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# Effects of Feeding Corn DDGS on Ammonia and Hydrogen Sulfide Emissions from Manure of Laying-hens

Maki IIDA; Hirokazu FUJISAKI; Yasushi HASHIMOTO;
Michito HANAZUMI; Chisato YONEMOCHI
Japan Scientific Feeds Association

Abstract

Feeding corn DDGS (DDGS) of laying-hens, we examined ammonia and hydrogen sulfide emissions from manure.

One hundred twenty white leghorns (Julia strain, aged 251 days) with stable egg laying record were used for the experiment. Control diet with no DDGS, as well as 10%, 20% and 30% DDGS diets that replaced corn and soybean meal, were all formulated to provide similar level of crude protein, metabolizable energy, phosphorous, calcium, methionine, lysine, tryptophan and threonine. Control, 10%, 20% or 30% diet was fed to three replicates of 10 hens each ad libitum for four weeks.

Egg production performance was investigated during the experiment period and yolk color evaluation was also conducted at the end of the experiment using the eggs produced by one of the replicates of each dietary treatment group. The all manure was collected from replicates on days 6-7, 13-24 and 27-28 after the start of the experiment and stored in buckets. Ammonia and hydrogen sulfide concentrations were measured in the empty space in the each bucket in 12, 24 and 48 hours later, followed by pH measurement of the manure. Manure water content was also measured using the manure produced on days 5, 12 and 26 after the start of the experiment and nitrogen and dry matter excretion rates were calculated for each dietary treatment.

The following results were obtained:

1) There was no difference in body weight gains during the period from the day of group assignment to the final day of the experiment between the control diet group and the 10% and 20% DDGS diet groups. Body weight gain of the 30% DDGS group was significantly lower than that of the control diet group. Except one of the laying hens fed 10% DDGS diet that stopped laying eggs and was culled, all the hens under the experiment were healthy and no abnormal health conditions were observed.
2) There was no difference in egg production rate, average egg weight, or daily egg production between the control diet group and the 10% DDGS group. The hens fed 20% and 30% DDGS diets showed the tendency of decrease in egg production rate, average egg weight and daily egg production at week 2 after the start of the experiment and thereafter compared to those fed the control diet. This tendency was pronounced in the 30% DDGS group.
3) There was no significant difference in feed intake in any weeks during the experiment period between

the control diet group and the other three dietary treatment groups. Although the weekly feed conversion rate of the DDGS diet groups tended to slightly decrease compared to the control diet group, there was no significant difference in the feed conversion rate throughout the experiment period between the control diet and the other three dietary treatments.

4) Egg yolk color significantly increased as dietary level of DDGS increased.
5) Adding DDGS to diets had no effect on the concentration of ammonia from manure at any time points. The DDGS inclusion did not affect the concentration of hydrogen sulfide at weeks 1 and 2 after the start of the experiment, however, the concentration of ammonia from the DDGS diet groups tended to decrease at week 4. This tendency was apparent in the treatment groups of 20 and 30% DDGS. Manure pH significantly decreased as dietary level of DDGS increased.
6) There was no difference in manure water content among treatment groups at week 1. It showed the tendency that manure water content decreases at weeks 2 and 4, almost directly correlating with the increase in dietary level of DDGS. Nitrogen and dry matter excretion rates showed negligible difference between the control diet and the 10% DDGS diet. These rates of the treatment groups of 20% and 30% DDGS tended to be higher than those of the control diet.

## 1. Materials and Methods

### 1) DDGS used in experiment

Corn DDGS (hereafter referred to as “DDGS”) purchased from the National Federation of Agricultural Cooperative Associations in Japan was used for the experiment

### 2) Animals used in experiment

One hundred twenty white leghorns (Julia strain, aged 251 days) were used. Their egg production rate continued at 82% or above for 4 weeks until the group assignment for the experiment. These laying hens were placed under preliminary feeding with the control diet as described later for two weeks after the group assignment so as to acclimate themselves to the experimental environment.

### 3) Design of experimental groups

This experiment used four dietary treatment groups: the control diet with no DDGS, and the other three groups of 10%, 20% and 30% DDGS diets in which DDGS was included instead of corn and soybean meal contained in the control diet. All these diets were formulated to provide similar levels of crude protein (CP), metabolizable energy (ME), non-phytic phosphorus (NpP), calcium (Ca), methionine (Met), lysine (Lys), tryptophan (Trp) and threonine (Thr).

The laying hens were divided into 12 groups of 10 each based on their egg production performance evaluated before the experiment. Those groups were divided into three replicates for each diet treatment and fed for four weeks.

**Table 1-1 Ingredients percentages of dietary treatments**

| Ingredient | DDGS inclusion level (%) | | | |
|---|---|---|---|---|
| | Control (0) | 10 | 20 | 30 |
| DDGS | - | 10.00 | 20.00 | 30.00 |
| Corn | 53.37 | 48.14 | 43.14 | 38.06 |
| Milo | 10.00 | 10.00 | 10.00 | 10.00 |
| Soybean meal | 14.60 | 9.90 | 5.00 | 0.15 |
| Canola meal | 4.00 | 4.00 | 4.00 | 4.00 |
| Fish meal (CP65%) | 1.50 | 1.50 | 1.50 | 1.50 |
| Corn gluten meal | 3.00 | 3.00 | 3.00 | 3.00 |
| Vegetable oil | 3.00 | 2.90 | 2.78 | 2.67 |
| Calcium carbonate | 8.29 | 8.53 | 8.76 | 9.00 |
| Dicalcium phosphate | 0.98 | 0.69 | 0.41 | 0.13 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin B family[1] | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin ADE[2] | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace minerals[3] | 0.25 | 0.25 | 0.25 | 0.25 |
| DL-methionine | 0.09 | 0.08 | 0.06 | 0.05 |
| L-lysine hydrochloride | 0.03 | 0.11 | 0.19 | 0.27 |
| L-tryptophan | 0.03 | 0.04 | 0.05 | 0.06 |
| Vitamin $K_3$10% product | 0.01 | 0.01 | 0.01 | 0.01 |
| Chromic oxide | 0.10 | 0.10 | 0.10 | 0.10 |

[1] In 1kg; Thiamin mononitrate 2.0g, Riboflavin 10.0g, Pyridoxine hydrochloride 2.0g, Nicotinic-acid amide 2.0g、D-calcium pantothenate 4.35g, Choline chloride138.0g, Folic acid 1.0g

[2] In 1g; Vitamin A oil 10,000IU, Vitamin $D_3$oil 2,000IU, DI-α- tocopherol acetate 10mg

[3] In 1kg; Manganese 80g, Copper 0.6g, Zinc 50g, Iodine 1g, Iron 6g

[4] Values calculated according to Standard Tables of Feed Composition in Japan (ver. 2009)

The dietary formulation of each treatment group is as shown in Table 1. The diets were so formulated that CP and ME were 104 to 105% of the feed requirements for the daily egg production of 56g and Lys, Met, Thr and Trp were at least 115% of the respective requirements (Table 1-2 as indicated by the Japan Feeding Standard [1)]).

When the experimental diets were formulated, the value of Standard Tables of Feed Composition in Japan [2)] was used for calculating ME. The contents of CP, P, Ca, methionine (Met), lysine (Lys), tryptophan (Trp) and threonine (Thr) in DDGS, corn (heat rolled corn), milo, soybean meal, canola meal, fish meal and corn gluten meal, and the contents of P and Ca in calcium carbonate and dicalcium phosphate were respectively analyzed in advance (Table 1-3). As to DDGS, NpP in total P was assumed at 90%, which was multiplied by the value from the analyzed data. As to other ingredients, the values from the analyzed data were used for the experimental diet formulation by multiplying them by the percentage of NpP contained in total P as indicated by the Standard Tables of Feed Composition in Japan [2)]. These components were analyzed in accordance with the Feed Analysis Standard[3)] for CP, P and Ca, and with the use of an automated amino acid analyzer for Met, Lys, Trp and Thr.

