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Original Article

Effects of a short-term molt using cassava meal, broken rice, or corn meal on plasma thyroxin concentrations, organ weights and intestinal histopathology in older (95 wk) laying hens

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ABSTRACT

The effects of a nonfasting induced molt were determined on the thyroxin concentration, organ weight and intestinal histopathology in 95-wk-old hens. Hens (60 birds each treatment) were randomly assigned to four treatments for 14 d: 1) molted by feeding broken rice (BRO), 2) fed corn (COR), 3) fed cassava (CAS), or 4) a non-molt control (NON). During the molt period, the BRO, COR and CAS groups were exposed to an 8 h light: 16 h dark photoperiod, whereas the control hens were fed a layer ration and provided with 16 h of light per day. The body weight loss in the CAS hens was 21.90% which was significantly higher than those of the BRO (6.01%) or COR hens (9.30%). The CAS hens completely stopped laying on d 7, whereas the BRO and COR birds exhibited reductions but continued laying. The egg weight of the COR treatment was significantly lower than those of the BRO and CAS treatments. At the end of the molt period, the hematocrit values of the BRO and CAS hens were significantly higher than those of the COR hens. The plasma thyroxin concentrations of the CAS treatment were significantly higher than those of the COR treatment, whereas the BRO hens had a value intermediate between the two groups. The CAS and COR hens had reduced liver weights compared with the BRO hens. However, the thyroid weights of the CAS and COR hens were significantly greater than those of the BRO hens. No inflammatory evidence was observed in any treatment from the examination of intestinal histopathology.

Introduction

Molting in avian species is characterized by the replacement of feathers in an orderly manner and is accompanied by the regression of reproductive organs and the cessation of egg laying (Johnson, 1986). Commercial laying hens also experience naturally occurring molts but these are usually incomplete, and hens continue to lay eggs at a low rate for a prolonged period. This creates a period of unprofitability for the commercial egg producer due to a reduction in egg production and leads to the end of the useful life of the flock (Berry, 2003). It is generally accepted that induced molting is an effective tool for the economic management of laying flocks to extend the productive life and improve the egg quality of aged hens (Roland and Brake, 1982; Christmas et al., 1985; Tona et al., 2002). The most commonly practiced method of induced molt is the withdrawal of feed (Park et al., 2004) accompanied by a reduction in the photoperiod relative to that of natural day length or less (Hembree et al., 1980).

As a consequence of feed deprivation, the molted hens lose body weight, relative liver weight and ovary and oviduct weight during prolonged fasting (Brake and Thaxton, 1979). Szabó et al. (2005) reported that the relative liver weight decreased 4.2% in laying hens fasted for 12 d, whereas the relative heart weight increased 3.2%. Landers et al. (2008) also found significantly lower relative weights of the liver, ovary, pancreas and heart in alfalfa-meal molted hens when compared to those of full fed control hens. Many of the investigations into the endocrine mechanisms of molting have focused on feather molting induction. Early research suggested that the thyroid gland was primarily responsible for feather loss and replacement (Zavadovsky, 1925; Cole and Hutt, 1928). Brake et al. (1979) observed an increased thyroxin (T_4) level during feed withdrawal. These workers reported that the T_4 increases during fasting coincided with active regression of the ovaries and that T_4 decreased sharply at the point of complete ovarian regression.

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Induced molting by feed withdrawal has been shown to increase the susceptibility of the birds to *Salmonella* infection (Holt, 1993; Ricke, 2003). Holt et al. (1994) reported that molted hens exhibited more extensive inflammation of the intestinal tract compared with the non-molted control hens. These workers also observed intermediate intestinal inflammation in the hens molted using a molt diet, indicating that induced molt using a molt diet has less detrimental effects of severe intestinal inflammation compared with the feed removal method.

