., Pseudomonas, and *Cytophaga*. Furthermore, the four isolates were, respectively, used as fermenters on the soybean husk from Tempe industry wastes derived from Usaha Tempe Rakyat Surabaya, Indonesia, at the next stage of the study.

<H2>Improving the quality of soybean husk waste

Improving the quality of soybean husk waste, which is the second stage of this research, is done through fermentation process with the addition of epidopt (Sugar Factory of Candi, Sidoarjo, Indonesia), urea (Petrokimia Gresik, Gresik, Indonesia), and various bacterial isolates which was obtained from first stage of the study (*Bacillus*, *Cellulomonas* sp., *Pseudomonas*, and *Cytophaga*) and compared with control (without addition of bacterial isolates). The results of this second stage study can be seen in Table-2.

<H2>The eggs quality of Mojosari duck (Anas javanica)

The egg's quality of Mojosari duck (Anas javanica) after feeding with a wide variety of complete feeds (both with the addition of soybean husk and the suspension of cellulolytic bacteria) compared with no addition was observed through cholesterol levels from egg yolks,

egg weight, eggshell thickness, and egg yolk color.

<H2>The cholesterol eggs level of duck

The cholesterol eggs level (mg/100 g) based on Liebermann–Burchard's method [12,13] was measured on day 7 before the end of the study. The mean and standard deviation of cholesterol eggs levels of duck which is given with various feeding complete either by addition of soybean husk fermented using suspension of *Cellulomonas* sp. bacteria from *S. litura* was compared with not addition. The cholesterol eggs level can be seen in Table-3.

<H2>Egg weight, eggshell thickness, and egg yolk color

Mean and standard deviation of duck egg quality, including egg weight, eggshell thickness, and egg yolk color which is given with various feeding complete either by addition of soybean husk waste fermentation using suspension of Cellulomonas sp. cellulose from *S. litura* was compared with not addition. Egg weight, eggshell thickness, and egg yolk color can be seen in Table-4.

<H1>Discussion

Based on the results of variance analysis, it was found that the content of crude fiber and CP

of soybean husk fermentation using 4 bacterium: Bacillus, *Cellulomonas* sp., Pseudomonas, and *Cytophaga* have shown significantly different results ($p<0.05$), while content of dry material was not showed significant difference ($p>0.05$). Based on Duncan's distance test for crude fiber content, the best result, the highest decrease of the crude fiber, was in T2 treatment, which was treated with a suspension of *Cellulomonas* sp.

According to Holt [17], the bacteria *Cellulomonas* sp. is Gram-positive, rod-shaped, and non-motile. The characteristic of this bacterium is as follows: Respiratory metabolism using oxygen as electron acceptor, catalase positive, lives at optimum temperature 300°C, and neutral pH, with growth rate 0.15-0.23/h. These bacteria have been known to digest cellulose, xylene, and starch. According to Gupta *et al*. [18], *Cellulomonas* sp., possesses extracellular enzymes that play a greater role in the breakdown of amorphous cellulose.

Observations on cholesterol levels were showed that feeding complete in T0, which is produce the highest cholesterol levels and significantly different ($p<0.05\%$) than T1, T2, and T3. The feeding complete in T4 was yielded the lowest cholesterol level compared with T3 treatment but significantly different ($p<0.05\%$) with T1 and T2 treatment, where between T1 and T2 treatment were not significantly different ($p>0.05\%$).

This result provides an opportunity to the utilization of complete feed with the addition of fermented soybean husk using *Cellulomonas* sp. bacteria suspension from which gives the best result as an the lowest cholesterol level.

Several other studies, such as the provision of katuk leaf flour which also contains high crude fiber as well as soybeans husk, showed that katuk leaf flour at level ≥5% was also able to decrease cholesterol levels of eggs Mojosari duck without decreasing percentage of egg yolk weight [19]. However, since the use of katuk leaf flour must compete with food consumed by humans, the utilization of the soybean husk waste can be an alternative to consider. Furthermore, in many other studies on the use of various foliage powders with a high content of crude fiber, egg cholesterol levels of duck cannot be reduced. A study conducted by Palupi *et al*. [20], eggs cholesterol level of duck with an additional meal of beluntas leaves up to 2%, the level had not effect on egg cholesterol level of duck, where cholesterol levels at the treatment were still at 27.79 mg/g egg yolks.

Table-4 shows that egg yolk color parameters on T0, T1, T2, and T3 treatments result in a significantly different color of egg yolk ($p<0.05$), whereas T4 yields a lower yolk color than the other four treatments. This is shows that the provision of soybean husk fermentation from

289 Tempe industry waste as much as 30% as a substitute for corn can affect the color of egg
290 yolks. Based on research by Subhan [21] was reported that the egg yolk colour of the Tegal
291 duck only 7.120, while Beardsworth and Hernandez [22] stated that the good egg yolk color
292 was in the range of 8-12. The good egg yolk color in the range of 8-12 was obtained with the
293 addition of corn to the feed. Corn is one of the agricultural commodities very important for
294 livestock. Corn is a high-energy feed ingredient, with a protein content of about 8.6-9.0%, but
295 corn protein cannot fermented or degraded by rumen microorganisms [23].

296 The parameter observation of egg weight and eggshell thickness was not showed significant
297 difference between treatments ($p>0.05$). This is shows that the utilization of soybean husk
298 waste fermented with *Cellulomonas* sp. bacteria up to 30% dose does not affect on egg
299 weight or eggshell thickness. Silversides and Villeneuve [24] reported that with increasing
300 age, the egg size will increase as a result of increased yolk weight.

301 In poultry, including ducks, the process of egg formation known as folliculogenesis, in
302 addition to affecting the development of the oocyte (egg cell), also affects the weight of the
303 egg yolk. The number of follicles during one cycle is influenced by factors such as animal
304 species, reproductive phase, circumstances, age, mother, genetic [25], and feed [26-28].

<H1>Conclusion

The fermentation of soybean husk from Tempe industry waste through the utilization of cellulolytic bacteria of *S. litura* added to complete feed can be done as an effort to improve the quality of Mojosari duck (Anas javanica) eggs in the form decrease of egg yolks cholesterol level without decreasing egg weight and eggshell thickness, although the decrease in yolk color is unavoidable statistically does not show significant differences ($p>0.05$).

<H1>Authors' Contributions

All the authors conceptualized the manuscript. Both SH and ES drafted the manuscript. SH: Research project leader and coordinating research, collected and processed samples. Carried out the data collection and gathering assay samples. DSN has done the statistical analysis part and critically reviewed the manuscript. ES: Assisted in manuscript preparation and corresponding author. All the authors have read and approved the final version of the manuscript.

<H1>Acknowledgments

The study was supported by funding from the Directorate General of Higher Education

(DIKTI) 2015. The National Education Ministry, Republic of Indonesia.

<H1>Competing Interests

The authors declare that they have no competing interests.

