ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN: 1682-8356
DOI: 10.3923/ijps.2017.125.131

Research Article
Nitrogen and Ammonia Mitigation on Laying Hen Farms: Effects of Low-protein Diet and Manure Filtering

[1]Rikardo Silaban, [1]Sumiati Sumiati, [2]Adrizal Adrizal, [2]Yusrizal Yusrizal, [2]Wiwaha Anas Sumadja, [2]Yatno Yatno, [2]Noferdiman Noferdiman, [3]Katsuki Koh and [3]Mustanur Rahman

[1]Faculty of Animal Sciences, Bogor Agricultural University, 16680 Bogor, Indonesia
[2]Faculty of Animal Sciences, University of Jambi, 36361 Jambi, Indonesia
[3]Faculty of Agriculture, Shinshu University, 399-4598 Nagano-Ken, Japan

Abstract

Background: Lowering dietary Crude Protein (CP) has been widely reported as a method to reduce nitrogen (N) excretion, but information on its effect in combination with filtering by *Azolla pinnata* to further mitigate N or ammonia (NH_3) volatilization from laying hen manure is limited. **Materials and Methods:** Two experimental diets containing 17% (control) and 15% (low) crude protein were assigned to 2 groups of 22 weeks old ISA Brown hens, so each treatment had 20 replicates with 6-7 birds each. The manure from each group was flushed into two terraced ponds, in which *Azolla pinnata* was grown in the lowest terrace to filter the N in the sludge. Fresh manure was collected when the hens were 29 weeks old to analyze the N and NH_3 contents and perform microbial counts and analyses of pond water quality (total dissolved solubles, temperature and pH), *Azolla* N and egg quality were conducted. Data were subjected to either a one or two-way ANOVA. **Results:** Lowering dietary crude protein to 15% resulted in a 21.83% ($p<0.05$) decrease in manure NH_3, but it did not alter the microbial counts or N content. Additionally, pond water quality was not affected by dietary CP, the presence of *Azolla* in the pond or the dietary crude protein × *Azolla* interaction and there was no difference in the N content of the *Azolla* biomass among dietary groups. Eggs laid by hens given the low-CP diet had comparable quality to those laid by the control hens. **Conclusion:** Reducing dietary crude protein to 15% lowered NH_3 volatilization and did not negatively affect egg quality. However, growing *Azolla* in a terraced pond failed to elicit an N-filtering effect.

Key words: Ammonia mitigation, nitrogen, low protein, manure filtering, laying hen

Received: November 18, 2016 Accepted: February 01, 2017 Published: March 15, 2017

Citation: Rikardo Silaban, Sumiati Sumiati, Adrizal Adrizal, Yusrizal Yusrizal, Wiwaha Anas Sumadja, Yatno Yatno, Noferdiman Noferdiman, Katsuki Koh and Mustanur Rahman, 2017. Nitrogen and ammonia mitigation on laying hen farms: Effects of low-protein diet and manure filtering. Int. J. Poult. Sci., 16: 125-131.

Corresponding Author: Adrizal Adrizal, Faculty of Animal Sciences, University of Jambi JL. Raya Jambi, km. 15, 36361 Jambi, Indonesia
Tel: +62 741 582907/+62 081366626937 Fax: +62 741 582907

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Manure accumulation during poultry feeding operations can potentially result in volatilized ammonia (NH_3), a pollutant under scrutiny by the National Research Council (NRC)[1] that may cause respiratory dysfunction among animals and farm workers[2]. In terms of environmental impacts, NH_3 not only contributes to acid rain and eutrophication[3], but it is also an indirect precursor of nitrous oxide, a gas that has been shown to degrade the ozone layer[4]. A mass balance study in chickens showed that 40% of the nitrogen (N) consumed by laying hens is lost to the air[5] in the form of NH_3 but that 25% is lost via manure in the form of ammonium or nitrate[6]. Therefore, there is great potential for N compounds to threaten the environment when manure is not properly managed.