Due to the increased public awareness of the animal welfare associated with feed deprivation, a wide variety of alternative molting procedures have been investigated. These alternate methods of induced molting use dietary manipulations to create an imbalance of a particular nutrient or nutrients (Berry and Brake, 1985; Breeding et al., 1992). Other non-fasting molt methods include the feeding of high wheat middlings (Biggs et al., 2003), a combination of wheat middlings and corn (Koelkebeck et al., 2006) or a whole-grain barley diet (Onbasilar and Erol, 2007). The degree of improvement in postmolt performance is associated with an increase in the number of days during which no eggs are produced (Berry, 2003). However, from an animal welfare standpoint, the use of a long-term forced molt period may actually be stressful and traumatic to the overall well-being of the birds. A previous study by the current authors reported that a short-term program of full feeding of aged laying hens with cassava meal could be an alternative method to fasting, without adverse effects on bone integrity, immunological organs or livability (Gongruttananun et al., 2013).

Investigations into the feasibility of alternative molting techniques have been focused on the effects of these methods on egg production and egg quality. Only a few studies have investigated the physiological effects and intestinal histopathology of these alternative induced molting techniques. The objective of this study was to determine what effects feeding cassava meal, broken rice or corn meal *ad libitum* to older laying hens for a short-term period would have on the plasma thyroxin concentrations, visceral organ weights and intestinal histopathology at the end of the molt period.

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 95 wk). The hens were housed in a caged layer shed of commercial design, with water and feed provided for *ad libitum* consumption, and the hens were exposed to a 16 h photoperiod (16 h light:8 h darkness) daily commencing before the start of the experiment. The mean temperature of the house was 20.7 °C, and the mean light intensity was 3.9 lux. The feed was a commercial layer diet calculated to contain 17% crude protein, 2800 kcal of metabolizable energy per kg of feed, and 3.5% calcium. Five replicate groups of 12 hens each (four adjacent cages containing three hens per cage, cage size 40 cm × 45 cm) were allotted to four treatments in a completely randomized design. The four treatments were designated as: non-molt control (NON), fully fed a broken rice molt diet (BRO), fully fed a corn mash molt diet (COR), or fully fed a cassava mash molt diet (CAS). Birds were weighed and allocated to each replicate to achieve a similar mean body weight (BW) for each treatment. Egg production and egg weight were measured for 2 wk (95–97 wk of age), in an attempt to keep a similar distribution of productive performance among the experimental treatments. At age 97 wk, the control group was moved carefully into a similar nearby house and maintained under an artificial lighting program of 16 h light:8 h darkness and provided access *ad libitum* to the commercial layer ration and drinking water throughout the experimental period, whereas the remaining groups were induced to molt for 14 d according to the molting procedure shown in Table 1. The daily egg production, egg weight, feed intake, body weight loss and mortality rate were recorded during the 14 d molt period. The ingredient composition and calculated nutrient analyses of the experimental molt diets are given in Table 2.

Hematological parameters

Blood samples (5 mL) were obtained from the brachial vein of two hens from each replicate at the end of the molt program (age 99 wk). The time of bleeding was between 0900 h and 1100 h. The hematocrit value was determined using heparinized microcapillary tubes by centrifuging in a microliter centrifuge (Hettich; Tuttlingen, Germany) for 5 min at 21,382 × *g* at 25 °C (Campbell, 1995). Next, the remainder of the blood was centrifuged for 15 min at 1090 × *g* at 25 °C. The plasma-ionized calcium concentration was recorded immediately on a CyberScan pH 5500/5000 (Eutech Instruments Pty Ltd; Singapore). The plasma thyroxin concentration was measured using chemiluminescent microparticle immunoassay on an ARCHITECT Total T_4 Model B7K660 (Abbott laboratories; Abbott Park, IL, USA). The intra-assay coefficient of variation (CV) was 5.9%, whereas the inter-assay CV was 5.05%.

Collection of visceral organs

At the end of the molt period (age 99 wk), one bird of each replicate was killed for observation of the morphological characteristics of the visceral organs. The birds were anaesthetized using an injection of nembutal pentobarbitone sodium, after which they were killed by exsanguination (jugular veins cut using a scalpel; bleed time 105 s), and the ovary, oviduct, crop, proventriculus, gizzard, heart, liver, kidney, spleen, pancreas, right adrenal gland and the entire intestine were excised aseptically and individually weighed. The crop and gizzard were cut, opened and rinsed of their contents, and the koilin (inner membrane) of the gizzard was removed. Relative organ weights (grams per kilogram of body weight) were calculated and presented.