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403 **Tables**

Table-1: Complete feed formulation was given to Mojosari duck (Anas javanica) using soybean husk waste fermented with *Cellulomonas* sp. bacteria suspension.					
Materials (%)	T0 (Control) complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension.	T1 (Treatment 1) complete feed + 15% soybean husk without *Cellulomonas* sp. bacteria suspension	T2 (Treatment 2) complete feed + 15% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension	T3 (Treatment 3) complete feed + 30% soybean husk without *Cellulomonas* sp. bacteria suspension.	T4 (Treatment 4) complete feed + 30% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension.
Yellow corn	61.00	46.00	46.00	31.00	31.00
Fish meal	13.80	13.75	13.80	13.80	13.75
Soy meal	5.60	5.60	5.60	5.60	5.60
Rice bran	14.70	14.70	14.70	14.70	14.70

Soybean	4.30	4.30	4.30	4.30	4.30
Coconut oil	0.30	0.30	0.30	0.30	0.30
Premix	0.30	0.30	0.30	0.30	0.30
Soybean husk	-	15	15	30	30
Cellulomonas sp. bacteria suspension	-	-	0.05	-	0.05
Total	100	100	100	100	100

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Table-2: The content of dry material (%), crude fiber (%), and CP (%) of fermented soybean husk using various cellulolytic bacteria isolates from *S. litura.*			
Treatment	Dry material (%)±SE	Crude fiber (%)±SE	CP (%)±SE

T0 (soybean husk + 1% molasses + 1% urea + without bacteria isolate)	78.15[a]±0.05	48.60[a]±0.14	15.63[a]±0.26
T1 (soybean husk + 1% molasses + 1% urea + 5% bacillus bacteria isolate)	78.79[a]±0.82	48.73[a]±0.53	15.85[a]±0.73
T2 (Soybean husk + 1% molasses + 1% urea + 5% *Cellulomonas* sp. bacteria isolate)	78.18[a]±0.20	43.81[b]±0.78	15.90[a]±0.55
T3 (soybean husk + 1% molasses + 1% urea + 5% *Pseudomonas* bacteria isolate)	78.67[a]±0.16	48.07[a]±0.50	17.10[b]±0.90
T4 (soybean husk + 1% molasses + 1% urea + 5% *Cytophaga* bacteria isolate)	78.40[a]±0.19	48.58[a]±1.38	17.57[b]±0.68
[a,b,c]Values in the same column with different superscripts indicate significant difference $p<0.05$ (n=4), CP=Crude protein, *S. litura*=*Spodoptera litura*,			

SE=Standard error

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Table-3: Mean and standard deviation of egg yolk cholesterol levels of Mojosari ducl (Anas javanica).	
Treatments	Mean±SE
T0 (complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension)	18.74^{a}±2.19
T1 (complete feed + soybean husk 15% without *Cellulomonas* sp. bacteria suspension)	15.61^{b}±2.12
T2 (complete feed + soybean husk 15% + 0.05% *Cellulomonas* sp. bacteria suspension)	16.53^{b}±2.52
T3 (complete feed + soybean husk 30% without *Cellulomonas* sp. bacteria suspension)	13.35^{c}±1.92
T4 (complete feed + soybean husk 30% + 0.05% *Cellulomonas* sp. bacteria suspension.)	12.69^{c}±2.23

[a,b,c]Values in the same column with different superscripts indicate significant difference p<0.05 (n=5), SE=Standard error

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Table-4: Mean and standard deviation of egg weight, egg yolk color, and egg shell thickness of duck.

Variable	Treatment				
	T0 (complete feed without soybean husk and *Cellulomonas* sp.	T1 (complete feed + 15% soybean husk without *Cellulomonas* sp.	T2 (complete feed + 15% soybean husk + 0.05% *Cellulomonas* sp.	T3 (complete feed + 30% soybean husk without *Cellulomonas* sp.	T4 (complete feed + soybean husk 30% + 0.05% *Cellulomonas* sp.

	bacteria suspension.)	bacteria suspension)	bacteria suspension)	bacteria suspension)	bacteria suspension.)
Egg weight (g)±SE	47.60[a]±4.07	50.96[a]±3.38	52.26[a]±2.48	42.17[a]±20.12	47.94[a]±9.37
Egg yolk color±SE	10.20[b]±1.79	8.20[ab]±1.92	9.40[b]±2.30	8.00[ab]±1.41	6.40[a]±2.30
Eggshell thickness (mm)±SE	0.55[a]±0.08	0.52[a]±0.08	0.53[a]±0.03	0.53[a]±0.07	0.53[a]±0.08
[a,b,c]Values in the same line with different superscripts indicate significant difference p<0.05 (n=5), SE=Standard error					

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Soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura* to improve the eggs quality of *Mojosari duck* (*Anas javanica*)

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Abstract

Background and Aim : This study was aimed to improve the eggs quality of *Mojosari duck* (*Anas javanica*) through complete feeding containing soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura*.

Materials and Methods : The first stages: isolation and identification of cellulolytic bacteria from *Spodoptera litura*; the second stage: the fermentation of soybean husk through the application of bacterial cellulolytic isolate from the first stage; the third stage: the application of the best complete feed formulation from the second stage to *Mojosari duck*.

Results: there are four dominant bacteria: *bacillus*, *Cellumonas sp*, *pseudomonas*, and *cytophaga*. Furthermore, the best reduction of the crude fiber of soybean husks is the use of *Cellumonas sp*. bacteria. The final of the study, the eggs quality of Mojosari duck was improved, as indicated by cholesterol decrease from the yolk without the decrease of egg weight and egg shell thickness, although the decrease in egg yolk colour was inevitable.

Conclusion : soy husk fermentation using cellulolytic bacteria of *Spodoptera litura* was added to complete feeding can be performed to improve the eggs quality of *Mojosari duck.*

Keywords: *Spodoptera litura*, cellulolytic bacteria, soybean husk fermentation, eggs quality of duck

Introduction

Soybean is an agricultural product that has been utilized to meet the needs of industry and food, such as *tempe*, tofu, soy sauce and soy milk. In general, the use and utilization of soybean is limited to seeds only, while the waste, such as soybean husk, is still discarded and has not been widely utilized. Analysis of dry matter (DM) = 91.11, crude protein (CP) = 5.04, ether extract (EE) = 1.65, nitrogen free extract (NFE), calcium (Ca) = 21, phosphorus (P) = 0.06, and gross energy (GE) = (kcal/g.DM) 3.98 according to the methods described in AOAC.

The analysis of neutral detergent fibre (NDF) = 60.15 and acid detergent fibre (ADF) = 42.08 was carried out according to Detergent method [1]. In other research, chemical composition of soybean husk comprises 47.01% crude fiber, 14.45% crude protein, 3.04% crude fat, 3.15% ash, and 3.060, 48 kcal kg of metabolic energy. Soybean husk contains 42 - 49% dry weight of cellulose, 29 - 34% hemicellulose, and 1 - 3% lignin, and has anti-nutritional antitrypsin substances [2].

On the other hand, *Spodoptera litura* is a pest of soybean crop that has a very high ability in damaging the plant. The leaves and pods attacked by *Spodoptera litura* become holes even then torn [3]. Based on its ability to damage the leaves and pods, allegedly the digestive tract of *Spodoptera litura* contains cellulolytic bacteria capable of digesting crude fibers well [4].

In general, cellulolytic bacteria have 3 cellulose enzymes called *endogluconase or carboxymethylcellulose (CMC-ase), exogluconase or cellulobiohidrolase* and *beta-glucosidase*. The enzymes can degrade cellulose into glucose [5]. CMC-ase breaks the hydrogen bonds present in the cellulose crystalline structure, forming single cellulose chains. Exogluconase cuts off the ends of single chains cellulose, producing disaccharides and tetrasaccharides, cellobiose, beta-glucosidase hydrolyzes disaccharides and tetrasaccharides into glucose [6]. Therefore, the utilization of cellulolytic microbes in the fermentation process of the feed material from the waste can allegedly improve the quality of complete feed formulation with the indication of the decrease of crude fiber and the increase of crude protein.

