한국가금학회지 제 51권 제 4호, 179~186 (2024)
Korean J. Poult. Sci. Vol.51, No.4, 179~186 (2024)
https://doi.org/10.5536/KJPS.2024.51.4.179

Evaluation of *Abeliophyllum distichum* Nakai Extract in Litter Quality and Footpad Dermatitis in Broiler Litter

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ABSTRACT The objective of this study was to investigate the effects of *Abeliophyllum distichum* Nakai (AN) extract in litter moisture, ammonia nitrogen (NH_3-N), pH, microflora, and FPD. A total of 180 one-day-old Arbor Acres (initial body weight of 37.51±0.17 g) were used for 28 d. Treatments were as follows: basal rice husk + 10 mL tap water (AN0), basal rice husk + 2% AN extract soluble (AN2; 0.2 g/mL), basal rice husk + 4% AN extract soluble (AN4; 0.4 g/mL). Each treatment had five replicates, with twelve birds per pen. At 0 to 2 weeks, the AN0 and AN2 significantly increased the feed intake (FI) compared with the AN4. There was no significant difference (P>0.05) in litter moisture during the experiment period. However, at week 4, the AN0 significantly increased (P<0.05) litter pH compared with the AN2 and AN4. At 3 and 4 weeks, the counts of *Salmonella* in the litter were significantly higher (P<0.05) in the AN0 compared with the AN2 and AN4. Also, the AN4 showed significantly lower (P<0.05) counts of *Salmonella* compared with the AN0 and AN4. Moreover, at 4 weeks, the AN0 showed significantly higher (P<0.05) counts of *E. coli* compared with the AN4. Also, AN4 showed significantly decreased (P<0.05) FPD scores compared with the AN0. Therefore, the result of this study could support the possibilities of AN extract in litter to alleviate the FPD.

(Key words: *Abeliophyllum distichum* Nakai, footpad dermatitis, litter)

INTRODUCTION

Footpad dermatitis (FPD), which is also known as pododermatitis and contact dermatitis, is a common skin problem, reflecting the impaired product quality and animal welfare (Bilgili et al., 2009; Kauonen et al., 2016). The occurrence of FPD is accompanied by a walking disorder through a excruciating skin ailment that led to synovitis and subsequent lameness (Michel et al., 2012; Park et al., 2023). Also, previous studies have reported that the painful footpad skin condition reduced the growth performance in broilers (Martland, 1985; Kaukonen et al., 2016).

Commonly, the main cause of FPD in broilers is impaired litter quality. As broiler spends most of their lifetime on litter, impaired litter quality increases the occurrence of FPD (Bilgili et al., 2009). When the litter could not play the moisture absorption, it induces the risk of 'wet litter'. According to Dunlop et al. (2016), once the litter moisture surpasses 25%, it could not provide the cushioning, insulation and water-holding capacity to broilers. Also, wet litter induces the microbial metabolism's volatilization of ammonia in the excreta, thereby increasing the bacterial pathogen activities and ammonia burns, which is another name for FPD (Kjaer et al., 2006; Garcia et al., 2012). Therefore, alternative strategies are needed for reducing the bacterial pathogens activities and concentration of ammonia.

Abeliophyllum distichum Nakai (AN) is the sole species within the genus *Abeliophyllum* in the world, which is mostly produced in Yeongdong region, Korea (Lee et al., 2020). It has been reported that AN could provide therapeutic value, such as anticancer (Yoo et al., 2000; Park et al., 2014), anti-inflammatory (Choi et al., 2017), antidiabetic (Li et al., 2013),

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and antihypertensive capacity (Oh et al., 2003). Especially, AN contains glycosides (acteoside, isoactoside, rutin, and hirsutrin) in its leaves, which shows the antibacterial and antioxidant effects (He et al., 2000; Song et al., 2021). Also, AN possesses numerous physiologically active polyphenols and flavonoids, exhibiting antibacterial and antioxidant effects (Ko et al., 2019). Moreover, previous studies have demonstrated the antibacterial effect of AN extract, by using a mice and *in-vitro* method (Go et al., 2021; Song et al., 2021).

However, to our knowledge, there are currently no studies identifying the effects of AN extract in broiler litter conditions. Therefore, the objective of this study was to identify the effects of AN extract in litter moisture, ammonia nitrogen (NH_3-N), pH, microflora, and FPD in broilers.

