Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

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Revista Brasileira de Ciência Avícola, vol. 15, núm. 3, julio-septiembre, 2013, pp. 239-245
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jul - Sept 2013 / v.15 / n.3 / 169-286

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Keywords

Heat stress, ovary, feed restriction, welfare

[1] Study funded by Fapesp. Process n.: 07/555970-3, 07/56447-2 e 10/01923-7.

Submitted: August/2012
Approved: May/2013

ABSTRACT

An experiment was conducted to evaluate the biometrics of organs (ovary, oviduct, liver, proventriculus and gizzard) of laying hens submitted to different forced-molting methods of molt and maintained at three different temperature. Organs were evaluated after molting and resting period. Six hundred birds were distributed according to a completely randomized experimental design in a 5x3 factorial arrangement (forced-molting methods x environmental temperatures) into 15 treatments with 5 replicates of 8 birds each. The molting methods applied were the dietary inclusion of 90%, 70%, 50% of alfalfa, 2,800 ppm zinc oxide, or total feed restriction. Birds were kept at ±20 °C, ±27 °C, or ±35 °C. Data were submitted to analysis of variance and means were compared by orthogonal and polynomial contrasts. The traditional forced-molting method (feed fasting) resulted in adequate performance. However, as the aim of the present study was to find an alternative method to feed fasting, the highest dietary alfafa inclusion level (90%) was shown to be efficient compared with other methods, particularly when hens were kept at high temperature. Therefore, this alternative forced-molting may be applied, especially when bird welfare is taken into consideration.

INTRODUCTION

At the end of the first laying cycle, eggshell thickness and strength are reduced, precisely when egg size is the largest. This problem may be solved by an economically feasible management practice, which is forced molting. Forced molting consists of a set of management practices applied to promote stress, inducing birds to stop producing eggs (Roland & Brake, 1982).

Several methods to force molting are applied, including feed and water fasting, lighting restriction, and the use of chemical agents. Feed withdrawal from the feeders for 10 to 12 days is the easiest method of inducing molting in layers, resulting in egg production cessation, reproductive tract involution, and feather loss (Brake, 1993). Molting induced by feed fasting causes about 25% weight loss in layers, and ¼ of this effect can be directly attributed to reductions in liver, ovary, and oviduct weights (Brake & Thaxton, 1979).

However, despite being easy to apply and producing the desired results, feed fasting is very aggressive, and therefore, taking into account the importance of animal welfare, molting methods that cause less stress, such as the dietary inclusion of insoluble fiber, have been investigated (Kwon *et al.*, 2001; Donalson *et al.*, 2005; Landers *et al.*, 2005, 2008).

Studies on alternative molting methods have frequently reported conflicting results, which may be attributed to the different environmental conditions in which birds are reared. Among the environmental factors, air temperature, humidity, and movement are highlighted, as they directly affect body temperature maintenance (Tinôco, 1995).

The present study aimed at evaluating the effect of different forced-molting methods applied at three different environmental temperatures on the performance and relative weights of the organs of commercial layers.

MATERIALS AND METHODS

The experimental period included the periods of molt and rest. Layers were selected according to body weight and egg production. The different methods of forced molting were applied for 14 days, after which there was a 28-day resting period for the recovery of body and organ weights.

In this trial, 600 commercial Isa Brown layers with 72 weeks of age were distributed, according to a completely randomized experimental design with a 5x3 factorial arrangement (forced molting methods x temperatures) into 15 treatments, with five replicates of eight birds each, totaling 75 experimental units.

During the molting period, a lighting program of 12h of light per day was adopted, and the period was light was gradually increased during the resting period until 17 hours of light per day. Feed and water were supplied *ad libitum.*

Birds were kept in three environmentally-controlled chambers during the molt and the rest periods. Using thermo-hygrometers, the following maximum and minimum temperature and humidity inside the chambers were recorded: 18.9-21ºC and 41.3-64.4% in the cold chamber, 25.9-28.5ºC and 29.5-55.8% in the thermoneutral chamber, and 29.1-32.4ºC and 39.9-52.3% in the hot chamber, respectively.