In many developing countries, flushing manure from poultry housing with water without further treatment is still practiced, which requires a large amount of water and flushing waste from the farm directly into waterways, the ground or surface water reduces water quality by increasing biochemical oxygen demand, chemical oxygen demand and Total Dissolved Solids (TDS), all of which harm the ecosystem[7]. However, some aquatic plants, including *Azolla* sp., have the capacity to absorb dissolved nitrates, phosphorus and heavy metals from water[8] and *Azolla* is a potential source of dietary protein that can enhance broiler production and egg yolk pigmentation in some countries[9,10]. Therefore, using *Azolla* as a biofilter to treat on-farm waste (manure) water can promote N mitigation and may serve as an alternative nutrient source for poultry diets.

Many studies have demonstrated that lowering dietary crude protein with an amino acid-balanced diet could reduce N excretion without impacting livestock performance[11,12] and a study of laying hens suggested that reducing crude protein from 17-15% would still meet the protein and N utilization requirements of the birds[13]. Although dietary crude protein reduction has the potential to reduce NH_3, the N that is contained in the manure is still a precursor of NH_3 synthesis, but mechanical and acid manure treatments can maintain manure N while reducing NH_3 emission[14,15]. However, introducing acid to liquid manure seems inappropriate, but the effectiveness of subsequently treating washed manure in reducing N or NH_3 is very limited. Therefore, the objectives of this study were to evaluate whether reducing dietary crude protein could lower NH_3 volatilization in the manure without affecting egg quality as well as gain preliminary insight into the manure N-filtering capacity of *Azolla*.

MATERIALS AND METHODS

Hens, experimental diet and the curtain-sided poultry house: The animal care and sampling protocol complied with the requirements of the Institute of Laboratory Animal Resources of the Commission on Life Sciences[16]. About 245 ISA Brown hens (22 weeks of age; average body weight [BW]: 1.343±38 g) were individually housed in wire-floored cages [36 cm (length)×32 cm (width)×35 cm (height in the back)×40 cm (height in the front)] and the lighting program was set at 16D:8L using LED electric bulbs suspended from the ceiling of the hen house and by opening and closing the sidewall curtain.

Two experimental diets containing 17% (control) and 15% (low) crude protein and other essential nutrients (Table 1) as recommended by the NRC[17] and Hy-Line[18] were formulated to be isocaloric (2,750 kcal ME kg^{-1}). The primary ingredients (corn, soybean meal, rice bran and palm kernel meal) were subjected to proximate (dry matter,

Table 1: Experimental diets containing two different levels of Crude Protein (CP)

	Dietary CP	
Ingredients[1]	Control (17%)	Low CP (15%)
Yellow corn (%)	45.56	53.22
Soybean meal (CP: 43%) (%)	23.37	17.18
Rice bran (%)	5.47	5.96
Palm kernel meal (%)	12.00	11.00
Palm oil (%)	3.80	2.14
DL-methionine (%)	0.11	0.16
L-lysine. HCl (%)	0.00	0.08
L-isoleucine (%)	0.04	0.15
L-valine (%)	0.00	0.10
Mono calcium phosphate (%)	0.82	0.86
$CaCO_3$ (%)	8.45	8.45
NaCl (%)	0.20	0.20
Sodium bicarbonate (%)	0.30	0.30
Vitamin-mineral premix[2] (%)	0.20	0.20
Nutrient composition (calculated)		
ME (kcal kg^{-1})	2750.00	2750.03
Crude protein (%)	17.00	15.01
Methionine (%)	0.38	0.41
Methionine+cysteine (%)	0.66	0.65
Lysine (%)	0.85	0.76
Crude fiber (%)	8.92	8.37
Ether extract (%)	5.93	4.74
Calcium (%)	3.61	3.60
Non-phytate phosphorus (%)	0.28	0.28
Na (%)	0.18	0.18