Histological examination of intestinal tissues

On the day of euthanasia, cecal tissues from the sacrificed hens were collected. The tissues were fixed in buffered neutral formalin, embedded in paraffin, sectioned at 3 μm and stained with hematoxylin and eosin. Histological sections of intestine from the control and molted hens were evaluated in blind fashion, scored on the degree of inflammation and numerically ranked according to the method of Porter and Holt (1993). The final score for each tissue was the sum of criteria 1, 2 and 3 plus whether heterophils were observed in the epithelial layer (yes = 1 and no = 0). Ten was the highest score and 0 was the lowest score. The mean inflammation scores of the different treatment groups were calculated.

Statistical analysis

The experiment was conducted as a completely randomized design with four treatments. The statistical comparisons were made among the three molt treatments, excluding the control treatment. 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 treatment on BW, egg weight, egg production, organ weight and hematological values. An arcsine transformation was used for all percentage data. When the means of the GLM procedure were statistically different, they were further

Table 1
Molting procedures used in the experiment.

Treatment	Feed	Water	Light (hr/d)	Length (d)
NON	Layer diet, *ad libitum*	Provided	16	Throughout the experimental period
BRO	Broken rice molt diet, *ad libitum*	Provided	8	14
COR	Corn mash molt diet, *ad libitum*	Provided	8	14
CAS	Cassava mash molt diet, *ad libitum*	Provided	8	14

NON = non-molt control; BRO = broken rice molt diet; COR = corn mash molt diet; and CAS = cassava mash molt diet.

Table 2
Ingredient composition and calculated nutrient analysis of the experimental diets.

Item	Broken rice molt diet (%)	Corn mash molt diet (%)	Cassava mash molt diet (%)	Layer diet[a] (%)
Ingredient				
Broken rice	96.72	–	–	
Corn mash	–	96.60	–	
Cassava mash	–	–	96.62	
Limestone	1.50	1.62	1.60	
Dicalcium phosphate	1.38	1.38	1.38	
Vitamin mineral premix[b]	0.40	0.40	0.40	
Calculated analysis				
ME[c] (kcal/kg)	3385.20	3255.40	3381.70	2800.00
Crude protein (%)	0.73	0.72	1.93	17.00
Crude fiber (%)	0.96	2.41	3.62	4.50
Calcium (%)	0.93	0.95	1.05	3.50
Available phosphorus (%)	0.24	0.25	0.29	0.64

[a] Commercial layer ration.
[b] Provided the following (per kilogram of diet): vitamin A, 20,000 International units (IU); vitamin D_3, 4800 IU; vitamin E, 16 IU; vitamin K_3, 2.4 mg; vitamin B_1, 32 mg; vitamin B_2, 8 mg; vitamin B_6, 4.8 mg; vitamin B_{12}, 0.01 mg; pantothenic acid, 15.04 mg; nicotinic acid, 20 mg; folic acid, 0.8 mg; biotin, 0.144 mg; selenium, 0.16 mg; copper, 16 mg; manganese, 96 mg; iron, 64 mg; zinc, 80 mg; cobalt, 0.32 mg; and iodine, 3.2 mg.
[c] Metabolizable energy.

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 internal organ parameters, only one sample per replicate was used. Data were presented as means and the pooled standard error of the means.

Results

Body weight, egg weight and egg production

At the beginning of the study, hens in all treatments had a similar value of body weight, egg weight and egg production (data not shown). The effects of molt diets on body weight, egg weight and egg production during the molt period are presented in Table 3. The CAS hens lost 21.90% of their original body weight, which was significantly greater than those of the BRO (6.01%) and COR (9.30%) treatments. The COR hens had a smaller egg size compared to the BRO and CAS hens ($p < 0.05$). The average egg production of the CAS treatment was 8.58%, which was significantly lower than that of the COR treatment (15.60%). The egg production rate of the BRO treatment (9.01%) was not significantly different from those of the COR or CAS treatments. The CAS hens exhibited more rapid reduction in egg production, reaching zero on d 7, and completely stopped laying, whereas neither of the other molted treatments totally ceased egg production.

