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REVIEW

Egg Production Performance, Nitrogen Excretion, and Environmental Gas Emissions from Manure Composting of Laying Hens Fed Low-Protein Diets Supplemented with Essential Amino Acids

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Abstract

This study reviews the effects of low-protein diets supplemented with essential amino acids (LPS diets) on egg production performance, nitrogen excretion, and environmental gas emissions from layer-manure composting for laying hens across the entire laying period, focusing mainly on our previous studies. Compared to hens fed control diets (Cont diets), hens fed LPS diets showed no significant differences in egg-laying rates or egg weights across the laying period. Furthermore, LPS diets decreased the nitrogen intake and nitrogen excretion of laying hens as well as the emissions of greenhouse gases and ammonia from layer-manure composting compared to Cont diets throughout the laying period. Integrating the results of the studies and calculating the effects across the entire laying period based on the experimental conditions in the studies (White Leghorn hens and manure composting without forced aeration), we found that feeding LPS diets with a 2% lower crude protein content to laying hens decreased nitrogen influx to Japan and nitrogen load in Japan associated with egg production by 11.7% and 19.7%, respectively, and emissions of greenhouse gases and ammonia from layer manure composting by 23.8% and 29.2%, respectively, compared to feeding Cont diets. Therefore, feeding LPS diets to laying hens throughout the laying period was suggested to reduce the nitrogen load and environmental gas emissions from manure composting without affecting egg production performance.

Discipline: Agricultural Environment
Additional key words: amino acid balance, ammonia, emission factor, greenhouse gas, nitrogen flow

Introduction

To develop sustainable livestock production systems, reducing environmental gas emissions, particularly greenhouse gas (GHG) emissions, is an important issue in the global livestock sector. Livestock manure management accounts for 19.8% of total GHG emissions from the agriculture sector in Japan (GIO & Ministry of the Environment, Japan 2023). Nitrous oxide (N_2O) is a potent GHG with 265 times the global warming potential of carbon dioxide (IPCC 2013) and is the largest contributor to GHG emissions from manure management. N_2O's ozone-depleting effects make it the single most important ozone-depleting substance (Ravishankara et al. 2009), and thus its reduction is necessary. Ammonia (NH_3) is not only a precursor of N_2O (IPCC 2019) but also one of the major odor compounds from manure composting (Kuroda et al. 2004). In particular, NH_3 emission factors from composting of layer and broiler manure are higher than those from composting of cattle and pig manure (Bernal et al. 2009, Sakai et al. 2004). From a global perspective, the release of reactive nitrogen

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to the environment has been reported to exceed the planetary boundary (Richardson et al. 2023), and agriculture, including livestock production, contributes significantly to it (Rockström et al. 2009). Thus, a strategy is needed to reduce emissions of N_2O and NH_3 from layer-manure composting.

Feeding a low-protein diet supplemented with adequate amounts of essential amino acids (LPS diet) to livestock reduces nitrogen excretion (Hristov et al. 2013, Saitoh 2001), a source of N_2O and NH_3. Osada et al. (2011) reported that pigs fed low-protein diets supplemented with Lys, Met, Thr, and Trp exhibited decreased nitrogen excretion into urine and decreased GHG emissions from urine wastewater treatment. Suto et al. (2016) fed LPS diets to pigs on a larger scale than Osada et al. (2011) and reported equivalent results. Yamazaki et al. (1998) and Kobayashi et al. (2013) reported that broilers fed LPS diets showed lower nitrogen levels in manure excreta and no change in productivity or meat quality. It has been reported that feeding low-protein diets to laying hens reduces nitrogen excretion while maintaining egg mass (egg weight × egg-laying rate) (Blair et al. 1999, Ji et al. 2014, Keshavarz & Austic 2004, Latshaw & Zhao 2011), whereas some of those studies reported a decrease in egg weight (Ji et al. 2014, Keshavarz & Austic 2004).