[1]All main ingredients (corn, rice bran and soybean meal) were subjected to proximate analysis (DM, CP, CF, EE, Ca and total P) prior to diet formulation, [2]Provided (kg^{-1}) as follows: Vitamin A 2,500 IU, vitamin D_3 500 IU, vitamin E 1.5 IU, vitamin K_3 0.4 mg, thiamine 0.3 mg, riboflavin 1 mg, pyridoxine 1 mg, cyanocobalamin 2.4 μg, vitamin C6 mg, niacin 7 mg, calcium-D-pantothenate 1 mg, manganese 20 mg, iron 5 mg, iodine 0.04 mg, zinc 20 mg, cobalt 0.04 mg, copper 0.6 mg, antioxidant 2 mg, methionine 7 mg and lysine 7 mg

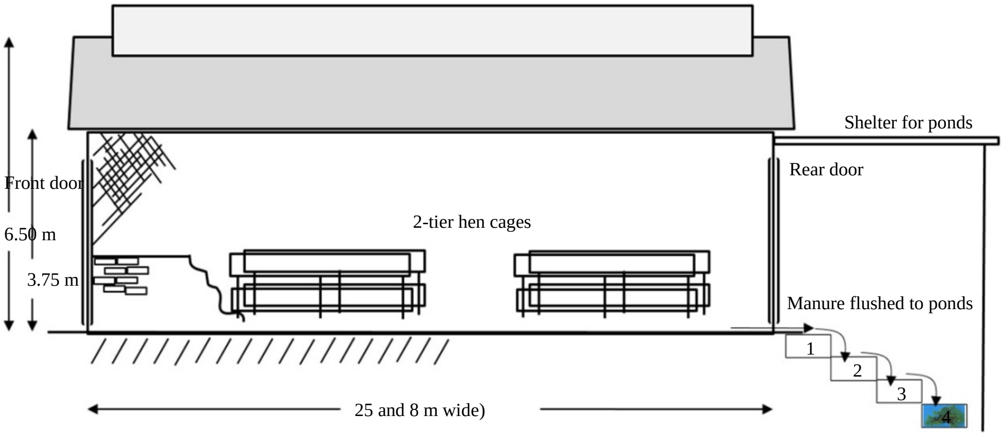

Fig. 1: Cross section of a curtain-sided laying hen house with cages and 4 terrace ponds (pond dimensions: 50 cm (length)×40 cm (width)×60 cm (depth)]

Crude Protein (CP), ether extract, crude fiber, calcium and phosphorus) and amino-acid analyses prior to diet formulation and diets were randomly assigned to cages so that each treatment consisted of 20 replicates with 6-7 birds per replicate. Diets and water were provided *ad libitum* from 22-29 weeks of age.

Manure that dropped on the floor from each dietary group was flushed with an equal volume of water through a drain into four terraced ponds (pond dimensions: 50 cm (length)×40 cm (width)×60 cm (depth); Fig. 1) once a week. *Azolla pinnata* was grown in two of the four terraced ponds for each dietary crude protein group (Fig. 2) and it was also grown in separate ponds filled with untreated water as a control.

Data collection and laboratory analysis: Fresh manure was collected over 24 h when the hens were 29 weeks of age to measure NH_3 and perform bacterial counts as described by Yusrizal *et al.*[19]. Briefly, the manure collected from the manure tray of each cage was immediately transferred into a plastic bag, sealed and then homogenized by gently squeezing the bag. All bags were transported to the laboratory within 15 min of homogenization and a 50 g subsample of each treatment replicate was placed into a pre-autoclaved 400 mL beaker. The beaker was immediately covered with paraffin film and incubated at room temperature for 24 h and a Kitagawa NH_3 tube (No. 105SC) connected to an aspirating pump (Model: AP-20, Komyo Rikagaku Kogyo K.K., Japan) was inserted into the beaker through the paraffin to sample the head-space gas. Once the NH_3 was measured, the pH of the manure was measured by inserting the probe of a pH meter (Model: HI 98107, Hanna Instruments, USA) into two distinct regions in the beaker; the reading for each beaker was the average of the two values. Immediately following the first set of measurements, the hole in the paraffin was sealed to take a second measurement (48 h of incubation). Approximately 1 g of manure was taken at the time of each sampling to analyze moisture, dry matter and N[20].

