Canadian Journal of Animal Science

Achyranthes Japonica Nakai root extract supplementation improves apparent nutrient digestibility, caecum microbiota, and excreta gas emission in broiler chicks

Journal:	<i>Canadian Journal of Animal Science</i>
Manuscript ID	CJAS-2020-0164.R2
Manuscript Type:	Article
Date Submitted by the Author:	05-Jul-2021
Complete List of Authors:	Dang, De Xin; Dankook University - Cheonan Campus, Yun, Kwan Sik; Weve The State Apt, Synergen Inc Kim, In Ho; Dankook University Department of Animal Resources Science, Department of Animal Resource, and Science
Keywords:	Achyranthes Japonica Nakai root extract, broiler chick, herbal extract, noxious gas emission, nutrient digestibility

SCHOLARONE™
Manuscripts

***Achyranthes Japonica Nakai* root extract supplementation improves apparent nutrient digestibility,
caecum microbiota, and excreta gas emission in broiler chicks**

De Xin Dang¹, Kwan Sik Yun², In Ho Kim^{1*}

Weve The State Apt, Bucheon-si, Gyeonggi-do, South Korea²

Department of Animal Resource & Science, Dankook University, Cheonan 31116, South Korea¹

*Corresponding author: Prof. In Ho Kim

Tel.: +82-41-550-3652; Fax: +82-41-559-7881

E-mail: inhokim@dankook.ac.kr

Abstract

This study investigated the effects of supplementing *Achyranthes Japonica Nakai* (AJN) root extract to the diet of broiler chicks on growth performance, nutrient digestibility, caecum microbiota, excreta gas emission, and relative weight of organs. A total of 270 1-day-old Ross 308 broiler chicks (42.11 ± 0.18 g) were randomly allotted into 3 dietary treatments according to the initial body weight. Each treatment had 5 replicate cages with 18 birds per cage. The experimental period was 35 days (starter, days 1-7; grower, days 8-21; finisher, days 22-35). Dietary treatments were corn-soybean meal-based basal diet supplemented with 0.000, 0.015, or 0.030 % AJN root extract. The apparent total tract digestibility of nitrogen ($P = 0.025$) increased linearly with the increase of the dosage of AJN root extract, while the counts of *E. coli* in caecum ($P = 0.038$) and excreta ammonia emission ($P = 0.003$) decreased linearly. However, the growth performance ($P > 0.05$) and the relative weight of organs ($P > 0.05$) did not differ among the dietary groups. In conclusion, AJN root extract could increase the nutrient digestibility and reduce the noxious gas emission by reducing the caecum harmful microbiota in a dose-dependent manner.

Keywords: *Achyranthes Japonica Nakai* root extract; broiler chick; herbal extract; noxious gas emission; nutrient digestibility

Introduction

Recently, due to the prohibition of antibiotic growth promoters, herbal extracts with antioxidant properties have attracted widespread attention as natural growth promoters. When supplementing herbal

extracts with antioxidant properties to the diet of broiler chicks, positive effects on growth performance, immune status, and intestinal health have been observed (Li et al. 2015; Park and Kim 2018).

Achyranthes Japonica Nakai (AJN) is an antioxidant herb that belongs to the Amaranthaceae family. The root of AJN contains several bioactive ingredients, such as saponins, triterpenoids, inokosterone, 20-hydroxyecdysone, and phytoecdysteroid (Liu et al. 2018). It is reported that AJN root extract has several positive physiological effects, including antioxidant, anti-inflammatory, and hepatoprotective properties (Bang et al. 2012). When used in animal husbandry, dietary supplementation of achyranthes extract has positive effects on the growth performance and intestinal health of animals (Chen et al. 2009; Liu et al. 2020; Dang et al. 2020). Park and Kim (2020) and Sun et al. (2020) reported that the growth performance, nutrient digestibility, and caecum microbiota community of broiler chicks were improved by supplementing 0.025, 0.050, or 0.100 % AJN root extract to the diet.

However, more studies are needed to evaluate the efficacy of AJN root extract at different dose levels. Thus, the objective of the present study was to test the hypothesis that broiler chicks fed a diet supplemented with 0.015 or 0.030 % AJN root extract would positively affect growth performance, apparent nutrient digestibility, caecum microbiota, excreta gas emission, and relative weight of organs.

Materials and Methods

The experimental procedures were reviewed and approved by the Animal Care and Use Committee of Dankook University (DK-1-1949-1). Birds were handled according to the guidelines described by the Canadian Council on Animal Care (2009).

Information on *Achyranthes Japonica Nakai* root extract

The commercial AJN root extract used in this study was the same as the study of Dang et al. (2020) and obtained from Synergen Inc. (Bucheon, Korea). Based on the information provided by the manufacturer, the AJN roots were washed three times with clean water and then ground with a mill (IKAM20; IKA, Staufen,

Germany). The sample was extracted with distilled water at 80°C and refluxed for 6 h to obtain the initial extract. The residues were extracted with distilled water (1:5) at 80°C for 2 h. The extract solution was filtered at low temperatures using a high-velocity centrifuge. The useful parts were collected by a centrifuge column and eluted with ethanol. After cooling to room temperature (25°C) and filtering (Whatman No.2; Whatman Ltd., Kent, UK), the samples were vacuum-dried at a temperature below 40°C. Then, the extracts were completely dried in a freeze-dryer (Dang et al. 2020).