Based on other study, the soybean husk waste fermented with aspergilus niger and lactobacillus was only able to decrease crude fiber from 44% to 40%. The decrease in crude fiber content is still relatively small. In addition to the decrease in crude fiber, fermentation process is also expected to increase crude protein from processed waste material [7]. Therefore, we need an alternative bacterium that has higher capability in breaking down crude fiber along with an increase in crude protein content of the soybean husk.

This study aims to determine the potential of cellulolytic bacteria was contained in *Spodoptera litura* as a source of probiotics that can reduce the soybean crude fiber derived from the *tempe* industry through the fermentation process, but followed by increased crude protein. If this is realized, then the quality of complete feed formulation on feed given to *Mojosari duck* (*Anas javanica*) will be improved. Furthermore, improving the quality of complete feed formulation on feed was given to *Mojosari* duck (Anas javanica) is expected to affect the quality of the eggs produced, such as low cholesterol levels with maintaining eggs' weight, yolk colour, and thickness of the shell.

Materials and methods

Stage of study

This study was consisted of three stages: The first stage, isolation and identification of cellulolytic bacteria from *Spodoptera litura* digestive tract [4, 8]; In total, 4 bacteria ie Bacillus, *Cellulomonas sp*, Pseudomonas and Cytophaga were characterized based on their colony color, morphological, biochemical and molecuar characteristics of bacteria. .

We explored the culturable bacterial community in the digestive tract of *Spodoptera litura* by using culture dependent technique based on 16S rRNA gene sequencing and screening of these four isolates. Bacterial isolation was performed on living larvae separately. The larva were homogenized in nutrient extract using a glass pounder and the homogenate is filtered two times to remove larvae debris than input into sterile tubes. The larvae extracts a number of 50 µl were placed on nutrient agar and incubated at 37°C in a humidified atmosphere containing at 5% CO2 moisture and allowed to increase the number of bacteria for 3 days. Isolates were distinguished based on colony color and morphology. After that, the pure cultures of bacterial colonies were added into 20% glycerol and prepared at the Laboratory of Microbiology of the Department of Microbiology, Faculty of Veterinary Medicine, Airlangga University.

Identification of bacterial isolates Bacterial isolates were identified by various tests, such as the utilization of organic compounds, spore formation, Gram staining, NaCl tolerance, optimum temperature, optimum pH, and catalase [4].

The isolate identification of four bacteria was confirmed using 16S rRNA gene sequencing. The standard protocol was used for confirm of total genomic DNA extraction. The isolated DNAs of each bacteria ie Bacillus, *Cellulomonas sp*, Pseudomonas and Cytophaga were stored at –20 °C until use. Furthermore, the PCR amplification of the 16S rRNA genes was performed using the universal primers UNI16S-L (5'-ATTCTAGAGTTTGATCATGGCTCA-3') as the forward primer and UNI16S-R (5'-ATGGTACCGTGTGA CGGGCGGTGTGTA-3') as the reverse primer and then Amplification process in a thermocycler (Eppendorf, Mastercycler Gradient, Hamburg, Germany for 36 reaction cycles. Reactions were routinely performed in 50 µL including 1.5 µL of 10 mM dNTP mix, 1.5 µL of 10 pmol each of the opposing amplification primers, 1 µL of 5 U/µL Taq DNA polymerase (Fermentas), 3 µL of MgCl2 , 5 µL of Taq DNA polymerase reaction buffer, 1 µL of genomic DNA, and 35.5 µL of dH2 O. PCR conditions were 5 min at 95 °C for the initial denaturation of template DNA, 36 amplification cycles (1 min at 94 °C, 1 min at 56 °C, 2 min at 72 °C), and 10 min at 72 °C for the final extension. PCR products were separated on 1.0% agarose gels, stained with ethidium bromide, and

viewed under UV light. After checking the PCR products, they were sent to Macrogen (the Netherlands) for sequencing. The obtained sequences were used to perform BLAST searches using the NCBI GenBank database. Additionally, sequences were used for phylogenetic analysis for further characterization [9].

The second stage, the process of soybean fermentation from *tempe* industry waste (Usaha *Tempe* Rakyat, Surabaya, Indonesia) with the addition of epidopt (Sugar Factory Candi, Sidoarjo, Indonesia), urea (Petrokimia Gresik, Gresik, Indonesia), with the addition of various bacterial isolates obtained from stage 1 studies compared with control (without addition of bacterial isolate). Fermentation is one of the major processes used in the production of food from soybeans. This fermentation changes the physico-chemical and organoleptic properties of soy products such as color, flavor and active components [10].

The second stage used complete randomized design with 5 treatments and 4 replicates [11]. The treatment was: T0: Soybean husk + 1% Molases + 1% urea + without bacterial isolate; T1: Soybean husk + 1% Molases + 1% urea + 5% Bacillus bacterial isolate; T2: Soybean husk + 1% Molases + 1% urea + 5% bacteria *Cellulomonas sp* isolate ; T3: Soybean husk + 1% Molases + 1% urea + 5% pseudomonas bacterial isolate, T4: Soybean husk+ 1% Molases + 1% urea + 5% cytophaga bacterial isolate.

The total of 20 samples of soybean husk, each weighing 200 grams, were randomly divided into 5 treatments with 4 replicates, 1% urea and epidopt and 5% of cellulolytic bacteria (108/ cc) dissolved in a diluent solution of sterile water as much as 30% of the sample weight. Subsequently, the solution was sprayed on the husk of the soybeans and inserted into a plastic bag (clear, hollow in some places and tied at the top), and fermented for 7 days. After the fermentation process ended, organoleptic examination was done, including colour, odor, texture, and pH measurement. Then, the fermented husk was aerated. Furthermore, to determine the content of dry matter, crude fiber, and crude protein, proximate analysis was performed according to the method recommended by Wendee [1]. The best results of this second stage were T2: Soybean husk + *Cellulomonas sp*. suspension (1% Molases + 1% urea + 5% isolate *Cellulomonas sp* as fermentor).

The third stage of this study was application of a complete feed formulation by adding fermentation of the best result of second stage : various % of soybean husk + *Cellulomonas sp.* suspension, compared with control (without *Cellulomonas sp*. suspension). Furthermore, prepared complete feed formulation was given as feed on the *Mojosari duck* (*Anas javanica*). The complete feed formulation can be seen in Table 1.

The third stage of this study was giving complete feed formulation to *Mojosari duck* (*Anas javanica*) in improving the quality of *Mojosari duck* (*Anas javanica*) egg. This study used 100 laying *Mojosari ducks* (*Anas javanica*), aged about 20 weeks, divided into 5 treatments in the form of 5 types of formula feed which were T0:

complete feed without soybean husk and *Cellulomonas sp* bacteria suspension; T1: complete feed + 15% soybean husk without *Cellulomonas sp* bacteria suspension; T2: complete feed + 15% soybean husk + 0.05% *Cellulomonas sp* bacteria suspension: T3: complete feed + 30% soyban husk without *Cellulomonas sp* bacteria suspension, and T4: complete feed + 30% soybean husk + 0.05% *Cellulomonas sp* bacteria suspension (Table 1). The experimental design was complete randomized design (5 × 5 replicates). Parameters to improve the quality of *Mojosari duck* (*Anas javanica*) eggs included egg cholesterol levels, egg weight, egg yolk, and eggshell thickness. Egg cholesterol (mg/ 100 g) levels was measured on day 7 before the end of the study. Cholesterol levels were tested using the Lieberman Burchard's method [12-13]. Egg weight is measured by weighing using digital scales. Evaluation of egg yolk colour estimated by the usual method applying La Roche scale (DSM Yolk Colour Fan) with spectrophotometric determination of β-carotene by AOAC method, and by new rapid analyser iCheckTM Egg photometr (Bio Analyt). The yolk colour varied between the values of 4–13 of La Roche scale. The carotenoid content expressed as β-carotene measured by AOAC method varied between 11–87 mg/kg. The carotenoid content expressed as β-carotene measured with the analyser Check TM Egg photometer was lower and varied between 7.5–68.5 mg/kg [14].