**Table 1-2 Formulation of dietary treatments**

| Ingredient | DDGS inclusion level (%) | | | |
|---|---|---|---|---|
| | Control (0) | 10 | 20 | 30 |
| Crude protein (%) | 16.1 (104) | 16.2 (104) | 16.2 (104) | 16.2 (105) |
| Metabolizable energy (kcal/kg) | 2,92 (104) | 2,92 (104) | 2,92 (104) | 2,92 (104) |
| Calcium (%) | 3.60 (108) | 3.60 (108) | 3.60 (108) | 3.60 (108) |
| Non Phytate Phosphorus (%) | 0.33 (110) | 0.33 (110) | 0.33 (110) | 0.33 (110) |
| Lys (%) | 0.75 (116) | 0.76 (116) | 0.76 (116) | 0.75 (116) |
| Met (%) | 0.38 (114) | 0.38 (114) | 0.37 (113) | 0.38 (115) |
| Trp (%) | 0.20 (116) | 0.20 (116) | 0.20 (116) | 0.19 (114) |
| Thr (%) | 0.60 (132) | 0.59 (132) | 0.59 (132) | 0.58 (130) |

**Table1-3 Analytical values of ingredients**

| Ingredient | CP (%) | P (%) | Ca (%) | Met (%) | Lys (%) | Trp (%) | Thr (%) |
|---|---|---|---|---|---|---|---|
| DDGS | 27.0 | 0.785 | 0.025 | 0.51 | 0.97 | 0.21 | 0.96 |
| Corn | 7.4 | 0.223 | 0.0039 | 0.15 | 0.20 | 0.05 | 0.24 |
| Milo | 8.6 | 0.289 | 0.0134 | 0.14 | 0.19 | 0.09 | 0.28 |
| Soybean meal | 46.3 | 0.656 | 0.341 | 0.62 | 2.87 | 0.62 | 1.83 |
| Canola meal | 35.8 | 1.02 | 0.654 | 0.71 | 2.02 | 0.50 | 1.58 |
| Fish meal | 67.9 | 2.53 | 4.25 | 1.53 | 4.51 | 0.66 | 2.67 |
| Corn gluten meal | 66.6 | 0.521 | 0.0132 | 1.71 | 1.19 | 0.39 | 2.27 |
| Calcium carbonate | - | 0.0032 | 38.5 | - | - | - | - |
| Dicalcium phosphate | - | 17.8 | 27.3 | - | - | - | - |

4) Feeding control

Each of the laying hens was controlled in a terraced single feeding cage installed in an open type layer house. Ten replicate cages were used for each treatment group. One empty cage was placed between one treatment group and another. Diets were fed ad libitum for respective groups and well water was freely taken. The lighting system was managed to ensure the light period of 14 hours (5:00 to 19:00) and the dark period of 10 hours (19:00 to 5:00).

2. Investigation Items

1) Body weight and body weight gain and health conditions

The weight of each laying hen was measured on the day of group assignment and the final day of the experiment, and body weight gain was investigated during the period between those days. The health conditions of each layer were daily checked. Every dead or culled layer was dissected to identify causes as clearly as possible.

2) Egg production performance

The egg production of each laying hen was investigated on a daily basis to calculate weekly egg production rate. The egg weight of each replicate of each treatment group was measured every day to

calculate average egg weight and daily egg production.

3) Feed intake and feed conversion rate
The feed intake of each replicate was investigated weekly to calculate feed intake and feed conversion rate per hen per day.

4) Egg yolk color
The eggs produced on day 28 after the start of the experiment were collected and stored at room temperature for one day to measure values of egg yolk color (equivalent to Roche Yolk Color Fan values) using an Egg Multitester EMT-5200 (Robotmation Co., Ltd.).

5) Concentrations of ammonia and hydrogen sulfide from manure and manure pH
All manure for each replicate was collected three times a day, that is, in the morning, at noon and in the early evening, at week 1 (day 6-7 after the start of the experiment), week 2 (day 13-14) and week 4 (day 27-28). Each time, it was separately stored in a bucket covered with a vinyl sheet for 48 hours after the final collection. Ammonia and hydrogen sulfide concentrations were measured in the empty space in each bucket in 12, 24 and 48 hours after the final collection, using a gas detector tube (GASTEC Corporation). After ammonia and hydrogen sulfide concentrations were measured in 48 hours after the final collection, the manure in each bucket was well mixed, and then manure pH was measured using a pH meter (main unit: D-51; electrode: 9621C; Horiba, Ltd.).

The buckets with manure at weeks 1 and 2 after the start of the experiment were stored in the chicken house (week 1 after the start of the experiment: 10.0 - 15.5°C; week 2: 4.0 - 17.5°C) and the buckets at week 4 after the start of the experiment was stored in a warmed room (16.5 - 23.0°C).

6) Manure water content and nitrogen and dry matter excretion rates
The entire amount of the manure, which was produced by the laying hens on days 5, 12 and 26 after the start of the experiment, was collected separately for each replicate and dehydrated through circulation drying at approximately 60°C and then by wind drying to measure water content based on the Feed Analysis Standard.[3] Then, nitrogen and dry matter excretion rates were calculated for each dietary treatment in accordance with the procedure set out in "Handling Total Digestible Nutrients or Metabolizable Energy for Feed Labeling"[4].

7) Statistical analysis
The variance was analyzed using a one way layout[5] for the obtained data. The items for which a difference was found within a significance level of 5% were verified for the significance of the difference between average values by using Tukey's multiple testing[5]. The angular transformation[5] applied to the egg production rate before it was analyzed.

3. Venue of Experiment
Scientific Feeds Research Center of Japan Scientific Feeds Association
(821, Yoshikura, Narita City, Chiba Prefecture)

4. Period of Experiment

From October 27 to December 10, 2011

5. Results of Experiment

1) Body weight gain and health conditions

Table 2 shows average body weights of the replicates in each treatment group on the day of group assignment and the final day of the experiment and body weight gains throughout the period between those days.

**Table 2 Body weight gain**

| Item | DDGS inclusion level (%) | | | |
|---|---|---|---|---|
| | Control (0) | 10 | 20 | 30 |
| Average weight (g/hen) | | | | |
| Date of assignment | 1666 ± 27 | 1659 ± 130 | 1744 ± 16 | 1655 ± 24 |
| Final date of experiment | 1735 ± 23 | 1702 ± 132 | 1796 ± 46 | 1664 ± 30 |
| Gain | $70^{a}$ ± 9 | $44^{ab}$ ± 13 | $52^{ab}$ ± 32 | $9^{b}$ ± 10 |

Note 1) Average weight ± Standard deviation (n = 3)
Note 2) a-b Significant differences between different superscripts ($p < 0.05$)

There was no significant difference in average weight both on the day of group assignment and the final day of the experiment among treatment groups. There was no significant difference in body weight gain during the period from the day of group assignment to the final day of the experiment between the control diet group and 10 and 20% DDGS groups. The body weight gain of the laying hens fed 30% DDGS, however, significantly decreased compared to those fed the control diets.

As a laying hen (No. 236) in the treatment group of 10% DDGS stopped laying for six days from day 14 after the start of the experiment, it was immediately culled and dissected, and no visual abnormality was found in its main internal organs. Any negative health conditions were not observed in other laying hens.

2) Egg production performance

Table 3 shows egg production rate, average egg weight and daily egg production.

The egg production rate, average egg weight and daily egg production of the laying hens fed 10% DDGS changed in a similar way to those of the control diet group, and there was no difference in the three egg production performance parameters between them throughout the period of the experiment. Feeding 20% and 30% DDGS diets reduced egg production rate, average egg weight and daily egg production at week 2 after the start of the experiment and thereafter compared to those of the control diet group. This tendency was pronounced in the group of 30% DDGS. These differences, however, were not significant.

