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new submission

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To: ivj83@yahoo.com

Sat, Jan 12, 2019 at 2:07 PM

Dear Editor Indian Veterinary Journal

I hereby send our manuscript research article with the title :

Phyllanthus niruri* Linn Extract As Antibiotic Growth Promoter (AGP) in Layer Chicken Performance was Infected by *Escherichia coli

Keywords: *Phyllanthus niruri* Linn, Antibiotic Growth Promoter, Layer Performance, *Escherichia coli*.

The article we sent has not been previously submitted and has not been submitted for evaluation in other journals.

Kindly consideration our article for published in Indian Veterinary Journal

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Dr. Safitri E., DVM, M.Si
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 Main Article OK.docx
34K

Phyllanthus niruri* Linn Extract As Antibiotic Growth Promoter (AGP) in Layer Chicken Performance was Infected by *Escherichia coli

Sri Hidanah, Emy Koestanti Sabdoningrum, M. Anam Al Arif, Rivienda S. Ratibawan,
Erma Safitri*

Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

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Abstract

The study purpose was to determine of effectiveness of *Phyllanthus niruri* Linn extract as AGP in layer chicken performance was infected by *Escherichia coli*. The research was using 25 experimental units of five treatment. T- = without *Escherichia coli* and *Phyllanthus niruri* Linn, T+,T1,T2,T3,T4 = *Escherichia coli* infected and added respectively as follows without *Phyllanthus niruri* Linn, 20% *Phyllanthus niruri* Linn, 25% *Phyllanthus niruri* Linn, 30% *Phyllanthus niruri* Linn, and 1%AGP. The results showed that 30% produced the highest feed consumption of 887.4 grams/head/week and produced the highest egg production of 430.8 grams/week and the lowest feed conversion is 2.08.

Keywords: *Phyllanthus niruri* Linn, Antibiotic Growth Promoter, Layer Performance, *Escherichia coli*

Losses due to *Escherichia coli* (*E. coli*) or Colibacillosis in the form of high chicken mortality can reach 30%. Colibacillosis affects chickens of all ages. Diagnosis and treatment as well as improper control of *E. coli* infection often occur resistance to antibiotics used (Hidanah *et al.*, 2018). Antibiotics are widely used in the livestock industry to prevent *E. coli* infection because although *E. coli* is a commensal bacterium, it can be fatal if septicemia can be followed by mycoplasmosis or viral infections such as bronchitis in chickens (Burch, 2000). According to Ragland *et al.* (2014) antibiotics can reduce feed consumption, but does not change performance. Antibiotics are expected to kill bacteria in the intestine, so the nutrients that will be lost to microbial fermentation can be used more efficiently (Hughes and Heritage, 2016) for chicken growth. However, it can also encourage the formation of antibiotic-resistant bacteria in the digestive tract and antibiotic residues in eggs (Feihgner and Dashkevics, 1987).

Natural feed additives such as medicinal plants or herbal plants can potentially replace commercial feed additives. *Phyllanthus niruri* Linn (*Pn*Linn) plant is one of the plants that can be used as a preventative and alternative treatment caused by *E. coli*. The chemicals contained in the *Pn* Linn include flavonoids and tannins. The function of flavonoids is as an immunomodulator which acts to enhance the immune system and improve the immune system whose function is impaired. Tannins are efficacious as antiseptics (prevent bacterial growth) and hemostatic (stop bleeding) (Mathivanan *et al.*, 2006). Another that *Pn* Linn contain Lignans, alkaloids, triterpenoids, fatty acids, vitamin C, potassium, resin, tannin and geranin. Essential fatty acids function to reduce cholesterol content (Astuti *et al.*, 2017). Vitamin C as proven to be a hot antistress potential. The benefits of other *Phyllanthus niruri* Linn extracts are terpenoids which can inhibit *Escherichia coli* and *Staphylococcus aureus* bacteria (Gunawan *et al.*, 2008).

This study aims to determine the effectiveness of the addition of *Phyllanthus niruri* Linn extract as a substitute for antibiotic growth promoter (AGP) on performance activity of laying hens infected with *Escherichia coli*.