When the hens were 29 weeks of age, another 50 g manure subsample was placed into a sterile, 50 mL conical tube for pathogenic (coliform/*E. coli*) and nonpathogenic (*Lactobacillus* sp.) microbial counts[19]. Briefly, MCA (McConkey Agar) (Difco, Becton, Dickinson Co., Sparks, MD, USA) was used as the medium to grow coliform/*E. coli*, whereas MRS (de Man Rogosa Sharpe agar) (Difco Laboratories, MI, USA) was used to grow *Lactobacillus* sp. Agar plates containing samples were anaerobically incubated at 37°C for 24 h for *E. coli* or 48 h for *Lactobacillus* sp. and the Colony Forming Units (CFU) of the bacteria were enumerated using a colony counter (Model: ProtoCOL-3, Synbiosis, Synoptics Ltd., Cambridge, UK).

The wastewater TDS, temperature and pH measurements in the lowest pond and in the untreated (control) ponds were performed when the hens were 25 and 29 weeks old using a multipurpose pH meter (Model: HI 98107, Hanna Instruments, USA). The probe of the pH meter was submerged in 3 different regions of each pond; the values were averaged to a final mean and the data were pooled by week for statistical analysis. *Azolla* biomass was collected from the treatment and control ponds when the hens were 29 weeks old and immediately dried in an oven at 30°C for 48 h to determine the dry matter content to analyze N.

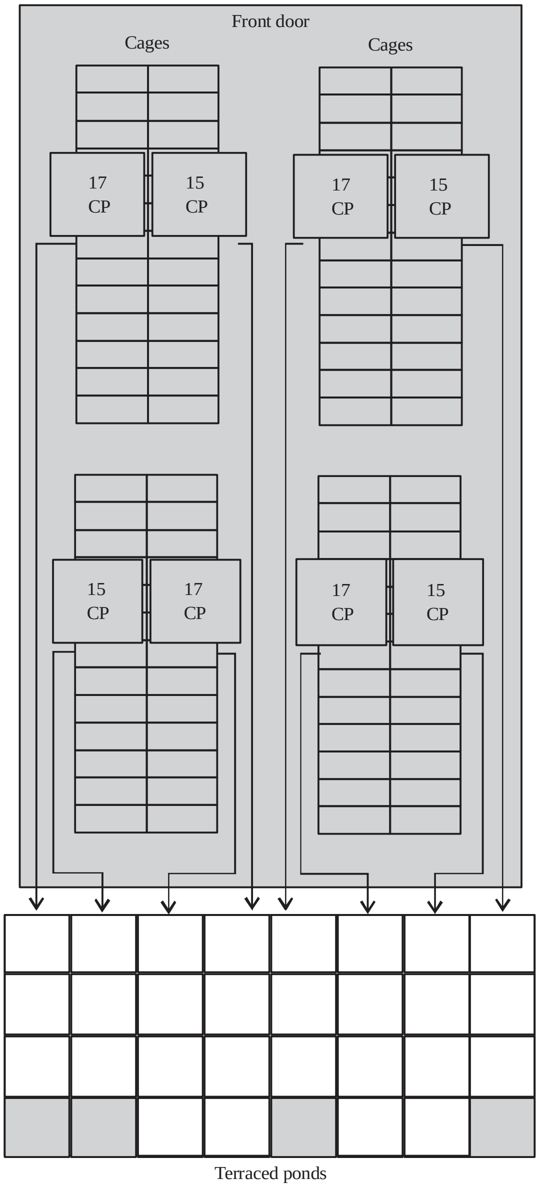

Fig. 2: Layout of cages and terraced ponds. Arrows indicate the respective drainage paths by which the manure dropped on the floor was washed out of the hen house and into the ponds; shaded ponds indicate the presence of *Azolla*

Eggs were collected during the last 3 days of both weeks 25 and 29 to measure egg quality (egg weight, eggshell weight, egg surface area, eggshell thickness, eggshell strength, albumen height, haugh unit and egg yolk color score) using an Egg Analyzer Unit (EAU) (Orka Food Technology Ltd®, UT 84010, USA). Following the measurement of egg surface area[21] and eggshell strength, the eggshell was then broken equatorially and the content was placed onto a flat glass surface of the egg analyzer unit to measure internal quality.