MATERIALS AND METHODS

1. Ethics Approval and Consent to Participate

The Institutional Animal Care and Use Committee of Chung-buk National University, Cheongju, Korea, reviewd and approved the study's protocol (approval no. CBNUA-24-0003-02).

2. Source of *Abeliophyllum distichum* Nakai Extract

The AN extract was supported by a Our Tree Farming Association (Chungcheongbuk-do, Goesan, Korea). The foreign body that contained in AN leaves and stems were washed with distilled water and then dried with using an agricultural product dryer at 40℃ to contain less than a 15% moisture content. Dried AN was extracted for 5 hours at 70℃, and then moved to a evaporator tank. Afterwards, it went through a filtration process with a 50μm filter and was concentrated to over 10 brix at an internal temperature of 70℃ before being stored.

3. Experimental Design, Animals, and Housing

A total of 180 mixed-sex one-day-old Arbor Acres (initial body weight of 37.51±0.17 g) were provided from a local hatchery (Dongsan hatchery, Cheonan, Korea) and used in this experiment for 28 d. A randomized complete block design was used to randomly assign each broiler to one of three treatments. Treatments were as follows: basal rice husk + 10 mL tap water (AN0), basal rice husk + 2% AN extract soluble (AN2; 0.2 g/mL), basal rice husk + 4% AN extract soluble (AN4; 0.4 g/mL). Each treatment had five replicates, with twelve birds per pen. The broilers were housed in pen (12 birds/ m^2) and fed *ad libitum* access to diet and water throughout the experiments. The stocking density per broiler has been set following Korean animal welfare standards. The 5 kg of rice husk was given to each pen as litter, and AN extract was sprayed on the surface of the litter weekly, by using a small hand pump. After starting experiment initiation temperature was 31±1℃, the temperature was gradually decreased to 21±1℃. For the first 7 d, the lighting schedule was 23L:1D at 30 lux, then 18L:6D at 20 lux.

Also, for the starter (1−7 d), grower (8−21 d), and finisher (22−28 d) periods, all diets were formulated to meet or exceed National Research Council (1994).

4. Litter Sample Collection

A litter sample was taken from each pen at five different locations (four on the edges and one in the middle), weekly. Following a thorough mixing process, the litter samples were refrigerated at 4℃ until analysis was completed.

5. Growth Performance

At initial, 7, 14, 28 d of age, body weight (BW), and feed consumption was determined per pen to calculate the average feed intake (FI), average daily gain (ADG), and feed conversion ratio (FCR) for each phase.

6. Litter Quality

To determine the pH of the litter, 20 g of samples was mixed with 100 mL of distilled water according to Brink et al. (2022) method. After a period of 30 m, the pH of the sample was measured by pH meter (Thermo Fisher Scientific, Orion Star A211 pH Benchtop Meter). To determine the litter moisture, 2 g of litter samples from each pen were dried in an oven for 8 h at 105℃ (AOAC, 2005).

7. NH_3-N Measurement

The intestinal content and the NH_3-N concentration in fresh faecal samples were assessed using a technique that Weatherburn (1967) method. Ten minutes were spent centrifuging

3,000 × g of 10% TCA (trichloroacetic acid) mixture over duplicate samples in a 1:1 (w/v) ratio. After that, samples were diluted five times (v/v) using distilled water. Following deproteinization, the liquid's ammonium was converted to indophenol blue in an alkaline solution containing phenol and hypochlorite. This reaction is named after Berthelot:

$2C_6H_5O^- + NH_3 + 3ClO^- \rightarrow OC_6H_4N = C_6H_4O + 2H_2O + OH^- + 3Cl^-$

The intensity of the blue colour formed in the above reaction is linear with the NH_3-N concentration. Utilizing a spectrophotometer (UVmini-1240, SHIMADZU, Kyoto, Japan), the absorbance at 623 nm was determined.

A 1 L volumetric flask filled to the mark with DW was used to dissolve 472 mg of ammonium sulphate [$(NH_4)_2SO_4$, Ajax Finechem, NSW, Australia] in order to create a standard ammonium stock solution [100 ppm (mg/L) NH_3-N]. The following concentrations were utilized five times for the calibration line: 0, 5, 10, 15, 20, 25, and 30 ppm NH_3-N. Using a UV-VIS Spectrophotometer (UVmini-1240, SHIMADZU, Kyoto, Japan), the standards were prepared by pipetting 0, 5, 10, 15, 20, 25, and 30 mL of the stock solution into 100 mL volumetric flasks, respectively. The samples were then well mixed and brought up to 100 mL with water. The measurements were taken at 623 nm against water.