The measurements of eggshell thickness was done by using ultrasonography (USG) technology. The measurements beginning from the large end of the egg and repeated at each parallel on 3 meridians. The measurements were taken with an electronic micrometer measurement (EMM) predominantly at the wider end of eggs [15].

Statistical analysis

Cholesterol, egg weight, and egg shell thickness were statistically analyzed using SPSS 13 for Windows XP with the confidence level of 99% ($\alpha = 0.01$) and the level of significance 0.05 ($p = 0.05$). Hypothesis tests were as follows: Normality test of the data with Kolmogorov-Smirnov test, homogeneity of variance test, analysis of variance, and post hoc test using Tukey test with very significant difference 5% [16].

Results

Isolation and Identification of Cellulolytic Bacteria of *Spodoptera litura*

The results of isolation and identification of the digestive tract of *Spodoptera litura*, which was the first stage of this study, it's found of 4 (four) isolates of cellulolytic bacteria, they are bacillus, *Cellulomonas sp*, pseudomonas and cytophaga. Furthermore, the four isolates were respectively used as fermenters on the soybean

158 husk from *tempe* industry wastes derived from Usaha *Tempe* Rakyat Surabaya, Indonesia, at the next stage of
159 the study.
160
161 **Improving the Quality of Soybean Husk Waste**
162 Improving the quality of soybean husk waste, which is the second stage of this research, is done
163 through fermentation process with the addition of epidopt (Sugar Factory of Candi, Sidoarjo, Indonesia), urea
164 (Petrokimia Gresik, Gresik, Indonesia), with the addition of various bacterial isolates was obtained from first
165 stage of the study (bacillus, *Cellulomonas sp*, pseudomonas and cytophaga) and compared with control (without
166 addition of bacterial isolates). The results of this second stage study can be seen in Table 2.
167
168 **The Eggs Quality of *Mojosari duck* (*Anas javanica*)**
169 The eggs quality of *Mojosari duck* (*Anas javanica*) after feeding with a wide variety of complete feeds
170 (both with the addition of soybean husk and the suspension of cellulolytic bacteria) compared with no addition,
171 were observed through cholesterol levels from egg yolks, egg weight, eggshell thickness and egg yolk colour .
172
173 **The Cholesterol Eggs Level of Duck**
174 The cholesterol eggs level (mg/ 100 g) based on Lieberman Burchard method [12-13] was measured on
175 day 7 before the end of the study. The mean and standard deviation of cholesterol eggs levels of duck wich is
176 given with various feeding complete either by addition of soybean husk fermented using suspension of
177 *cellulomonas sp.* bacteria from *Spodoptera litura* was compared with not addition. The cholesterol eggs level
178 can be seen in Table 3.
179
180 **Egg Weight, Eggshell Thickness And Egg Yolk Colour**
181 Mean and standard deviation of duck egg quality, including egg weight, eggshell thickness and egg
182 yolk colour which is given with various feeding complete either by addition of soybean husk waste fermentation
183 using suspension of Cellulomonas sp. cellulose from Spodoptera litura was compared with not addition. Egg
184 weight, eggshell thickness and egg yolk colour can be seen in Table 4.
185
186 **Discussion**

Based on the results of variance analysis, it was found that the content of crude fiber and crude protein of soybean husk fermentation using 4 bacterium : bacillus, *Cellulomonas sp*, pseudomonas, and cytophaga have showed significantly different results ($P < 0.05$), while content of dry material was not showed significant difference ($P > 0.05$). Based on Duncan's distance test for crude fiber content, the best result, the highest decrease of the crude fiber, was in T2 treatment, which was treated with suspension of Cellulomonas sp.

According to [17], the bacteria *Cellulomonas sp*. is gram-positive, rod-shaped and non-motile. The characteristic of this bacterium is as follows: respiratory metabolism using oxygen as electron acceptor, catalase positive, lives at optimum temperature 300^{o} C and neutral pH, with growth rate 0.15 – 0.23/hrs. These bacteria have been known to digest cellulose, xylene and starch. According to [18], *Cellulomonas sp* possesses extracellular enzymes that play a greater role in the breakdown of amorphous cellulose.

Observations on cholesterol levels was showed that feeding complete in T0, which is produce the highest cholesterol levels and significantly different ($p < 0.05\%$) than T1, T2 and T3. The feeding complete in T4 was yielded the lowest cholesterol level compared with T3 treatment but significantly different ($p < 0,05\%$) with T1 and T2 treatment, where between T1 and T2 treatment were not significantly different ($p > 0.05\%$).

This result provides an opportunity to utilization of complete feed with the addition of fermented soybean husk using cellulomonas sp bacteria suspension from which gives the best result as an the lowest cholesterol level.

Several other studies, such as the provision of *katuk* leaf flour which also contains high crude fiber as well as soybeans husk, showed that *katuk* leaf flour at level $\geq$ 5% was also able to decrease cholesterol levels of eggs *Mojosari* duck without decreasing percentage of egg yolk weight [19]. However, since use of katuk leaf flour must compete with food consumed by humans, the utilization of the soybean husk waste can be an alternative to consider. Furthermore, in many other studies on the use of various foliage powders with a high content of crude fiber, egg cholesterol levels of duck cannot be reduced. In a study conducted by [20], eggs cholesterol level of duck with additional meal of beluntas leaves up to 2%, the level had not effect on egg cholesterol level of duck, where cholesterol levels at the treatment were still at 27.79 mg/g egg yolks.

The Table 4 was showed that egg yolk colour parameters on T0, T1, T2 and T3 treatments result in a significantly different colour of egg yolk ($p < 0.05$), whereas T4 yields a lower yolk colour than the other four treatments. This is shows that the provision of soybean husk fermentation from *tempe* industry waste as much as 30% as a substitute for corn can affect the colour of egg yolks. Based on research by [21] was reported that the egg yolk colour of the *Tegal* duck only 7.120, while [22] stated that the good egg yolk colour was in the range

of 8 to 12. The good egg yolk colour in the range of 8 – 12 was obtained with the addition of corn to the feed. Corn is one of the agricultural commodities very important for livestock. Corn is a high-energy feed ingredient, with a protein content of about 8.6 to 9.0%, but corn protein can not fermented or degraded by rumen microorganisms [23].

The parameter observation of egg weight and eggshell thickness was not showed significant difference between treatments ($p > 0.05$). This is shows that the utilization of soybean husk waste fermented with Cellulomonas sp bacteria up to 30% dose does not affect on egg weight or eggshell thickness. By [24] was reported that with increasing age, the egg size will increase as a result of increased yolk weight.

In poultry, including ducks, the process of egg formation known as folliculogenesis, in addition to affecting the development of the oocyte (egg cell), also affects the weight of the egg yolk. The number of follicles during one cycle is influenced by factors such as animal species, reproductive phase, circumstances, age, mother, genetic [25] and feed [26-28].

Conclusion

Conclusion of the research: The fermentation of soybean husk from tempe industry waste through the utilization of cellulolytic bacteria of *Spodoptera litura* added to complete feed can be done as an effort to improve the quality of Mojosari duck (*Anas javanica*) eggs in the form decrease of egg yolks cholesterol level without decreasing egg weight and eggshell thickness, although the decrease in yolk colour is unavoidable but statistically does not show significant differences ($p > 0.05$).