Plasma calcium and thyroxin concentrations and hematocrit values

The treatment effects on the hematocrit, plasma ionized calcium and thyroxin concentrations of the experimental birds are given in Table 4. There was a significantly higher value of packed cell volume in the BRO (34.27%) and CAS (32.71%) treatments compared to that of the COR treatment (26.88%). Hens in the CAS treatment had a significantly ($p = 0.004$) higher level of plasma thyroxin concentration than that of hens in the COR treatment (1.67 *vs.* 1.17 μg/dL), whereas the mean of the BRO treatment was not significantly different from those of the CAS or COR treatments ($p > 0.05$). There was no significant difference in the concentration of plasma-ionized calcium among the molted treatment groups.

Table 3
Body weight loss, egg weight and egg production (means of 5 replicates of 12 hens each) by treatment during the 14 d molting period (97–99 wk of age).

Item	Initial BW (kg)	BW loss (%)	Feed consumption (g/hen per day)	Egg weight (g)	Egg production (% hen-day)
Treatment					
NON	1.92	0.85	130.7	70.07	44.61
BRO	1.97	6.01[b*]	125.9[a]	69.85[a]	9.01[ab]
COR	1.95	9.30[b]	107.8[a]	67.24[b]	15.60[a]
CAS	2.01	21.90[a]	70.8[b]	69.96[a]	8.58[b]
Pooled SE	78.30	2.80	10.9	1.73	3.71
p-value	0.39	<0.001	<0.001	0.049	0.021

BW = body weight; NON = non-molt control; BRO = broken rice molt diet; COR = corn mash molt diet; and CAS = cassava mash molt diet.
[*] Means within the same column without a common lowercase superscript are significantly different ($p < 0.05$). The statistical comparisons were made among the three molt treatments, excluding the control treatment.

Table 4
Effect (means of 10 samples) of non-molted and molted treatments on hematocrit values, plasma-ionized calcium and thyroxin concentrations measured at the termination of the 14 d molting period.

Item	Hematocrit (%)	Ca^{2+} (mole/L × 10^3)	Thyroxin concentration (μg dL^{-1})
Treatment			
NON	26.07	2.88	1.39
BRO	34.27[a*]	2.97	1.35[ab]
COR	26.88[b]	3.09	1.17[b]
CAS	32.71[a]	3.08	1.67[a]
Pooled SEM	3.94	0.07	0.31
p-value	0.028	0.060	0.004

NON = non-molt control; BRO = broken rice molt diet; COR = corn mash molt diet; and CAS = cassava mash molt diet.
* Means within the same column without a common lowercase superscript are significantly different ($p < 0.05$). The statistical comparisons were made among the three molt treatments, excluding the control treatment.

Table 5
Effect of non-molted and molted treatments on the relative organ weight (grams of organ weight per kilogram of body weight) at the end of the 14 d molting period.

Organ	Treatment[c]				SEM[c]	*p*-value
	NON	BRO	COR	CAS		
Ovary	25.06	12.08	2.98	2.75	6.28	0.060
Oviduct	32.07	17.16	10.64	10.75	7.38	0.314
Crop	3.65	4.30	3.99	4.80	0.85	0.353
Proventriculus	4.23	3.37	3.73	3.86	0.88	0.670
Gizzard	12.89	14.99	17.89	19.56	3.78	0.197
Heart	3.73	4.08	4.70	4.44	0.84	0.524
Liver	24.88	28.93[a*]	22.51[b]	21.19[b]	3.20	0.005
Gall bladder	0.74	0.80	0.79	0.76	0.30	0.973
Kidneys (both sides)	8.17	8.21	9.11	10.36	1.99	0.270
Pancreas	1.87	1.29	1.77	1.78	0.34	0.074
Intestine	19.27	22.75	19.47	20.70	3.00	0.259
Adrenal gland (right side)	0.12	0.08	0.13	0.12	0.06	0.473
Thyroid gland (both sides)	0.10	0.07[b]	0.16[a]	0.13[a]	0.03	0.016

NON = non-molt control; BRO = broken rice molt diet; COR = corn mash molt diet; and CAS = cassava mash molt diet.
* Means within the same row without a common superscript are significantly different ($p < 0.05$). The statistical comparisons were made among the 3 molt treatments, excluding the control treatment.
[c] SEM = pooled standard error of the mean (5 replicates of 1 sample each per treatment).