Materials and Methode

This type of research was experimental using a completely randomized design with 25 five treatment trial units. Before treatment, bacterial suspension is done by making Phosphate Buffer Saline solution and centrifuge and added with 0.5 level Mc. Farland solution. After that, making of *Pn* Linn Extract was as follows: 1000 grams of *Pn* Linn herbs were added plus 2 liters of 95% ethanol macerated for 3 days. Next, application in laying chicken has been infected by *E. coli*. The research was using 25 experimental units of five treatment. T- = without *E.coli* and *Pn* Linn, T+,T1,T2,T3,T4 = *E. coli* infected and added respectively as follows without *Pn* Linn, 20% *Pn* Linn, 25% *Pn* Linn, 30% *Pn* Linn, and 1% AGP. Data collection as follow:

1. Feed consumption (g) = feed given (g) – left over feed (g); 2. Feed Conversion Ratio

2. (FCR) = $\frac{\text{amount of feed consumed (gram/head/ week)}}{\text{Egg production (gram / head / week)}}$

3. Hen Day Production (%) \rightarrow HDP = $\frac{\text{The number of eggs that day} \times 100\%}{\text{Laying chicken population}}$

Results and Discussion

This study shows the results of feed consumption, egg production, feed conversion and hen day can be seen in Table I

Table 1 Average Feed Consumption, Egg Production, Feed conversion & Hen Day in Laying Chicken given *Pn* Linn Extract as Substitute for AGP & Infected with *E. coli*

Treatment	Feed Consumption (grams/head/week)	Egg Production (grams/head/week)	Feed Conversion (consumtion / egg production)	Hen Day (%)
T-	854.6 ^{ab} ± 47.5	383.6 ^b ± 15.22	2.2324 ^a ± 0.18	91 ^b ± 8.21
T+	829.2 ^a ± 25.63	205.2 ^a ± 129	3.4510 ^b ± 0.99	74 ^a ± 9.61
T1	858.40 ^{ab} ± 33.76	402.61 ^b ± 23.9	2.1397 ^a ± 0.17	94 ^b ± 8.21
T2	871.20 ^{ab} ± 38.5	410 ^b ± 12.4	2.1274 ^a ± 0.13	95 ^b ± 7.07
T3	887.40 ^b ± 25.3	430.8 ^b ± 45.8	2.0840 ^a ± 0.28	90 ^b ± 9.35
T4	862.0 ^{ab} ± 51.7	337.6 ^b ± 35.98	2.569 ^a ± 0.22	85 ^b ± 0.00

Description: Different superscripts in the same column show a very significant difference (p <0.05)

Feed consumption, the average value of feed consumption ranges from 830 - 887 grams / head / week. Nurcholis *et al.* (2009) stated the consumption of laying hens that are producing between 100 g-120 grams / head / day. This shows that the *Pn* Linn extract can replace the use of AGP, because the presence of *Pn* Linn content, namely alkaloids, saponins, flavonoids, and tannins which are active can inhibit pathogenic bacteria in the intestine so that it can improve the physiological performance of the intestine and can increase the digestibility of food substances in chickens.

Based on these results showed that the adding of *Pn* Linn extract in laying chickens infected with *E. coli* can increase egg production in the laying hens. Increased egg production

is also influenced by the presence of terpenoids as an antibacterial so that it can inhibit *E. coli* that have been infected in the laying hens (Gunawan *et al.*, 2008).

Based on these results showed that the adding of *Pn* Linn extract in laying chickens infected with *E.coli* can reduce feed conversion ratio in the laying hens. The feed conversion rate is lower. This is due to the presence of *Pn* Linn flavonoid content according to Sharififar *et al.* (2009) as an antioxidant and enhancing immunomodulator. The content of flavonoids in *Pn* Linn extract, *E. coli* can be inhibited and chickens still form good antibodies so as to produce high egg production and low feed consumption.

