Agriculture and Natural Resources 50 (2016) 400–407

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Responses of laying hens to induce molting procedures using cassava meal of variable length with or without recovery period

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ARTICLE INFO

Article history:
Received 5 May 2015
Accepted 14 June 2016
Available online 24 December 2016

Keywords:
Albumen quality
Cassava
Eggshell ultrastructure
Laying hen
Molting

ABSTRACT

The effects were determined of varying the length of the recovery period on postmolt performance in laying hens induced to molt by cassava meal. The hens were randomly assigned to one of five groups: group 1 (NC), the control group, whereas groups 2 (IM_0), 3 (IM_1), 4 (IM_2) and 5 (IM_3) were subjected to an induced molt program. The procedure included a 14 d stress period, during which the birds were induced to molt with cassava meal, and recovery periods varying from 0 wk to 3 wk. Hens in the IM_0, IM_1, IM_2 and IM_3 groups had recovery periods of 0 wk, 1 wk, 2 wk and 3 wk, respectively, during which they were fed a pullet developer diet. During the early postmolt period, the egg production of the IM_0 group was significantly lower than that of the control group. The IM_1 hens consumed significantly more feed and had a significantly higher feed conversion ratio than the control birds. The albumen height was significantly improved by the IM_0 and IM_1 treatments. No consistent differences were observed among treatments for any parameter of postmolt performances, except for improved albumen weight and mortality rate, which were observed in the IM_3 group. Scanning electron microscopy illustrated evidence of erosion in the mammillary layer of the shell in the control hens. The density of the mammillary knobs in all molted groups was significantly lower than that of the control group. The mammillary knob diameter of the IM_2 group was significantly greater than that of the control group.

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Introduction

Induced molting of hens, when appropriately done, can provide a way of recycling old hens by improving the rate of egg production, albumen quality and eggshell quality during the postmolt period (as reviewed by Berry, 2003). The degree of improvement in postmolt productive performance is associated with an increase in the induced molting period (Koelkebeck et al., 1992). Much of the research efforts in the past have been focused on the effect of induced molt on the performance of hens by comparing the data collected before and after induced molt as well as the comparative performance of hens molted by different methods (Gilbert and Blair, 1975; Rolon et al., 1993; Mejia et al., 2011).

The most common procedure of induced molt is based on a short period of fasting, which results in cessation of egg production, regression of the reproductive tract and loss of primary feathers. The feed removal phase (a stress period) is followed by a variable-length "recovery" period during which the birds are kept on a regimen of restricted protein or total feed, which allows the maintenance of body weight but not resumption of egg laying (as reviewed by Brake, 1993). In recent years, induced molting using the fasting method has become the target of vigorous criticism with regard to animal welfare (Keshawarz and Quimby, 2002). Therefore, alternative methods for molt induction that avoid feed withdrawal have been investigated including: feeding a low-Na diet and high-dietary Zn (Berry and Brake, 1985), low-Ca diets (Breeding et al., 1992) or high-fiber and low-energy diets (Woodward et al., 2005). Other nonfasting molt methods include the feeding of wheat middlings (Biggs et al., 2003), a combination of wheat middlings and corn (Mazzuco and Hester, 2005), various ratios of alfalfa or layer ration (Donalson et al., 2005), a whole-grain barley diet (Onbasilar and Erol, 2007), a combination of soy-hulls based diet and corn (Mazzuco et al., 2011) or feeding broken rice, rice bran or cassava meal for a short period (Gongruttananun et al., 2013).

An ideal molting method should be simple to apply, be low in cost, result in low mortality and lead to high subsequent performance. Cassava (*Manihot esculenta*) is a woody shrub of the *Euphorbiaceae* family, native to South America. It has been grown extensively as an important economic root crop in Southeast Asia,

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http://dx.doi.org/10.1016/j.anres.2016.06.008
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tropical Africa and Central America (as reviewed by Chauynarong et al., 2009). The abundant availability of cassava in certain regions and its low cost make it a good alternative to maize and other cereal grains for feeding livestock and poultry. Cassava root meal contains a range of metabolizable energy (ME) values for poultry from 2.87 kcal ME/g to 4.27 kcal ME/g (Maust et al., 1972), very low levels of protein, 2.5% crude protein, and is deficient in essential amino acids, fat, minerals and vitamins (Muller et al., 1974). An important limitation of using cassava as animal feed is that fresh cassava roots contain cyanogenic-glycosides, which become hydrolyzed to hydrocyanic acid (prussic acid) when the root is cut into pieces. The hydrocyanic acid depresses poultry performance and feed consumption due to palatability problems (Vogt, 1966). Khajarern et al. (1982) observed a reduction of the hydrocyanic acid content from 111.63 ppm to 22.97 ppm after sun drying of cassava roots for 6 d. Gomez et al. (1984) reported that after sun drying, more than 86% of hydrocyanic acid present in cassava root meal was lost due to the evaporation of free cyanide at a temperature of 28 °C. Panigrahi et al. (1992) reported that cassava root meal with a total level of cyanide content less than 40 ppm could be fed to chickens without any adverse effects. Gongruttananun et al. (2013) reported that full feeding of aged laying hens with cassava meal for 2 wk without the recovery period was an effective method for the induction of molt with advantages of improved albumen quality. However, the investigators did not find any improvements in egg production or eggshell quality during the subsequent postmolt laying period. Hurwitz et al. (1995) suggested that maximal improvements in postmolt egg production and egg quality could be achieved when the birds had a recovery period of 14–21 d. Information regarding the effects of induced molting using cassava meal on postmolt productive performance and the structure of the eggshell of laying hens is limited. Therefore, the present study was undertaken to determine the effects of feeding cassava meal as a molt diet followed by a variable-length recovery period on the postmolt productive performance and eggshell ultrastructure in laying hens.