The Japanese Feeding Standard for Poultry (NARO 2011) divides the laying period into two phases, an early laying period and a later laying period, and defines separate crude protein (CP, total nitrogen × 6.25) contents for each. We have reported that feeding laying hens with diets containing lower CP content supplemented with amino acids in the early laying period (Iio et al. 2021) and the later laying period (Iio et al. 2023) reduced nitrogen excretion while not affecting egg production performance. In addition, feeding laying hens a 2%-lower CP diet than control diets reduced N_2O and NH_3 emissions from manure composting in those studies. More experimental data is needed to confirm the effects of the LPS diets on the laying performance, nitrogen excretion, and environmental gas emissions of laying hens and to examine the relationship between the reduced CP content in layer diets and the reduction of nitrogen excretion.

The objectives of this study are 1) to review the effects of LPS diets on laying performance across the entire laying period based on data in two published papers and new data; 2) to examine the relationship between reduced CP content in layer diets and decreased nitrogen excretion, utilizing not only our data but also data reported by other authors; and 3) to analyze the effects of LPS diets on environmental gas emissions from layer-manure composting and the nitrogen flow in egg production in Japan.

Additional experiments on feeding low-protein diets to laying hens

Besides previous studies (Iio et al. 2021, 2023), additional experiments were conducted on feeding low-protein diets to laying hens in the early and late laying periods. These experiments were performed in accordance with the requirements of The Animal Care and Use Committee of the Ibaraki Prefectural Livestock Research Center (ACUC-ILRC-2018-7, ACUC-ILRC-2020-5, ACUC-ILRC-2021-4). The compositions of the diets are shown in Table 1.

A total of 275 White Leghorn (Julia) laying hens aged 150 days (for the early laying period), 254 Julia laying hens aged 330 days (for the late laying period) for measurements of laying performance and nitrogen balance, and 164 Julia laying hens also aged 330 days for measurements of environmental gas emissions from layer-manure composting were used in the experiments in the early and late laying periods.

Laying performance, nitrogen balance, and environmental gas emissions from layer-manure composting were measured as described in Iio et al. (2021, 2023).

Effects of low-protein diets supplemented with amino acids on laying performance and nitrogen balance

While the emphasis on amino acid sufficiency, including that of nonessential amino acids, for laying hens has been increasing, the Japanese Feeding Standard for Poultry continues to specify not only the amino acid requirements but also the CP requirements for laying hens (NARO 2011). Furthermore, the feeding manuals for major commercial breeds, such as White Leghorn, set even higher CP requirements for laying hens. Thus, there is room to reduce CP content in layer diets by supplementing with synthetic amino acids.

Regarding the laying performance in our studies, neither the egg-laying rate nor egg weight differed significantly between the laying hens fed control diets (Cont group) and those fed lower CP diets (LPS group) in all our studies considered in this review (Table 2). In particular, in the early laying period, the egg-laying rate and egg weight were unaffected even when CP content decreased by 4 percentage points.

Regarding the nitrogen balance in our studies, nitrogen intake, nitrogen excretion of manure, and total nitrogen output (the sum of nitrogen excretion and nitrogen outputs as eggs) were lower in the LPS group compared to the Cont group (Table 3). The nitrogen

Table 1. Ingredients and chemical composition of the diets for additional experiments

Ingredient (%)	Early laying period			Later laying period	
	Cont	LPS		Cont	LPS
	CP19%	CP17%	CP15%	CP16%	CP14%
Corn	59.58	63.87	67.53	64.91	69.22
Soybean meal	18.20	16.20	15.90	16.12	13.60
Corn gluten meal (CP60%)	7.50	5.00	1.50	3.00	1.00
White fish meal (CP60%)	2.00	2.00	2.00	2.50	2.50
Animal fat	1.00	1.00	1.00	1.00	1.00
Calcium carbonate	9.30	9.30	9.29	10.26	10.25
Dicalcium phosphate	1.45	1.49	1.50	1.25	1.30
Sodium chloride	0.39	0.39	0.39	0.34	0.34
L-lysine HCl	0.13	0.20	0.24	0.15	0.23
DL-methionine	0.21	0.29	0.38	0.22	0.29
L-tryptophan	0.05	0.07	0.08	0.06	0.07
Vitamin premix	0.20	0.20	0.20	0.20	0.20
Calculated chemical composition (as-fed basis)					
ME (kcal/g)	2.8	2.8	2.8	2.8	2.8
CP (%)	19.0	17.0	15.0	16.0	14.0
Lys (%)	1.0	1.0	1.0	0.9	0.9
Met (%)	0.6	0.6	0.6	0.5	0.5
Met+Cys (%)	0.9	0.9	0.9	0.8	0.8
Trp (%)	0.2	0.2	0.2	0.2	0.2
Ca (%)	4.10	4.10	4.10	4.45	4.45
Non-phytate P (%)	0.44	0.44	0.44	0.42	0.42
Analytical value					
CP (% FM)	19.8	18.1	15.8	16.7	14.8