Based on these results showed that the adding of *Pn* Linn extract in laying chickens infected with *E.coli* bacteria can increase the production of hen day or daily production in the laying hens. Hen day (HD) is the percentage of egg production produced by productive chickens per day (Leke, 2015). The average production HD layer during his lifetime is 80% with HD reaching a peak of production at 95% and production persistence (long holding HD peak > 90%) for 23-24 weeks (average strain of laying hens). Hen Day is a comparison of the number of eggs produced against the number of chicken populations in a cage (Abbas, *et al.* 2010). According to Utomo (2017), the difference in the high and low productivity of broiler eggs is influenced by several factors, including; genetic, the quality and quantity of rations provided, the temperature of the environment, the health of livestock and the activities or handling of livestock kept. *Pn* Linn extract can be used as a substitute for antibiotic growth promoter (AGP) because it contains active substances tannin, alkaloids, saponins and flavonoids which are antimicrobial.

The conclusion, addition of *Pn* Linn extract as a substitute for Antibiotic Growth Promoter (AGP) on the performance activity of layer chickens infected by *E.coli* with 20% *Pn* Linn extract, which showed the best result can replace AGP in the future.

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Acknowledgement Letter # 14/19

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Fri, Jan 18, 2019 at 3:29 PM

ACKNOWLEDGEMENT

Reg. No: 14/19

Dated : 18/01/2019

Dear Dr. Erma Safitri,

We acknowledge the receipt of the following articles entitled "PhyllanthusniruriLinn Extract As Antibiotic Growth Promoter (AGP) in Layer Chicken Performance was Infected by Escherichia coli." (Erma Safitri, et al.).

For any further correspondence, please always quote the Registration Number of the Article.

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Referee comments for Article # 14/19 is attached for revision & IVJ Guidelines

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Sat, Feb 2, 2019 at 1:32 PM

ARTICLE # 14/19

TITLE : Phyllanthusniruri Linn extract ...

Corresponding author : Erma Safitri

REFeree COMMENTS

1. The article needs drastic corrections pointed out in the manuscript.
2. Title of the article to be revised as shown.
3. Introduction without subtitle should not exceed 5 lines and Materials and Methods 10 lines.
4. Results and Discussion to be abridged.
5. References should follow IVJ format.
6. The design experiment may be given clearly indicating no. allotted per negative control and positive control for experimental groups.
7. The total number of birds used and the total duration of the experiment, number of eggs produced per each treatment groups, feed consumption per group during the experimental period (duration) has to be furnished so as to check the results presented in the Table.
8. The Pn Linn extract given to the birds to be specifically mentioned in quarterly as ml or gram.
9. In an perception the experimental design should be as follows. If there is any deviation please explain.

Experimental Design

Experimental Group	E.coli	ADG	Pn Linn extract
T1 Negative control	-	-	-
T2 Positive control	+	+	+
T3 20% Pn Linn	+	-	+
T4 25 Pn Linn	+	-	+
T5 30 Pn Linn	+	-	+

10. Only if all the corrections, suggestions pointed out are meticulously carried out, revised article and a soft copy prepared and submitted as short communication of 3 pages after going through the IVJ guidelines enclosed, the article shall be taken up for further action.

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Thu, Feb 7, 2019 at 1:26 PM

ARTICLE # 14/19TITLE : *Phyllanthusniruri* Linn extract ...

Dear Editor,

Aurhor Answer of Refere COMMENTS

1. We have corrected this article .
2. Title of the article have revised according refere sugestion.
3. We have revised the Introduction (without subtitle and not exceed 5 lines and Materials and Methods 10 lines)
4. Results and Discussion have abridged.
5. References have revised and following IVJ format.
6. We have revised the design experiment
7. We have added he total number of birds used and the total duration of the experiment, number of eggs produced per each treatment groups, feed consumption per group during the experimental period (duration).
8. The Pn Linn extract given to the birds to be specifically mentioned in quarterly as gram.
9. We have revised the experimental design.
10. We have revised and abridged 3 pages and following the IVJ guideines

Thank you for your considered and chance for me to revise our article

Erma Safitri

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14/19
11

Phyllanthus niruri Linn Extract As Antibiotic Growth Promoter (AGP) in Layer Chicken Performance was Infected by *Escherichia coli*