Experimental design and statistical analysis: Manure N, NH_3, moisture, pH, microbial counts and egg quality data were subjected to one-way ANOVA with 20 replicates per treatment, whereas the N content of the *Azolla* biomass had 2 replicates per treatment. Pond water quality data (TDS, temperature and pH) were subjected to a two-way ANOVA with 2 replicated terraced ponds per treatment. Significant differences among treatment means ($p<0.05$) were determined with a t- or Tukey's test[22].

Table 2: Concentration of ammonia (NH_3), pH, moisture and nitrogen (N) and the bacterial counts in the manure of laying hens fed two different levels of protein (CP) from 22-29 weeks of age

		Dietary CP			
Parameter[1]	Incubation time (h)	Control (17%)	Low (15%)	SEM[2]	Probability
NH_3 (ppm)	24	137.25	114.40	9.66	0.10
	48	375.00[a]	293.15[b]	27.15	0.04
pH	24	7.60	7.58	0.11	0.89
	48	7.94	7.70	0.15	0.26
Moisture (%)	24	80.39	79.52	0.44	0.17
	48	80.40	80.02	0.38	0.48
N (%)		14.50	12.50	0.10	0.81
Escherichia coli/Coliform ($\times 10^7$ CFU g^{-1} of fresh manure)		2.22	1.47	0.67	0.43
Lactobacillus sp. ($\times 10^{11}$ CFU g^{-1} of fresh manure)		7.79	7.30	1.09	0.75

[a, b] Means within a row with no common superscript letters differ significantly ($p<0.05$), [1]Manure was collected at 29 weeks of age for the all parameter measurements and NH_3 measurements were performed using an NH_3 tube with an aspirating pump, [2]Standard error of the means of 20 replicates

Table 3: Total Dissolved Solids (TDS) concentration, temperature and pH of the wastewater (manure sludge) in the pond water and the nitrogen (N) content of the *Azolla* biomass

	Pond water and *Azolla*[1]			
Factor	TDS (ppm)	Temperature (°C)	pH	*Azolla* N[2] (%)
CP (%)				
15	649	27.1	7.39	3.51
17	903	27.2	7.81	3.59
***Azolla*[3]**				
(-)	762	27.2	7.60	-
(+)	790	27.1	7.60	-
CP × *Azolla*				
15×(-)	547	26.96	7.12	-
15×(+)	751	27.17	7.66	-
17×(-)	977	27.34	8.08	-
17×(+)	830	27.09	7.54	-
SEM[4]	153	0.14	0.29	0.06
Probability				
Sources of variance:				
CP	0.25	0.21	0.31	0.35
Azolla	0.90	0.29	0.99	-
CP×*Azolla*	0.43	0.08	0.20	-

[1]All parameters were measured at the last pond (fourth level) of each 4 terrace pond. Pond water data were pooled from two measurements (at 25 and 29 weeks of age), [2]Dry-air basis (*Azolla* was collected from treatment or control ponds when the hens were 29 weeks old, dried in an oven at 30°C for 48 h and then allowed to adjust to ambient temperature for 48 h prior to dry matter and N analysis. The dry matter contents of the control, the 15% CP and the 17% CP samples were 99.58, 99.71 and 99.77%, respectively; the N content of the control was 3.44%, [3]Absence and presence of *Azolla* in the pond is indicated by "(-)" and "(+)", respectively, [4]Standard error of the means of 2 replicates

RESULTS

Lowering dietary crude protein to 15% did not significantly influence the manure NH_3 concentration at 24 h of incubation, but a reduction in NH_3 was observed at 48 h ($p<0.05$) (Table 2). Additionally, lowering the dietary crude protein from 17-15% did not clearly affect manure pH, moisture, N content or the microbial count.