Materials and methods

Experimental birds and molt procedure

All animal care procedures were approved by the Animal Ethics Committee of Kasetsart University, Thailand. An experiment was conducted using 240 H&N Brown hens (aged 94 wk). The hens were housed in a caged layer house of commercial design, with water and feed provided for *ad libitum* consumption, and the hens were exposed to a light (L) to darkness (D) photoperiod of 16L:8D daily before the start of the experiment. The feed was a commercial layer diet. The mean temperature of the house was 23.7 °C, and the mean light intensity was 3.9 lux. Four replicate groups of 12 hens each (four adjacent cages containing three hens per cage, cage size 40 cm × 45 cm) were allotted to five treatments in a completely randomized design. Treatment 1 (NC) was the non-molt control group, whereas treatments 2 (IM_0), 3 (IM_1), 4 (IM_2) and 5 (IM_3) were induced to molt for 2 weeks and had recovery periods of 0–3 wk, respectively. Birds were weighed and allocated to each replicate to achieve a similar mean body weight for each treatment. Egg production, egg weight and egg quality were measured for 2 wk (age 94–96 wk) in an attempt to keep a similar distribution of production rate, egg weight and egg quality among the experimental treatments.

At age 96 wk, birds in groups IM_0, IM_1, IM_2 and IM_3 were moved carefully into a windowless molting house equipped with mechanical ventilation. The mean temperature of the house was 28.1 °C, and the mean light intensity was 3.5 lux. Birds in each replicate of the molted treatments were housed together in one of the 16 pens located in the molting house. Each pen was 2.9 m × 3.0 m × 2.9 m (width × length × height). The induced molt period was divided into a 2 wk "stress period" and a 0 wk, 1 wk, 2 wk or 3 wk "recovery period". During the 2 wk stress period, hens in all molted groups were provided with cassava meal and drinking water at all times. At the end of the stress period, the birds were weighed and body weight loss was calculated. Thereafter, birds in the IM_0, IM_1, IM_2 and IM_3 groups had recovery periods 0 wk, 1 wk, 2 wk and 3 wk, respectively, during which they were provided with a pullet developer diet and drinking water at all times. At the end of each recovery period, the birds were weighed and moved to their original house, where the control group was being kept, and maintained under the same management regime throughout the experimental period. Egg production and mortality were recorded daily throughout the induced molt period.

Photoperiod during the induced molt period was 8L:16D instead of the usual 16L:8D per day. The molting procedure is shown in Table 1. Birds in the control group were weighed at ages 96 wk, 98 wk, 99 wk, 100 wk and 101 wk. The cassava molt diet was processed as follows: the fresh cassava roots were peeled, washed, chipped into small pieces, and sun-dried on a concrete floor for 7 d at a 31.4 °C average temperature; thereafter, the dried cassava chips were ground in a hammer mill into powdered cassava root meal. Proximate composition analysis of the experimental diets was performed using the procedure of Association of Official Analytical Chemists (1990). The ingredient composition and nutrient analyses of the molt diets are given in Table 2.

Post-molt productive performance and egg quality

Egg production was recorded daily and expressed as a percentage of hen-day egg production on a weekly basis for the 16 wk

Table 1
Molting procedure used in the experiment.

Treatment*	Feed (*ad libitum*)	Water	Light (h/d)	Recovery period (wk)
NC	Control—hens not induced to molt, on a layer diet under a lighting program of 16 light (L):8 darkness (D)	Provided	16	Throughout the experimental period
IM_0	Cassava meal offered for 2 wk, then returned to a layer diet and a lighting program of 16L:8D	Provided	8	0
IM_1	Cassava meal offered for 2 wk and a pullet developer diet for another 1 wk, then returned to a layer diet and a lighting program of 16L:8D	Provided	8	1
IM_2	Cassava meal offered for 2 wk and a pullet developer diet for another 2 wk, then returned to a layer diet and a lighting program of 16L:8D	Provided	8	2
IM_3	Cassava meal offered for 2 wk and a pullet developer diet for another 3 wk, then returned to a layer diet and a lighting program of 16L:8D	Provided	8	3

* NC = non-molt control; IM_0 = induced molt with cassava meal for 2 wk without a recovery period; IM_1 = induced molt with cassava meal for 2 wk and a recovery period for 1 wk; IM_2 = induced molt with cassava meal for 2 wk and a recovery period for 2 wk; IM_3 = induced molt with cassava meal for 2 wk and a recovery period for 3 wk.