Sri Hidanah, Emy Koestanti Sabdoningrum, M. Anam Al Arif, Rivienda S. Ratibawan,
Erma Salitri*

Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

Corresponding Author, Email : erma-s@fkh.unair.ac.id

Abstract

The study purpose was to determine of effectiveness of *Phyllanthus niruri* Linn extract as AGP in layer chicken performance was infected by *Escherichia coli*. The research was using 25 experimental units of five treatment. T=₀ without *Escherichia coli* and *Phyllanthus niruri* Linn, T₁, T₂, T₃, T₄ = *Escherichia coli* infected and added respectively as follows without *Phyllanthus niruri* Linn, 20% *Phyllanthus niruri* Linn, 25% *Phyllanthus niruri* Linn, 30% *Phyllanthus niruri* Linn, and 1% AGP. The results showed that 30% produced the highest feed consumption of 887.4 grams/head/week and produced the highest egg production of 430.8 grams/week and the lowest feed conversion is 2.08.

Keywords: *Phyllanthus niruri* Linn, Antibiotic Growth Promoter, Layer Performance, *Escherichia coli*

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The T₁ group was used as negative control with *Pn* linn extract. The T₂ was used as positive control with AGP. The T₁, T₂, T₃ and T₄ were given 20, 25, 30 and 1% of *Pn* linn extract and T₄ with 1% antibiotic growth promoter.

1. Feed consumption (g) = feed given (g) - leftover feed (g); 2. Feed Conversion Ratio

2. (FCR) = $\frac{\text{amount of feed consumed (gram/head/ week)}}{\text{Egg production (gram / head / week)}}$

3. Hen Day Production (%) → HDP = $\frac{\text{The number of eggs that day} \times 100\%}{\text{Laying chicken population}}$

→ Delete

modulation on feed consumption, egg production, feed conversion ratio and on egg production were collected and analyzed.

Results and Discussion

This study shows the results of feed consumption, egg production, feed conversion and

hen day can be seen in Table 1

Table 1. Average Feed Consumption, Egg Production, Feed conversion & Hen Day in Laying Chicken given *Pn* Linn Extract as Substitute for AGP & Infected with *E. coli* (mean ± SE)

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T-	854.6 ^{ab} ± 47.5	383.6 ^b ± 15.22	2.2324 ^a ± 0.18	91 ^b ± 8.21
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Description: Different superscripts in the same column show a very significant difference (p < 0.05) means bearing all Hen Day and egg production as a column all Hen Day significant (p < 0.05)

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Based on these results showed that the adding of *Pn* Linn extract in laying chickens infected with *E. coli* can increase egg production in the laying hens. Increased egg production

As per materials and method there were only 5 experimental groups, however there were six experimental groups which is missing leading to explanation.

is also influenced by the presence of terpenoids as an antibacterial so that it can inhibit *E. coli* that have been infected in the laying hens (Gunawan *et al.*, ^{loc cit} 2008).

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References

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ARTICLE # 14/19

TITLE : Phyllanthusniruri Linn extract ...

Corresponding author : Erma Safitri

REFEREE COMMENTS

1. The article needs drastic corrections pointed out in the manuscript.
2. Title of the article to be revised as shown.
3. Introduction without subtitle should not exceed 5 lines and Materials and Methods 10 lines.
4. Results and Discussion to be abridged.
5. References should follow IVJ format.
6. The design experiment may be given clearly indicating no. allotted per negative control and positive control for experimental groups.
7. The total number of birds used and the total duration of the experiment, number of eggs produced per each treatment groups, feed consumption per group during the experimental period (duration) has to be furnished so as to check the results presented in the Table.
8. The Pn Linn extract given to the birds to be specifically mentioned in quarterly as ml or gram.
9. In an perception the experimental design should be as follows. If there is any deviation please explain.