**Table 3 Egg production performance**

| Item | | DDGS inclusion level (%) | | | |
|---|---|---|---|---|---|
| | | Control (0) | 10 | 20 | 30 |
| Egg production rate (%) | Week 1 | 95.7 ± 4.3 | 95.2 ± 3.5 | 98.1 ± 0.9 | 94.3 ± 1.4 |
| | Week 2 | 98.6 ± 1.5 | 98.6 ± 1.5 | 96.2 ± 2.2 | 98.1 ± 2.2 |
| | Week 3 | 98.1 ± 0.9 | 98.0 ± 1.0 | 97.2 ± 2.5 | 96.2 ± 2.2 |
| | Week 4 | 98.6 ± 1.5 | 99.0 ± 0.9 | 95.2 ± 0.8 | 93.3 ± 4.3 |
| | Throughout experiment | 97.7 ± 1.3 | 97.8 ± 1.0 | 96.6 ± 0.8 | 95.5 ± 1.2 |
| Average egg weight (g/egg) | Week 1 | 64.5 ± 1.1 | 64.5 ± 2.5 | 63.5 ± 2.0 | 63.1 ± 0.6 |
| | Week 2 | 64.8 ± 0.8 | 64.5 ± 2.2 | 63.6 ± 1.7 | 62.9 ± 0.7 |
| | Week 3 | 64.7 ± 1.4 | 65.1 ± 1.6 | 64.0 ± 1.9 | 63.3 ± 0.3 |
| | Week 4 | 64.8 ± 1.2 | 65.0 ± 2.1 | 64.6 ± 1.3 | 63.9 ± 0.4 |
| | Throughout experiment | 64.7 ± 1.1 | 64.8 ± 2.1 | 64.0 ± 1.7 | 63.3 ± 0.2 |
| Daily egg production (g/day/hen) | Week 1 | 61.8 ± 3.6 | 61.4 ± 4.5 | 62.3 ± 2.0 | 59.5 ± 1.3 |
| | Week 2 | 63.9 ± 1.5 | 63.6 ± 2.5 | 61.2 ± 2.9 | 61.7 ± 2.0 |
| | Week 3 | 63.5 ± 1.9 | 63.8 ± 1.3 | 62.2 ± 3.4 | 60.9 ± 1.3 |
| | Week 4 | 63.8 ± 1.7 | 64.4 ± 2.5 | 61.6 ± 1.3 | 59.6 ± 2.6 |
| | Throughout experiment | 63.3 ± 1.8 | 63.4 ± 2.6 | 61.8 ± 2.1 | 60.4 ± 0.9 |

Note) Average value ± Standard deviation (n = 3)

3) Feed intake and feed conversion rate

Table 4 shows feed intake and feed conversion rate.

There was no significant difference in feed intake at any weeks between the control diets and the other dietary treatments. The feed conversion rates of the laying hens fed DDGS diets tended to slightly decrease compared to that of the control diet group. The feed conversion rate of the laying hens fed 30 % DDGS diet was significantly lower than that of the hens fed the control diet at week 4. There was no significant difference in feed conversion rate throughout the period of the experiment between the control diet and the other dietary treatments.

**Table 4 Feed intakes and feed conversion rates**

| Item | | DDGS inclusion level (%) | | | |
|---|---|---|---|---|---|
| | | Control (0) | 10 | 20 | 30 |
| Feed intake (g/day/hen) | Week 1 | 116.2 ± 3.8 | 118.2 ± 7.2 | 120.4 ± 5.7 | 118.0 ± 2.1 |
| | Week 2 | 114.3 ± 3.3 | 115.8 ± 4.8 | 117.8 ± 3.7 | 117.3 ± 0.9 |
| | Week 3 | 111.9 ± 1.9 | 112.2 ± 11.0 | 118.3 ± 6.6 | 113.9 ± 2.2 |
| | Week 4 | 114.6 ± 2.4 | 119.4 ± 8.4 | 118.5 ± 5.1 | 117.0 ± 1.3 |
| | Throughout experiment | 114.3 ± 2.1 | 116.4 ± 7.4 | 118.8 ± 5.2 | 116.6 ± 1.0 |
| Feed conversion rate | Week 1 | 1.88 ± 0.05 | 1.93 ± 0.08 | 1.93 ± 0.03 | 1.99 ± 0.08 |
| | Week 2 | 1.79 ± 0.08 | 1.82 ± 0.08 | 1.93 ± 0.10 | 1.90 ± 0.06 |
| | Week 3 | 1.76 ± 0.06 | 1.76 ± 0.16 | 1.90 ± 0.08 | 1.87 ± 0.06 |
| | Week 4 | $1.80^{a}$ ± 0.01 | $1.85^{ab}$ ± 0.07 | $1.93^{ab}$ ± 0.05 | $1.96^{b}$ ± 0.07 |
| | Throughout experiment | 1.81 ± 0.03 | 1.84 ± 0.10 | 1.92 ± 0.05 | 1.93 ± 0.04 |

Note1) Average value ± Standard deviation (n = 3)
Note 2) a-b Significant differences between different superscripts ($p < 0.05$)

4) Egg yolk color value

Table 5 shows the yolk color values of the eggs collected on the final day of the experiment. The color value significantly increased with increasing level of DDGS in the diets.

**Table 5 Yolk color value**

| DDGS inclusion level (%) | | | |
|---|---|---|---|
| Control (0) | 10 | 20 | 30 |
| $5.7 \pm 0.5^{a}$ | $6.2^{ab} \pm 0.6$ | $6.9^{bc} \pm 0.3$ | $7.3^{c} \pm 0.8$ |

Note 1) Average value ± Standard deviation (n = 3)
Note 2) a-b Significant differences between different superscripts ($p < 0.05$)

5) Concentrations of ammonia and hydrogen sulfide from manure and manure pH

Table 6 shows the concentrations of ammonia and hydrogen sulfide from manure and manure pH.

**Table 6 Ammonia and hydrogen sulfide from manure (ppm) and manure pH**

| Item | Time of measurement | | DDGS inclusion level (%) | | | |
|---|---|---|---|---|---|---|
| | | | Control (0) | 10 | 20 | 30 |
| Ammonia (ppm) | Week 1 | After 12 hrs. | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | | After 24hrs. | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | | After 48 hrs. | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | Week 2 | After 12 hrs. | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | | After 24hrs. | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | | After 48 hrs. | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | Week 4 | After 12 hrs. | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | | After 24hrs. | < 0.5 ~ 0.5 | < 0.5 | < 0.5 ~ 0.5 | < 0.5 |
| | | After 48 hrs. | < 0.5 ~ 0.9 | < 0.5 | < 0.5 ~ 0.5 | < 0.5 ~ 0.5 |
| Hydrogen sulfide (ppm) | Week 1 | After 12 hrs. | 0.5 ± 0.0 | 1.3 ± 0.3 | 1.3 ± 0.5 | 1.3 ± 0.6 |
| | | After 24hrs. | 0.4 ± 0.1 | 1.3 ± 0.4 | 1.3 ± 0.6 | 1.2 ± 0.4 |
| | | After 48 hrs. | 3.6 ± 2.5 | 4.9 ± 1.7 | 2.8 ± 1.3 | 3.1 ± 1.0 |
| | Week 2 | After 12 hrs. | 1.1 ± 0.5 | 1.5 ± 0.6 | 1.2 ± 0.2 | 1.3 ± 0.3 |
| | | After 24hrs. | 0.5 ± 0.4 | 0.7 ± 0.3 | 0.5 ± 0.2 | 0.6 ± 0.2 |
| | | After 48 hrs. | 0.7 ± 0.8 | 0.7 ± 0.3 | 0.5 ± 0.3 | 0.7 ± 0.4 |
| | Week 4 | After 12 hrs. | 13.8 ± 9.8 | 8.5 ± 1.8 | 8.8 ± 1.8 | 10.0 ± 3.1 |
| | | After 24hrs. | 90.8 ± 87.4 | 38.3 ± 12.6 | 28.7 ± 11.0 | 33.3 ± 7.6 |
| | | After 48 hrs. | 76.7 ± 68.3 | 98.3 ± 52.5 | 69.3 ± 46.8 | 58.3 ± 38.2 |
| pH | Week 1 | After 48 hrs. | $6.43^{b}$ ± 0.07 | $6.35^{ab}$ ± 0.30 | $6.02^{ab}$ ± 0.11 | $5.97^{a}$ ± 0.12 |
| | Week 2 | After 48 hrs. | $6.42^{b}$ ± 0.05 | $6.58^{b}$ ± 0.06 | $6.34^{ab}$ ± 0.14 | $6.08^{a}$ ± 0.12 |
| | Week 4 | After 48 hrs. | $6.64^{c}$ ± 0.23 | $6.31^{c}$ ± 0.06 | $6.14^{b}$ ± 0.03 | $5.75^{a}$ ± 0.10 |