Table 4: Egg quality of laying hens fed two different levels of protein (CP) from 22-29 weeks of age

	Dietary CP			
Parameter[1]	Control (17%)	Low (15%)	SEM[2]	Probability
At 25 weeks of age				
Egg weight (g egg^{-1})	47.28	47.40	0.65	0.91
Egg surface area (cm^2)	62.08	62.17	0.56	0.91
Eggshell thickness (mm)	0.468	0.471	0.006	0.77
Eggshell force (kg cm^{-2})	4.45	4.23	0.17	0.36
Albumen height (mm)	4.08	4.07	0.12	0.95
Yolk color score	5.34	5.50	0.25	0.66
Haugh unit	65.27	65.11	1.17	0.92
At 29 weeks of age				
Egg weight (g egg^{-1})	53.43	52.78	0.84	0.59
Egg surface area (cm^2)	67.31	66.72	0.72	0.56
Eggshell thickness (mm)	0.502	0.489	0.009	0.30
Eggshell force (kg cm^{-2})	4.29	4.54	0.16	0.26
Albumen height (mm)	5.02	4.78	0.14	0.23
Yolk color score	6.00	6.25	0.20	0.38
Haugh unit	70.90	68.34	1.33	0.18

[1]All parameters were measured using an EAU (ORKA Food Technology Ltd®, UT 84010, USA), except egg surface area[21], [2]Standard error of the means of 20 replicates

No effect of dietary crude protein on pond water TDS, temperature, pH and *Azolla* N biomass was observed, nor were there effects from the presence of *Azolla* in the pond or the interaction between dietary crude protein and *Azolla* on pond water parameters (Table 3). However, reducing dietary crude protein to 15% did not negatively affect egg quality compared to the 17% crude protein diet (Table 4).

DISCUSSION

The significant reduction in manure NH_3 (21.83% at 48 h) due to the low-CP diet confirmed previous findings for hens and broilers as reviewed by Patterson and Adrizal[23], but the factors that might influence such a decline, such as lowered pH and moisture content[6] were not obvious in the present study. Whether the reduction in manure N (13.8%) or coliform counts (33.8%) was due to lowering the dietary crude protein is unclear, but slightly lowered coliform counts (Table 2) likely indicated competitive exclusion of pathogenic by nonpathogenic microbes[24,25], resulting in unfavorable conditions for pathogenic microbes including NH_3-synthesizing bacteria.

There were no effects of diet or *Azolla* treatments on pond water parameters, which may have been related to the effect of the terraced pond system, so the potential dissolved-N filtering effect of *Azolla* as observed by Little[8], was not particularly apparent. Overall, relative to the standard water TDS values of less than 1,000 ppm, pH of 6.8-7.5 and temperature of 25°C[26,27], the results demonstrated that the water sample from the lowest pond could potentially to be used or be further recycled prior to use, on another poultry farm (e.g., for cleaning the cage floor) or flushed directly into the waterways. The pond design and its capacity to allow *Azolla* growth were probably not appropriate for detecting an aquatic plant filtering effect in the present study.

The findings of the study, particularly the N excretion results, indicated that lowering dietary crude protein from 17-15% resulted in similar amino acid utilization, so comparable egg albumen heights, Haugh units and yolk color scores might be expected from the two dietary crude protein groups. Junqueira *et al.*[28] reported a similar outcome when reducing dietary crude protein from 18-16% in post-molted hens and another study of 52-60 weeks old Leghorn hens given a diet containing 13.5% crude protein also showed comparable egg production and quality compared with hens given a 16.5% crude protein diet[29]. The researchers concluded that amino acid supplementation of the low-CP diet might play a significant role in meeting amino requirements in addition to overcoming nutritional stress. Bunchasak and Silapasorn[30] reported that supplementation of the 14% crude protein diet with methionine had a favorable effect on eggshell thickness of ISA Brown hens at 22-24 weeks of age. Overall, the present study confirms that supplementing a low-CP diet with essential amino acids is important for egg production and quality, but it is also important to note that meeting the minimum total N requirements on the low-CP diet is necessary for the synthesis of certain amino acids that are not readily available from the diet. Although the present study failed to demonstrate an N-filtering capacity in *Azolla*, a study connecting the dietary crude protein strategy with subsequent manure-N filtering by aquatic plants needs to be conducted with an appropriate pond design. The successful demonstration of this concept will inform a holistic strategy for N and NH_3 mitigation on poultry farms.