Experimental Design			
Experimental Group	E.coli	ADG	Pn Linn extract
T1 Negative control	-	-	-
T2 Positive control	+	+	+
T3 20% Pn Linn	+	-	+
T4 25 Pn Linn	+	-	+
T5 30 Pn Linn	+	-	+

10. Only if all the corrections, suggestions pointed out are meticulously carried out, revised article and a soft copy prepared and submitted as short communication of 3 pages after going through the IVJ guidelines enclosed, the article shall be taken up for further action.



erma safitri <erma-s@fkh.unair.ac.id>

Referee comments for Article # 14/19 is attached for revision & IVJ Guidelines

erma safitri <erma-s@fkh.unair.ac.id>
To: Ind Vet Journal <ivj83@yahoo.com>

Thu, Feb 7, 2019 at 1:26 PM

ARTICLE # 14/19

TITLE : Phyllanthusniruri Linn extract ...

Dear Editor,

Aurhor Answer of Refere COMMENTS

1. We have corrected this article .
2. Title of the article have revised according refere sugestion.
3. We have revised the Introduction (without subtitle and not exceed 5 lines and Materials and Methods 10 lines)
4. Results and Discussion have abridged.
5. References have revised and following IVJ format.
6. We have revised the design experiment
7. We have added he total number of birds used and the total duration of the experiment, number of eggs produced per each treatment groups, feed consumption per group during the experimental period (duration).
8. The Pn Linn extract given to the birds to be specifically mentioned in quarterly as gram.
9. We have revised the experimental design.
10. We have revised and abridged 3 pages and following the IVJ guideines

Thank you for your considered and chance for me to revise our article

Erma Safitri
[Quoted text hidden]



Article # 14-19 Rev.docx
35K

Phyllanthus niruri* Linn Extract As Antibiotic Growth Promoter (AGP) in Layer Chicken Infected by *Escherichia coli

Sri Hidanah, Emy K. Sabdoningrum, M.A. Al Arif, Rivienda S. Ratibawan, Erma Safitri*
Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

Abstract

The study purpose was to determine of effectiveness of *Phyllanthus niruri* Linn (*Pn* Linn) extract as AGP in layer chicken performance was infected by *Escherichia coli*. The research was using 25 experimental units of five treatment. T1 = without *Escherichia coli* and *Phyllanthus niruri* Linn, T2,T3,T4,T5 = *Escherichia coli* infected and added respectively as follows AGP 1%, 20% *Pn* Linn, 25% *Pn* Linn, 30% *Pn* Linn. The results showed that 30% produced the highest feed consumption of 887.4 grams/head/week and produced the highest egg production of 430.8 grams/week and the lowest feed conversion is 2.08.

Keywords: *Pn* Linn, AGP, Layer Performance, *Escherichia coli*

Diagnosis and treatment as well as improper control of *E. coli* infection often occur resistance to antibiotics used (Hidanah *et al.*, 2018). Natural feed additives such as *Pn* Linn can used as medicinal herbal for a preventative or alternative treatment caused by *E. coli* (Astuti *et al.*, 2017; Gunawan *et al.*, 2008). This study aims to determine the effectiveness of *Pn* Linn extract as a substitute for AGP on laying hens performance was infected with *E. coli*.

Materials and Methode

A completely randomized design with treatment groups in five hens per treatment were allotted. The 25 total number hens were used during 7 days for treatment, and number of eggs produced per each treatment groups are 25 eggs. The feed consumption (grams/ hen/ week) per group T1, T2, T3, T4 and T5 during experimental periode are respectively as follows : 854,6^{ab}± 47.5; 862.0^{ab} ± 51.7; 858.4^{ab} ± 33.76; 871.20^{ab} ± 38.5; 887.40^b ± 25.3 (Tabel I). Before teatment, bacterial suspension is done by making Phosphate Buffer Saline solution and centrifuge and added with 0.5 level Mc. Farland solution. *Pn* Linn Extract was

made follows: 1000 grams of *Pn* Linn herbs were added plus 2 liters of 95% ethanol macerated for 3 days. The laying were infected by *E. coli*. The T1 group was used as negative control, T2 was used as positive control with AGP with in *Pn* Linn extract, The T3, T4 and T5 were given 20, 25, 30% *Pn* Linn extract.