Note 1) Detection limit for ammonia measurement: 0.5 ppm
Note 2) Average value ± Standard deviation (n = 3)
Note 3) a-b Significant differences between different superscripts ($p < 0.05$)

Ammonia was not detected at any time points at weeks 1 and 2 when manure was stored in the chicken house (detection limit: 0.5 ppm). It was not detected when manure was stored for 12 hours in a warmed room at week 4. Even when manure was stored for 24 hours or 48 hours, 0.5 - 0.9 ppm ammonia was detected only one of the three replicates of each dietary treatment group.

The concentrations of hydrogen sulfide at weeks 1 and 2 were generally low, and there was little difference between the control diet and the three DDGS dietary treatments. Adding DDGS to layer diets tended to reduce the concentration of hydrogen sulfide at any time points at week 4, and this reduction tendency was apparent in the groups of 20 and 30% DDGS diets. These differences, however, were not significant.

Manure pH significantly decreased as dietary DDGS level increased.

6) Manure water content and nitrogen and dry matter excretion rates

Table 7 shows manure water content and nitrogen and dry matter excretion rates.

There was no difference in manure water content at week 1 among the dietary treatment groups. Manure water content showed the tendency of decrease at weeks 2 and 4, almost correlating with the increase in dietary level of DDGS. The manure water contents in the dietary treatment groups of 20% and 30% DDGS were significantly lower at week 4 than that of the control diet group.
Except the dry matter excretion rate at week 2, there was no significant difference in nitrogen excretion rate or dry matter excretion rate at any time points between the control diet group and the 10% DDGS diet group. However, those of the treatment groups of 20% and 30% DDGS tended to increase compared to the control diet, and there was significant difference at any time points except the nitrogen excretion rate at week 2.

**Table 7 Manure water content and nitrogen and dry matter excretion rates**

| Item | Time of measurement | DDGS inclusion level (%) | | | |
|---|---|---|---|---|---|
| | | Control (0) | 10 | 20 | 30 |
| Manure water content (%) | Week 1 | 78.2 ± 0.5 | 78.0 ± 1.1 | 76.4 ± 0.7 | 79.9 ± 3.3 |
| | Week 2 | 78.1 ± 0.1 | 77.7 ± 1.1 | 76.2 ± 0.7 | 76.6 ± 1.3 |
| | Week 4 | $78.8^{b}$ ± 0.7 | $78.1^{ab}$ ± 1.1 | $76.5^{a}$ ± 0.1 | $76.3^{a}$ ± 0.7 |
| Nitrogen excretion rate (%) | Week 1 | $52.9^{a}$ ± 5.5 | $55.3^{ab}$ ± 3.8 | $62.8^{bc}$ ± 0.9 | $66.6^{c}$ ± 1.9 |
| | Week 2 | 54.1 ± 4.4 | 60.2 ± 6.1 | 61.7 ± 6.3 | 63.7 ± 4.4 |
| | Week 4 | $52.6^{a}$ ± 4.5 | $52.9^{a}$ ± 2.3 | $59.3^{b}$ ± 2.5 | $59.1^{b}$ ± 1.8 |
| Dry matter excretion rate (%) | Week 1 | $26.4^{a}$ ± 1.0 | $28.0^{a}$ ± 0.9 | $32.4^{b}$ ± 0.5 | $36.6^{c}$ ± 0.8 |
| | Week 2 | $26.9^{a}$ ± 1.1 | $31.2^{b}$ ± 1.6 | $32.3^{b}$ ± 0.8 | $35.9^{c}$ ± 1.2 |
| | Week 4 | $26.7^{a}$ ± 1.0 | $28.0^{a}$ ± 0.6 | $30.6^{b}$ ± 0.6 | $33.3^{c}$ ± 0.9 |

Note 1) Average value ± Standard deviation (n = 3)
Note 2) a-c Significant differences between different superscripts ($p < 0.05$)

6. Reference


1) National Agriculture and Bio-oriented Research Organization (ed.), J*apan Feeding Standard for Poultry (Version of 2004)*. Tokyo: Japan Livestock Industry Association, 2004
2) National Agriculture and Food Research Organization (ed.). *Standard Tables of Feed Composition in Japan (Version of 2001).* Tokyo: Japan Livestock Industry Association, 2001, Feed Composition
3) *Feed Analysis Standard.* Notification from the Director-General of Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries dated April 1, 2008, Notice Number, 19, Consumer Products Safety Act No. 14729
4) *Enforcement of the Ministerial Ordinance on the Partial Revision of Ministerial Ordinance Concerning Ingredient Standards for Feeds and Feed Additives", Separate Paragraph 3: "Handling of Total Digestible Nutrients or Metabolizable Energy for Feed Labeling*. Notification from the Director of Livestock Industry Bureau and the Director-General of the Fisheries Agency, Ministry of Agriculture, Forestry and Fisheries dated July 27, 1981, Notice Number, 56 Livestock B 1594
5) YOSHIDA Minoru. *Chikuasan o Chushintosuru Jikken Keikaku-ho (Design of Experiments Mainly for Livestock Industry)*". Tokyo: Yokendo, 1983

**Appendix table1-1 Body weight and body weight gain of each hen**

| DDGS inclusion level (%) | Replicate | Hen number | Body weight (g) | | Gain (g) |
|---|---|---|---|---|---|
| | | | Assignment date | Final date | |
| Control (0) | 1 | 34 | 1625 | 1681 | 56 |
| | | 35 | 1729 | 1862 | 133 |
| | | 36 | 1606 | 1604 | -2 |
| | | 37 | 1818 | 1879 | 61 |
| | | 38 | 1690 | 1736 | 46 |
| | | 39 | 1818 | 1792 | -26 |
| | | 40 | 1323 | 1367 | 44 |
| | | 41 | 1409 | 1600 | 191 |
| | | 42 | 1842 | 2005 | 163 |
| | | 43 | 1738 | 1851 | 113 |
| | | Average | 1660 | 1738 | 78 |
| | 2 | 174 | 1469 | 1483 | 14 |
| | | 175 | 1675 | 1732 | 57 |
| | | 176 | 1455 | 1468 | 13 |
| | | 177 | 1680 | 1852 | 172 |
| | | 178 | 1731 | 1866 | 135 |
| | | 179 | 1698 | 1786 | 88 |
| | | 180 | 1760 | 1710 | -50 |
| | | 181 | 1710 | 1838 | 128 |
| | | 182 | 1545 | 1616 | 71 |
| | | 183 | 1682 | 1758 | 76 |
| | | Average | 1641 | 1711 | 70 |
| | 3 | 244 | 1895 | 1974 | 79 |
| | | 245 | 1658 | 1716 | 58 |
| | | 246 | 1772 | 1802 | 30 |
| | | 247 | 1721 | 1861 | 140 |
| | | 248 | 1874 | 1881 | 7 |
| | | 249 | 1591 | 1675 | 84 |
| | | 250 | 1500 | 1567 | 67 |
| | | 251 | 1579 | 1663 | 84 |
| | | 252 | 1618 | 1676 | 58 |
| | | 253 | 1748 | 1753 | 5 |
| | | Average | 1696 | 1757 | 61 |
| | Group average | | 1666 | 1735 | 70 |