Table 2
Ingredient and nutrient composition of experimental diets.

Item	Cassava molt diet	Commercial pullet developer diet	Commercial layer diet
Ingredient			
Cassava mash (%)	100		
Chemical analysis			
Gross energy (kcal/kg)	3305	3881	3780
Crude protein (%)	2.04	15.88	20.79
Crude fiber (%)	5.11	4.05	2.50
Calcium (%)	0.46	1.45	3.68
Total phosphorus (%)	0.009	0.11	0.09

of the postmolt period. Eggs from each replicate laid on three consecutive days in each week were weighed and the average was calculated for each replicate. The weekly feed intake and feed conversion ratio were calculated during the postmolt period. All eggs from each replicate laid on the last day of the week were collected in each 2 wk period and measured for egg and eggshell quality. Shell strength was measured using an Eggshell Destruction Strength Meter (Model EFG-0503; Robotmation Co., Ltd.; Tokyo, Japan). Thereafter, the eggs were broken at the equatorial region and the interior contents were allowed to drain out. The internal quality of eggs was assessed according to albumen height and Haugh units using specialized equipment (Technical Services and Supplies; York, UK.). The yolk weight was determined after it was separated from the adhering albumen and then weighed on an electrical balance (Model PB 1501; Mettler-Toledo; OH, USA.). The eggshell along with membranes was washed with tap water and dried at room temperature (around 28 °C) for 1 wk. After drying, the eggshell was weighed and the shell thickness was measured in millimeters using a digimatic micrometer (Mitutoyo Corporation; Kanagawa, Japan). Three measurements were taken on the equatorial region of each eggshell; the mean of three measurements was calculated. The albumen weight was determined by subtracting the yolk plus shell weight from the total egg weight. The daily mortality rate was recorded throughout the postmolt period.

Preparation of samples for eggshell ultrastructural examination

At the termination of the trial, at age 114 wk, two eggs of each replicate laid on the same day were randomly collected to examine the fine structure of the eggshell. The selected eggs were broken, the interior contents were removed and the shells were cleaned with tap water. The specimens were prepared by cutting a piece (1 cm^2) of shell from the equatorial region of each egg. The shell membranes were carefully removed by soaking in tap water. The loosely adhering membranes were peeled from the edge of the sample inwards. The samples were then soaked in 1.0N NaOH for 72 h according to the method of Kaplan and Siegesmund (1973). The purpose of this treatment with a base was to eliminate any proteinous materials incorporated in the shell. Thereafter, the samples were rinsed in distilled water and left to dry at room temperature. All samples were mounted inner side uppermost on aluminum stubs and coated with gold using an ion coater (Engineering IB-2, Eiko Engineering Co. Ltd; Ibaraki, Japan) for direct observation using a scanning electron microscope (JSM-5600LV; JEOL Ltd.; Oxford, UK) operated at 10 kV, at magnifications of ×100 and ×200. The incidence of ultrastructural variants at the level of the mammillary layer was assessed according to the method and terminology developed by the Poultry Research Unit, University of Glasgow, UK (Solomon, 1991). Photographs of replica surfaces were made to facilitate counting the number of mammillary knobs per unit eggshell interior surface area. The density of mammillae of each shell was expressed as the number of knobs per unit. The average diameter of the mammillary knobs was estimated from the measured mammillary knob density, assuming regular circular geometry according to the method of Van Toledo et al. (1982).

Statistical analysis

The experiment was conducted as a completely randomized design with five treatments. Data were analyzed using the statistical software package SAS, version 9.0 (SAS Institute, 2002). The GLM procedure was used to analyze the effect of the induced molt period on productive performance, egg and eggshell quality and the ultrastructure of the eggshell. The arcsine transformation was used for all percentage data. When the means of the GLM procedure were statistically different, these means were further compared between the control and the experimental groups using Duncan's multiple range test. Significance was based on $p < 0.05$. The experimental unit was a group of 12 hens for all traits studied. For the determination of the morphology of the eggshell, only two samples of shell per replicate were used. Data were presented as means and the pooled standard error of the mean (SEM).