Cont, control diet; LPS, low-protein diet; FM, fresh matter

outputs as egg, calculated as described in Ogino et al. (2017), did not differ significantly between the groups, consistent with a previous report (Saitoh 2001). Furthermore, the feed intake and the amount of excreta were also not significantly different, except in one experiment during the late laying period in which the amount of excreta was lower for the LPS group (Table 3). Collectively, feeding these diets to laying hens therefore reduces nitrogen excretion without affecting feed intake amount or laying performance.

It has been reported that feeding a 17% CP diet supplemented with smaller amounts of Met and Trp or a 13.5% CP diet supplemented with additional Met, Lys, Trp, and Thr to laying hens aged 28-36 weeks showed no difference in the egg-laying rate, egg weight, or egg mass, but feeding the 13.5% CP diet reduced nitrogen excretion in laying hens (Blair et al. 1999). Keshavarz & Austic (2004) conducted a study in which a 16.5% CP diet with Met supplementation or a 13% CP diet supplemented with additional amino acids was fed to laying hens aged 36-48 weeks. Their study showed that nitrogen excretion and egg weight were lower in laying hens fed the 13% CP diet. There was no difference in the egg-laying rate or egg mass between the 16.5% CP diet and the 13% diet supplemented with additional Met, Lys, Trp, Ile, and Val, whereas the egg-laying rate and egg mass were lower in laying hens fed the 13% CP diet supplemented with only additional Met and Lys compared to the 16.5% CP diet.

In Latshaw & Zhao (2011), laying hens aged 29-33 weeks were fed diets that provided 13, 15, or 17 g of protein/d, supplemented with additional Met, Lys, Thr, Trp, Ile, Val, and Phe in the lower-protein die. Nitrogen excretion was reduced in the lower-protein groups, whereas no difference was observed in the egg-laying rate, egg mass, or egg weight between the higher- and lower-protein groups. Ji et al. (2014) studied feeding laying hens aged 21-34 weeks diets with varying CP contents ranging from 18% to 16% in 0.5-point

Table 2. Effects of low-protein diets supplemented with essential amino acids on laying performance of laying hens

	CP	Egg-laying rate (%)	Egg weight (g)	References
Early laying period				
Cont	CP19%	97.2±0.2	58.7±0.5	This study
LPS	CP17%	96.5±0.3	58.3±0.5	This study
	CP15%	97.3±0.4	58.2±0.4	This study
Cont	CP19%’	95.0±2.2	59.1±0.6	Iio et al. 2021
LPS	CP17%’	96.0±1.1	59.2±0.3	Iio et al. 2021
Later laying period				
Cont	CP16%	91.8±2.8	62.7±0.4	This study
LPS	CP14%	87.2±3.7	62.5±0.4	This study
Cont	CP16%†	91.3±0.9	62.7±0.4	Iio et al. 2023
LPS	CP14%†	91.1±1.0	62.4±0.3	Iio et al. 2023

The data represent the mean ± SEM.
Egg-laying rate represents the hen-day average calculated by the number of eggs laid during the period and the number of laying hens during the same period. Egg weight represents the weight of one egg.
Egg-laying rate: n= 6/group for CP16%† and CP14%†, and n= 4/group for others. Egg weight: CP19%, n= 92; CP17%, n= 94; CP15%, n= 89; CP19%’, n= 404; CP17%’, n= 404; CP16%, n= 132; CP14%, n= 122; CP16%†, n= 180; CP14%†, n= 179.
Cont, control diet; LPS, low-protein diet
†Supplemented with phytase.