**Appendix table 1-2 Body weight and body weight gain of each hen**

| DDGS inclusion level (%) | Replicate | Hen number | Body weight (g) | | Gain (g) |
|---|---|---|---|---|---|
| | | | Assignment date | Final date | |
| 10 | 1 | 23 | 1775 | 1953 | 178 |
| | | 24 | 1732 | 1759 | 27 |
| | | 25 | 1531 | 1560 | 29 |
| | | 26 | 1561 | 1514 | -47 |
| | | 27 | 1747 | 1712 | -35 |
| | | 28 | 1491 | 1577 | 86 |
| | | 29 | 1405 | 1494 | 89 |
| | | 30 | 1298 | 1311 | 13 |
| | | 31 | 1496 | 1476 | -20 |
| | | 32 | 1489 | 1496 | 7 |
| | | Average | 1553 | 1585 | 33 |
| | 2 | 163 | 1603 | 1629 | 26 |
| | | 164 | 1941 | 2020 | 79 |
| | | 165 | 1931 | 1953 | 22 |
| | | 166 | 1861 | 1866 | 5 |
| | | 167 | 2061 | 2081 | 20 |
| | | 168 | 1458 | 1545 | 87 |
| | | 169 | 1845 | 1943 | 98 |
| | | 170 | 1842 | 1861 | 19 |
| | | 171 | 1770 | 1811 | 41 |
| | | 172 | 1728 | 1740 | 12 |
| | | Average | 1804 | 1845 | 41 |
| | 3 | 233 | 1580 | 1673 | 93 |
| | | 234 | 1588 | 1586 | -2 |
| | | 235 | 1648 | 1660 | 12 |
| | | 236* | 1719 | 淘汰 | - |
| | | 237 | 1488 | 1547 | 59 |
| | | 238 | 1658 | 1673 | 15 |
| | | 239 | 1587 | 1654 | 67 |
| | | 240 | 1556 | 1653 | 97 |
| | | 241 | 1526 | 1618 | 92 |
| | | 242 | 1936 | 2029 | 93 |
| | | Average | 1619 | 1677 | 58 |
| | Group average | | 1659 | 1702 | 44 |

*: No.236 was culled and its data were retroactively excluded from the calculation of average values.

**Appendix table 1-3 Body weight and body weight gain of each hen**

| DDGS inclusion level (%) | Replicate | Hen number | Body weight (g) | | Gain (g) |
|---|---|---|---|---|---|
| | | | Assignment date | Final date | |
| 20 | 1 | 12 | 1631 | 1607 | -24 |
| | | 13 | 1796 | 1890 | 94 |
| | | 14 | 1620 | 1673 | 53 |
| | | 15 | 1672 | 1738 | 66 |
| | | 16 | 1653 | 1808 | 155 |
| | | 17 | 1960 | 2093 | 133 |
| | | 18 | 2011 | 1988 | -23 |
| | | 19 | 1782 | 1942 | 160 |
| | | 20 | 1682 | 1800 | 118 |
| | | 21 | 1698 | 1781 | 83 |
| | | Average | 1751 | 1832 | 82 |
| | 2 | 152 | 1673 | 1668 | -5 |
| | | 153 | 1990 | 1974 | -16 |
| | | 154 | 1988 | 2116 | 128 |
| | | 155 | 1774 | 1956 | 182 |
| | | 156 | 1772 | 1833 | 61 |
| | | 157 | 1876 | 1922 | 46 |
| | | 158 | 1550 | 1574 | 24 |
| | | 159 | 1562 | 1546 | -16 |
| | | 160 | 1833 | 1904 | 71 |
| | | 161 | 1534 | 1613 | 79 |
| | | Average | 1755 | 1811 | 55 |
| | 3 | 222 | 1760 | 1726 | -34 |
| | | 223 | 1819 | 1891 | 72 |
| | | 224 | 1901 | 1906 | 5 |
| | | 225 | 1804 | 1708 | -96 |
| | | 226 | 1699 | 1811 | 112 |
| | | 227 | 1418 | 1489 | 71 |
| | | 228 | 1776 | 1814 | 38 |
| | | 229 | 1725 | 1780 | 55 |
| | | 230 | 1720 | 1656 | -64 |
| | | 231 | 1624 | 1656 | 32 |
| | | Average | 1725 | 1744 | 19 |
| | Group average | | 1744 | 1796 | 52 |

**Appendix table1-4 Body weight and body weight gain of each hen (g)**

| DDGS inclusion level (%) | Replicate | Hen number | Weight (g) | | Gain (g) |
|---|---|---|---|---|---|
| | | | Assignment date | Final date | |
| 30 | 1 | 1 | 1755 | 1760 | 5 |
| | | 2 | 1459 | 1596 | 137 |
| | | 3 | 1972 | 2104 | 132 |
| | | 4 | 1535 | 1446 | -89 |
| | | 5 | 1474 | 1474 | 0 |
| | | 6 | 1655 | 1666 | 11 |
| | | 7 | 1786 | 1739 | -47 |
| | | 8 | 1776 | 1734 | -42 |
| | | 9 | 1600 | 1625 | 25 |
| | | 10 | 1620 | 1699 | 79 |
| | | Average | 1663 | 1684 | 21 |
| | 2 | 141 | 1498 | 1483 | -15 |
| | | 142 | 1809 | 1759 | -50 |
| | | 143 | 1820 | 1852 | 32 |
| | | 144 | 1375 | 1434 | 59 |
| | | 145 | 1649 | 1611 | -38 |
| | | 146 | 1596 | 1613 | 17 |
| | | 147 | 1724 | 1780 | 56 |
| | | 148 | 1514 | 1484 | -30 |
| | | 149 | 1741 | 1770 | 29 |
| | | 150 | 1555 | 1503 | -52 |
| | | Average | 1628 | 1629 | 1 |
| | 3 | 211 | 1621 | 1714 | 93 |
| | | 212 | 1623 | 1555 | -68 |
| | | 213 | 1799 | 1612 | -187 |
| | | 214 | 1615 | 1630 | 15 |
| | | 215 | 1659 | 1758 | 99 |
| | | 216 | 1887 | 1930 | 43 |
| | | 217 | 1965 | 1896 | -69 |
| | | 218 | 1539 | 1617 | 78 |
| | | 219 | 1497 | 1553 | 56 |
| | | 220 | 1543 | 1519 | -24 |
| | | Average | 1675 | 1678 | 4 |
| | Group average | | 1655 | 1664 | 9 |

**Appendix table2 Egg production rate (%)**

| DDGS inclusion level (%) | Replicate | Preliminary feeding period | Experimental period | | | | |
|---|---|---|---|---|---|---|---|
| | | | Week 1 | Week 2 | Week 3 | Week 4 | Throughout experiment |
| Control (0) | 1 | 97.1 | 100.0 | 100.0 | 98.6 | 97.1 | 98.9 |
| | 2 | 98.6 | 91.4 | 98.6 | 97.1 | 98.6 | 96.4 |
| | 3 | 98.6 | 95.7 | 97.1 | 98.6 | 100.0 | 97.9 |
| | Average | 98.1 | 95.7 | 98.6 | 98.1 | 98.6 | 97.7 |
| 10 | 1 | 96.4 | 91.4 | 98.6 | 98.6 | 98.6 | 96.8 |
| | 2 | 100.0 | 95.7 | 97.1 | 98.6 | 100.0 | 97.9 |
| | 3 | 96.0 | 98.4 | 100.0 | 96.8 | 98.4 | 98.8 |
| | Average | 97.5 | 95.2 | 98.6 | 98.0 | 99.0 | 97.8 |
| 20 | 1 | 98.6 | 98.6 | 95.7 | 98.6 | 95.7 | 97.1 |
| | 2 | 99.3 | 97.1 | 98.6 | 98.6 | 94.3 | 97.1 |
| | 3 | 95.0 | 98.6 | 94.3 | 94.3 | 95.7 | 95.7 |
| | Average | 97.6 | 98.1 | 96.2 | 97.2 | 95.2 | 96.6 |
| 30 | 1 | 99.3 | 92.9 | 98.6 | 94.3 | 94.3 | 95.0 |
| | 2 | 97.1 | 94.3 | 100.0 | 95.7 | 97.1 | 96.8 |
| | 3 | 98.6 | 95.7 | 95.7 | 98.6 | 88.6 | 94.6 |
| | Average | 98.3 | 94.3 | 98.1 | 96.2 | 93.3 | 95.5 |