8. Litter Microflora

Every week, litter samples were gathered in conical tubes for analysis of the counts of litter microflora. From the sample, 1 g of the sample was combined with 9 mL of 1 × PBS buffer and vortexed for a duration of one minute. By plating tenfold serial dilutions (10^{-5} to 10^{-8}) onto MacConkey agar (MB cell, Seoul, Korea) and BG sulfa (MB cell, Seoul, Korea) agar to isolate *E. coli* and *Salmonella*, the counts of bacteria in the litter samples were measured. The MacConkey agar and BG sulfa agar were cultured for 24 h at 37℃. Following the incubation times, the respective bacteria colonies were counted, and their counts were expressed as the logarithm of colony-forming unit per gram (log CFU/g).

9. Footpad Dermatitis Score

Following the Martrenchar et al. (2002) method, footpad dermatitis was scored based on the type of lesion in all birds at the end of the experiment. Following the broiler euthanasia, the footpad lesion score was: A lesion covering less than 25% of the sole is scored 0, a big area lesion encompassing between 25% and 50% of the sole is scored 2, and a lesion covering more than 50% of the plantar surface is scored 3.

10. Statistical Analysis

Using each pen as the experimental unit, all data were analyzed using SAS's general linear model techniques (SAS Institute, Cary, NC, USA). Differences between treatment means were analyzed by using Tukey's multiple range test. A level of $0.05 \leq P < 0.10$ was shown to be statistically significant, and a probability level of $P < 0.05$ was shown to be statistically significant.

RESULTS

1. Growth Performance

The effects of spraying AN extract in the litter on growth performance is presented in Table 1. At 0 to 2 weeks, the AN0 and AN2 significantly increased the feed intake (FI) compared with the AN4. However, there was no significant difference ($P > 0.05$) in body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR) during experiment period.

2. Litter Quality

The effects of spraying AN extract in the litter on moisture and pH are presented in Table 2. There was no significant difference ($P > 0.05$) in litter moisture during experiment period. However, at weeks 4, the AN0 significantly increased ($P < 0.05$) litter pH compared with the AN2 and AN4.

3. NH_3-N Measurement

The effects of spraying AN extract in the litter on NH_3-N is presented in Table 3. During the experiment periods, the AN0 showed significantly highest ($P < 0.05$) NH3-N levels compared with the AN2 and AN4. Also, the AN2 significantly increased ($P < 0.05$) NH3-N levels compared with the AN4 during experiment period.

Table 1. Effect of spraying AN extract on growth performance in broiler's litter[1]

Items	AN0	AN2	AN4	SE	*P*-value
BW, kg					
Initial	37.32	37.75	37.45	0.173	0.243
d 7	136.12	124.76	129.93	7.256	0.557
d 14	433.00	412.00	398.00	14.341	0.260
d 21	849.00	854.00	855.00	13.988	0.949
d 28	1,392.00	1,390.00	1,398.00	15.895	0.934
BWG, g					
d 0−14	395.68	374.25	360.55	14.321	0.256
d 15−28	959.00	978.00	1,000.00	27.234	0.581
d 0−28	1,354.68	1,352.25	1,360.55	15.961	0.932
FI, g					
d 0−14	547.54[a]	565.55[a]	544.40[b]	4.976	0.011
d 15−28	1672.56	1699.53	1728.54	29.906	0.441
d 0−28	2,231.10	2,265.09	2,295.94	32.000	0.388
FCR, g/g					
d 0−14	1.44	1.52	1.53	0.062	0.553
d 15−28	1.75	1.74	1.74	0.058	0.990
d 0−28	1.65	1.68	1.69	0.031	0.654

[1]AN0, basal rice husk + 10 mL; AN2, basal rice husk + 2% *Abeliophyllum distichum* Nakai; AN4, basal rice husk + 4% *Abeliophyllum distichum* Nakai; AN, *Abeliophyllum distichum* Nakai; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; SE, standard error.

[a,b] Means within column with different superscripts differ significantly (P<0.05).