Table 3. Effects of low-protein diets supplemented with essential amino acids on nitrogen balance in laying period

	CP	Feed intake (g FM/layer/day)	Excreta (g DM/layer/day)	Nitrogen intake (g/layer/day)	Nitrogen excretion (g/layer/day)	References
Early laying period						
Cont	CP19%	103.7±3.1	26.8±1.1	3.28±0.10A	2.13±0.09A	This study
LPS	CP17%	106.4±2.5	27.0±1.0	3.09±0.07A	1.71±0.08B	This study
	CP15%	107.9±2.1	25.0±0.8	2.73±0.05B	1.36±0.06C	This study
Cont	CP19%’	98.2±5.2	26.3±2.2	3.17±0.17a	1.87±0.09a	Iio et al. 2021
LPS	CP17%’	91.8±4.5	23.8±2.2	2.64±0.13b	1.52±0.11b	Iio et al. 2021
Later laying period						
Cont	CP16%	106.4±2.4	24.6±1.2	2.85±0.06A	1.43±0.06A	This study
LPS	CP14%	104.6±1.7	22.2±0.9	2.48±0.04B	1.14±0.08B	This study
Cont	CP16%†	106.0±5.3	25.5±0.9a	2.85±0.14a	1.43±0.05a	Iio et al. 2023
LPS	CP14%†	106.6±2.9	21.7±0.6b	2.52±0.07b	1.13±0.03b	Iio et al. 2023

The data represent the mean ± SEM.
CP19%, n= 12; CP17%, n= 12; CP15%, n= 11; CP19%’, n= 5; CP17%’, n= 5; CP16%, n= 11; CP14%, n= 11; CP16%†, n= 10; CP14%†, n= 12.
Values with different superscripts are significantly different (P < 0.05, Tukey–Kramer test, Student’s t-test, or Welch’s t-test).
Cont, control diet; LPS, low-protein diet; DM, dry matter
†Supplemented with phytase.

increments, supplemented with additional amino acids to meet their requirements. They reported that nitrogen excretion and egg weight were reduced in the lower-CP diet groups, whereas neither the egg-laying rate nor the egg mass differed between the higher- and lower-CP diet groups. It should be noted that a low-protein diet could be deficient in nitrogen for the synthesis of nonessential amino acids, although the conventional layer diet contained sufficient essential and nonessential amino acids (Macelline et al. 2021). Macelline et al. (2021) also mentioned that protein-bound amino acids (amino acids taken as protein) could be more effective than non-bound amino acids in some stages of laying. Zhou et al. (2021) also reported that a lower-CP diet supplemented with Ser and essential amino acids recovered the egg-laying rate depending on the Ser concentration. In our studies, we designed the experimental diets to contain adequate amounts of essential amino acids and Gly + Ser. For these reasons, laying hens fed the LPS diets showed laying performance comparable to laying hens fed the Cont diets. Heo et al. (2023) conducted a study in which laying hens aged 19-32 weeks were fed diets with varying CP contents ranging from 19% to 13% in 2-percentage point increments, supplemented with additional amino acids to meet their requirements. They reported that nitrogen excretion and egg weight were reduced in the lower-CP diet groups, whereas the egg-laying rate, egg mass, or feed conversion ratio did not differ between the higher- and lower-CP diet groups.

Based on the above data (Blair et al. 1999, Heo et al. 2023, Ji et al. 2014, Keshavarz & Austic 2004, Latshaw & Zhao 2011) and our data (Table 3), we plotted the relationships between the reductions of CP in layer diets and that of nitrogen excretion compared to the control diet group of each study (Fig. 1). The following regression equation was developed using the lm function of the R (ver. 4.3.0) software program (R-Development-Core-Team 2023).

$Y = 8.71X + 1.72$ ($n = 14$, $R^2 = 0.718$, $P < 0.001$)
Y: reduction rate of layer nitrogen excretion (%)
X: reduction of CP content in layer diets (percentage points)

This regression equation indicates that the reduction rate of nitrogen excretion increases by 8.7% per percentage point reduction of CP content in layer diets.