Authors' Contribution

All the authors conceptualized the manuscript. Both SH and ES drafted the manuscript. SH **:** Research project leader and coordinating research, collected and processed samples. carried out the data collection and gathering assay samples. DSN has done the statistical analysis part and critically reviewed the manuscript. ES : assisted in manuscript preparation and corresponding author. All the authors have read and approved the final version of the manuscript.

Acknoledgment

The study was supported by funding from the Directorate General of Higher Education (DIKTI) 2015. The National Education Ministry. Republic of Indonesia.

247

248 **Competing Interests**

249 The authors declare that they have no competing interests.

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251 **References**

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Table 1. Complete feed formulation was given to *Mojosari duck* (*Anas javanica*) using soybean husk waste fermented with *Cellulomonas sp.* bacteria suspension.

MATERIALS (%)	**T0 (Control)** complete feed without soybean husk and *Cellulomonas sp* bacteria suspension.	**T1 (Treatment 1)** complete feed + 15% soybean husk without *Cellulomonas sp* bacteria suspension	**T2 (Treatment 2)** complete feed + 15% soybean husk + 0.05% *Cellulomonas sp* bacteria suspension	**T3 (Treatment 3)** complete feed + 30% soybean husk without *Cellulomonas sp* bacteria suspension.	**T4 (Treatment 4)** complete feed + 30% soybean husk + 0.05% *Cellulomonas sp* bacteria suspension.
Yellow corn	61.00%	46.00%	46.00%	31.00%	31.00%
Fish meal	13.80%	13.75%	13.80%	13.80%	13.75%
Soy meal	5.60%	5.60%	5.60%	5.60%	5.60%
Rice bran	14.70%	14.70%	14.70%	14.70%	14.70%
Soybean	4.30%	4.30%	4.30%	4.30%	4.30%
Coconut oil	0.30%	0.30%	0.30%	0.30%	0.30%
Premix	0.30%	0.30%	0.30%	0.30%	0.30%
Soybean husk	-	15%	15%	30%	30%
Cellulomonas sp bacteria suspension	-	-	0.05%	-	0.05%
Total	**100**	**100**	**100**	**100**	**100**

Table 2. The content of dry material (%), crude fiber (%), and crude protein (%) of fermented soybean husk using various cellulolytic bacteria isolates from *Spodoptera litura*

Treatment	Dry Material (%) ± SE	Crude Fiber (%) ± SE	Crude Protein (%) ± SE
T0 (Soybean husk + 1% Molases + 1% urea + without bacteria isolate)	78.15[a] ± 0.05	48.60[a] ± 0.14	15.63[a] ± 0.26
T1 (Soybean husk + 1% Molases + 1% urea + 5% *bacillus* bacteria isolate)	78.79[a] ± 0.82	48.73[a] ± 0.53	15.85[a] ± 0.73
T2 (Soybean husk + 1% Molases + 1% urea + 5% *Cellulomonas sp* bacteria isolate)	78.18[a] ± 0.20	43.81[b] ± 0.78	15.90[a] ± 0.55
T3 (Soybean husk + 1% Molases + 1% urea + 5% *pseudomonas* bacteria isolate)	78.67[a] ± 0.16	48.07[a] ± 0.50	17.10[b] ± 0.90
T4 (Soybean husk + 1% Molases + 1% urea + 5% *cytophaga* bacteria isolate)	78.40[a] ± 0.19	48.58[a] ± 1.38	17.57[b] ± 0.68

[a,b,c] Values in the same column with different superscripts indicate significant difference $p < 0.05$ (n=4).

289 Table 3. Mean and standard deviation of egg yolk cholesterol levels of *Mojosari duck* (*Anas*

290 *javanica*)

Treatments	Mean ± SE
T0 (*complete feed* without soybean husk and *Cellulomonas sp* bacteria suspension.)	$18.74^{a} \pm 2.19$
T1 (*complete feed* + soybean husk 15% without *Cellulomonas sp* bacteria suspension)	$15.61^{b} \pm 2.12$
T2 (*complete feed* + soybean husk 15% + 0.05% *Cellulomonas sp* bacteria suspension)	$16.53^{b} \pm 2.52$
T3 (*complete feed* + soybean husk 30% without *Cellulomonas sp* bacteria suspension)	$13.35^{c} \pm 1.92$
T4 (*complete feed* + soybean husk 30% + 0.05% *Cellulomonas sp* bacteria suspension.)	$12.69^{c} \pm 2.23$

291 [a,b,c] Values in the same column with different superscripts indicate significant difference $p<0.05$ (n=5).

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303 Table 4. Mean and standard deviation of egg weight, egg yolk colour, and egg shell thickness

304 of duck

Variabel	Treament				
	T0 (complete feed without soybean husk and *Cellulomonas sp* bacteria suspension.)	T1 (complete feed + 15% soybean husk without *Cellulomonas sp* bacteria suspension)	T2 (complete feed + 15%soybean husk + 0.05% *Cellulomonas sp* bacteria suspension)	T3 (complete feed + 30% soybean husk without *Cellulomonas sp* bacteria suspension)	T4 (complete feed + soybean husk 30% + 0.05% *Cellulomonas sp* bacteria suspension.)
Egg weight (g) ± SE	$47.60^{a} \pm 4.07$	$50.96^{a} \pm 3.38$	$52.26^{a} \pm 2.48$	$42.17^{a} \pm 20.12$	$47.94^{a} \pm 9.37$
Egg yolk colour± SE	$10.20^{b} \pm 1.79$	$8.20^{ab} \pm 1.92$	$9.40^{b} \pm 2.30$	$8.00^{ab} \pm 1.41$	$6.40^{a} \pm 2.30$
Egg shell thickness (mm) ± SE	$0.55^{a} \pm 0.08$	$0.52^{a} \pm 0.08$	$0.53^{a} \pm 0.03$	$0.53^{a} \pm 0.07$	$0.53^{a} \pm 0.08$

305 ^{a,b,c} Values in the same line with different superscripts indicate significant difference $p<0.05$ (n=5).

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RESEARCH ARTICLE
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The improvement of eggs quality of Mojosari duck (*Anas javanica*) with soybean husk fermentation using cellulolytic bacteria of *Spodoptera litura*

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Received: 06-03-2018, **Accepted:** 25-04-2018, **Published online:** ***

doi: 10.14202/vetworld.2018.720-725 **How to cite this article:** Hidanah S, Nazar DS, Safitri E (2018) The improvement of eggs quality of Mojosari duck (*Anas javanica*) with soybean husk fermentation using cellulolytic bacteria of *Spodoptera litura*, *Veterinary World*, 11(5): 720-725.

Abstract

Aim: This study was aimed to improve the quality of the eggs of Mojosari duck (*Anas javanica*) through complete feeding containing soybean husk was fermented using cellulolytic bacteria of *Spodoptera litura*.

Materials and Methods: This study consisted of three stages: The first stages, isolation and identification of cellulolytic bacteria from *S. litura*; the second stage, the fermentation of soybean husk through the application of bacterial cellulolytic isolate from the first stage; and the third stage, the application of the best complete feed formulation from the second stage to Mojosari duck.

Results: There are four dominant bacteria: *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp. Furthermore, the best reduction of the crude fiber of soybean husks is the use of *Cellulomonas* sp. bacteria. The final of the study, the quality of the eggs of *Anas javanica*, was improved, as indicated by cholesterol decrease from the yolk without the decrease of egg weight and eggshell thickness, although the decrease in egg yolk color was inevitable.