Organ weights

A summary of internal organ weights of the experimental birds is shown in Table 5. The organ weights were calculated based on a body weight percentage for molted and non-molted hens sacrificed at the end of the molt period. Significant differences were observed in the liver and thyroid weights among the three molted treatments. The liver weight of the BRO treatment (28.92%) was significantly greater ($p = 0.005$) than those of the COR (22.51%) or CAS (21.19%) treatments. The thyroid glands removed from the COR and CAS hens were significantly greater in weight percentages than those of the BRO hens. However, there were no significant differences in the relative weights of the ovary, oviduct, crop, proventriculus, gizzard, heart, gall bladder, kidneys, pancreas, intestine or adrenal gland among the molted treatments.

Intestinal histological changes

The cecal tissue from the molted hens exhibited normal characteristics compared with those of the control hens (Fig. 1). No evidence of inflammation was observed in the intestinal sections of any samples of the molted birds. However, examination at a magnification of ×400 showed a small number of heterophils infiltrating the lamina propria in one sample of the NON treatment and a sample of the CAS treatment (Fig. 2).

Discussion

The results obtained from the present study indicated that cassava meal can be used as an alternative molt diet in laying hens without any effect on internal organs or adverse effects on the intestinal tissue. During the molt period, hens fed the cassava molt diet lost approximately 22% of their body weight, whereas the body weight loss of the BRO and COR hens was only 6.01 and 9.30%, respectively. In addition, hens in the CAS treatment group exhibited more rapid reductions in egg production, reaching zero on d 7, and they stopped laying completely, whereas neither of the other two molted treatments totally ceased egg production. Scheideler and Beck (2002) stated that the goals of a successful molt using a non-fasting program are: 1) about 20–25% body weight loss; 2) cessation of laying long enough for total regression of the reproductive tract; and 3) acceptable and persistent second cycle performance.

During the molt period, the COR hens produced eggs with a smaller size than those laid by the BRO hens suggesting that hens fed the COR molt diet had lower energy intakes compared with the BRO hens. The calculated energy consumption of the COR hens during the molt program was 350.93 kcal/hen/d, whereas that of the BRO hens was 426.19 kcal/hen/d. As expected, the CAS hens had the highest level of blood thyroxin concentrations compared to hens in the other molted groups, suggesting that hens in the CAS treatment group molted more completely than birds in the BRO and COR groups. In birds, as in other vertebrates, thyroxin (T4) and triiodothyronine (T3) are considered thyroid hormones and these hormones are important in several organismal-level processes such as molt and reproduction in birds (McNabb, 2000). Estrogen decreases appear to be important in the initiation of molt, whereas an increase in the thyroid hormone/estrogen ratio appears to be important in new feather formation as reviewed by Decuypere and Verheyen (1986). An increase in plasma thyroxin, during the molt period, of laying hens induced to molt by fasting has been reported by a number of investigators (Brake et al., 1979; Hoshino et al., 1988; Davis et al., 2000; Khajali et al., 2008). In the present trial, an increase in the thyroid gland weights of birds in the COR and CAS groups would have resulted from the increased activities of the endocrine glands of birds in those groups during the molt period.

It is of interest to note that hens in all molted groups, especially the BRO and CAS treatment groups, had a higher value of hematocrit than hens in the control non-molt group indicating that the packed cell volume increased during the period of reproductive involution. This could have been due to the removal of estrogen inhibition of red blood cell formation, reduced vascular volume due to involution of the ovary and oviduct, and presumably to a decreased fluid intake leading to hemoconcentration (Berry, 2003). An increase in hematocrit in laying hens molted using feed deprivation has been reported previously by some investigators (Brake and Baker, 1982; Khajali et al., 2008).