Animals, housing, and diets

A total of 270 1-day-old (as hatched) Ross 308 broiler chicks with 42.11 ± 0.18 g live weight were used for a 35-day trial. The experimental period was 35 days and divided into the starter period (days 1-7), grower period (days 8-21), and finisher period (days 22-35). All birds were divided into 3 dietary treatments according to the initial body weight. There were 5 replicate cages in each diet group and 18 birds per cage. Dietary composition in the dietary groups were corn-soybean meal-based diet supplemented with 0.000, 0.015, or 0.030 % AJN root extract. The recommendation of the National Research Council (NRC 1994) was referenced to formulate the feed ingredients and to calculate the nutritional composition in the basal diet (Table 1).

The birds were housed with a 3-floor battery cages ($1.55 \times 0.75 \times 0.55$ m/cage) in an environmentally controlled room (temperature was started at 32°C and reduced by 2°C every week up to 24°C, and 65 % relative humidity). Each cage was equipped with 2 feeders (one feeder on each side) and 2 nipple drinkers to provide feed and water *ad libitum* to birds.

Sampling and measurements

Body weight (BW) of each replicate cage was recorded on the 1st, 8th, 22nd, and 35th day, and the feed consumption was calculated daily to measure the body weight gain (BWG), average feed intake (FI), and feed conversion ratio (FCR).

On the 28th day, 0.2 % chromium oxide was added to the diets as an inert marker to determine apparent

total tract digestibility (ATTD) of dry matter (DM), nitrogen, and energy. The feed samples were taken after mixed and stored in the -20°C freezer. During days 30 to 32, excreta samples were collected from each replicate cage and immediately stored in a freezer at -20°C until analysis. On the 35th day, feed and excreta samples were thawed and dried at 60°C for 72 h, then finely ground to pass through a 1-mm sieve and collected. Both feed and excreta powder samples were analyzed for energy (Parr Instrument, Moline, IL, USA), DM (methods 934.01), and nitrogen (methods 968.06) using standard procedures of AOAC (2000). Chromium concentration was determined by the method provided by Sun and Kim (2019) using UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan).

The ATTD of DM, nitrogen, and energy were calculated according to the following equation:

$$\text{Digestibility (\%)} = \left(1 - \frac{N_f \times C_d}{N_d \times C_f}\right) \times 100,$$

where N_f is the nutrient concentration in excreta (% DM), N_d is the nutrient concentration in the diet (% DM), C_f is the chromium concentration in excreta (% DM), and C_d is the chromium concentration in the diet (% DM).

On days 32 to 34, approximately 300 g excreta samples were collected from each replicate cage for quantifying ammonia (NH₃), hydrogen sulfide (H₂S), and total mercaptan (R-SH). The excreta samples were kept in 2-L sealed plastic containers for 3 days at room temperature (25°C). After the fermentation period, a Gastec (model GV-100) gas sampling pump was used for gas detection (Gastec Corp., Tokyo, Japan). The NH₃, H₂S, and R-SH concentrations were measured within the scope of 5.0-100.0 ppm (No. 3La, detector tube; Gastec Corp.), 2.0-20.0 ppm (No. 4LK, detector tube; Gastec Corp.), and 0.5-120.0 ppm (No. 70 and 70-L, detector tubes; Gastec Corp.), respectively. The adhesive plaster was punctured, and 100 mL of head-space air was sampled at approximately 3 cm above the excreta surface.

At the end of the experimental period, 20 birds (4 birds per replicate) were randomly selected from each treatment (total 60) were slaughtered by cervical dislocation. The gizzard, breast meat, bursa of fabricius, liver, spleen, and abdominal fat were removed and weighed to calculate the relative weight of

organs. The organ indexes were calculated using the following equation:

$$\text{Organ indexes} = \frac{\text{Organ weight}}{\text{Live body weight}} \times 100 \%$$

After slaughtering the birds, fresh digesta content samples were collected from the caecum into micro-tubes. The caecum microbiota analysis is based on the method provided by Sun and Kim (2019). One gram of digesta sample was blended with 9 mL sterile peptone water and mixed for 1 min on a vortex stirrer. Counts of viable bacteria in the caecum samples were determined by plating serial 10-fold dilutions (10^{-3} to 10^{-6}) onto Lactobacilli MRS agar (Difco Laboratories, Detroit, MI, USA), MacConkey agar (Difco Laboratories, Detroit, MI, USA), and Salmonella-Shigella (SS) agar (Difco Laboratories, Detroit, MI, USA) plates to isolate *Lactobacillus*, *Escherichia coli* (*E. coli*), and *Salmonella*, respectively. The lactobacilli agar plates were then incubated for 24 h at 37°C under anaerobic conditions. The MacConkey and SS agar plates were incubated for 24 h at 37°C under aerobic conditions. After the incubation periods, colonies of the respective bacteria were counted and expressed as the logarithm of colony-forming units per gram ($\log_{10}$ CFU/g).