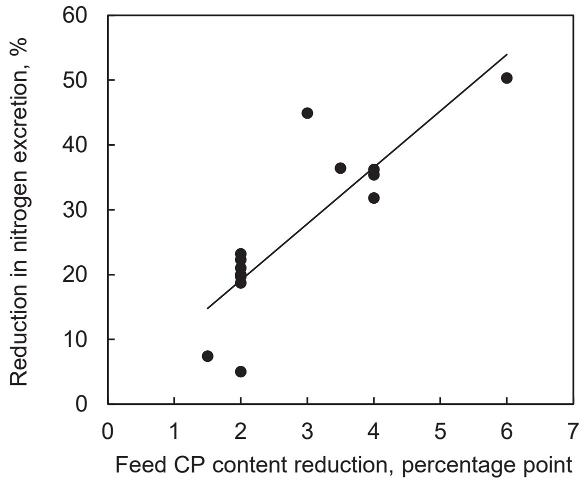

Fig. 1. Relationship between reduction in CP content in layer diets and reduction in nitrogen excretion
The solid line denotes the linear regression curve.

Effects of low-protein diets supplemented with amino acids on environmental gas emissions from manure composting

The LPS group showed lower environmental gas emissions per laying hen during the laying period and per egg compared to the Cont group in all our studies, and reduction rates of the LPS group compared to the Cont group during the entire laying period are 23.8% for GHG emissions and 29.2% for NH_3 emission (Tables 4, 5). N_2O and NH_3 are known to be generated from nitrogen compounds such as the urea and uric acid contained in excreta (Hristov et al. 2013). In pigs, low-protein diets supplemented with essential amino acids have been shown to reduce nitrogen excretion in urine, whereas no differences in nitrogen excretion in feces were observed compared to the control diets (Osada et al. 2011). Furthermore, feeding pigs low-protein diets supplemented with essential amino acids reduced GHG emissions, mainly N_2O emissions from urine wastewater treatment (Osada et al. 2011, Suto et al. 2016). It is known that feeding low-protein diets supplemented with essential amino acids to pigs more effectively decreases nitrogen excretion into urine than into feces (Bernal et al. 2009). Since poultry manure is a mixture of feces and urine, nitrogen excretion in manure and GHG emissions from manure composting appeared to be reduced in our studies. Phytase, the enzyme that enhances the availability of phytate phosphorus in poultry feed ingredients, is often added to layer feed. Adding phytase did not hinder the reduction of nitrogen excretion and environmental gas emissions from manure composting associated with the LPS diet (Iio et al. 2023).

Ammonia is a primary odor compound (Kuroda et al. 2004, Sakai et al. 2004) and a pollutant that causes acidification from poultry manure composting. In our study, the LPS group decreased NH_3 emissions per laying hen by 29.2% and NH_3 emissions per egg by 30.5% during the laying period compared to the Cont group. Therefore, the LPS group reduces both odor and GHG emissions from manure composting. For these reasons, environmental gas emissions could be reduced in the LPS group compared to the Cont group because of the reduced nitrogen excretion. Regarding the emission factors (EFs) for environmental gases, the EFs for NH_3 obtained in the

Table 4. Effects of low-protein diets supplemented with essential amino acids on environmental gas emissions per laying hen during laying period

		CP	N_2O	CH_4	NH_3	GHG‡	References
			(g/layer/laying period)				
Early laying period	Cont	CP19%	1.56	5.53	122.7	1,158.0	Iio et al. 2021
	LPS	CP17%	1.42	2.87	82.8	855.0	Iio et al. 2021
Later laying period	Cont	CP16%	1.97	3.44	136.6	1,273.1	This study
	LPS	CP14%	1.78	2.07	97.7	998.8	This study
	Cont	CP16%†	1.34	3.63	158.6	1,218.5	Iio et al. 2023
	LPS	CP14%†	1.21	2.14	119.3	954.1	Iio et al. 2023
Entire laying period	Cont		3.22	9.06	270.3	2,403.8	
	LPS		2.92	4.97	191.3	1,831.4	

The data were calculated using the data on environmental gas emissions and laying periods described below.
The early laying period was defined as days 133-356.
The later laying period was defined as days 357-560.
The entire laying period was defined as days 133-560.
Cont, control diet; LPS, low-protein diet
†Supplemented with phytase.
‡g CO_2 equivalent values calculated using the global warming potential shown in IPCC (2013) (N_2O: 265, CH_4: 28), including indirect N_2O emission derived from NH_3 emission.