CONCLUSION AND FUTURE RECOMMENDATIONS

Lowering the dietary crude protein from 17-15% could be an upstream strategy for N and NH_3 mitigation that does not affect egg quality. Under the experimental conditions and pond dimensions in this study, treating liquid manure with *Azolla* failed to elicit a clear nitrogen-filtering effect by the plant. Further study is needed to reevaluate the effect of filtering manure with *Azolla* because there is potential for further on-farm N and NH_3 mitigation to improve the farm environment.

SIGNIFICANCE STATEMENTS

- Feeding chickens low-protein diets in previous studies reduced manure nitrogen but had variable impacts on performance
- This study was aimed at evaluating the effect of a low-protein diet on both manure N as well as ammonia and gaining insights into filtering the manure flushed into ponds for further N mitigation
- The present study confirmed that lowering the dietary crude protein from 17-15% reduces manure N and ammonia without affecting egg quality

ACKNOWLEDGEMENTS

We would like to acknowledge and are grateful for the Japan Society for the Promotion of Sciences-Directorate General of Higher Education of Indonesia (JSPS-DGHE) Joint Research 2013-2015 Grant for the current study. Additionally, the assistance of our students in the field during the experiment was very helpful and the technical review of the manuscript and suggestions by Dr. Paul Patterson and Amy Mayer of Pennsylvania State University, USA were greatly appreciated.

REFERENCES

1. NRC., 2003. Air Emissions from Animal Feeding Operations Current Knowledge Future Needs. National Academy Press, Washington, DC., USA.
2. Donham, K.J., D. Cumro and S. Reynolds, 2002. Synergistic effects of dust and ammonia on the occupational health effects of poultry production workers. J. Agromedicine, 8: 57-76.