The following five methods were applied to induce molting: 90% alfalfa and 10% feed (A90); 70% alfalfa and 30% feed (A70); 50% alfalfa and 50% feed (A50); dietary addition of 2,800 ppm zinc (Zn); or feed fasting (control).

The three different environmental temperatures were: cold temperature (CT): ± 20° C; thermoneutral temperature (NT): ± 27° C; or hot temperature (HT): ± 32° C.

During the experimental period, four birds per treatment were sacrificed after molting and four after resting in order to determine relative ovary, oviduct, liver, proventriculus, and gizzard weights.

Feeds were based on corn and soybean meal, and formulated according to the recommendations of Rostagno *et al.* (2005). (2005).

Table 1 - Ingredient composition of the experimental feeds supplied during the different experimental phases.

Ingredients (%)	Basal diet	
	Molting methods	Resting
Ground corn	72.80	72.32
Soybean meal 45%	23.60	23.60
Limestone	1.54	1.54
Dicalcium phosphate	1.43	1.43
Mineral and vitamin supplement*	0.50	0.50
Salt	0.02	0.50
DL-Methionine	0.11	0.11
Total	100	100

*Supplied per kg feed: 7,812 IU vitamin A, 3,125 IU vitamin D_3, 15.6 mg vitamin E, 1.2 mg vitamin K_3, 1.9 mg vitamin B_1, 4.3 mg vitamin B_2, 1.2 mg vitamin B_6, 25 mcg vitamin B_{12}, 0.3 mg folic acid, 0.1 mg biotin, 12.4 mg niacin, 0.06 mg calcium pantothenate 9.4 mg copper, 0.8 mg iodine, 57.2 mg manganese, 75.3 mg zinc, 0.3 mg selenium, 0.3 g choline chloride 50%, 1.8 g methionine, 62.5 mg growth promoter, 0.5 mg antioxidant.

Table 2 - Nutritional composition of the experimental feeds supplied during the different experimental phases.

	Molting methods				Resting
	A90	A70	A50	Zn	
Metabolizable energy (kcal/kg)	1.184	1.592	2.000	3.025	3.008
Crude protein (%)	17.00	17.00	17.00	17.00	17.00
Crude fiber (%)	22.82	18.45	14.09	-	-
Calcium (%)	1.270	1.210	1.150	1.020	1.020
Available phosphorus (%)	0.110	0.170	0.230	0.380	0.380
Total phosphorus (%)	0.267	0.341	0.415	0.603	0.602
Sodium (%)	0.077	0.071	0.065	0.050	0.230
Total lysine (%)	0.796	0.808	0.820	0.855	0.856
Dig. Lysine (%)	0.595	0.645	0.695	0.816	0.818
Total methionine	0.264	0.292	0.320	0.389	0.390
Dig. methionine (%)	0.218	0.254	0.290	0.376	0.377
Total met+cys (%)	0.466	0.518	0.570	0.698	0.698
Dig. met+cys (%)	0.428	0.484	0.540	0.680	0.680
Total threonine	0.733	0.718	0.703	0.658	0.658
Dig. threonine (%)	0.630	0.490	0.350	0.570	0.570
Total tryptophan (%)	0.258	0.234	0.210	0.151	0.151
Dig. tryptophan (%)	0.230	0.210	0.190	0.137	0.137

* A90 = 90% alfalfa and 10% basal diet; A70 = 70% alfalfa and 30% basal diet; A50 = 50% alfalfa and 50% basal diet; Zn = basal diet with the addition of 2,800 ppm zinc.

The obtained data were submitted to analysis of variance using the General Linear Models (GLM) procedure of SAS® statistical package (SAS Institute, 2002). When effect was significant, means were compared, at 5% probability level, by orthogonal and polynomial contrasts, as contrast 1: comparison between the sum of A90, A70, and A50 means vs. the sum of Zn and control (feed fasting) means; contrast 2: linear effect of alfalfa levels; contrast 3: quadratic effect of alfalfa levels; contrast 4: comparison between Zn and control means; contrast 5: linear effect of temperatures; and contrast 6: quadratic effect of temperatures.