In the present study, the relative liver weight decreased 14.83% in the CAS hens compared to that of the control birds, whereas the other visceral organs were not affected by the treatment. Similar decreases in liver weights have been reported for feed-deprived molted hens (Szabó et al., 2005) and alfalfa molted hens (Landers et al., 2008). The results indicated that the liver plays a general protective role for other organs during negative energy balance. The photographs from an examination of histology of the intestinal tissues at the end of the molting period, illustrated normal characteristics of the intestinal tract in all treatment groups. The results

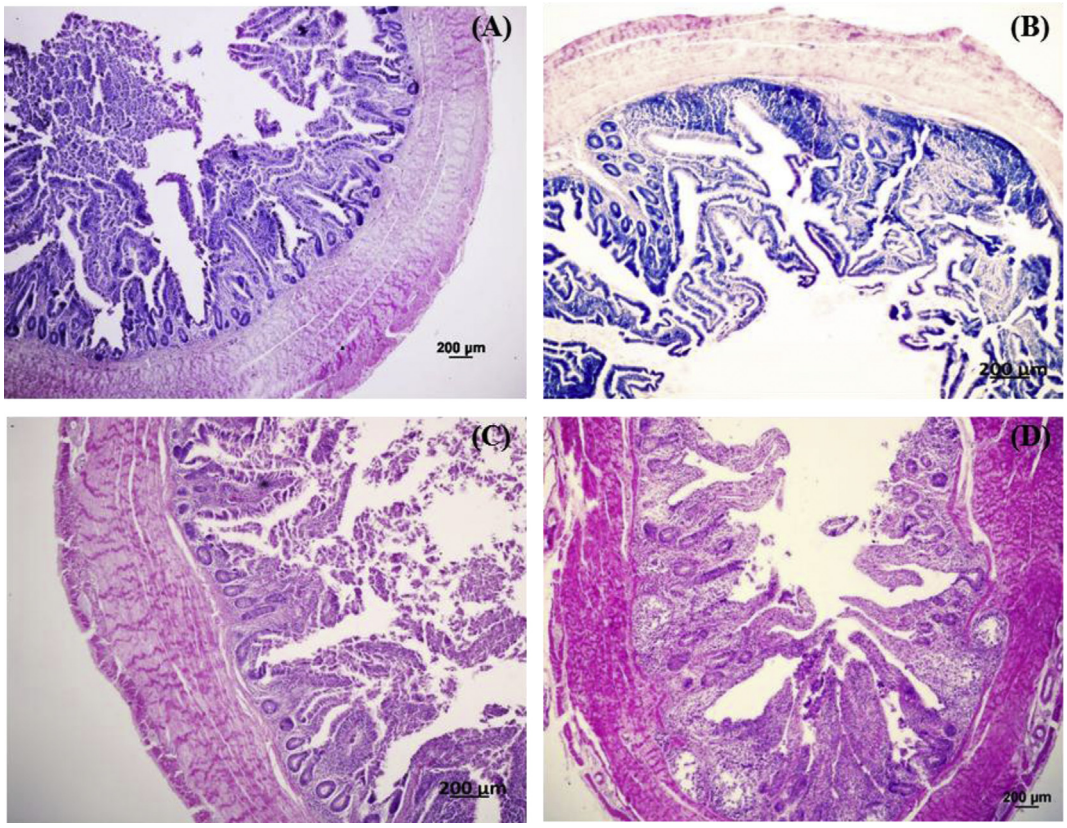

Fig. 1. Sections of the cecal tissue using hematoxylin and eosin 4 × staining from hens in treatments: (A) non-molt control; (B) broken rice molt diet; (C) corn mash molt diet; (D) cassava mash molt diet.