Table 2. Effect of spraying AN extract on litter moisture and pH in broiler's litter[1]

Items (%)	AN0	AN2	AN4	SE	*P*-value
Moisture					
d 7	14.56	15.84	15.71	1.131	0.689
d 14	27.76	22.70	22.24	1.870	0.109
d 21	28.83	28.54	30.71	4.188	0.925
d 28	32.67	30.91	30.79	4.743	0.952
pH					
d 7	6.83	6.86	6.85	0.228	0.996
d 14	7.24	7.08	7.16	0.161	0.798
d 21	7.40	7.31	7.27	0.117	0.716
d 28	7.89[a]	7.39[b]	7.38[b]	0.077	0.001

[1]AN0, basal rice husk + 10 mL; AN2, basal rice husk + 2% *Abeliophyllum distichum* Nakai; AN4, basal rice husk + 4% *Abeliophyllum distichum* Nakai; AN, *Abeliophyllum distichum* Nakai; SE, standard error.

[a,b] Means within column with different superscripts differ significantly (P<0.05).

Table 3. Effect of spraying AN extract on litter microflora content in broiler's litter[1]

Items (%)	AN0	AN2	AN4	SE	*P*-value
E. coli					
d 7	5.48	5.44	5.38	0.367	0.983
d 14	5.72	5.58	5.43	0.392	0.874
d 21	6.20	5.89	5.79	0.144	0.156
d 28	6.33[a]	6.05[ab]	5.95[b]	0.084	0.018
Salmonella					
d 7	4.42	4.20	4.26	0.339	0.899
d 14	4.50	4.55	4.40	0.208	0.876
d 21	5.31[a]	4.52[b]	4.48[b]	0.194	0.016
d 28	5.35[a]	4.69[b]	4.67[b]	0.158	0.015

[1]AN0, basal rice husk + 10 mL; AN2, basal rice husk + 2% *Abeliophyllum distichum* Nakai; AN4, basal rice husk + 4% *Abeliophyllum distichum* Nakai; AN, *Abeliophyllum distichum* Nakai; *E. coli*, *Escherichia coli*; SE, standard error.

[a,b] Means within column with different superscripts differ significantly (P<0.05).

4. Litter Microflora

The effects of spraying AN extract in the litter on microflora is presented in Table 4. At 3 and 4 weeks, the counts of *Salmonella* in the litter were significantly highest (P<0.05) in the AN0 compared with the AN2 and AN4. Also, the AN4 showed significantly lowest (P<0.05) counts of *Salmonella* compared with the AN0 and AN4. Moreover, at 4 weeks, the AN0 showed significantly higher (P<0.05) counts of *E. coli* compared with the AN4.

Table 4. Effect of spraying AN extract on litter NH_3-N content in broiler's litter[1]

Items (%)	AN0	AN2	AN4	SE	*P*-value
NH_3-N					
d 7	384.20[a]	354.20[b]	325.00[c]	3.636	<0.001
d 14	406.80[a]	379.43[b]	301.88[c]	3.361	<0.001
d 21	434.20[a]	376.40[b]	328.60[c]	3.691	<0.001
d 28	463.20[a]	393.20[b]	344.00[c]	2.743	<0.001

[1]AN0, basal rice husk + 10 mL; AN2, basal rice husk + 2% *Abeliophyllum distichum* Nakai; AN4, basal rice husk + 4% *Abeliophyllum distichum* Nakai; AN, *Abeliophyllum distichum* Nakai; NH_3-N, ammonia nitrogen; SE, standard error.

[a−c] Means within column with different superscripts differ significantly (P<0.05).

5. Footpad Dermatitis

The effects of spraying AN extract in the litter on FPD is presented in Table 5. As shown in Table 5, AN4 showed significantly decreased (P<0.05) FPD scores compared with the AN0.

DISCUSSION

Nowadays, broilers are developed to gain rapid growth in breast muscle as well as for increased total BW than the increase of leg muscle (Miller et al., 1968; Clayton et al., 1978). In this regard, the increased BW induces higher pressure on leg muscles, thereby resulting in lower mobility and intensive contact with the foot litter (Škrbić et al., 2014). Therefore, several researchers suggested that the increased BW could result a higher incidence and severity of FPD in broilers (Nestor et al., 1985; Škrbić et al., 2014). However, in this study, there were no significant differences in BW between AN0, AN2, and AN4. This result confirmed that BW did not affect the incidence and severity of FPD in the present study.

Litter pH is a major factor in modulating ammonia volatilization. According to Reece et al. (1980), a low level of ammonia was released when the litter pH was under 7, whereas a higher level of ammonia was released in a litter with a pH of more than 8. Also, Carr et al. (1990) have reported that the higher litter pH level increases the release of ammonia. In this study, we observed the decreased litter pH by spraying AN extract in broiler litter. Previous studies have demonstrated that supplying antibacterial effects could decrease litter pH by reducing ammonia-producing bacteria (Oliveira et al., 2003; Park et al., 2023). It is well known that AN extract contains high levels of flavonoids and polyphenols, which produce an antibacterial effect (Ko et al., 2019). Also, this result was consistent with Go et al. (2021), who have reported decreased pH by using AN extract (0.0125, 0.05, and 0.5%) in mice. Therefore, decreased litter pH might be due to the antibacterial effect of AN extract.