Statistical analysis

All data were subjected to statistical analysis in a randomized completely block design using the General Linear Model procedure (SAS Inst. Inc., Cary, NC, USA). The replicate cage was used as the experimental unit. Orthogonal contrasts were used to examine the linear and quadratic effects in response to increasing the dietary supplementation of AJN root extract. Variability in the data was expressed as the standard error of means (SEM), $P < 0.05$ is considered as statistically significant and $P < 0.10$ indicating trends.

Results

Effects of dietary supplementation of AJN root extract on growth performance of broiler chicks were shown in Table 2. A trend towards a linear increase in BWG ($P = 0.086$) and FI ($P = 0.070$)

during days 1-35 were observed. However, there was no significant difference in the FCR among the different dietary groups ($P > 0.05$).

As shown in Table 3, there was a linear increase in the apparent total tract digestibility of nitrogen ($P < 0.05$) with an increase in the dose of AJN root extract. Moreover, the statistical difference in the apparent total tract digestibility of nitrogen was observed in broiler chicks consuming 0.03 % AJN root extract containing diet compared with those consuming control diet ($P = 0.025$). However, the supplementation of AJN root extract in the diet of broiler chicks did not affect the apparent total tract digestibility of DM and energy ($P > 0.05$).

The caecum microbiota of the analyzed community of the dietary groups was shown in Table 4. The counts of *E. coli* in the caecum ($P < 0.05$) decreased linearly with increasing doses of AJN root extract. Moreover, the counts of *E. coli* in the caecum of broiler chicks fed a diet supplemented with 0.03 % AJN root extract was lower than those fed a control diet ($P = 0.038$). There was no difference in the counts of *Lactobacillus* and *Salmonella* in caecum among all dietary groups ($P > 0.05$).

The excreta ammonia emission ($P < 0.01$) decreased linearly with the increase of the dosage of AJN root extract. Moreover, the excreta ammonia emission in broiler chicks consuming a 0.03 % AJN root extract containing diet was lower than those consuming the control diet ($P = 0.003$). The excreta hydrogen sulfide and total mercaptans emission were not affected by the supplementation of AJN root extract ($P > 0.05$) (Table 5).

Dietary supplementation of AJN root extract had no effects on the relative weight of breast muscle, liver, spleen, abdominal fat, bursa of fabricius, and gizzard ($P > 0.05$) (Table 6).

Discussion

The mechanisms for the positive effects of supplementing herbal extracts with antioxidant properties to the diet on the growth performance of broiler chicks are manifold. Paraskeuas et al. (2017)

mentioned that herbal complexes extract supplementation in the diet of broiler chicks has positive effects on the growth performance, which was thought to be due to the improvement of nutrient digestibility. Abolfathi et al. (2019) noted that the increase of BWG caused by dietary supplementation of herbal extracts with antioxidant properties was due to the reduction of FCR. Johannah et al. (2018) observed that broiler chicks fed with herbal extracts with antioxidant properties containing diet increased FI, thereby leading to a high BWG. Therefore, the positive effects of dietary supplementation of herbal extracts with antioxidant properties on the growth performance of broiler chicks were mainly focused on the improvement of nutrient digestibility, FCR, and/or FI (Habibian et al. 2019; Long et al. 2020). Studies by Park and Kim (2020) reported a linear increase in evaluated growth performance parameters and nutrient digestibility by supplementing 0.025, 0.050, or 0.100 % AJN root extract to the diet of broiler chicks. Sun et al. (2020) also reported a linear increase in BWG and nitrogen digestibility, and a linear decrease in FCR, with an increasing dose of AJN root extract (0.025, 0.050, or 0.100 %) in the diet of broiler chicks. However, the above research on supplementing AJN root extract was not entirely similar to the results of this study. This study observed that with the increase of the dosage of AJN root extract, there was no significant difference in evaluated growth performance, but there was a trend of increasing BWG and FI, and the nitrogen digestibility increased linearly. Unlike the above experimental results, the dosage of the AJN root extract in this study was low. In addition, different replicate cages used in the study may also be why the supplementation of AJN root extract has insignificant effects on growth performance.

The activity of the herbal extracts in regulating the intestinal microbiota community is related to its bioactive ingredients (Cho et al. 2014). It is reported that the AJN root extract is rich in saponins, triterpenoids, phytoecdysteroid, 20-hydroxyecdysone, and inokosterone (Liu et al. 2018). The regulation effects on the intestinal microbiota community by supplementing AJN root extract have been reported by Dang et al. (2020) and Liu et al. (2020). Abolfathi et al. (2019) mentioned that herbal

extract with similar bioactive ingredients to the AJN root extract supplementation in the diet increased beneficial bacteria counts and decreased harmful bacteria counts in the caecum of broiler chicks. Pérez-Burillo et al. (2020) also observed the regulation effects of supplementing herbal extracts with antioxidant properties to the diet of animals on the intestinal microbiota community. Therefore, it is speculated that the decrease in the counts of *E. coli* by supplementing AJN root extract was related to its phytochemical composition.