**Appendix table3 Average egg weight (g/egg)**

| DDGS inclusion level (%) | Replicate | Preliminary feeding period | Experimental period | | | | |
|---|---|---|---|---|---|---|---|
| | | | Week 1 | Week 2 | Week 3 | Week 4 | Throughout experiment |
| Control (0) | 1 | 64.1 | 65.0 | 65.6 | 65.2 | 65.2 | 65.3 |
| | 2 | 62.5 | 63.3 | 64.1 | 63.2 | 63.4 | 63.5 |
| | 3 | 64.3 | 65.3 | 64.7 | 65.8 | 65.7 | 65.4 |
| | Average | 63.6 | 64.5 | 64.8 | 64.7 | 64.8 | 64.7 |
| 10 | 1 | 60.8 | 61.6 | 62.0 | 63.3 | 62.7 | 62.4 |
| | 2 | 65.3 | 66.2 | 65.5 | 65.8 | 66.8 | 66.1 |
| | 3 | 64.3 | 65.7 | 66.0 | 66.3 | 65.5 | 65.9 |
| | Average | 63.5 | 64.5 | 64.5 | 65.1 | 65.0 | 64.8 |
| 20 | 1 | 65.1 | 65.3 | 64.4 | 65.5 | 65.9 | 65.3 |
| | 2 | 63.3 | 63.8 | 64.8 | 64.6 | 64.6 | 64.5 |
| | 3 | 62.2 | 61.4 | 61.6 | 61.9 | 63.4 | 62.1 |
| | Average | 63.5 | 63.5 | 63.6 | 64.0 | 64.6 | 64.0 |
| 30 | 1 | 62.9 | 63.1 | 62.6 | 63.2 | 63.4 | 63.1 |
| | 2 | 63.3 | 62.5 | 63.7 | 63.6 | 64.0 | 63.5 |
| | 3 | 63.6 | 63.6 | 62.5 | 63.0 | 64.2 | 63.3 |
| | Average | 63.3 | 63.1 | 62.9 | 63.3 | 63.9 | 63.3 |

**Appendix table4 Daily egg production (g/hen/day)**

| DDGS inclusion level (%) | Replicate | Preliminary feeding period | Experimental period | | | | |
|---|---|---|---|---|---|---|---|
| | | | Week 1 | Week 2 | Week 3 | Week 4 | Throughout experiment |
| Control (0) | 1 | 62.2 | 65.0 | 65.6 | 64.3 | 63.3 | 64.6 |
| | 2 | 61.6 | 57.9 | 63.2 | 61.4 | 62.5 | 61.2 |
| | 3 | 63.4 | 62.5 | 62.8 | 64.9 | 65.7 | 64.0 |
| | Average | 62.4 | 61.8 | 63.9 | 63.5 | 63.8 | 63.3 |
| 10 | 1 | 58.6 | 56.3 | 61.1 | 62.4 | 61.8 | 60.4 |
| | 2 | 65.3 | 63.4 | 63.6 | 64.9 | 66.8 | 64.7 |
| | 3 | 61.7 | 64.6 | 66.0 | 64.2 | 64.5 | 65.1 |
| | Average | 61.9 | 61.4 | 63.6 | 63.8 | 64.4 | 63.4 |
| 20 | 1 | 64.2 | 64.4 | 61.6 | 64.6 | 63.1 | 63.4 |
| | 2 | 62.9 | 61.9 | 63.9 | 63.7 | 60.9 | 62.6 |
| | 3 | 59.1 | 60.5 | 58.1 | 58.4 | 60.7 | 59.4 |
| | Average | 62.1 | 62.3 | 61.2 | 62.2 | 61.6 | 61.8 |
| 30 | 1 | 62.5 | 58.6 | 61.7 | 59.6 | 59.8 | 59.9 |
| | 2 | 61.5 | 58.9 | 63.7 | 60.9 | 62.1 | 61.5 |
| | 3 | 62.7 | 60.9 | 59.8 | 62.1 | 56.9 | 59.9 |
| | Average | 62.2 | 59.5 | 61.7 | 60.9 | 59.6 | 60.4 |

**Appendix table5 Feed intake changes (g/hen/day)**

| DDGS inclusion level (%) | Replicate | Preliminary feeding period | Experimental period | | | | |
|---|---|---|---|---|---|---|---|
| | | | Week 1 | Week 2 | Week 3 | Week 4 | Throughout experiment |
| Control (0) | 1 | 116.0 | 119.0 | 113.2 | 114.0 | 113.7 | 115.0 |
| | 2 | 115.4 | 111.9 | 111.7 | 111.1 | 112.8 | 111.9 |
| | 3 | 114.5 | 117.6 | 118.0 | 110.5 | 117.4 | 115.9 |
| | Average | 115.3 | 116.2 | 114.3 | 111.9 | 114.6 | 114.3 |
| 10 | 1 | 102.5 | 111.0 | 111.6 | 109.6 | 112.6 | 111.2 |
| | 2 | 113.4 | 125.4 | 121.1 | 124.3 | 128.7 | 124.9 |
| | 3 | 104.5 | 118.2 | 114.7 | 102.7 | 116.8 | 113.1 |
| | Average | 106.8 | 118.2 | 115.8 | 112.2 | 119.4 | 116.4 |
| 20 | 1 | 119.2 | 126.8 | 122.1 | 125.8 | 123.5 | 124.6 |
| | 2 | 108.3 | 118.5 | 115.7 | 115.6 | 118.7 | 117.1 |
| | 3 | 102.8 | 115.9 | 115.6 | 113.4 | 113.4 | 114.6 |
| | Average | 110.1 | 120.4 | 117.8 | 118.3 | 118.5 | 118.8 |
| 30 | 1 | 113.4 | 119.3 | 118.3 | 115.8 | 117.0 | 117.6 |
| | 2 | 101.7 | 119.1 | 116.8 | 111.5 | 118.2 | 116.4 |
| | 3 | 104.5 | 115.6 | 116.8 | 114.5 | 115.7 | 115.7 |
| | Average | 106.5 | 118.0 | 117.3 | 113.9 | 117.0 | 116.6 |