Conclusion: Soy husk fermentation using cellulolytic bacteria of *S. litura* was added to complete feeding can be performed to improve the quality of the eggs of Mojosari duck.

Keywords: cellulolytic bacteria, eggs quality of duck, soybean husk fermentation, *Spodoptera litura.*

Introduction

Soybean is an agricultural product that has been utilized to meet the needs of industry and food, such as Tempe, tofu, soy sauce, and soy milk. In general, the use and utilization of soybean are limited to seeds only, while the waste, such as soybean husk, is still discarded and has not been widely utilized. Analysis of dry matter (DM)=91.11, crude protein (CP)=5.04, ether extract=1.65, nitrogen-free extract, calcium=21, phosphorus=0.06, and gross energy=(kcal/g. DM) 3.98 according to the methods described in AOAC. The analysis of neutral detergent fiber=60.15 and acid detergent fiber=42.08 was carried out according to detergent method [1]. In other research, the chemical composition of soybean husk comprises 47.01% crude fiber, 14.45% CP, 3.04% crude fat, 3.15% ash, and 3.060, 48 kcal kg of energy metabolism. Soybean husk contains 42-49% dry weight of cellulose, 29-34% hemicellulose, and 1-3% lignin and has anti-nutritional antitrypsin substances [2].

On the other hand, *Spodoptera litura* is a pest of soybean crop that has a very high ability in damaging the plant. The leaves and pods attacked by *S. litura* become holes even then torn [3]. Based on its ability to damage the leaves and pods, allegedly the digestive tract of *S. litura* contains cellulolytic bacteria capable of digesting crude fibers well [4].

In general, cellulolytic bacteria have three cellulose enzymes called endoglucanase or carboxymethylcellulose (CMC-ase), exoglucanase or cellobiohydrolase, and beta-glucosidase. The enzymes can degrade cellulose into glucose [5]. CMC-ase breaks the hydrogen bonds present in the cellulose crystalline structure, forming single cellulose chains. Exoglucanase cuts off the ends of single chains cellulose, producing disaccharides and tetrasaccharides, cellobiose, beta-glucosidase hydrolyzes disaccharides, and tetrasaccharides into glucose [6]. Therefore, the utilization of cellulolytic microbes in the fermentation process of the feed material from the waste can allegedly improve the quality of complete feed formulation with the indication of the decrease of crude fiber and the increase of CP.

Based on another study, the soybean husk waste fermented with *Aspergillus niger* and Lactobacillus was only able to decrease crude fiber from 44% to 40%.

The decrease in crude fiber content is still relatively small. In addition to the decrease in crude fiber, the fermentation process is also expected to increase CP from processed waste material [7]. Therefore, we need an alternative bacterium that has the higher capability in breaking down crude fiber along with an increase in CP content of the soybean husk.

This study aims to determine the potential of cellulolytic bacteria was contained in *S. litura* as a source of probiotics that can reduce the soybean crude fiber derived from the Tempe (Tempe is a traditional soy product originating from Indonesia. It is made by a natural culturing and controlled fermentation process that binds soybeans into a cake form) industry through the fermentation process, but followed by increased CP. If this is realized, then the quality of complete feed formulation on feed given to *Anas javanica* will be improved. Furthermore, improving the quality of complete feed formulation on feed was given to *Anas javanica* is expected to affect the quality of the eggs produced, such as low cholesterol levels with maintaining eggs' weight, yolk color, and thickness of the shell.

Materials and Methods

Ethical approval

The present study was approved by ethical committee vide Ethical Clearance KE (Komisi Etik Penelitian), Animal Care and Use Committee (ACUC). Veterinary Medicine Faculty, Universitas Airlangga, Surabaya, Indonesia.

Stage of study

This study consisted of three stages.

First stage

The first stage, isolation and identification of cellulolytic bacteria from *S. litura* digestive tract [4,8]; in total, 4 bacteria, i.e., *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp.were characterized based on their colony color, morphological, biochemical, and molecular characteristics of bacteria.

We explored the culturable bacterial community in the digestive tract of *S. litura* using a culture-dependent technique based on 16S rRNA gene sequencing and screening of these four isolates. Bacterial isolation was performed on living larvae separately. The larva was homogenized in nutrient extract using a glass pounder, and the homogenate is filtered 2 times to remove larvae debris than input into sterile tubes. The larvae extract a number of 50 µL were placed on nutrient agar and incubated at 37°C in a humidified atmosphere containing at 5% CO_2 moisture and allowed to increase the number of bacteria for 3 days. Isolates were distinguished based on colony color and morphology. After that, the pure cultures of bacterial colonies were added into 20% glycerol and prepared at the Laboratory of Microbiology of the Department of Microbiology, Faculty of Veterinary Medicine, Airlangga University.

Identification of bacterial isolates was identified by various tests, such as the utilization of organic compounds, spore formation, Gram staining, NaCl tolerance, optimum temperature, optimum pH, and catalase [4].

The isolate identification of four bacteria was confirmed using 16S rRNA gene sequencing. The standard protocol was used for confirm of total genomic DNA extraction. The isolated DNAs of each bacteria, i.e., *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp. were stored at −20°C until use. Furthermore, the polymerase chain reaction (PCR) amplification of the 16S rRNA genes was performed using the universal primers UNI16S-L (5'-ATTCTAGAGTTTGATCATGGCTCA-3') as the forward primer and UNI16S-R (5'-ATGGTACCGTGTGA CGGGCGGTGTGTA-3') as the reverse primer and then Amplification process in a thermocycler (Eppendorf, Mastercycler Gradient, Hamburg, Germany) for 36 reaction cycles. Reactions were routinely performed in 50 µL including 1.5 µL of 10 mM dNTP mix, 1.5 µL of 10 pmol each of the opposing amplification primers, 1 µL of 5 U/µL Taq DNA polymerase (Fermentas), 3 µL of MgCl2, 5 µL of Taq DNA polymerase reaction buffer, 1 µL of genomic DNA, and 35.5 µL of dH2 O. PCR conditions were 5 min at 95°C for the initial denaturation of template DNA, 36 amplification cycles (1 min at 94°C, 1 min at 56°C, and 2 min at 72°C), and 10 min at 72°C for the final extension. PCR products were separated on 1.0% agarose gels, stained with ethidium bromide, and viewed under ultraviolet light. After checking the PCR products, they were sent to Macrogen (the Netherlands) for sequencing. The obtained sequences were used to perform BLAST searches using the NCBI GenBank database. In addition, sequences were used for phylogenetic analysis for further characterization [9].

Second stage

The second stage, the process of soybean fermentation from Tempe industry waste (Usaha Tempe Rakyat, Surabaya, Indonesia), with the addition of Epidopt (Sugar Factory Candi, Sidoarjo, Indonesia), urea (Petrokimia Gresik, Gresik, Indonesia), and various bacterial isolates obtained from Stage 1 studies compared with control (without addition of bacterial isolate). Fermentation is one of the major processes used in the production of food from soybeans. This fermentation changes the physicochemical and organoleptic properties of soy products such as color, flavor, and active components [10].

The second stage used complete randomized design with 5 treatments and 4 replicates [11]. The treatment was: T0: Soybean husk + 1% molasses + 1% urea + without bacterial isolate; T1: Soybean husk + 1% molasses + 1% urea + 5% bacillus sp. bacterial isolate; T2: Soybean husk + 1% molasses + 1% urea + 5% bacteria *Cellulomonas* sp. isolate; T3: Soybean

husk + 1% molasses + 1% urea + 5% pseudomonas sp. bacterial isolate; and T4: Soybean husk+ 1% molasses + 1% urea + 5% *Cytophaga* sp. bacterial isolate.