The intestinal microbiota plays an essential role in the health of broiler chicks. When harmful microbiota, such as *E. coli*, are dominant in the intestine, the digestion of nutrients would be impaired (Wang et al. 2018). Jeong and Kim (2014) reported that the counts of *E. coli* in caecum decreased in broiler chicks fed a diet supplemented with herbal complexes extract with antioxidant properties resulting in a high apparent total tract digestibility. Nilkanth et al. (2020) reported that dietary supplementation of herbal extracts with antioxidant properties decreased the counts of *E. coli* and *Salmonella* in caecum, thereby increasing the apparent total tract digestibility of nitrogen in broiler chicks. Marcin et al. (2016) and Rezvani et al. (2018) also reported the strategies of improving nutrient digestibility by reducing the counts of harmful bacteria in the intestine. Therefore, the increase of nitrogen digestibility observed in the study was related to reducing caecum *E. coli* counts.

The source of excreta noxious gas came from the fermentation of unabsorbed nutrient ingredients by intestinal microbiota (Ospina-Rojas et al. 2014). Improving the nutrient digestibility could reduce the concentration of unabsorbed nutrient ingredients, thus reducing the noxious gas emission (Siegert et al. 2016). As Park et al. (2018) reported, supplementing herbal extracts with antioxidant properties to the diet of broiler chicks increased the apparent total tract digestibility of nitrogen, thus reducing the excreta ammonia emission. Dang et al. (2020) also reported the strategy for reducing excreta gas emission by improving nutrient digestibility. Therefore, the excreta ammonia emission decreased in broiler chicks fed a diet supplemented with 0.015 or 0.030 % AJN root extract was associated with the

increase in the apparent total tract digestibility of nitrogen in the current study.

High breast yield along with low abdominal fat is the goal of broiler industry (Santoso et al. 2015). In the present study, the relative weight of breast muscle and abdominal fat were not affected by AJN root extract supplementation. Ecdysterone has anabolic effects on animals (Gorelick-Feldman et al. 2010) and has been identified in the extract of AJN root (Al-Mijan et al. 2018). Gorelick-Feldman et al. (2008) and Parr et al. (2014) mentioned that phytoecdysteroids increased protein synthesis in skeletal muscle cells of mice in a dose-dependent manner. However, the anabolic effect of ecdysterone in AJN root extract have not been verified in broiler chicks. Park and Kim (2020) and Sun et al. (2020) reported that the relative weight of breast muscle increased linearly with the increase of the dose of AJN root extract (0.025, 0.050, or 0.10 %). Therefore, the anabolic effects of ecdysterone in AJN root extract seems to be effective in broiler chicks. No improvement in the relative weight of breast muscle was observed in this study, which may be due to the low dose of AJN root extract used in this study. The relative weight of abdominal fat was related to the relative weight of the liver (Mohiti-Asli et al. 2012; Awad et al. 2014), since liver is the place where lipogenesis occurs (Kamran et al. 2008). A large number of fat deposits lead to an increase in liver weight (Hada et al. 2013). Lipiński et al. (2019) observed that the relative weight of liver in broiler chicks fed a diet supplemented with herbal complexes extract with antioxidant properties was low, which corresponded to a low relative weight of abdominal fat. On the other hand, the increase of feed intake means enhancing the energy intake in animals. Excessive energy intake will lead to an increase in heat production and/or fat deposition (Swennen et al. 2007), thereby increasing the relative weight of liver and abdominal fat (Ghasemi et al. 2014). Therefore, the relative weight of abdominal fat did not increase, because the addition of AJN root extract in the diet of broiler chicks had no effects on the feed intake and the relative weight of liver (Richards et al., 2003; Hada et al. 2013). The shrinkage of the gizzard means the grinding ability to feed damage, thus impairing the nutrient digestibility (Jeong and Kim 2014). The shrinkage of the bursa of fabricius and spleen means immunosuppression (Barekatin et al. 2019). As the results observed in the

present study, dietary supplementation of AJN root extract at 0.015 or 0.030 % did not affect the relative weight of gizzard, bursa of fabricius, and spleen, which was consistent with the studies of Park and Kim (2020) and Sun et al. (2020). Therefore, we considered that dietary supplementation of 0.015 or 0.030 % AJN root extract could not impair the nutrient digestibility and immune status through decreasing the relative weight of gizzard, bursa of fabricius, and spleen.

Conclusion

The results observed in the present study partially verified the previous assumptions. AJN root extract supplementation dose-dependently decreased the caecal *E. coli* count, increased the ATTD of nitrogen which resulted in a significant decrease in the excreta ammonia volatilization. In addition, AJN root extract supplementation had no effects on the growth performance and the relative weight of organs. Therefore, dietary AJN root extract supplementation at 0.03% level might improve the broiler house environment, increase nitrogen digestibility, and improve gut microbiota.

References

- AOAC. 2000. Official method of analysis. 17th ed. Association of Official Analytical Chemists, Washington, D.C., USA.
- Awad, E.A., Fadlullah, M., Zulkifli, I., Farjam, A.S., and Chwen, L.T. 2014. Amino acids fortification of low-protein diet for broilers under tropical climate: ideal essential amino acids profile. Ital. J. Anim. Sci. **13**(2): 3166. doi:<https://doi.org/10.4081/ijas.2014.3166>.
- Al-Mijan, M., Park, S.H., Lee, Y.M., and Lim, B.O. 2018. Evaluation of the antioxidant and anti-inflammatory potential of fermented *Achyranthes Japonica* Nakai extract. Nat. Prod. Chem. Res. **6**(5): 337-343. doi:[10.4172/2329-6836.1000337](https://doi.org/10.4172/2329-6836.1000337).