Results and discussion

Performance and egg production during the molt period

The data collected during the premolt period (age 94–96 wk) are listed in Table 3 for reference. There were no significant differences in any of the traits measured among the treatment groups, which indicated that the birds had been adequately randomized. During the induced molt periods, birds in the IM_0, IM_1, IM_2 and IM_3 treatments maintained significantly low levels of egg production (Table 4). Egg production dropped to zero on the 7th day of induced molt in each molted treatment (data not shown). Cessation of egg production within 4–7 d following forced molt has been reported previously (Nordstrom, 1980; Lee, 1982; Gongruttananun et al., 2013). As expected, birds in the IM_0 treatment were significantly lighter in body weight than the control birds at the end of the molting period. Body weights of birds in the IM_1, IM_2 and IM_3 were similar to those of the control birds as a result of birds in these molted groups having had recovery periods, after the stress period, by feeding with a pullet developer diet. The results indicated that feeding with nourishment during the recovery period is necessary for molted hens in order to recover the body weight loss that has occurred during the stress period. In agreement with previous studies (Lee, 1982; Gongruttananun et al., 2013), mortality during the induced molt period of non-fasting methods was unaffected by molt treatments.

Postmolt productive performance

Egg production data collected during the first 4 wk postmolt period showed that only hens in the IM_0 group laid significantly fewer eggs on a hen-day basis than birds in the control group (Table 5). It is possible that the birds in the IM_0 treatment were stimulated too early to lay when they were not able to replenish their loss body store and, consequently, they returned to egg production at a slower rate than hens under the other molt treatments. During the early phase of postmolt (age 99–103 wk), birds in the IM_1 group consumed significantly more feed per bird per day and had a significantly higher feed conversion ratio than birds in the control group, which suggested that hens may need more than a 1 wk recovery period to replenish their loss body stores completely. The albumen height, expressed as Haugh units, was improved by all molted treatments compared to the control group (Table 6).

Table 3
Performance of the experimental hens during the premolt period (age 94–96 wk).

Treatment*	Body weight (kg)	Hen-day egg production (%)	Egg weight (g)	Yolk weight (%)	Albumen weight (%)	Albumen height (HU†)	Shell weight (%)	Shell thickness (mm)
NC	1.80	54.6	65.6	26.3	64.2	83.5	9.5	0.316
IM_0	1.83	50.0	64.7	26.0	64.1	81.7	9.7	0.301
IM_1	1.81	52.5	66.2	25.0	65.4	81.6	9.6	0.312
IM_2	1.78	52.5	65.2	26.1	64.3	78.0	9.3	0.300
IM_3	1.81	56.1	65.4	25.3	65.4	87.1	9.1	0.294
SEM‡	0.05	10.0	2.5	0.9	1.1	5.7	0.4	0.023
p-value	0.81	0.92	0.93	0.29	0.23	0.31	0.39	0.64

* NC = non-molt control; IM_0 = induced molt with cassava meal for 2 wk without a recovery period; IM_1 = induced molt with cassava meal for 2 wk and a recovery period for 1 wk; IM_2 = induced molt with cassava meal for 2 wk and a recovery period for 2 wk; IM_3 = induced molt with cassava meal for 2 wk and a recovery period for 3 wk.
† HU = Haugh units.
‡ SEM = pooled standard error of the mean (4 replicates of 12 hens each per treatment).

Table 4
Performance of the experimental hens during the molt period.

Treatment*	Body weight† (kg)	Hen-day egg production (%)	Mortality rate (%)
Age 96–98 wk			
NC	1.806[a‡]	62.52[a]	6.24
IM_0	1.440[b]	2.42[b]	7.68
Pooled SEM§	0.061	4.51	6.82
Age 96–99 wk			
NC	1.804	62.31[m]	6.24
IM_1	1.728	1.75[n]	7.68
Pooled SEM	0.111	3.75	6.82
Age 96–100 wk			
NC	1.862	61.73[p]	10.41
IM_2	1.732	2.08[q]	9.61
Pooled SEM	0.120	4.95	4.58
Age 96–101 wk			
NC	1.890	61.04[x]	12.24
IM_3	1.780	2.29[y]	9.61
Pooled SEM	0.015	5.47	4.78

* NC = non-molt control; IM_0 = induced molt with cassava meal for 2 wk without a recovery period; IM_1 = induced molt with cassava meal for 2 wk and a recovery period for 1 wk; IM_2 = induced molt with cassava meal for 2 wk and a recovery period for 2 wk; IM_3 = induced molt with cassava meal for 2 wk and a recovery period for 3 wk.
† Measured at the end of each induced molt period.
‡ [a,b,m,n,p,q,x,y] means within a column within an age group range with different lowercase superscripts are significantly different ($p < 0.05$).
§ Pooled standard error of mean.

However, these differences were not statistically significant for the IM_2 and IM_3 groups due to variations within treatments.