Table 5. Effects of low-protein diets supplemented with essential amino acids on environmental gas emissions per egg and emission factor

		CP	N_2O	CH_4	NH_3	GHG‡	N_2O	CH_4	NH_3	References
			Gas emissions (mg/egg)				Emission factor (%)§			
Early laying period	Cont	CP19%	6.97	24.66	547.4	5,165.6	0.476	0.151	48.3	Iio et al. 2021
	LPS	CP17%	6.33	12.74	368.0	3,800.3	0.531	0.075	39.9	Iio et al. 2021
Later laying period	Cont	CP16%	8.90	15.59	618.1	5,761.3	0.551	0.122	49.5	This study
	LPS	CP14%	7.70	8.96	422.7	4,320.0	0.651	0.086	46.2	This study
	Cont	CP16%†	7.15	19.32	845.0	6,493.6	0.388	0.101	59.3	Iio et al. 2023
	LPS	CP14%†	6.53	11.54	642.1	5,135.1	0.389	0.058	49.5	Iio et al. 2023
Entire laying period	Cont		7.47	21.25	634.7	5,621.5	0.472	0.125	52.4	
	LPS		6.63	11.44	441.3	4,195.6	0.524	0.073	45.2	

The data were calculated using the data on environmental gas emissions, the number of eggs during manure collection, and the egg-laying rates and laying periods described below.
The early laying period was defined as days 133-356.
The later laying period was defined as days 357-560.
The entire laying period was defined as days 133-560.
Cont, control diet; LPS, low-protein diet
† Supplemented with phytase.
‡ g CO_2 equivalent values calculated using the global warming potential shown in IPCC (2013) (N_2O: 265, CH_4: 28), including indirect N_2O emission derived from NH_3 emission.
§ g N_2O-N/g N for N_2O, g CH_4/g organic matter for CH_4, g NH_3-N/g N for NH_3.

present study were of comparable magnitude with those of previously reported NH_3 (from 42% to 62%) (Kithome 1999, Morand 2005). The EFs for CH_4 and N_2O obtained in the present study were similar to those described in the National Inventory Report (GIO 2023). The LPS group tended to have slightly lower EFs for NH_3 and CH_4 than the Cont group ($P < 0.1$), whereas EFs for N_2O did not differ between the two groups ($P > 0.1$). The higher C/N ratio of composting material has been reported to result in lower NH_3 emissions from composting in experiments using pig feces (Jiang et al. 2011) or organic waste (Pagans et al. 2006). The C/N ratios of composting material were higher in the LPS group than in the Cont group, as shown in Table 6, and this seemed to result in lower NH_3 emission factors in the LPS group.

In our studies, the CH_4 emissions from manure

Table 6. Effects of low-protein diets supplemented with essential amino acids on the characteristics of composting manure

		CP	pH	EC (mS/cm)	T-N (% DM)	T-P_2O_5 (% DM)	T-K_2O (% DM)	C/N Ratio	References
Early laying period	Cont	CP19%	8.8	3.8	2.3	8.2	4.6	10.8	Iio et al. 2021
	LPS	CP17%	8.7	3.7	2.3	7.4	4.2	11.3	Iio et al. 2021
Later laying period	Cont	CP16%	8.8	6.3	1.5	6.9	4.5	12.2	This study
	LPS	CP14%	8.7	5.5	1.3	6.2	3.0	14.1	This study
	Cont	CP16%†	8.5	5.8	1.6	8.7	4.4	17.6	Iio et al. 2023
	LPS	CP14%†	8.4	5.6	1.4	8.5	4.5	21.3	Iio et al. 2023