- Abolfathi, M.E., Tabeidian, S.A., Shahraki, A.D.F., Tabatabaei, S.N., and Habibian, M. 2019. Effects of Ethanol extract of elecampane (*Inula helenium* L.) Rhizome on Growth performance, diet digestibility, gut health, and antioxidant status in broiler chickens. *Livest. Sci.* **223**(2019): 68-75. doi:<https://doi.org/10.1016/j.livsci.2019.03.006>.
- Bang, S.Y., Kim, J.H., Kim, H.Y., Lee, Y.J., Park, S.Y., Lee, S.J., and Kim, Y.H. 2012. *Achyranthes japonica* exhibits anti-inflammatory effect via NF- κ B suppression and HO-1 induction in macrophages. *J. Ethnopharmacol.* **144**(1): 109-117. doi:<https://doi.org/10.1016/j.jep.2012.08.037>.
- Barekatin, R., Nattrass, G., Tilbrook, A.J., Chousalkar, K., and Gilani, S. 2019. Reduced protein diet and amino acid concentration alter intestinal barrier function and performance of broiler chickens with or without synthetic glucocorticoid. *Poult. Sci.* **98**(9): 3662-3675. doi:<https://doi.org/10.3382/ps/pey563>.
- Chen, Q., Liu, Z., and He, J. 2009. *Achyranthes bidentata* polysaccharide enhances immune response in weaned piglets. *Immunopharm. Immunot.* **31**(2): 253-260. doi:<https://doi.org/10.1080/08923970802439795>.
- Cho, J.H., Kim, H.J., and Kim, I.H. 2014. Effects of phytogetic feed additive on growth performance, digestibility, blood metabolites, intestinal microbiota, meat color and relative organ weight after oral challenge with *Clostridium perfringens* in broilers. *Livest. Sci.* **160**(2014): 82-88. doi:<https://doi.org/10.1016/j.livsci.2013.11.006>.
- Dang, D.X., Kim, Y.M., and Kim, I.H. 2020. Effects of a root extract from *Achyranthes Japonica* Nakai on the growth performance, blood profile, fecal microbial community, fecal gas emission, and meat quality of finishing pigs. *Livest. Sci.* **239**(2020): 104160. doi:<https://doi.org/10.1016/j.livsci.2020.104160>.
- Gorelick-Feldman, J., MacLean, D., Ilic, N., Poulev, A., Lila, M.A., Cheng, D., and Raskin, I. 2008. Phytoecdysteroids increase protein synthesis in skeletal muscle cells. *J. Agr. Food. Chem.* **56**(10): 3532-3537. doi:<https://doi.org/10.1021/jf073059z>.

- Gorelick-Feldman, J., Cohick, W., and Raskin, I. 2010. Ecdysteroids elicit a rapid Ca^{2+} flux leading to Akt activation and increased protein synthesis in skeletal muscle cells. *Steroids*. **75**(10): 632-637. doi:<https://doi.org/10.1016/j.steroids.2010.03.008>.
- Ghasemi, H.A., Ghasemi, R., and Torki, M. 2014. Periodic usage of low-protein methionine-fortified diets in broiler chickens under high ambient temperature conditions: effects on performance, slaughter traits, leukocyte profiles and antibody response. *Int. J. Biometeorol.* **58**(7): 1405-1414. doi:<https://doi.org/10.1007/s00484-013-0741-0>.
- Hada, F.H., Malheiros, R.D., Silva, J.D.T., Marques, R.H., Gravena, R.A., Silva, V.K., and Moraes, V.M.B. 2013. Effect of protein, carbohydrate, lipid, and selenium levels on the performance, carcass yield, and blood changes in broilers. *Braz. J. Poultry Sci.* **15**(4): 385-394. doi:<https://doi.org/10.1590/S1516-635X2013000400014>.
- Habibian, M., Sadeghi, G., and Karimi, A. 2019. Comparative effects of powder, aqueous and methanolic extracts of purslane (*Portulaca oleracea L.*) on growth performance, antioxidant status, abdominal fat deposition and plasma lipids in broiler chickens. *Anim. Prod. Sci.* **59**(1): 89-100. doi:<https://doi.org/10.1071/AN17352>.
- Jeong, J.S., and Kim, I.H. 2014. Effect of fermented medicinal plants (*Gynura procumbens*, *Rehmannia glutinosa*, *Scutellaria baicalensis*) as alternative performance enhancers in broilers. *J. Poult. Sci.* **52**(2): 119-216. doi:<https://doi.org/10.2141/jpsa.0140061>.
- Johannah, N.M., Joseph, A., Maliakel, B., and Krishnakumar, I.M. 2018. Dietary addition of a standardized extract of turmeric (TurmaFEEDTM) improves growth performance and carcass quality of broilers. *J. Anim. Sci. Technol.* **60**(5): 1-9. doi:<https://doi.org/10.1186/s40781-018-0167-7>.
- Kamran, Z., Sarwar, M., Nisa, M., Nadeem, M.A., Mahmood, S., Babar, M.E., and Ahmed, S. 2008. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics

- of broiler chickens from one to thirty-five days of age. *Poult. Sci.* **87**(3): 468-474.
doi:<https://doi.org/10.3382/ps.2007-00180>.
- Li, H.L., Zhao, P.Y., Lei, Y., Hossain, M.M., and Kim, I.H. 2015. Phytoncide, phytogenic feed additive as an alternative to conventional antibiotics, improved growth performance and decreased excreta gas emission without adverse effect on meat quality in broiler chickens. *Livest. Sci.* **181**(2015): 1-6.
doi:<https://doi.org/10.1016/j.livsci.2015.10.001>.
- Liu, Z., Wang, X., Ou, S., Arowolo, M., Hou, D.X., and He, J. 2018. Effects of *Achyranthes bidentata* polysaccharides on intestinal morphology, immune response, and gut microbiome in yellow broiler chickens challenged with *Escherichia coli* K88. *Polymers.* **10**(11): 1233-1247.
doi:<https://doi.org/10.3390/polym10111233>.
- Lipiński, K., Antoszkiewicz, Z., Kotlarczyk, S., Mazur-Kuśnerek, M., Kaliniewicz, J., and Makowski, Z. 2019. The effect of herbal feed additive on the growth performance, carcass characteristics and meat quality of broiler chickens fed low-energy diets. *Arch. Anim. Breed.* **62**(1): 33-40.
doi:<https://dx.doi.org/10.5194%2Faab-62-33-2019>.
- Liu, X., Lee, S.I., and Kim, I.H. 2020. *Achyranthes japonica* extracts supplementation to growing pigs positively influences growth performance, nutrient digestibility, fecal microbial shedding, and fecal gas emission [online]. *Asian-Austral. J. Anim.* doi:<https://doi.org/10.5713/ajas.20.0012>.
- Long, S.F., He, T.F., Wu, D., Yang, M., and Piao, X.S. 2020. *Forsythia suspensa* extract enhances performance via the improvement of nutrient digestibility, antioxidant status, anti-inflammatory function, and gut morphology in broilers. *Poult. Sci.* **99**(9): 4217-4226.
doi:<https://doi.org/10.1016/j.psj.2020.05.011>.
- Mohiti-Asli, M., Shivazad, M., Zaghari, M., Rezaian, M., Aminzadeh, S., and Mateos, G.G. 2012. Effects of feeding regimen, fiber inclusion, and crude protein content of the diet on performance and egg quality

- and hatchability of eggs of broiler breeder hens. *Poult. Sci.* **91**(12): 3097-3106.
doi:<https://doi.org/10.3382/ps.2012-02282>.
- Marcin, A., Levkut, M., Revajová, V., Šoltysová, B., and Nad, P. 2016. Influence of *Salvia Officinalis* Essential Oil on Digestion Parameters and Intestinal Microflora of Broiler Chickens. *Folia. Vet.* **60**(1): 5-14. doi:<https://doi.org/10.1515/fv-2016-0001>.
- NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. National Academies Press, Washington, D.C., USA.
- Nilkanth, R.H., Vipin, K.K., and Kumar, P. 2020. Effect of feeding of oregano oil with probiotic on gut microbiota and nutrients digestibility of broiler chicken. *J. Pharm. Innov.* **9**(3): 660-664.
doi:<https://doi.org/10.22271/tpi.2020.v9.i3l.4657>.
- Ospina-Rojas, I.C., Murakami, A.E., Duarte, C.R.A., Eyng, C., Oliveira, C.A.L., and Janeiro, V. 2014. Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. *Brit. Poultry Sci.* **55**(6): 766-773.
doi:<https://doi.org/10.1080/00071668.2014.970125>.
- Parr, M.K., Zhao, P., Haupt, O., Ngueu, S.T., Hengevoss, J., Fritzemeier, K.H., Piechotta, M., Schlörer, N., Muhn, P., Zheng, W., Xie, M., and Diel, P. 2014. Estrogen receptor beta is involved in skeletal muscle hypertrophy induced by the phytoecdysteroid ecdysterone. *Mol. Nutr. Food. Res.* **58**(9): 1861-1872.
doi:<https://doi.org/10.1002/mnfr.201300806>.
- Paraskeuas, V., Fegeros, K., Palamidi, I., Hunger, C., and Mountzouris, K.C. 2017. Growth performance, nutrient digestibility, antioxidant capacity, blood biochemical biomarkers and cytokines expression in broiler chickens fed different phytogetic levels. *Anim. Nutr.* **3**(2): 114-120.
doi:<https://doi.org/10.1016/j.aninu.2017.01.005>.