Due to the differences in the length of the induced molt periods, direct comparison of data among all treatments was possible only after age 101 wk. During the postmolt period (age 101–114 wk), hens in all molted groups tended to produce more eggs and had better feed efficiency than hens in the control group (Table 7). The average values of albumen height, measured seven times during this phase, of all molted treatments were numerically higher than that of the control treatment (Table 8). There were no significant differences in the shell weight, shell thickness or shell strength among the treatment groups throughout the postmolt period. Body weight loss is a major factor correlating with successful results of an induced molting procedure (Baker et al., 1983; Herremans et al., 1988). Baker and Brake (1981) reported that a body weight loss of 30% was needed for optimum 20 wk postmolt performance. In the present study, during the 2 wk stress period, the body weight loss of hens in all molted treatments varied from 21.3% to 21.8% (data not shown). This may explain the general lack of treatment effect on the egg production and eggshell quality during the postmolt period. Progressive improvement in egg production, albumen quality and eggshell quality, compared to nonmolted hens, with increasing severity of the molting treatment was reported by some investigators (Koelkebeck et al., 1992). However, during the postmolt period, the IM_3 treatment was the only treatment that had a significant improvement in the albumen weight and mortality rate

Table 5
Effects of induced molt period on the first 4 wk postmolt laying performance and mortality rate.

Treatment*	Feed consumption (g/hen/day)	Egg production (%)	Egg weight (g)	Feed conversion ratio (g feed/g egg weight)	Mortality rate (%)
Age 98–102 wk					
NC	115.2	60.0[a†]	67.6	2.89	6.2
IM_0	125.0	38.9[b]	67.3	5.52	2.2
Pooled SEM‡	8.3	10.8	1.9	2.01	6.4
Age 99–103 wk					
NC	113.0[n]	59.5	67.5	2.88[n]	4.7
IM_1	124.1[m]	57.0	65.4	4.95[m]	10.0
Pooled SEM	5.5	7.7	2.1	0.89	3.9
Age 100–104 wk					
NC	114.3	60.9	66.9	2.87	7.2
IM_2	113.5	51.9	66.8	3.68	7.0
Pooled SEM	5.1	11.1	1.7	0.83	4.7
Age 101–105 wk					
NC	116.2	63.0	66.5	2.83	9.5[x]
IM_3	113.9	53.6	67.5	3.74	0.0[y]
Pooled SEM	2.9	8.1	2.1	0.55	0.3

* NC = non-molt control; IM_0 = induced molt with cassava meal for 2 wk without a recovery period; IM_1 = induced molt with cassava meal for 2 wk and a recovery period for 1 wk; IM_2 = induced molt with cassava meal for 2 wk and a recovery period for 2 wk; IM_3 = induced molt with cassava meal for 2 wk and a recovery period for 3 wk.
† [a,b,m,n,x,y] means within a column within an age group range with different lowercase superscripts are significantly different ($p < 0.05$).
‡ Pooled standard error of mean.

Table 6
Effects of induced molt period on egg quality during the first 4 wk postmolt laying period.

Treatment*	Yolk weight (%)	Albumen weight (%)	Albumen height (HU†)	Shell weight (%)	Shell thickness (mm)	Shell strength (N)
Age 98–102 wk						
NC	25.7	64.3	78.7[b,‡]	9.5	0.335	34.8
IM_0	25.6	65.2	86.0[a]	9.1	0.319	36.6
Pooled SEM[§]	0.6	0.8	4.1	0.1	0.114	3.3
Age 99–103 wk						
NC	25.4	64.7	77.8[n]	9.5	0.335	36.2
IM_1	25.9	64.4	86.4[m]	9.5	0.327	38.2
Pooled SEM	0.4	0.3	4.0	0.1	0.013	3.5
Age 100–104 wk						
NC	25.1	65.2	74.9	9.5	0.335	37.0
IM_2	25.2	65.2	84.5	9.5	0.334	38.7
Pooled SEM	0.6	0.8	7.0	0.3	0.015	6.3
Age 101–105 wk						
NC	25.2[x]	65.1[y]	76.6	9.5	0.337	35.9
IM_3	24.1[y]	66.2[x]	80.9	9.5	0.334	37.6
Pooled SEM	0.3	0.5	6.1	0.3	0.013	2.8

* NC = non-molt control; IM_0 = induced molt with cassava meal for 2 wk without a recovery period; IM_1 = induced molt with cassava meal for 2 wk and a recovery period for 1 wk; IM_2 = induced molt with cassava meal for 2 wk and a recovery period for 2 wk; IM_3 = induced molt with cassava meal for 2 wk and a recovery period for 3 wk.
† Haugh units.
‡ [a,b,m,n,x,y] means within a column within an age group range with different lowercase superscripts are significantly different ($p < 0.05$).
§ Pooled standard error of mean.

Table 7
Effects of induced molt period on productive performance and mortality rate during the postmolt laying period (age 101–114 wk).