A total of 20 samples of soybean husk, each weighing 200 g, were randomly divided into 5 treatments with 4 replicates, 1% urea + epidopt and 5% of cellulolytic bacteria (108/cc) dissolved in a diluent solution of sterile water as much as 30% of the sample weight. Subsequently, the solution was sprayed on the husk of the soybeans and inserted into a plastic bag (clear, hollow in some places, and tied at the top), and fermented for 7 days. After the fermentation process ended, the organoleptic examination was done, including color, odor, texture, and pH measurement. Then, the fermented husk was aerated. Furthermore, to determine the content of DM, crude fiber, and CP, the proximate analysis was performed according to the method recommended by Sruamsiri and Silman [1]. The best results of this second stage were T2: Soybean husk + *Cellulomonas* sp. suspension (1% Molasses + 1% urea + 5% isolate *Cellulomonas* sp. as fermenter).

Third stage

The third stage of this study was the application of a complete feed formulation by adding fermentation of the best result of the second stage: Various percentage of soybean husk + *Cellulomonas* sp. suspension, compared with control (without *Cellulomonas* sp. suspension). Furthermore, prepared complete feed formulation was given as feed on the *Anas javanica*. The complete feed formulation is shown in Table-1.

The third stage of this study was giving complete feed formulation to *Anas javanica* in improving the quality of *Anas javanica* egg. This study used 100 laying *Anas javanica*, aged about 20 weeks, divided into 5 treatments in the form of 5 types of formula feed which were T0: Complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension; T1: Complete feed + 15% soybean husk without *Cellulomonas* sp. bacteria suspension; T2: Complete feed + 15% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension; T3: Complete feed + 30% soybean husk without *Cellulomonas* sp. bacteria suspension; and T4: Complete feed + 30% soybean husk + 0.05% *Cellulomonas* sp. bacteria suspension (Table-1). The experimental design was complete randomized design (5×5 replicates). Parameters to improve the quality of *Anas javanica* eggs included egg cholesterol levels, egg weight, egg yolk, and eggshell thickness. Egg cholesterol (mg/100 g) levels were measured on day 7 before the end of the study. Cholesterol levels were tested using the Liebermann–Burchard's method [12,13]. Egg weight is measured by weighing using digital scales. Evaluation of egg yolk color estimated by the usual method applying La Roche scale (DSM Yolk Color Fan) with spectrophotometric determination of β-carotene by AOAC method, and by new rapid analyzer iCheckTM Egg photometer (BioAnalyt). The yolk color varied between the values of 4 and 13 of La Roche scale. The carotenoid content expressed as β-carotene measured by AOAC method varied between 11 and 87 mg/kg. The carotenoid content expressed as β-carotene measured with the analyzer Check TM Egg photometer was lower and varied between 7.5 and 68.5 mg/kg [14].

The measurements of eggshell thickness were done using ultrasonography technology. The measurements beginning from the large end to small end of the egg and repeated at each on 3 meridians in parallel. The measurements were taken with an electronic micrometer measurement predominantly at the wider end of eggs [15].

Statistical analysis

Cholesterol, egg weight, and eggshell thickness were statistically analyzed using SPSS 13 for Windows XP with the confidence level of 99% (α=0.01) and the level of significance 0.05 (p=0.05). Hypothesis tests were as follows: Normality test of the data with

Table-1: Complete feed formulation was given to *Anas javanica* using soybean husk waste fermented with *Cellulomonas* sp. bacteria suspension.

Materials (%)	T0 (Control) complete feed without soybean husk and *Cellulomonas* sp. bacteriasuspension	T1 (Treatment 1) complete feed+15% soybean husk without *Cellulomonas* sp. bacteria suspension	T2 (Treatment 2) complete feed+15% soybean husk+0.05% *Cellulomonas* sp. bacteria suspension	T3 (Treatment 3) complete feed+30% soybean husk without *Cellulomonas* sp. bacteria suspension	T4 (Treatment 4) complete feed+30% soybean husk+0.05% *Cellulomonas* sp. bacteria suspension
Yellow corn	61.00	46.00	46.00	31.00	31.00
Fish meal	13.80	13.75	13.80	13.80	13.75
Soy meal	5.60	5.60	5.60	5.60	5.60
Rice bran	14.70	14.70	14.70	14.70	14.70
Soybean	4.30	4.30	4.30	4.30	4.30
Coconut oil	0.30	0.30	0.30	0.30	0.30
Premix	0.30	0.30	0.30	0.30	0.30
Soybean husk	-	15	15	30	30
Cellulomonas sp. bacteria suspension	-	-	0.05	-	0.05
Total	100	100	100	100	100

Kolmogorov–Smirnov test, homogeneity of variance test, analysis of variance, and *post hoc* test using Tukey test with very significant difference 5% [16].

Results

Isolation and identification of cellulolytic bacteria of *S. litura*

The results of isolation and identification of the digestive tract of *S. litura*, which was the first stage of this study, it is found of 4 isolates of cellulolytic bacteria, they are *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp. Furthermore, the four isolates were, respectively, used as fermenters on the soybean husk from Tempe industry wastes derived from Usaha Tempe Rakyat Surabaya, Indonesia, at the next stage of the study.

Improving the quality of soybean husk waste

Improving the quality of soybean husk waste, which is the second stage of this research, is done through fermentation process with the addition of epidopt (Sugar Factory of Candi, Sidoarjo, Indonesia), urea (Petrokimia Gresik, Gresik, Indonesia), and various bacterial isolates which was obtained from first stage of the study (*Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp.) and compared with control (without addition of bacterial isolates). The results of this second stage study can be seen in Table-2.

The eggs quality of *Anas javanica*

The egg's quality of *Anas javanica* after feeding with a wide variety of complete feeds (both with the addition of soybean husk and the suspension of cellulolytic bacteria) compared with no addition was observed through cholesterol levels from egg yolks, egg weight, eggshell thickness, and egg yolk color.

The cholesterol eggs level of duck

The cholesterol eggs level (mg/100 g) based on Liebermann–Burchard's method [12,13] was measured on day 7 before the end of the study. The mean and standard deviation of cholesterol eggs levels of duck which is given with various feeding complete either by addition of soybean husk fermented using suspension of *Cellulomonas* sp. bacteria from *S. litura* was compared with not addition. The cholesterol eggs level can be seen in Table-3.

Egg weight, eggshell thickness, and egg yolk color

Mean, and standard deviation of duck egg quality, including egg weight, eggshell thickness, and egg yolk color which is given with various feeding complete either by addition of soybean husk waste fermentation using suspension of *Cellulomonas* sp. cellulose from *S. litura* was compared with not addition. Egg weight, eggshell thickness, and egg yolk color can be seen in Table-4.

Discussion

Based on the results of variance analysis, it was found that the content of crude fiber and CP of soybean husk fermentation using 4 bacterium: *Bacillus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Cytophaga* sp. have shown significantly different results ($p<0.05$), while the content of dry material was not showed a significant difference ($p>0.05$). Based on Duncan's distance test for crude fiber content, the best result, the highest decrease of the crude fiber, was in T2 treatment, which was treated with a suspension of *Cellulomonas* sp.

Table-2: The content of dry material (%), crude fiber (%), and CP (%) of fermented soybean husk using various cellulolytic bacteria isolates from *S. litura*.