RESULTS AND DISCUSSION

Ovary weight loss is concomitant with body weight loss, and this process is directly linked to the recovery of the hen's reproductive tract during the molting period (Braker, 1983). Souza *et al.* (2010) reported that many studies in literature found that reproductive tract involution during molt is required to ensure adequate performance during the second laying cycle. Therefore, morphometric characteristics of some organs of the hens submitted to molting were determined (Tables 3, 4, and 5).

Ovary and oviduct relative weights linearly increased with temperature ($p<0.05$; $y = 0.0292x – 0.0623$, $R^2 = 0.80$; $y = 0.0378x + 0.6448$, $R^2 = 0.97$, respectively) as shown in Table 3, with birds maintained at cold temperature presenting the lightest ovaries and oviducts.

The contrast between dietary alfalfa inclusion means and zinc oxide and feed fasting means (C1) was significant ($p<0.05$) for oviduct relative weight. Average oviduct relative weight of hens submitted to the treatments with alfalfa inclusion (1.82%) was higher than the average relative weight of hens in the zinc oxide and feed fasting groups (1.46%), mainly because of the higher oviduct relative weights of hens fed 70 and 50% of alfalfa (2.07% and 1.93%, respectively).

The low feed intake, stress, and nutritional unbalance that result in the involution of the reproductive tract cause a cascade of neuroendocrine effects. Feed fasting or the supply of diets which

Table 3 - Mean relative weight of organs after molting, including probability and contrasts.

Treatments[1]	Parameters				
	Ovary (%)	Oviduct (%)	Liver (%)	Gizzard (%)	Proventriculus (%)
	Molting methods (MM)[1]				
A90	0.63	1.46	1.23	2.53	0.45
A70	0.83	2.07	1.48	2.45	0.43
A50	0.96	1.93	1.38	2.66	0.43
Zn	0.59	1.37	1.49	2.63	0.53
Feed fasting	0.66	1.55	1.25	2.26	0.40
	Temperature (T)[1]				
CT	0.59	1.37	1.36	2.57	0.49
NT	0.60	1.72	1.43	2.50	0.44
HT	1.02	1.94	1.32	2.45	0.42
	p - value at analysis of variance				
Molting method	0.2829 NS	0.0340*	0.4195 NS	0.0429*	0.0247*
Temperature	0.0143*	0.0214*	0.6930 NS	0.4835 NS	0.0803 NS
MM x T interaction	0.8402 NS	0.7863 NS	0.4350 NS	0.0895 NS	0.4860 NS
CV (%)	44.33	24.25	19.90	8.73	14.23
	p - value for contrasts[2]				
C1	-	0.0305*	-	0.2084 NS	0.2609 NS
C2	-	0.0642 NS	-	0.3321 NS	0.7558 NS
C3	-	0.0779 NS	-	0.2100 NS	0.8573 NS
C4	-	0.4585 NS	-	0.0110*	0.0021*
C5	0.0099*	0.0067*	-	-	-
C6	0.1200 NS	0.6966 NS	-	-	-

NS not significant, *$p< 0.05$. [1]A90=90 % alfalfa and 10 % feed, A70 =70% alfalfa and 30% feed, A50=50% alfalfa and 50% feed, Zn=feed with zinc oxide; CT=cold temperature; NT=neutral temperature; HT=hot temperature. [2]C1=means of treatments with alfalfa versus means of the other treatments (zinc and feed fasting); C2=linear effect of alfalfa levels; C3=quadratic effect of alfalfa levels; C4= comparison between zinc and feed fasting; C5=linear effect of temperature; and C6=quadratic effect of temperature.

Table 4 - Mean organ relative weights after resting, including probability and contrasts.