Treatment*	Feed consumption (g/hen/d)	Egg production (%)	Egg weight (g)	Feed conversion ratio (g feed/g egg weight)	Mortality rate (%)
NC	119.0	64.3	67.0	2.80	21.5[a†]
IM_0	113.7	65.6	68.0	2.76	14.7[ab]
IM_1	119.2	77.0	66.7	2.34	11.8[ab]
IM_2	112.0	65.1	67.9	2.61	13.2[ab]
IM_3	112.5	69.2	68.8	2.67	6.8[b]
SEM‡	6.0	11.7	1.9	0.49	1.9
p-value	0.28	0.55	0.57	0.62	0.02

* NC = non-molt control; IM_0 = induced molt with cassava meal for 2 wk without a recovery period; IM_1 = induced molt with cassava meal for 2 wk and a recovery period for 1 wk; IM_2 = induced molt with cassava meal for 2 wk and a recovery period for 2 wk; IM_3 = induced molt with cassava meal for 2 wk and a recovery period for 3 wk.
† [a,b] means within a column with different lowercase superscripts are significantly different ($p < 0.05$).
‡ SEM = pooled standard error of the mean (4 replicates of 12 hens each per treatment).

Table 8
Effects of induced molt period on egg and eggshell quality during the postmolt laying period (age 101–114 wk).

Treatment*	Yolk weight (%)	Albumen weight (%)	Albumen height (HU†)	Shell weight (%)	Shell thickness (mm)	Shell strength (N)
NC	26.0[a,‡]	64.4[b]	78.6	9.5	0.343	35.5
IM_0	25.0[b]	65.4[ab]	85.2	9.4	0.339	33.4
IM_1	25.8[ab]	64.5[b]	87.1	9.5	0.348	35.8
IM_2	25.3[ab]	65.1[ab]	86.4	9.4	0.345	35.6
IM_3	24.3[b]	66.2[a]	84.0	9.4	0.344	36.1
SEM§	0.4	0.6	4.0	0.2	0.008	2.2
p-value	0.001	0.005	0.07	0.97	0.92	0.49

* NC = non-molt control; IM_0 = induced molt with cassava meal for 2 wk without a recovery period; IM_1 = induced molt with cassava meal for 2 wk and a recovery period for 1 wk; IM_2 = induced molt with cassava meal for 2 wk and a recovery period for 2 wk; IM_3 = induced molt with cassava meal for 2 wk and a recovery period for 3 wk.
† HU = Haugh units.
‡ [a,b] means within a column with different lowercase superscripts are significantly different ($p < 0.05$).
§ SEM = pooled standard error of the mean (4 replicates of 12 hens each per treatment).

Table 9
Means of mammillary density and mammillary knob diameter of the eggshell produced by the experimental hens as affected by molt treatment (aged 114 wk).

	Treatment*						
	NC	IM_0	IM_1	IM_2	IM_3	SEM†	*p*-value
Number of samples	8	8	8	8	8		
Mammillary density (knobs/mm^2)	223.6[a‡]	147.1[bc]	176.6[b]	139.7[c]	153.8[bc]	19.8	<0.001
Mammillary knob diameter (mm)	0.080[b]	0.098[ab]	0.089[b]	0.102[a]	0.087[b]	0.007	0.001

* NC = non-molt control; IM_0 = induced molt with cassava meal for 2 wk without a recovery period; IM_1 = induced molt with cassava meal for 2 wk and a recovery period for 1 wk; IM_2 = induced molt with cassava meal for 2 wk and a recovery period for 2 wk; IM_3 = induced molt with cassava meal for 2 wk and a recovery period for 3 wk.
† SEM = pooled standard error of the mean (4 replicates of 2 samples each per treatment).
‡ [a,b,c] means within a row with different lowercase superscripts are significantly different ($p < 0.05$).

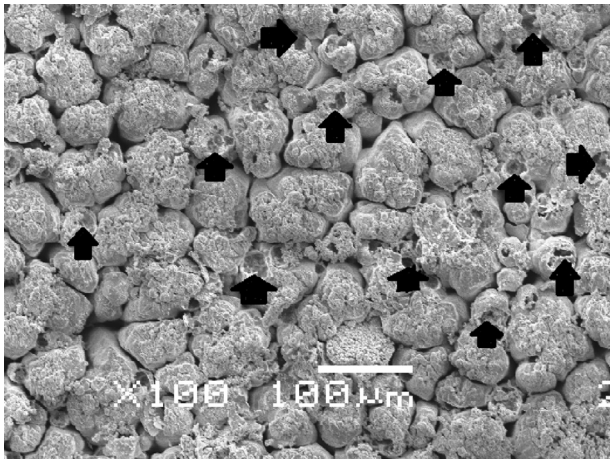

Fig. 1. Scanning electron micrograph showing ultrastructure of the mammillary layer of birds in the control group. (Note the mammillary knobs showing erosion (arrows); ×100 and scale bar = 100 µm).