3. Galloway, J.N. and E.B. Cowling, 2002. Reactive nitrogen and the world: 200 years of change. Ambio, 31: 64-71.
4. Brennan, R.B., M.G. Healy, O. Fenton and G.J. Lanigan, 2015. The effect of chemical amendments used for phosphorus abatement on greenhouse gas and ammonia emissions from dairy cattle slurry: Synergies and pollution swapping. PloS One, Vol. 10. 10.1371/journal.pone.0111965.
5. Patterson, P.H. and E.S. Lorenz, 1996. Manure nutrient production from commercial White Leghorn hens. J. Applied Poult. Res., 5: 260-268.
6. Koerkamp, P.W.G.G., 1994. Review on emissions of ammonia from housing systems for laying hens in relation to sources, processes, building design and manure handling. J. Agric. Eng. Res., 59: 73-87.
7. Steinfeld, H., P. Gerber, T. Wassenaar, V. Castel, M. Rosales and C. de Haan, 2006. Livestock's Long Shadow: Environmental Issues and Options. Food and Agricultural Organization, Rome, Italy, ISBN-13: 9789251055717, Pages: 390.
8. Little, E.C.S., 1979. Little Handbook of Utilization of Aquatic Plants: A Review of World Literature. Food and Agriculture Organization of the United Nations, Bay of Islands, New Zealand.
9. Basak, B., M.A.H. Pramanik, M.S. Rahman, S.U. Tarafdar and B.C. Roy, 2002. Azolla (*Azolla pinnata*) as a feed ingredient in broiler ration. Int. J. Poult. Sci., 1: 29-34.
10. Alalade, O.A., E.A. Iyayi and T.O. Alalade, 2007. The nutritive value of azolla (*Azolla pinnata*) meal in diets for growing pullets and subsequent effect on laying performance. J. Poult. Sci., 44: 273-277.
11. Aftab, U., M. Ashraf and Z. Jiang, 2006. Low protein diets for broilers. World's Poult. Sci. J., 62: 688-701.
12. Adrizal, A., C. Angel and A. Markant, 2008. Low protein, hydroxy- and keto-amino acid analog supplemented diets for broiler chickens: 2. Manure nitrogen. Poult. Sci., 87 (Suppl. 1): 22-22.
13. Meluzzi, A., F. Sirri, N. Tallarico and A. Franchini, 2001. Nitrogen retention and performance of brown laying hens on diets with different protein content and constant concentration of amino acids and energy. Br. Poult. Sci., 42: 213-217.
14. Moore, P.A., T.C. Daniel, D.R. Edwards and D.M. Miller, 1995. Effect of chemical amendments on ammonia volatilization from poultry litter. J. Environ. Qual., 24: 293-300.
15. Moore, P.A., T.C. Daniel and D.R. Edwards, 2000. Reducing phosphorus runoff and inhibiting ammonia loss from poultry manure with aluminum sulfate. J. Environ. Qual., 29: 37-49.
16. NRC., 1996. Institute of Laboratory Animal Resources Commission on Life Sciences. National Academy Press, Washington, DC., USA.
17. NRC., 1994. Nutrient Requirements of Poultry. 9th Edn., National Academy Press, Washington, DC., USA., ISBN-13: 9780309048927, Pages: 155.
18. Hy-Line, 2007. Hy-Line variety brown-commercial management guide 2005-2007. Hy-Line International, Iowa.
19. Yusrizal, Y., R. Angel, A. Adrizal, B.E. Wanto, S. Fakhri and Y. Yatno, 2013. Feeding native laying hens diets containing palm kernel meal with or without enzyme supplementations. 2. Excreta nitrogen, ammonia and microbial counts. J. Applied Poult. Res., 22: 269-278.
20. AOAC., 2005. Official Methods of Analysis of the Association of Analytical Chemists International. 18th Edn., Association of Official Analytical Chemists, Gathersburg, MD., USA.
21. Paganelli, C.V., A. Olszowka and A. Ar, 1974. The avian egg: Surface area, volume and density. Condor, 79: 319-325.
22. SAS., 2008. JMP 8 for Windows. SAS Institute Inc., North Carolina, USA.
23. Patterson, P.H. and Adrizal, 2005. Management strategies to reduce air emissions: Emphasis-dust and ammonia. J. Applied Poult. Res., 14: 638-650.
24. Yang, Y., P.A. Iji and M. Choct, 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J., 65: 97-114.
25. Molnar, A.K., B. Podmaniczky, P. Kurti, I. Tenk, R. Glavits, G.Y. Virag and Z.S. Szabo, 2011. Effect of different concentrations of *Bacillus subtilis* on growth performance, carcase quality, gut microflora and immune response of broiler chickens. Br. Poult. Sci., 52: 658-665.
26. NRC., 1974. Nutrients and Toxic Substances in Water for Livestock and Poultry. National Academy Press, Washington, DC., USA.
27. Plumber, H.S. and B.H. Kiepper, 2011. Impact of poultry processing by-products on waste water generation, treatment and discharges. Proceedings of the 2011 Georgia Water Research Conference, April 11-13, 2011, Georgia, pp: 1-5.
28. Junqueira, O.M., A.C. de Laurentiz, R. da Silva Filardi, E.A. Rodrigues and E.M.C. Casartelli, 2006. Effects of energy and protein levels on egg quality and performance of laying hens at early second production cycle. J. Applied Poult. Res., 15: 110-115.
29. Torki, M., A. Mohebbifar, H.A. Ghasemi and A. Zardast, 2015. Response of laying hens to feeding low-protein amino acid-supplemented diets under high ambient temperature: Performance, egg quality, leukocyte profile, blood lipids and excreta pH. Int. J. Biometeorol., 59: 575-584.
30. Bunchasak, C. and T. Silapasorn, 2005. Effects of adding methionine in low-protein diet on production performance, reproductive organs and chemical liver composition of laying hens under tropical conditions. Int. J. Poult. Sci., 4: 301-308.