The data represent the analytical values.
Cont, control diet; LPS, low-protein diet; DM, dry matter
† Supplemented with phytase.

composting were slightly lower in the LPS group than in the Cont group, although the CH_4 emissions were at low levels in both groups. Anaerobic conditions tend to cause higher CH_4 emissions, and higher moisture content is likely to result in anaerobic conditions. Since it is important to supply air to the composting material to decrease anaerobic conditions and promote fermentation, its moisture content must be appropriately adjusted. It is recommended that the composting material be adjusted to a moisture content of 62% or less with sawdust as a bulking agent or a volumetric weight of 700 kg/m^3 for pig, layer, and broiler (LEIO, 2004). The composting experiments in our studies used sawdust as a bulking agent and adjusted the moisture content according to the recommendation. Although the moisture content of the composting material was slightly higher in the LPS group in the study for the early laying period (54.8% vs. 52.9%, Iio et al. 2021) and in the Cont group in the study for the late laying period (63.3% vs. 60.2%, Iio et al. 2023), the CH_4 emissions were higher in the Cont group in both studies. Therefore, in our studies, the moisture content of the composting material did not seem to affect CH_4 emissions. During the composting process, the pH remained between 8.1 and 9.6, and nitrate was detected in the finished compost. Our composting temperature reached 60°C in our studies. The composting processes in our studies were thus considered sufficiently aerated, and compost was produced successfully.

Synthetic amino acid manufacturing produces more GHG emissions than the production of main feed ingredients such as corn and soybean. Life-cycle assessment studies showed that feeding a low-protein diet supplemented with amino acids reduced GHG emissions in pig and broiler production compared to conventional diets (Ogino et al. 2013, 2021). Future work should also investigate life-cycle GHG emissions from egg production using low-protein diets.

Effects of low-protein diets supplemented with amino acids on the characteristics of manure compost and nitrogen flow

The total nitrogen (T-N) content of manure compost of the LPS group was similar to that of the Cont group (Table 6). The LPS group exhibited similar N_2O EFs, approximately 10% lower NH_3 EFs, and about 20% less nitrogen excretion than the Cont group. The calculations based on N_2O EFs, NH_3 EFs, and nitrogen excretion showed similar nitrogen residuals and were consistent with the T-N content in the manure compost. Furthermore, the manure compost of the LPS group showed a similar germination percentage of komatsuna (*Brassica rapa* var. *perviridis*) and performance in the komatsuna growth test compared to the Cont group (Iio et al. 2021, 2023).

According to the nitrogen cycle model in Japan reported by Matsumoto et al. (2017), in 2007, the nitrogen flow derived from imported food and feed supply was 2.5 times greater than that derived from domestic food and feed supply. This implies that the nitrogen supply to farmland and the environment derived from manure compost and animal by-products is much greater than that required for domestic food and feed supply, resulting in an excessive nitrogen balance in Japanese agriculture (Matsumoto et al. 2017). If all feed ingredients for laying hens are imported, feeding LPS diets with 2% lower CP for laying hens during the entire laying period reduced the nitrogen inflow in Japan by 11.7%. Furthermore, feeding LPS diets with 2% lower CP for laying hens throughout the laying period reduced the nitrogen load for the environment by 19.7% (Table 7 and Fig. 2). Assuming that all laying hens are fed LPS diets with 2% lower CP throughout the laying period, the nitrogen inflow and nitrogen load for the environment in Japan were reduced by 16,750 and 15,971 tonnes per year, respectively, as calculated using statistical data for 2023

Table 7. Effects of low-protein diets supplemented with essential amino acids on the nitrogen flow in laying period

		CP	Nitrogen intake (g/layer/laying period)	Nitrogen excretion (g/layer/laying period)	References
Early laying period	Cont	CP19%	735.0	476.7	This study
	LPS	CP17%	691.1	381.9	This study
	Cont	CP19%	709.6	419.3	Iio et al. 2021
	LPS	CP17%	591.4	341.6	Iio et al. 2021
Later laying period	Cont	CP16%	581.3	291.2	This study
	LPS	CP14%	505.7	233.2	This study
	Cont	CP16%†	581.0	292.3	Iio et al. 2023
	LPS	CP14%†	513.1	231.4	Iio et al. 2023
Entire laying period	Cont		1,303.4	739.7	
	LPS		1,150.7	594.0	

