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Evaluation of hypocaloric and hyperfibrous diets on molting induced in laying hens

Evaluación de dietas hipocalóricas e hiperfibrosas sobre la muda inducida en gallinas en postura

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ABSTRACT

The effect of low-energy and high-fiber diets on induced molting in laying hens was evaluated. Forty Rhode Island Red hens were divided into four feeding schemes (FS): I) alfalfa meal (AM), II) wheat bran (WB), III) AM/WB (1: 1); *ad libitum* and, IV) control diet (30 g·day^{-1}). Feed intake (FI), laying cessation (LC), body weight loss (BWL), energy balance (EB), laying restart (LR), weight and egg production were evaluated. The information was analyzed using mixed models. FI·hen^{-1} was lower in FS I ($P < 0.05$). The LC was higher ($P < 0.05$) in FS II (7.3 days). The AM led to the ideal BWL (25-30%) faster (16.9 days) with respect to the other FS ($P < 0.05$): range between 22.9 to 32.4 days. The LR was faster in FS II ($P < 0.05$): 12.3 days. Egg weight and production were not affected by FS ($P > 0.05$). Inducing molt in laying hens using AM-based FS offers advantages over conventional methods, accelerates body weight loss, ovarian reactivation time is minimized, and productivity is not altered.

Keywords: molting, weight loss, energetic balance, ovarian reactivation, egg production.

RESUMEN

Se evaluó el efecto de dietas bajas en energía y altas en fibra sobre la muda inducida en gallinas en postura. Cuarenta gallinas Rhode Island Red fueron divididas en cuatro esquemas de alimentación (EA): I) harina de alfalfa (HA), II) salvado de trigo (ST), III) HA/ST (1:1); *ad libitum* y, IV) dieta testigo (30 g·día^{-1}). Se evaluó, consumo de alimento (CA), cese de postura (CP), pérdida de peso corporal (PPC), balance energético (BE), reinicio de postura (RP), peso y producción de huevo. La información se analizó mediante los modelos mixtos. El CA·gallina^{-1} fue menor en el EA I ($P < 0.05$). El CP fue más prolongado ($P < 0.05$) en el EA II (7.3 días). La HA propicio la PPC ideal (25-30%) más rápido (16.9 días) con respecto los demás EA ($P < 0.05$): rango entre 22.9 a 32.4 días. El RP fue más rápido en el EA II ($P < 0.05$): 12.3 días. El peso y producción de huevo no se afectó por el EA ($P > 0.05$). Inducir la muda en gallinas mediante EA a base de HA ofrece ventajas sobre métodos convencionales, acelera la pérdida de peso corporal, se minimiza el tiempo de reactivación ovárica y no se altera la productividad.

Palabras clave: pelecha, pérdida de peso, balance energético, reactivación ovárica, producción de huevo.

INTRODUCTION

Most species of birds undergo natural molting; therefore, laying hens are not exempt from this physiological process (Berry, 2003); molting implies an increase in metabolic rate and protein synthesis, loss of adipose tissue, bone mass, suppression of the immune system (Mumma *et al.,* 2006); as well as alteration of the hen's endocrine system (Davis *et al.,* 2000). This is typically associated with reproductive or migratory processes carried out by birds in the wild and which are reflected in minimal food consumption, replacement of feathers (generally incomplete) and irregularities in the laying rate (Koelkebeck and Anderson, 2007). However, in current egg production systems, incomplete molting (natural molting) means an unprofitable period, due to the reduction in production and the end of the hen's productive life (Berry, 2003).

In order to increase the productive life of hen, the egg-producing industry generally extends the productive period of the birds from 80 weeks (a productive cycle) up to 140 weeks through the use of induced molting (Bell, 2003); since it has been observed that post-induction molt, production and egg quality improve (Webster, 2003). Molting has traditionally been induced by fasting for a period of up to ten days, water withdrawal for two days, or both; along with a reduction in photoperiod. However, such practices have been banned by organizations dedicated to animal welfare and in the USA and the EU they have already been eliminated (Mazzuco *et al.,* 2011); practices that in Mexico are surely close to being abolished. Because prolonged fasting not only stimulates molting in birds; it also favors an increase in diseases due to the suppression of the immune system (Ricke, 2003). All this due to the fact that they induce a cascade of physiological adaptations to restore homeostasis; such is the case of the mobilization of immune system cells into the bloodstream (Mumma *et al.,* 2006), increase in the proportion of circulating heterophiles-lymphocytes (Campo *et al.,* 2008) and changes in the ethology of birds (Dunkley *et al.,* 2008).