- Park, J.H., and Kim, I.H. 2018. Effects of a protease and essential oils on growth performance, blood cell profiles, nutrient retention, ileal microbiota, excreta gas emission, and breast meat quality in broiler chicks. *Poult. Sci.* **97**(8): 2854-2860. doi:<https://doi.org/10.3382/ps/pey151>.
- Park, J.H., Lee, S.I., and Kim, I.H. 2018. Effect of dietary *Spirulina (Arthrospira) platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. *Poult. Sci.* **97**(7): 2451-2459. doi:<https://doi.org/10.3382/ps/pey093>.
- Park, J.H., and Kim, I.H. 2020. Effects of dietary *Achyranthes japonica* extract supplementation on the growth performance, total tract digestibility, cecal microflora, excreta noxious gas emission, and meat quality of broiler chickens. *Poult. Sci.* **99**(1): 463-470. doi:<https://doi.org/10.3382/ps/pez533>.
- Pérez-Burillo, S., Hinojosa-Nogueira, D., Pastoriza, S., and Rufián-Henares, J.A. 2020. Plant extracts as natural modulators of gut microbiota community structure and functionality. *Heliyon*. **6**(11): e05474. doi:<https://doi.org/10.1016/j.heliyon.2020.e05474>.
- Richards, M.P., Poch, S.M., Coon, C.N., Rosebrough, R.W., Ashwell, C.M., and McMurtry, J.P. 2003. Feed Restriction Significantly Alters Lipogenic Gene Expression in Broiler Breeder Chickens. *J. Nutr.* **133**(3):707-715. doi:<https://doi.org/10.1093/jn/133.3.707>.
- Rezvani, M.R., Sayadpour, N., and Saemi, F. 2018. The effect of adding pomegranate seed extract to fat-containing diets on nutrients digestibility, intestinal microflora and growth performance of broilers. *Iran. J. Vet. Res.* **19**(4), 310-317. doi:<https://dx.doi.org/10.22099/ijvr.2018.5061>.
- Swennen, Q., Decuypere, E., and Buyse, J. 2007. Implications of dietary macronutrients for growth and metabolism in broiler chickens. *World. Poultry. Sci. J.* **63**(4): 541-556. doi:<https://doi.org/10.1017/S0043933907001602>.

- Santoso, U., Kususiyah, K., and Suharyanto, S. 2015. The effect of *Sauropus androgynus* leaves extract plus turmeric powder on fat deposition, carcass quality and blood profile in broilers fed low protein diets. J. Indones. Trop. Anim. Agric. **40**(2): 121-130. doi:<https://doi.org/10.14710/jitaa.40.2.121-130>.
- Siegert, W., Wild, K.J., Schollenberger, M., Helmbrecht, A., and Rodehutsord, M. 2016. Effect of glycine supplementation in low protein diets with amino acids from soy protein isolate or free amino acids on broiler growth and nitrogen utilisation. Brit. Poultry. Sci. **57**(3): 424-434. doi:<https://doi.org/10.1080/00071668.2016.1163523>.
- Sun, H.Y., and Kim, I.H. 2019. Evaluation of an emulsifier blend on growth performance, nutrient digestibility, blood lipid profiles, and fecal microbial in growing pigs fed low energy density diet. Livest. Sci. **227**(2019): 55-59. doi:<https://doi.org/10.1016/j.livsci.2019.06.016>.
- Sun, H.Y., Kim, Y.M., and Kim, I.H. 2020. Evaluation of *Achyranthes japonica Nakai* extract on growth performance, nutrient utilization, cecal microbiota, excreta noxious gas emission, and meat quality in broilers fed corn–wheat–soybean meal diet. Poult. Sci. **99**(11): 5728-5735. doi:<https://doi.org/10.1016/j.psj.2020.07.023>.
- Wang, S., Yao, J., Zhou, B., Yang, J., Chaudry, M.T., Wang, M., Xiao, F., Li, Y., and Yin, W. 2018. Bacteriostatic Effect of quercetin as an antibiotic alternative in vivo and its antibacterial mechanism in vitro. J. Food. Prot. **81**(1): 68-78. <https://doi.org/10.4315/0362-028X.JFP-17-214>.

Table 1. Ingredients and calculated chemical composition of diets, (% as fed).

Items	Feeding phases		
	Starter (days 1-7)	Grower (days 8-21)	Finisher (days 22-35)
Ingredients (%)			
Corn (CP 7.5%)	54.19	55.38	56.77
Soybean meal (CP 45%)	33.80	26.10	18.23
Canola meal (CP 38%)	5.00	10.00	15.00
Soybean oil	2.10	3.62	5.07
Monocalcium phosphate	-	1.28	1.12
Dicalcium phosphate	1.70	-	-
Limestone	1.15	1.34	1.22
L-Lysine	0.50	0.65	0.81
DL-Methionine	0.46	0.47	0.52
L-Threonine	0.20	0.25	0.32
L-Tryptophan	-	0.01	0.04
NaHCO ₃	0.10	0.10	0.10
Salt	0.30	0.30	0.30
Mineral mix ¹	0.20	0.20	0.20
Vitamin mix ²	0.20	0.20	0.20
Choline	0.10	0.10	0.10
Total	100.00	100.00	100.00
Analyzed composition			
Crude protein, %	23.00	21.50	20.00
Ca, %	0.96	0.87	0.81
Available P, %	0.48	0.44	0.41
Lys, %	1.50	1.40	1.30
Met + Cys, %	1.08	0.99	0.94
Metabolizable energy, kcal/kg	3,000	3,100	3,200

¹ Provided per kg of complete diet: 12mg Cu (as CuSO₄•5H₂O); 85mg Zn (as ZnSO₄); 8mg Mn (as MnO₂); 0.28 mg I (as KI); 0.15mg Se (as Na₂SeO₃•5H₂O).

² Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D₃; 44 IU vitamin E; 4.4 mg vitamin K; 8.3mg riboflavin; 50mg niacin; 4mg thiamine; 29mg d-pantothenic; 166mg choline; 33µg vitamin B₁₂.

Table 2. Effects of supplementation of *Achyranthes japonica Nakai* (AJN) root extract in the diet of broiler chicks on the growth performance¹

Items	AJN root extract, %			SEM ²	P-value	
	0.000	0.015	0.030		Linear	Quadratic
IBW ³ , g	42.83	42.86	42.93	0.178	0.698	0.901
BWG ⁴ , g/d/bird						
Days 1-7	139.56	139.57	139.69	2.029	0.964	0.983
Days 8-21	592.71	603.77	619.47	17.921	0.312	0.918
Days 22-35	1072.11	1085.73	1107.55	23.072	0.299	0.887
Days 1-35	1804.38	1829.07	1866.71	23.594	0.086	0.826
FI ⁵ , g/d/bird						
Days 1-7	168.89	176.11	177.33	5.448	0.295	0.661
Days 8-21	949.75	971.50	979.22	20.574	0.331	0.786
Days 22-35	1915.51	1952.82	1955.23	28.039	0.336	0.621
Days 1-35	3034.15	3100.43	3111.78	27.650	0.070	0.433
FCR ⁶						
Days 1-7	1.21	1.26	1.27	0.047	0.427	0.733
Days 8-21	1.61	1.61	1.59	0.040	0.740	0.741
Days 22-35	1.79	1.80	1.77	0.041	0.712	0.696
Days 1-35	1.68	1.70	1.67	0.018	0.582	0.391

¹Valus represent the means of five cages (n = 5) per treatment.

²SEM, standard error of the mean.

³IBW, initial body weight.

⁴BWG, body weight gain.

⁵FI, feed intake.

⁶FCR, feed conversion ratio.

Table 3. Effects of supplementation of *Achyranthes japonica Nakai* (AJN) root extract in the diet of broiler chicks on nutrient digestibility¹

Items, %	AJN root extract, %			SEM ²	<i>P</i> -value	
	0.000	0.015	0.030		Linear	Quadratic
DM ³	74.46	76.30	77.73	1.667	0.180	0.920
Nitrogen	72.68 ^b	74.68 ^{ab}	77.73 ^a	1.483	0.025	0.776
Energy	75.18	75.74	76.42	1.464	0.556	0.974

¹Values represent the means of five cages (n = 5) per treatment.

²SEM, standard error of the mean.

³DM, dry matter.

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

Table 4. Effects of supplementation of *Achyranthes japonica Nakai* (AJN) root extract in the diet of broiler chicks on caecum microbiota¹

Items, log ₁₀ cfu/g	AJN root extract, %			SEM ²	P-value	
	0.000	0.015	0.030		Linear	Quadratic
<i>Lactobacillus</i>	9.41	9.43	9.44	0.082	0.797	0.982
<i>E. coli</i>	5.64 ^a	5.14 ^{ab}	5.01 ^b	0.193	0.038	0.447
<i>Salmonella</i>	3.17	3.07	3.06	0.394	0.838	0.921

¹Valus represent the means of five cages with 4 birds per replicate cages (n = 20) per treatment.

²SEM, standard error of the mean.

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

Table 5. Effects of supplementation of *Achyranthes japonica* Nakai (AJN) root extract in the diet of broiler chicks on excreta gas emission¹

Items, ppm	AJN root extract, %			SEM ²	<i>P</i> -value	
	0.000	0.015	0.030		Linear	Quadratic
NH ₃ ³	15.00 ^a	12.00 ^{ab}	9.50 ^b	0.957	0.003	0.836
H ₂ S ⁴	6.25	5.75	5.00	0.874	0.338	0.910
R-SH ⁵	6.75	6.75	6.00	1.328	0.699	0.823

¹Values represent the means of five cages (n = 5) per treatment.

²SEM, standard error of the mean.

³NH₃, ammonia.

⁴H₂S, hydrogen sulfide.

⁵R-SH, total mercaptan.

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

Table 6. Effects of supplementation of *Achyranthes japonica Nakai* (AJN) root extract in the diet of broiler chicks on relative weight of organs¹

Items	AJN root extract, %			SEM ²	P-value	
	0.000	0.015	0.030		Linear	Quadratic
Breast muscle	17.15	17.78	18.03	1.505	0.689	0.921
Liver	2.11	2.15	2.06	0.157	0.819	0.760
Spleen	0.11	0.12	0.13	0.012	0.194	0.757
Abdominal fat	1.47	1.46	1.39	0.152	0.703	0.875
Bursa of fabricius	0.13	0.13	0.14	0.017	0.739	0.763
Gizzard	1.39	1.40	1.58	0.101	0.227	0.516

¹Valus represent the means of five cages with 4 birds per replicate cages (n = 20) per treatment.

¹SEM, standard error of the mean.