**Appendix table6 Feed conversion rate changes**

| DDGS inclusion level (%) | Replicate | Preliminary feeding period | Experimental period | | | | |
|---|---|---|---|---|---|---|---|
| | | | Week 1 | Week 2 | Week 3 | Week 4 | Throughout experiment |
| Control (0) | 1 | 1.86 | 1.83 | 1.73 | 1.77 | 1.80 | 1.78 |
| | 2 | 1.87 | 1.93 | 1.77 | 1.81 | 1.80 | 1.83 |
| | 3 | 1.81 | 1.88 | 1.88 | 1.70 | 1.79 | 1.81 |
| | Average | 1.85 | 1.88 | 1.79 | 1.76 | 1.80 | 1.81 |
| 10 | 1 | 1.75 | 1.97 | 1.83 | 1.76 | 1.82 | 1.84 |
| | 2 | 1.74 | 1.98 | 1.90 | 1.92 | 1.93 | 1.93 |
| | 3 | 1.69 | 1.83 | 1.74 | 1.60 | 1.81 | 1.74 |
| | Average | 1.73 | 1.93 | 1.82 | 1.76 | 1.85 | 1.84 |
| 20 | 1 | 1.86 | 1.97 | 1.98 | 1.95 | 1.96 | 1.97 |
| | 2 | 1.72 | 1.91 | 1.81 | 1.81 | 1.95 | 1.87 |
| | 3 | 1.74 | 1.92 | 1.99 | 1.94 | 1.87 | 1.93 |
| | Average | 1.77 | 1.93 | 1.93 | 1.90 | 1.93 | 1.92 |
| 30 | 1 | 1.81 | 2.04 | 1.92 | 1.94 | 1.96 | 1.96 |
| | 2 | 1.65 | 2.02 | 1.83 | 1.83 | 1.90 | 1.89 |
| | 3 | 1.67 | 1.90 | 1.95 | 1.84 | 2.03 | 1.93 |
| | Average | 1.71 | 1.99 | 1.90 | 1.87 | 1.96 | 1.93 |

**Appendix table7 Yolk color value**

| DDGS inclusion level (%) | Hen number | Yolk color value (equivalent to RCF) |
|---|---|---|
| Control (0) | 34 | 6 |
| | 35 | 6 |
| | 36 | 6 |
| | 37 | 6 |
| | 38 | 6 |
| | 39 | 6 |
| | 40 | 5 |
| | 41 | 5 |
| | 42 | 5 |
| | 43 | 6 |
| | Average | 5.7 |
| 10 | 23 | 7 |
| | 24 | 7 |
| | 25 | 6 |
| | 26 | 5 |
| | 27 | 6 |
| | 28 | 6 |
| | 29 | 7 |
| | 30 | 6 |
| | 31 | 6 |
| | 32 | 6 |
| | Average | 6.2 |
| 20 | 12 | 7 |
| | 13 | 7 |
| | 14 | 7 |
| | 15 | 7 |
| | 16 | 7 |
| | 17 | 6 |
| | 18 | 7 |
| | 19 | 7 |
| | 20 | 7 |
| | 21 | 7 |
| | Average | 6.9 |
| 30 | 1 | 7 |
| | 2 | 8 |
| | 3 | 8 |
| | 4 | 7 |
| | 5 | 8 |
| | 6 | 6 |
| | 7 | 8 |
| | 8 | 8 |
| | 9 | 6 |
| | 10 | 7 |
| | Average | 7.3 |

**Appendix table8-1 Concentrations of ammonia and hydrogen sulfide from manure and manure pH (Week 1)**

| DDGS inclusion level (%) | Replicate | Ammonia (ppm) | | | Hydrogen sulfide (ppm) | | | pH (After 48 hrs.) |
|---|---|---|---|---|---|---|---|---|
| | | After 12 hrs. | After 24 hrs. | After 48 hrs. | After 12 hrs. | After 24 hrs. | After 48 hrs. | |
| Control (0) | 1 | < 0.5 | < 0.5 | < 0.5 | 0.5 | 0.5 | 3.7 | 6.43 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 0.5 | 0.5 | 6.0 | 6.50 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 0.5 | 0.3 | 1.0 | 6.36 |
| | Average | < 0.5 | < 0.5 | < 0.5 | 0.5 | 0.4 | 3.6 | 6.43 |
| 10 | 1 | < 0.5 | < 0.5 | < 0.5 | 1.2 | 1.3 | 6.3 | 6.32 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 1.6 | 1.7 | 5.4 | 6.07 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 1.0 | 1.0 | 3.0 | 6.66 |
| | Average | < 0.5 | < 0.5 | < 0.5 | 1.3 | 1.3 | 4.9 | 6.35 |
| 20 | 1 | < 0.5 | < 0.5 | < 0.5 | 1.0 | 1.0 | 2.3 | 6.14 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 1.0 | 1.0 | 1.8 | 5.92 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 1.9 | 2.0 | 4.2 | 6.00 |
| | Average | < 0.5 | < 0.5 | < 0.5 | 1.3 | 1.3 | 2.8 | 6.02 |
| 30 | 1 | < 0.5 | < 0.5 | < 0.5 | 1.0 | 1.0 | 3.7 | 6.06 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 2.0 | 1.7 | 3.6 | 6.02 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 1.0 | 1.0 | 2.0 | 5.84 |
| | Average | < 0.5 | < 0.5 | < 0.5 | 1.3 | 1.2 | 3.1 | 5.97 |

Note) Detection limit of ammonia measurement: 0.5 ppm

**Appendix table8-2 Concentrations of ammonia and hydrogen sulfide from manure and manure pH (Week 2)**

| DDGS inclusion level (%) | Replicate | Ammonia (ppm) | | | Hydrogen sulfide (ppm) | | | pH (After 48 hrs.) |
|---|---|---|---|---|---|---|---|---|
| | | After 12 hrs. | After 24 hrs. | After 48 hrs. | After 12 hrs. | After 24 hrs. | After 48 hrs. | |
| Control (0) | 1 | < 0.5 | < 0.5 | < 0.5 | 1.0 | 0.3 | 0.3 | 6.42 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 0.7 | 0.2 | 0.2 | 6.37 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 1.7 | 0.9 | 1.7 | 6.46 |
| | Average | < 0.5 | < 0.5 | < 0.5 | 1.1 | 0.5 | 0.7 | 6.42 |
| 10 | 1 | < 0.5 | < 0.5 | < 0.5 | 1.0 | 0.5 | 0.6 | 6.56 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 1.2 | 0.7 | 0.5 | 6.53 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 2.2 | 1.0 | 1.0 | 6.65 |
| | Average | < 0.5 | < 0.5 | < 0.5 | 1.5 | 0.7 | 0.7 | 6.58 |
| 20 | 1 | < 0.5 | < 0.5 | < 0.5 | 1.4 | 0.5 | 0.5 | 6.17 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 1.2 | 0.4 | 0.3 | 6.42 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 1.1 | 0.7 | 0.8 | 6.42 |
| | Average | < 0.5 | < 0.5 | < 0.5 | 1.2 | 0.5 | 0.5 | 6.34 |
| 30 | 1 | < 0.5 | < 0.5 | < 0.5 | 1.6 | 0.8 | 1.2 | 6.04 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 1.3 | 0.5 | 0.5 | 5.98 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 1.1 | 0.4 | 0.5 | 6.21 |
| | Average | < 0.5 | < 0.5 | < 0.5 | 1.3 | 0.6 | 0.7 | 6.08 |