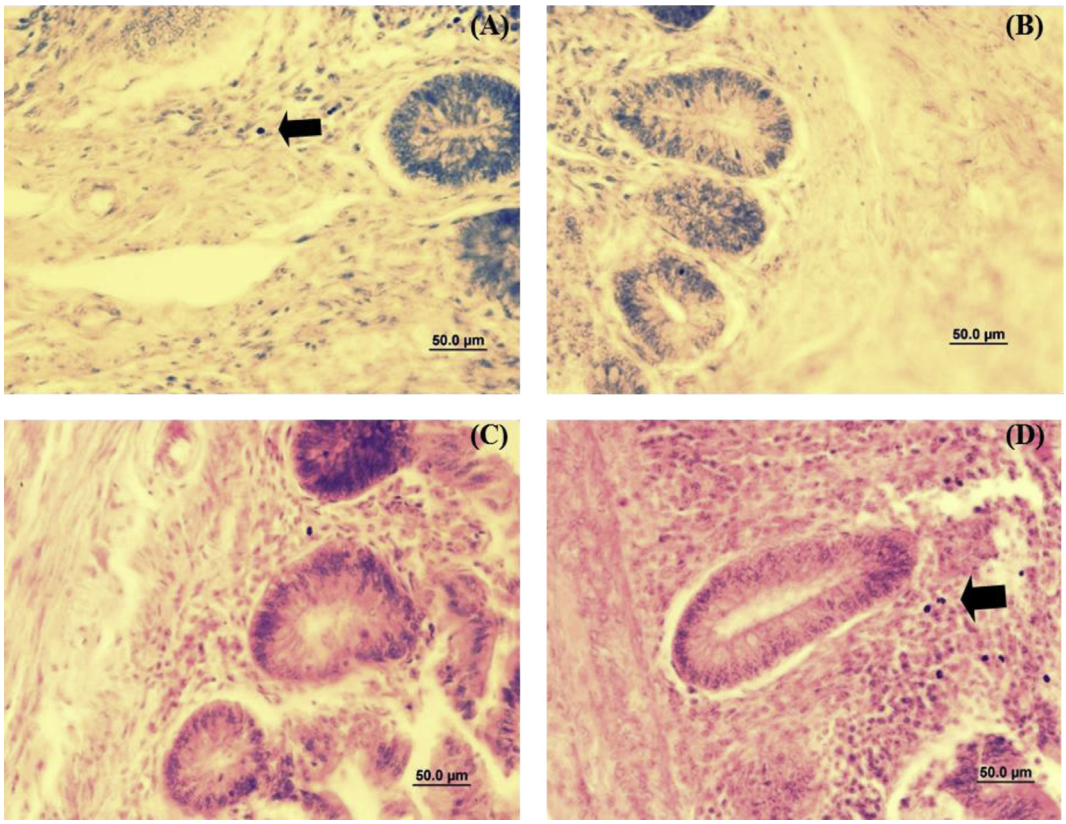

Fig. 2. Higher magnification of the cecal sections using hematoxylin and eosin 40 × staining showing a small number of heterophils (at arrowheads) infiltrating the lamina propria in: (A) one sample of the non-molt control diet; and (D) a sample of the cassava mash molt diet. However, sections of ceca were normal in: (B) broken rice molt diet; and (C) corn mash molt diet.

indicated that the non-feed removal molting method used in this study had less detrimental effects on the intestinal tract of the birds, in agreement with the results reported by Holt et al. (1994).

Feeding of aged laying hens with cassava molt diets for 14 d resulted in body weight losses of approximately 22% and increased thyroxin concentrations, without adverse effects on the structure of the intestine or other internal organs except for the liver weight. The results indicated that full feeding with cassava meal is an effective method for the induction of molting and could be used as an alternative method to fasting.

Other investigators have reported that induced molt using cassava meal as a molt diet did not have any deleterious effects on immune tissues, blood heterophil:lymphocyte ratios, bone integrity and mortality of laying hens (Gongruttananun et al., 2013). In addition, Gongruttananun and Saengkudrua (2016) have reported that induced molt using cassava meal with a recovery period of 3 wk could improve the mortality rate and albumen quality of the birds during the postmolt period. Therefore, induced molt using cassava meal is an alternative to improve egg product and egg quality in old laying hens without any tremendous effects on animal welfare.

Conflict of interest

The authors declare no conflict of interest.

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