Table 5. Effect of spraying AN extract on footpad dermatitis in broiler[1]

Score[2]	AN0	AN2	AN4	SE	*P*-value
Average	2.00[a]	1.50[ab]	1.40[b]	0.141	0.024

[1]AN0, basal rice husk + 10 mL; AN2, basal rice husk + 2% *Abeliophyllum distichum* Nakai; AN4, basal rice husk + 4% *Abeliophyllum distichum* Nakai; AN, *Abeliophyllum distichum* Nakai; SE, standard error.

[2] Lesion score: Lesion score was determined as follow: 0, no lesion; 1, lesion covering less than 25% of the sole of the foot large area lesion; 2, covering between 25% and 50% of the sole of the foot; 3, more than 50% of the lesion of the plantar.

[a,b] Means within column with different superscripts differ significantly (P<0.05).

The *E. coli* and *Salmonella* are pathogenic bacteria, which commonly emerged in impaired quality of litter (Sahoo et al., 2016). When the *E. coli* and *Salmonella* contact with the wound on the skin and mucous membranes, it induces the infiltration into bloodstream, thereby increasing the FPD incidence in broilers (Xavier et al., 2010). In this study, spraying AN extract reduced the counts of *E. coli* and *Salmonella*. Previous studies have reported that AN extract possesses antibacterial effects, due to its high amount of flavonoid and polyphenol (Ji et al., 1993; Song et al., 2021). Also, decreased litter pH induces unfavorable conditions for bacterial pathogens to survive and growth in broiler litter (Pope and Cherry, 2000). In agreement with this result, previous studies have supported that decreased litter pH condition results in reduced the counts of *E. coli* and *Salmonella* (Mcward and Taylor, 2000; Line, 2002). Therefore, the antibacterial effect and decreased pH litter condition might be attributed to the reduction of the counts of *E. coli* and *Salmonella*.

Nitrogen is a major source of producing ammonia, which is accumulated by the feces and urines in broiler litter (Vilela et al., 2020). Especially, nitrogen is converted into NH_3-N through various factors, such as urea hydrolysis, bacterial activity, moisture, and pH in the litter (Kelleher et al., 2002; Liu et al., 2007). Previous studies have reported that the increase of NH_3-N accelerates broiler health problems and high levels of ammonia emission (Tucker and Walker, 1992; Wang et al., 2006; Liu et al., 2007). In the current study, spraying AN extract showed a reduction of NH_3-N in litter. In accordance with our result, Nahm (2005) have reported that reduction of litter pH decreased litter NH_3-N by inhibiting urea hydrolysis. Also, Li et al. (2012) have supported that decreased bacterial activity could reduce the litter NH_3-N. These results could support the decrease of litter NH_3-N, by spraying AN extract in broiler litter.

The present study represented that spraying AN extract in broiler litter significantly reduced the FPD scores with the decrease of litter pH, litter microflora, and litter NH_3-N. As these factors modulate the severity of FPD in broilers, a decrease in FPD scores might be reasonable in this study (Martins et al., 2013; Soliman et al., 2018). Consistently, previous studies have reported that a decrease in litter pH, litter microflora, and litter NH_3-N could alleviate the FPD in broilers (Liu et al., 2007; Xavier et al., 2010; Kaukonen et al., 2016). However, in our knowledge, no research has been conducted the evaluation of spraying AN extract in the broilers. Therefore, to identify the exact mechanisms of alleviating FPD by spraying AN extract, additional studies are needed.

SUMMARY

The result of this study could suggest the effects of spraying AN extract in litter to alleviate the FPD. In this study, spraying 2 and 4% of AN extract decreased the litter pH, the counts of pathogenic bacteria count, NH_3-N levels, and FPD score. However, research on the spraying of AN extract in broiler litter is insufficient. Thus, additional research is needed to identify the exact mechanisms of AN extract in broiler litter.

ACKNOWLEDGEMENTS

This research was supported by “Regional Innovation Strategy (RIS)” through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (MOE) (2021RIS-001).

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Received Jul. 24, 2024, Revised Sep. 5, 2024, Accepted Sep. 19, 2024