Treatments[1]	Parameters				
	Ovary (%)	Oviduct (%)	Liver (%)	Gizzard (%)	Proventriculus (%)
	Molting methods (MM)[1]				
A90	0.97	1.81	0.33	0.98	1.15
A70	1.32	2.08	0.33	1.17	1.23
A50	1.41	2.13	0.41	1.26	1.17
Zn	1.50	2.11	0.35	1.17	1.17
Feed fasting	1.35	2.09	0.31	1.30	1.15
	Temperature (T)[1]				
CT	1.46	2.01	0.31	1.23	1.19
NT	1.24	2.13	0.37	1.19	1.26
HT	1.23	1.99	0.35	1.11	1.07
	p - value at analysis of variance				
Molting method	0.0014*	0.1729 NS	0.0134*	0.2717 NS	0.7910 NS
Temperature	0.0204*	0.3468 NS	0.0258*	0.5461 NS	0.0066*
MM x T interaction	0.0007*	0.048*	0.0238*	0.5730 NS	0.0573 NS
CV (%)	13.80	11.60	13.26	21.27	10.01
	p - value for contrasts[2]				
C5	-	-	-	-	0.0300*
C6	-	-	-	-	0.0105*

NS not significant, *p< 0.05. 1A90=90 % alfalfa and 10 % feed, A70 =70% alfalfa and 30% feed, A50=50% alfalfa and 50% feed, Zn=feed with zinc oxide; CT=cold temperature; NT=neutral temperature; HT=hot temperature. [2]C5=linear effect of temperature and C6=quadratic effect of temperature.

composition does not supply the nutrients required for body maintenance, cause stress and, consequently hypothalamus hyperactivity. The hypothalamus than releases corticotropic (ACTHRF) and thyrotropic (TSHRF) hormones (Girardon, 2011), which act on the pituitary, which then secretes ACTH and TSH. The increase in the blood levels of these hormones leads to adrenal and thyroid hypertrophy and hyperfunction, causing gonadotropic hormone unbalance (Garcia, 2004).

Woodward *et al.* (2005) fed Leghorn layers a regular feed, 100% alfalfa or fasted the hens during a 9-day experimental period. The authors observed that ovary weight loss was similar between the fasted and the alfalfa-fed hens. This was also observed by Donalson *et al.* (2005) and Landers *et al.* (2005) when comparing feed fasting and feeding 100% alfalfa as forced-molting methods. Landers *et al.* (2008) found a 10-fold ovary reduction in commercial layers submitted to forced molting by feeding 100% alfalfa or feed fasting.

The results of the present study are consistent with literature reports that show that the supply of high fiber levels to induce molting causes similar reproductive tract weight loss as feed fasting (Table 3). According to Landers *et al.* (2005), in order to be accepted by the poultry industry, methods to induce molting must provide a stimulus substantial enough to cause suficient reproductive tract regression during molting and result in egg production and quality in the second cycle similar to those obtained with the feed-fasting method.

The contrast between the zinc oxide and the feed fasting methods was significant ($p<0.05$) when gizzard and proventriculus relative weights were considered, with heavier weights for the birds fed zinc oxide. These findings are consistent with those reported by Souza *et al.* (2010), who observed severe gizzard regression and lower gizzard weight when hens were submitted to feed fasting (1.17%).

There was no effect of molting method or temperature ($p>0.05$) on liver relative weight.

Table 4 shows the effects of molting methods applied at different temperatures on ovary, oviduct, liver, gizzard, and proventriculus relative weights of hens sacrificed after the resting period.

Temperature had a quadratic effect ($p<0.05$) on proventriculus relative weight, which was the heaviest at thermoneutral temperature ($y = -0.0022x^2 + 0.1157x - 0.225$; $R^2 = 0.99$), as shown in Table 4.

Gizzard relative weight was not influenced ($p>0.05$) by molting method or temperature during the resting period.

There was a significant interaction ($p<0.05$) between treatments in relation to ovary, oviduct, and liver relative weights.

Mean relative weight, F value of orthogonal and polynomial contrasts of the organs that presented significant interaction during the resting period are shown in Table 5.