Results and Discussion

The data on feed consumption, feed conversion ratio, egg production and hen day were collected and analysed. The data can be seen in Table I

Table I Feed Consumption, Egg Production, Feed conversion & Hen Day Egg Production Layers Prepared Use as Substitute for AGP & Infected with *E. coli*

Treatment	Feed Consumption (grams/hen/week)	Egg Production (grams/hen/week)	Feed Conversion (consumtion / egg production)	Hen Day (%)
T1	854.6 ^{ab} ± 47.5	383.6 ^b ± 15.22	2.2324 ^a ± 0.18	91 ^b ± 8.21
T2	862.0 ^{ab} ± 51.7	337.6 ^b ± 35.98	2.569 ^a ± 0.22	85 ^b ± 0.00
T3	858.40 ^{ab} ± 33.76	402.61 ^b ± 23.9	2.1397 ^a ± 0.17	94 ^b ± 8.21
T4	871.20 ^{ab} ± 38.5	410 ^b ± 12.4	2.1274 ^a ± 0.13	95 ^b ± 7.07
T5	887.40 ^b ± 25.3	430.8 ^b ± 45.8	2.0840 ^a ± 0.28	90 ^b ± 9.35

Means denote different superscripts in the same column show a significant difference (p <0.05)

Feed consumption, the average value of feed consumption ranges from 830 - 887 grams/ hen/ week. Nurcholis *et al.* (2009) stated the consumption of laying hens that are producing between 100-120 gams/ hen/ day. This shows that the *Pn* Linn extract can replace the use of AGP, because the presence of *Pn* Linn content, namely alkaloids, saponins, flavonoids, and tannins which are active can inhibit pathogenic bacteria in the intestine so that it can improve the physiological performance of the intestine and can increase the digestibility of food substances in chickens.

Increased egg production is also influenced by the presence of terpenoids as an antibacterial so that it can inhibit *E. coli* that have been infected in the laying hens (Ragland *et al.*, 2014). According to Sharififar *et al.* (2009) as an antioxidant and enhancing

immunomodulator. The content of flavonoids in *Pn* Linn extract can be *E. coli* inhibited and hen still form good antibodies so as to produce high egg production, low feed consumption and increase the production of hen day or daily production in the laying hens. Hen day is the percentage of egg production produced by productive chickens per day (Abbas, 2010).

The conclusion, addition of *Pn* Linn extract as a substitute for Antibiotic Growth Promoter (AGP) on the performance activity of layer chickens infected by *E.coli* with 30% *Pn* Linn extract, which showed the best result can replace AGP in the future.

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Demand Letter # 14/19

3 messages

Ind Vet Journal <ivj83@yahoo.com>
Reply-To: Ind Vet Journal <ivj83@yahoo.com>
To: erma-s@fkh.unair.ac.id

Thu, Feb 21, 2019 at 11:18 AM

Dear Dr. Erma Safitri,

We wish to inform that the under mentioned article has been accepted for publication (14/19)
"Phyllanthusniruri Linn Extract As Antibiotic Growth Promoter (AGP) in Layer Chicken Infected by Escherichia coli."

Please remit a sum of USD 220 towards the following charges drawn in favour of the "Editor, Indian Veterinary Journal" and payable at Chennai.

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DEMAND LETTER Dated 21/02/2019

Dear **Dr. Erma Safitri**,
We wish to inform that the under mentioned article has been accepted for publication (14/19)
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Tue, Feb 26, 2019 at 3:42 PM

Sir / Madam,

The following article has been accepted and will be published in JUNE, 2019 issue of Indian Veterinary Journal.

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Dated : February 26, 2019

ACCEPTANCE LETTER

The following article has been accepted and will be published in **JUNE, 2019** issue of Indian Veterinary Journal.

Article No.	Title	Author (s)
14/19	<i>Phyllanthusniruri</i> Linn Extract As Antibiotic Growth Promoter (AGP) in Layer Chicken Infected by <i>Escherichia coli</i>	Sri Hidanah, Emy K.Sabdoningrum, M.A. Al Arif, Rivienda S.Ratibawan, Erma Safitri

Sd/-

**Managing Editor,
Indian Veterinary Journal**

To,

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