The data were calculated using the data in Table 2 and laying periods described below.
The early laying period was defined as days 133-356.
The later laying period was defined as days 357-560.
The entire laying period was defined as days 133-560.
Cont, control diet; LPS, low-protein diet
†Supplemented with phytase.

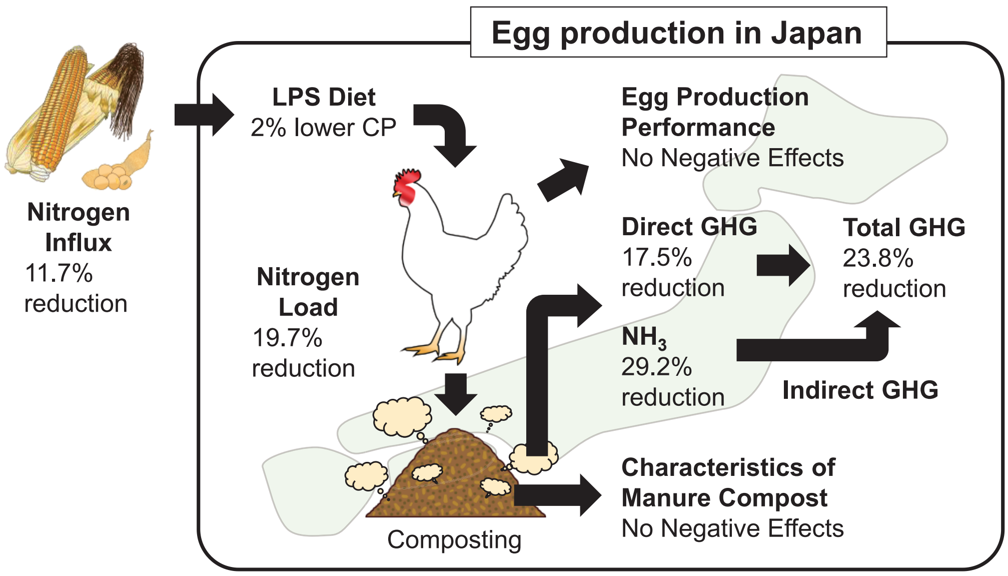

Fig. 2. Effects of low-protein diets on egg production in Japan
GHG: greenhouse gas

from the Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF 2023). LPS diets therefore were suggested to reduce the nitrogen load associated with egg production without affecting the characteristics of the manure compost from laying hens fed the LPS diets.

Considering that the release of reactive nitrogen to the environment has been reported to exceed the planetary boundary (Rockström et al. 2009, Richardson et al. 2023), researchers have been investigating nitrogen footprints of products, including animal products, to quantify and reduce nitrogen emissions (Leip et al. 2014, Shibata et al. 2017). Low-protein diets are expected to improve the egg nitrogen footprint and reduce nitrogen release associated with egg production by reducing the nitrogen excretion of laying hens without affecting laying performance.

Conclusion

In this paper, we reviewed the effects of LPS diets on the laying performance and nitrogen balance of laying hens across the entire laying period. LPS diets designed to contain adequate amounts of essential and nonessential amino acids did not affect laying performance and led to a decrease in nitrogen excretion. We developed a regression equation to estimate the reduction rate of layer nitrogen excretion resulting from decreased CP content in layer diets. The effects of LPS diets on the environmental gas emissions from manure composting and the characteristics of the manure compost were also summarized, revealing that feeding the LPS diets resulted in reduced environmental gas emissions from manure composting. Mean emission factors for environmental gases were also calculated. The results are summarized in Figure 2. Laying hens need more than 100 days to mature into the laying period. Thus, there is a need to investigate the effects of LPS diets on growth and environmental gas emissions from manure composting in chicks in the future.

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