It was observed that, when hens were kept in the hot temperature environment, the average ovary relative weight of hens fed alfalfa was lower than that of those fed zinc oxide and feed fasted (C1), with 0.95% and 1.66%, respectively.

Also for hens kept at hot temperature, ovary and oviduct relative weights were quadratically ($p<0.05$) affected by alfalfa levels (C3) with higher relative ovary and oviduct relative weights for molting methods A50 and A70, respectively ($y = -0.0009x^2 + 0.1005x - 1.3738$; $R^2 = 0.99$, $y = -0.0018x^2 + 0.229x - 4.9225$; $R^2 = 0.99$, respectively).

When molting methods were considered, temperature had a quadratic effect ($p<0.05$)on the

Table 5 - Mean relative weight, F value of orthogonal and polynomial contrasts of the organs that presented significant interaction during the resting period and contrast probabilities**.**

Ovary (%)	Temperature (T)[1]					
Molting methods (MM)[1]	CT	NT	HT	Mean	C5	C6
A90	1.35	1.28	0.28	0.97	0.0003*	0.0020*
A70	1.52	1.25	1.19	1.32	0.3258 NS	0.695 NS
A50	1.48	1.39	1.37	1.41	0.6643 NS	0.8713 NS
Zn	1.50	1.22	1.79	1.50	0.1567 NS	0.0501*
Feed fasting	1.46	1.05	1.53	1.35	0.3618 NS	0.0051*
Mean	1.46	1.24	1.23	1.31		
C1	0.8396NS	0.0703NS	0.0005*			
C2	0.5933NS	0.3863NS	0.0006*			
C3	0.6313NS	0.4358NS	0.0305*			
C4	0.8812NS	0.2025NS	0.1304NS			
Oviduct (%)	**Temperature (T)[1]**					
Molting methods (MM)[1]	CT	NT	HT	Mean	C5	C6
A90	1.87	2.26	1.31	1.81	0.0829 NS	0.0371*
A70	1.85	1.97	2.41	2.08	0.1232 NS	0.5214 NS
A50	2.17	2.14	2.09	2.13	0.6517 NS	0.9395 NS
Zn	2.01	2.23	2.07	2.11	0.7269 NS	0.2457 NS
Feed fasting	2.17	2.07	2.03	2.09	0.7333 NS	0.9269 NS
Mean	2.01	2.13	1.99	2.04		
C1	0.5744NS	0.7776NS	0.4244NS			
C2	0.3979NS	0.4644NS	0.1112NS			
C3	0.5940NS	0.1401NS	0.0033*			
C4	0.6533NS	0.3260NS	0.8683NS			
Liver (%)	**Temperature (T)[1]**					
Molting methods (MM)[1]	CT	NT	HT	Mean	C5	C6
A90	0.27	0.42	0.29	0.33	0.5157 NS	0.0044*
A70	0.49	0.29	0.39	0.33	0.0652 NS	0.1683 NS
A50	0.41	0.45	0.37	0.41	0.4642 NS	0.2107 NS
Zn	0.27	0.43	0.37	0.35	0.2155 NS	0.1218 NS
Feed fasting	0.31	0.28	0.34	0.31	0.6455 NS	0.4425 NS
Mean	0.31	0.37	0.35	0.34		
C1	0.3143NS	0.2093 NS	0.9807 NS			
C2	0.0450*	0.4187 NS	0.1557 NS			
C3	0.0329*	0.0044*	0.2154 NS			
C4	0.4667NS	0.0070*	0.6441 NS			

NS not significant, *p< 0.05. 1A90=90 % alfalfa and 10 % feed, A70 =70% alfalfa and 30% feed, A50=50% alfalfa and 50% feed, Zn=feed with zinc oxide; CT=cold temperature; NT=neutral temperature; HT=hot temperature. C1=means of treatments with alfalfa versus means of the other treatments (zinc oxide and feed fasting); C2=linear effect of alfalfa levels; C3=quadratic effect of alfalfa levels; C4= comparison between zinc and feed fasting; C5=linear effect of temperature; and C6=quadratic effect of temperature.

ovary and oviduct relative weights of birds fed 90% alfalfa, with higher ovary and oviduct relative weights ($y = -0.0077x^2 + 0.3503x - 2.59$; $R^2 = 0.99$, $y = -0.0116x^2 + 0.6024x - 5.525$; $R^2 = 0.99$, respectively) obtained in hens kept at thermoneutral temperature.

