Agriculture and Natural Resources 51 (2017) 526–531

Contents lists available at ScienceDirect

Agriculture and Natural Resources

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

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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ARTICLE INFO

Article history:
Received 4 August 2016
Accepted 27 July 2017
Available online 21 May 2018

Keywords:
Intestine
Laying hen
Molt
Organ
Thyroxin

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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https://doi.org/10.1016/j.anres.2018.05.005

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