Note) Detection limit of ammonia measurement: 0.5 ppm

**Appendix table8-3 Concentrations of ammonia and hydrogen sulfide from manure and manure pH (Week 4)**

| DDGS inclusion level (%) | Replicate | Ammonia (ppm) | | | Hydrogen sulfide (ppm) | | | pH (After 48 hrs.) |
|---|---|---|---|---|---|---|---|---|
| | | After 12 hrs. | After 24 hrs. | After 48 hrs. | After 12 hrs. | After 24 hrs. | After 48 hrs. | |
| Control (0) | 1 | < 0.5 | < 0.5 | < 0.5 | 6.5 | 57.5 | 65.0 | 6.86 |
| | 2 | < 0.5 | < 0.5 | 0.9 | 25.0 | 190.0 | 150.0 | 6.41 |
| | 3 | < 0.5 | 0.5 | < 0.5 | 10.0 | 25.0 | 15.0 | 6.65 |
| | Average | < 0.5 | < 0.5~ 0.5 | < 0.5~ 0.9 | 13.8 | 90.8 | 76.7 | 6.64 |
| 10 | 1 | < 0.5 | < 0.5 | < 0.5 | 8.0 | 25.0 | 45.0 | 6.24 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 7.0 | 40.0 | 100.0 | 6.35 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 10.5 | 50.0 | 150.0 | 6.35 |
| | Average | < 0.5 | < 0.5 | < 0.5 | 8.5 | 38.3 | 98.3 | 6.31 |
| 20 | 1 | < 0.5 | < 0.5 | < 0.5 | 8.8 | 35.0 | 92.5 | 6.16 |
| | 2 | < 0.5 | 0.5 | 0.5 | 7.0 | 16.0 | 15.5 | 6.15 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 10.5 | 35.0 | 100.0 | 6.11 |
| | Average | < 0.5 | < 0.5~ 0.5 | < 0.5~ 0.5 | 8.8 | 28.7 | 69.3 | 6.14 |
| 30 | 1 | < 0.5 | < 0.5 | 0.5 | 7.5 | 25.0 | 25.0 | 5.73 |
| | 2 | < 0.5 | < 0.5 | < 0.5 | 13.5 | 40.0 | 50.0 | 5.66 |
| | 3 | < 0.5 | < 0.5 | < 0.5 | 9.0 | 35.0 | 100.0 | 5.85 |
| | Average | < 0.5 | < 0.5 | < 0.5~ 0.5 | 10.0 | 33.3 | 58.3 | 5.75 |

Note) Detection limit of ammonia measurement: 0.5 ppm

**Appendix table9-1 Composition of manure; nitrogen and dry matter excretion rates (Week 1)**

| DDGS inclusion level (%) | Replicate | Water content (%) | N (DM, %) | $Cr_2O_3$ (DM, %) | N excretion rate (%) | DM excretion rate (%) |
|---|---|---|---|---|---|---|
| Control (0) | Feed | - | 2.91 | 0.105 | - | - |
| | Manure, Group 1 | 78.7 | 5.68 | 0.415 | 49.4 | 25.3 |
| | Manure, Group 2 | 78.2 | 5.48 | 0.395 | 50.1 | 26.6 |
| | Manure, Group 3 | 77.8 | 6.34 | 0.386 | 59.3 | 27.2 |
| | Average | 78.2 | - | - | 52.9 | 26.4 |
| 10 | Feed | - | 2.96 | 0.103 | - | - |
| | Manure, Group 1 | 78.6 | 5.78 | 0.373 | 53.9 | 28.2 |
| | Manure, Group 2 | 78.6 | 6.25 | 0.365 | 59.6 | 28.8 |
| | Manure, Group 3 | 76.7 | 5.85 | 0.389 | 52.3 | 27.0 |
| | Average | 78.0 | - | - | 55.3 | 28.0 |
| 20 | Feed | - | 2.95 | 0.105 | - | - |
| | Manure, Group 1 | 76.9 | 5.82 | 0.326 | 63.5 | 32.2 |
| | Manure, Group 2 | 76.8 | 5.68 | 0.327 | 61.8 | 32.1 |
| | Manure, Group 3 | 75.6 | 5.63 | 0.318 | 63.0 | 33.0 |
| | Average | 76.4 | - | - | 62.8 | 32.4 |
| 30 | Feed | - | 2.97 | 0.107 | - | - |
| | Manure, Group 1 | 79.2 | 5.44 | 0.288 | 68.1 | 36.5 |
| | Manure, Group 2 | 83.5 | 5.24 | 0.293 | 64.4 | 35.8 |
| | Manure, Group 3 | 77.1 | 5.24 | 0.281 | 67.2 | 37.4 |
| | Average | 79.9 | - | - | 66.6 | 36.6 |

**Appendix table9-2 Composition of manure; nitrogen and dry matter excretion rates (Week 2)**

| DDGS inclusion level (%) | Replicate | Water content (%) | N (DM, %) | $Cr_2O_3$ (DM, %) | N excretion rate (%) | DM excretion rate (%) |
|---|---|---|---|---|---|---|
| Control (0) | Feed | - | 2.91 | 0.105 | - | - |
| | Manure, Group 1 | 78.0 | 6.05 | 0.378 | 57.8 | 27.8 |
| | Manure, Group 2 | 78.2 | 5.57 | 0.408 | 49.3 | 25.7 |
| | Manure, Group 3 | 78.0 | 5.89 | 0.385 | 55.2 | 27.3 |
| | Average | 78.1 | - | - | 54.1 | 26.9 |
| 10 | Feed | - | 2.96 | 0.103 | - | - |
| | Manure, Group 1 | 79.0 | 5.93 | 0.322 | 64.1 | 32.6 |
| | Manure, Group 2 | 77.4 | 6.07 | 0.334 | 63.2 | 31.4 |
| | Manure, Group 3 | 76.8 | 5.44 | 0.356 | 53.2 | 29.5 |
| | Average | 77.7 | - | - | 60.2 | 31.2 |
| 20 | Feed | - | 2.95 | 0.105 | - | - |
| | Manure, Group 1 | 75.5 | 6.02 | 0.318 | 67.4 | 33.0 |
| | Manure, Group 2 | 76.4 | 5.69 | 0.323 | 62.7 | 32.5 |
| | Manure, Group 3 | 76.8 | 5.14 | 0.333 | 54.9 | 31.5 |
| | Average | 76.2 | - | - | 61.7 | 32.3 |
| 30 | Feed | - | 2.97 | 0.107 | - | - |
| | Manure, Group 1 | 75.7 | 5.02 | 0.302 | 59.9 | 34.8 |
| | Manure, Group 2 | 78.1 | 5.14 | 0.295 | 62.8 | 35.6 |
| | Manure, Group 3 | 76.1 | 5.36 | 0.282 | 68.5 | 37.2 |
| | Average | 76.6 | - | - | 63.7 | 35.9 |

**Appendix table9-3 Water content in manure; nitrogen and dry matter excretion rates (Week 4)**

| DDGS inclusion level (%) | Replicate | Water content (%) | N (DM, %) | $Cr_2O_3$ (DM, %) | N excretion rate (%) | DM excretion rate (%) |
|---|---|---|---|---|---|---|
| Control (0) | Feed | - | 2.91 | 0.105 | - | - |
| | Manure, Group 1 | 78.1 | 5.91 | 0.386 | 55.2 | 27.2 |
| | Manure, Group 2 | 79.4 | 5.39 | 0.410 | 47.4 | 25.6 |
| | Manure, Group 3 | 78.8 | 5.85 | 0.383 | 55.1 | 27.4 |
| | Average | 78.8 | - | - | 52.6 | 26.7 |
| 10 | Feed | - | 2.96 | 0.103 | - | - |
| | Manure, Group 1 | 79.2 | 5.42 | 0.367 | 51.4 | 28.6 |
| | Manure, Group 2 | 77.0 | 5.64 | 0.379 | 51.8 | 27.7 |
| | Manure, Group 3 | 78.0 | 6.07 | 0.380 | 55.6 | 27.6 |
| | Average | 78.1 | - | - | 52.9 | 28.0 |
| 20 | Feed | - | 2.95 | 0.105 | - | - |
| | Manure, Group 1 | 76.4 | 5.79 | 0.343 | 60.1 | 30.6 |
| | Manure, Group 2 | 76.5 | 6.03 | 0.350 | 61.3 | 30.0 |
| | Manure, Group 3 | 76.5 | 5.37 | 0.338 | 56.5 | 31.1 |
| | Average | 76.5 | - | - | 59.3 | 30.6 |
| 30 | Feed | - | 2.97 | 0.107 | - | - |
| | Manure, Group 1 | 76.8 | 5.18 | 0.306 | 61.0 | 34.3 |
| | Manure, Group 2 | 76.5 | 5.07 | 0.318 | 57.4 | 33.0 |
| | Manure, Group 3 | 75.5 | 5.26 | 0.321 | 59.0 | 32.7 |
| | Average | 76.3 | - | - | 59.1 | 33.3 |