compared to the control group. The results indicated that optimum postmolt performance would be achieved when the birds had a recovery period of at least 3 wk. Improved albumen quality as well as mortality, during the 20 wk postmolt period has been reported by Lee (1982). A significant reduction in the yolk percentage was observed in the IM_0 and IM_3 groups compared to the control group. One possible explanation for this may be that the efficiency in albumen synthesis of the oviduct of hens in these groups was increased, whereas their efficiencies in yolk and shell formation were comparable to those of the control hens. Hens in the IM_0 and IM_3 groups produced larger eggs containing greater albumen content but less yolk and shell composition compared to those of hens in the control group (Tables 7 and 8).

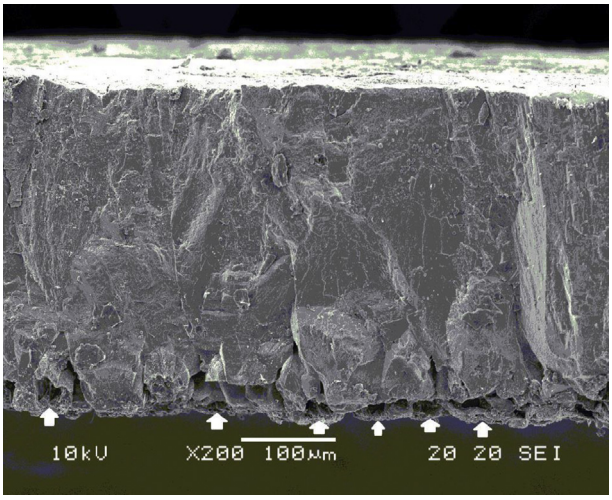

Fig. 3. Scanning electron micrograph showing transverse view of an egg produced from a hen in the control group. (Note the mammillary knobs showing evidence of erosion (arrows); ×200 and scale bar = 50 µm).

Eggshell ultrastructural observation

Table 9 summarizes the density and diameter of the mammillary knobs of selected eggshells. It was apparent that the density of the mammillary knobs of the shells selected from the control group was significantly higher than that of the shells collected from all molted groups. Conversely, the diameter of the mammillary knobs of the shells in the control group was lower than that of the shells in the molted groups. Mammillary density refers to the number of mammillae per unit area. Van Toledo et al. (1982) reported that shells with greater numbers of mammillary knobs per unit interior

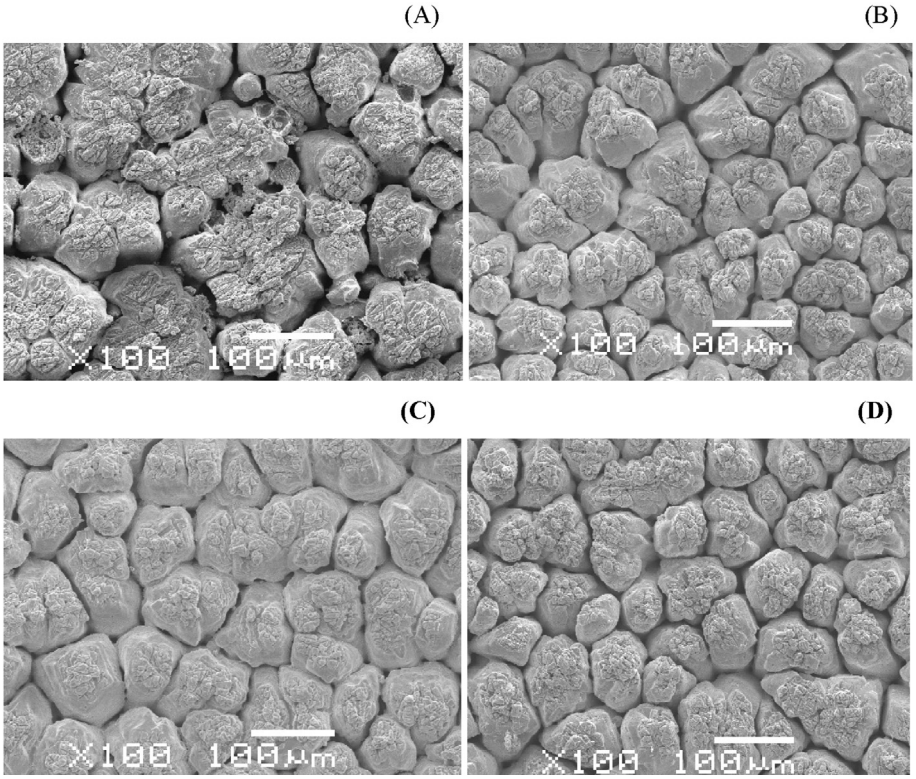

Fig. 2. Scanning electron micrographs showing ultrastructure of the mammillary layer of birds in the IM_0 (A); IM_1 (B); IM_2 (C); IM_3 (D) groups. (×100 and scale bar = 100 µm).