Treatment	Dry material (%)±SE	Crude fiber (%)±SE	CP (%)±SE
T0 (soybean husk+1% molasses+1% urea+without bacteria isolate)	78.15^{a}±0.05	48.60^{a}±0.14	15.63^{a}±0.26
T1 (soybean husk+1% molasses+1% urea+5% *Bacillus* sp. bacteria isolate)	78.79^{a}±0.82	48.73^{a}±0.53	15.85^{a}±0.73
T2 (Soybean husk+1% molasses+1% urea+5% *Cellulomonas* sp. bacteria isolate)	78.18^{a}±0.20	43.81^{b}±0.78	15.90^{a}±0.55
T3 (soybean husk+1% molasses+1% urea+5% *Pseudomonas* sp. bacteria isolate)	78.67^{a}±0.16	48.07^{a}±0.50	17.10^{b}±0.90
T4 (soybean husk+1% molasses+1% urea+5% *Cytophaga* sp. bacteria isolate)	78.40^{a}±0.19	48.58^{a}±1.38	17.57^{b}±0.68

^{a,b,c}Values in the same column with different superscripts indicate significant difference $p<0.05$ (n=4), CP=Crude protein, *S. litura*=*Spodoptera litura*, SE=Standard error

Table-3: Mean and standard deviation of egg yolk cholesterol levels of *Anas javanica*.

Treatments	Mean±SE
T0 (complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension)	18.74^{a}±2.19
T1 (complete feed+soybean husk 15% without *Cellulomonas* sp. bacteria suspension)	15.61^{b}±2.12
T2 (complete feed+soybean husk 15% + 0.05% *Cellulomonas* sp. bacteria suspension)	16.53^{b}±2.52
T3 (complete feed+soybean husk 30% without *Cellulomonas* sp. bacteria suspension)	13.35^{c}±1.92
T4 (complete feed+soybean husk 30% + 0.05% *Cellulomonas* sp. bacteria suspension.)	12.69^{c}±2.23

^{a,b,c}Values in the same column with different superscripts indicate significant difference $p<0.05$ (n=5), SE=Standard error

Table-4: Mean and standard deviation of egg weight, egg yolk color, and egg shell thickness of duck.

Variable	Treatment				
	T0 (complete feed without soybean husk and *Cellulomonas* sp. bacteria suspension)	T1 (complete feed+15% soybean husk without *Cellulomonas* sp. bacteria suspension)	T2 (complete feed+15% soybean husk+0.05% *Cellulomonas* sp. bacteria suspension)	T3 (complete feed+30% soybean husk without *Cellulomonas* sp. bacteria suspension)	T4 (complete feed+soybean husk 30% + 0.05% *Cellulomonas* sp. bacteria suspension)
Egg weight (g)±SE	47.60^{a}±4.07	50.96^{a}±3.38	52.26^{a}±2.48	42.17^{a}±20.12	47.94^{a}±9.37
Egg yolk color±SE	10.20^{b}±1.79	8.20a^{b}±1.92	9.40^{b}±2.30	8.00^{ab}±1.41	6.40^{a}±2.30
Eggshell thickness (mm)±SE	0.55^{a}±0.08	0.52^{a}±0.08	0.53^{a}±0.03	0.53^{a}±0.07	0.53^{a}±0.08

^{a,b,c}Values in the same line with different superscripts indicate significant difference $p<0.05$ (n=5), SE=Standard error

According to Holt [17], the bacteria *Cellulomonas* sp. is Gram-positive, rod-shaped, and non-motile. The characteristic of this bacterium is as follows: Respiratory metabolism using oxygen as electron acceptor, catalase positive, lives at optimum temperature 300°C, and neutral pH, with growth rate 0.15-0.23/h. These bacteria have been known to digest cellulose, xylene, and starch. According to Gupta *et al*. [18], *Cellulomonas* sp. possesses extracellular enzymes that play a greater role in the breakdown of amorphous cellulose.

Observations on cholesterol levels were showed that feeding complete in T0, which produces the highest cholesterol levels and significantly different (p<0.05%) than T1, T2, and T3. The feeding complete in T4 has yielded the lowest cholesterol level compared with T3 treatment but significantly different (p<0.05%) with T1 and T2 treatment, whereas between T1 and T2 treatment did not significantly different (p> 0.05%). This result provides an opportunity to the utilization of complete feed with the addition of fermented soybean husk using *Cellulomonas* sp. bacteria suspension from which gives the best result as the lowest cholesterol level.

Several other studies, such as the provision of katuk leaf flour which also contains high crude fiber as well as soybeans husk, showed that katuk leaf flour at level ≥5% was also able to decrease cholesterol levels of eggs Mojosari duck without decreasing percentage of egg yolk weight [19]. However, since the use of katuk leaf flour must compete with food consumed by humans, the utilization of the soybean husk waste can be an alternative to consider. Furthermore, in many other studies on the use of various foliage powders with a high content of crude fiber, egg cholesterol levels of duck cannot be reduced. A study conducted by Palupi *et al*. [20], eggs cholesterol level of duck with an additional meal of beluntas leaves up to 2%; the level had no effect on egg cholesterol level of duck, where cholesterol levels at the treatment were still at 27.79 mg/g egg yolks.

Table-4 shows that egg yolk color parameters on T0, T1, T2, and T3 treatments result in a significantly different color of egg yolk (p<0.05), whereas T4 yields a lower yolk color than the other four treatments. This shows that the provision of soybean husk fermentation from Tempe industry waste as much as 30% as a substitute for corn can affect the color of egg yolks. Subhan [21] reported that the score of egg yolk color of the *Anas javanicus* from Tegal region, Indonesia was < 7.5, while Beardsworth and Hernandez [22] stated that the good egg yolk color was in the range of 8-12. The good egg yolk color in the range of 8-12 was obtained with the addition of corn to the feed. Corn is one of the agricultural commodities very important for livestock. Corn is a high-energy feed ingredient, with a protein content of about 8.6-9.0%, but corn protein cannot ferment or degraded by rumen microorganisms [23].

The parameter observation of egg weight and eggshell thickness was not showed a significant difference between treatments (p>0.05). This shows that the utilization of soybean husk waste fermented with *Cellulomonas* sp. bacteria up to 30% dose does not affect egg weight or eggshell thickness. Silversides and Villeneuve [24] reported that with increasing age, the egg size would increase as a result of increased yolk weight.

In poultry, including ducks, the process of egg formation known as folliculogenesis, in addition to affecting the development of the oocyte (egg cell), also affects the weight of the egg yolk. The number of follicles during one cycle is influenced by factors such as animal species, reproductive phase, circumstances, age, mother, genetic [25], and feed [26-28].

Conclusion

The fermentation of soybean husk from Tempe industry waste through the utilization of cellulolytic bacteria of *S. litura* added to complete feed can be done as an effort to improve the quality of *Anas javanic*a eggs in the form decrease of egg yolks cholesterol level without decreasing egg weight and eggshell thickness, although the decrease in yolk color is unavoidable statistically does not show significant differences (p>0.05).

Authors' Contributions

All the authors conceptualized the manuscript. SH and ES drafted the manuscript. SH: Research

project leader and coordinating research, collected and processed samples. Carried out the data collection and gathering assay samples. DSN has done the statistical analysis part and critically reviewed the manuscript. ES: Assisted in manuscript preparation and corresponding author. All the authors have read and approved the final version of the manuscript.

Acknowledgments

The study was supported by funding from the Directorate General of Higher Education (DIKTI) 2015 with number 519/UN3/2015, Date March, 26, 2015. The National Education Ministry, Republic of Indonesia.

Competing Interests

The authors declare that they have no competing interests.

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