When molting was induced with zinc oxide and feed fasting, temperature had a quadratic effect on ovary relative weight ($p<0.05$; $y = 0.0074x^2 - 0.3886x + 6.305$; $R^2 = 0.99$, $y = 0.0079x^2 - 0.4301x + 6.9$; $R^2 = 0.99$, respectively), with the heaviest ovary relative weight obtained in hens kept in the hot temperature environment.

The recovery of the reproductive tract after the resting period is extremely important for egg production during the second laying cycle. One of the main functions of the ovary is to produce steroidal hormones, which are essential for reproductive tract growth and function and depend on the bird's nutritional status. The reproductive system is regulated by the hypothalamic-pituitary-gonadal axis. The gonadotropin-releasing hormone (GnRH) stimulates the pituitary to secrete the luteinizing hormone (LH), which is responsible for stimulating progesterone production by the mature follicle that consequently breaks and releases the ovum. The pituitary also produces the follicle-stimulating hormone (FSH). FSH may stimulate the production of steroidal hormones by the developing follicle cell, particularly in the small follicles (Leite & Viveiros, 2009).

The details of the interactions found in the present study showed that, when hens are maintained at hot temperature, the most efficient molting methods in terms of recovery of the reproductive tract were A50, A70, zinc oxide, and feed fasting, whereas when temperature was thermoneutral, A90 promoted the best recovery of the ovary and of the oviduct.

There is little information in literature describing the effects of alternative molting methods on the recovery of organs after the resting period, particularly when different environmental temperatures were used.

There was a quadratic effect ($p<0.05$) of the interaction (C3) of cold temperature with dietary alfalfa levels ($y = -0.0004x^2 + 0.049x - 1.1025$; $R^2 = 0.99$), with hens submitted to molting methods A70 and A50 presenting the heaviest livers. When birds were kept at thermoneutral temperature, the contrast among alfalfa levels (C3) had a quadratic effect ($p<0.05$) on liver relative weight ($y = 0.0004x^2 - 0.0515x + 2.1187$; $R^2 = 0.99$), with birds submitted to A90 and A50 presenting heavier livers. Also when kept under thermoneutral temperature, when the methods of feed fasting and zinc oxide were compared, hens submitted to the zinc oxide method presented higher liver relative weight ($p<0.05$).

Considering the molting methods applied, only liver relative weight of hens submitted to A90 was influenced by temperature ($p<0.05$; $y = -0.0025x2 + 0.1395x - 1.515$; $R^2 = 0.99$), with the heaviest livers obtained at thermoneutral temperature.

Better liver weight recovery indicates that more energy sources, such as glycogen and lipids, are being metabolized in the liver (Berry & Brake, 1985). Liver recovery is also an indication that the hormones required for egg production after molting are being produced. Egg production depends on ovarian steroids (Berry & Brake, 1985), such as estrogen, which stimulate yolk production through the synthesis of phosphoproteins that are produced in the liver (Berry & Brake, 1985). Therefore, when hens were submitted to different forced-molting methods and kept at thermoneutral temperature, the method of the dietary inclusion of 90% alfalfa promoted the best liver recovery, which may aid egg production during the second laying cycle.

CONCLUSIONS

The traditional forced-molting method of feed fasting resulted in good performance. However, as the purpose of the present study was to find an alternative method to feed fasting, the dietary inclusion of the highest alfalfa level, that is, the supply of 90% alfalfa and 10% feed was shown to be efficient compared with the other tested methods, particularly when birds were kept under hot temperature. Therefore, the dietary inclusion of 90% alfalfa may be used as an alternative forced-molting method relative to feed fasting, especially when bird welfare is taken into consideration.

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