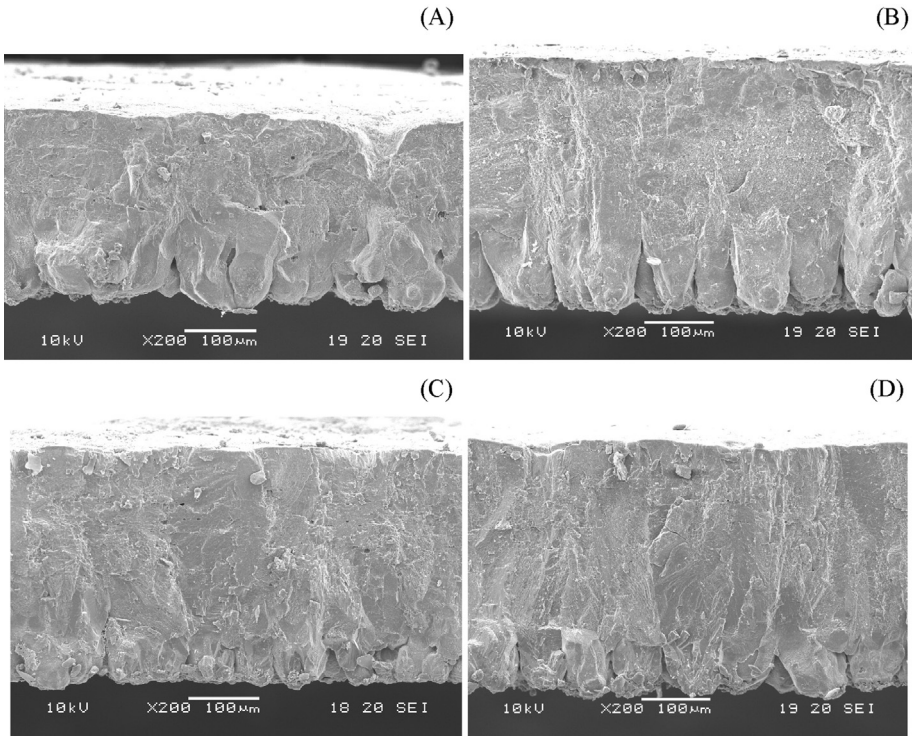

Fig. 4. Scanning electron micrographs showing transverse view of an egg produced from a hen in the IM_0 (A), IM_1 (B), IM_2 (C) and IM_3 (D) groups. (×300 and scale bar = 50 µm).

surface area had a greater relative interstitial area between mammillary formations. The investigators proposed that the increased interstitial area appeared to make the shell more susceptible to cracks. In the present study, however, no significant differences were noticed in the shell strength among the treatment groups (Table 8). Interestingly, scanning electron microscopy inspection at a magnification of ×100 revealed evidence of erosion in the mammillary layer in five samples of the shells taken from the control group (Fig. 1), whereas the examination showed normal formation of mammillae with rounded caps in all samples of the shells taken from the molted groups (Fig. 2). The incidence of erosion at the mammillary layer of the eggshell is normally found in aged hens, which creates areas of weakness within the shell (Roberts and Brackpool, 1994). Figs. 3 and 4 show a transverse view of an egg selected from the control and molted groups, respectively. The palisade layer in the selected shell taken from the IM_0 treatment was clearly shorter than those of the other treatments, suggesting that the IM_0 hens were photostimulated too early during the postmolt productive period. The results agreed with those of Gongruttananun et al. (2013) who found a significant reduction in the palisade layer in eggshells produced by hens that had been induced to molt for 2 wk without the recovery period. Heryanto et al. (1997b) reported that during oviducal regression in induced molting hens, apoptosis was induced in the early stage of oviduct regression and autolysis occur thereafter, resulting in the disappearance of the tubular gland cells of the shell gland. Remodeling of the shell gland tissues was found at 16 d after termination of egg production (Heryanto et al., 1997a). In the present study, hens in the IM_0 treatment were photostimulated during the postmolt period at 7 d after cessation of production when the recovery of the shell gland tissues was not yet accomplished. This was confirmed by a decrease in the thickness of the eggshells produced by the IM_0 hens as shown in Table 8.

The results indicated that feeding the cassava molt diet was an effective nonfasting method for induced molting in laying hens. A recovery period of at least 3 wk was necessary for improving of postmolt albumen quality and mortality rate. Further studies need to be undertaken to enhance the efficiency of the technique for the improvement of egg production and eggshell quality during the subsequent productive cycle.

Conflict of interest

A recovery period of at least 3 wk was necessary for improving of postmolt albumen quality and mortality rate.

Acknowledgements

The research project was funded by the Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Bangkok, Thailand.

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