Due to the aforementioned, it has been decided to investigate new, less aggressive alternatives to induce molting in laying hens and that in turn accelerate the stressful transition of the neuroendocrine mechanism for egg formation and oviposition (Buxade, 2000). In this regard, Guzmán et al. (2016) suggests the existence of a molt stimulation relationship between time and type of food; According to the fiber-energy relationship, thyroid weight loss and hypofunction that induces molt is reported. Other alternative methods to induce molt include modifying dietary minerals, reducing Ca or Na and increasing Zn, or providing low-calorie diets (Woodward *et al.,* 2005). Likewise, melengestrol acetate has been implemented in order to suppress ovarian activity (Koch *et al*., 2005; Koch *et al*. 2007). There are reports (Donalson *et al.* 2005; Petek and Alpay, 2008) that diets high in fiber (wheat bran, rice husk or cotton seed) promote satiety in the bird, due to the fact that fiber digestion is partial and slower, which determines less food consumption. In addition, the lower energy intake of fibrous diets compared to a conventional diet (2200 *vs*. 2800 kcal kg^{-1}) causes loss of body weight without depressing the immune system (Gordon *et al.,* 2009). Donalson *et al.* (2005) when evaluating the addition of alfalfa with commercial feed on the molting and production of eggs, they report that when supplying 90% alfalfa and 10% commercial feed, the laying ceased six days after treatment. Likewise, Landers *et al.* (2005) indicate that feeding hens with alfalfa favors ovarian reactivation and return of laying at a rate similar to that of hens subjected to prolonged fasting.

The effect of different levels (100, 60 and 40%) of commercial feed restriction for laying hens, added with nopal (*Opuntia ficus-indica*), on the induced molt was recently tested (Juárez *et al.,* 2018); observing that the restriction of 60% of diet, added with 24 g of nopal, was the most favorable alternative to induce molting, since there was no total restriction of food and the objectives of the molt were met, reincorporation of hen as soon as possible to the next laying cycle. Therefore, it is necessary to explore other alternatives such as the use of hypocaloric and hyperfiberic diets, to induce molting in laying hens, promoting the least possible stress.

The objective of the present work was to evaluate the effect of low-energy and high-fiber diets on the induced molting in laying hens and its relationship with productivity.

MATERIAL AND METHODS

The research was carried out in the Poultry Sector of the Zootechnical Post, belonging to the Faculty of Veterinary Medicine and Zootechnics of Michoacana University of San Nicolás de Hidalgo (UMSNH), Michoacán, Mexico. The procedure followed in the handling of the birds complied with NOM-062-ZOO-1999, technical specifications for the production, care and use of laboratory animals.

Animals, diets and accommodations

40 dual-purpose hens of the Rhode Island Red genotype were used, with 52 weeks of laying and 72 of age. The total number of animals was distributed according to a completely randomized design in individual conventional battery-type cages, with dimensions of 46x40x43 cm (length, width and height respectively); in four feeding schemes (FS, n = 10 birds FS^{-1}): FS I, animals that consumed alfalfa meal (AM) *ad libitum*; FS II, birds that consumed wheat bran (WB) *ad libitum*; FS III, animals that consumed AM and WB in proportions 50 to 50% *ad libitum* and; FS IV, birds that consumed a control diet (CD) for "layers", restricted to 30 g day^{-1}. Such diets were fed to the birds until achieving an approximate weight loss between 25 to 30%. After this, the birds of all diets were given CD: 120 g day^{-1} and they were monitored until 18 weeks after the experiment start. Table 1 summarizes the composition and nutritional value of the diet and inputs used.

Table 1. Composition and nutritional value of the diet and inputs used

Ingredients, %	Control Diet (CD)			
Corn	69.00			
Soybean paste	18.88			
Limestone	8.55			
Dicalcium phosphate	1.35			
Kaolin	0.59			
Soybean oil	0.53			
Minerals + vitamins premix [&]	0.50			
Salt (NaCl)	0.43			
DL-methionoine -98%	0.12			
L-lysine -78%	0.03			
Butylhydroxytoluene	0.02			
Nutrient analysis	CD	AM	WB	AM/WB
Metabolizable energy, kcal/kg	2800	900	1640	1207
Crude protein, %	14.50	14.78	15.40	14.34
Crude fiber, %	4.00	11.11	26.6	17.91
Total calcium, %	3.65	1.30	0.14	0.68
Total phosphorus, %	0.34	0.18	1.00	0.56
Digestible arginine, %	0.84	0.60	1.00	0.76
Digestible lysine, %	0.64	0.63	0.61	0.59
Digestible methionine, %	0.34	0.21	0.23	0.21
Met + digestible Cis, %	0.56	0.36	0.55	0.43

[&] Levels per Kg of diet: A Vit - 8000 IU; D3 Vit -2000 IU; E Vit - 50 mg; K Vit - 3 mg; B1 Vit - 1.5 mg; B2 Vit - 4 mg; B6 Vit - 0.12 mg; B12 Vit - 15 mg; Folic acid – 0.6 mg; Pantothenic acid–10 mg; Niacin–30 mg; Biotin – 0.1 mg; Choline–300 mg; Iron–50 mg; Copper – 10 mg; Zinc–70 mg; Manganese – 100 mg; Iodine–1 mg; Selenium – 0.3 mg; Antioxidants 50 mg.
AM= alfalfa meal; WB= wheat bran.

Experimental procedure

The variables evaluated were: initial live weight (ILW) and final (FLW) and body weight loss (BWL) in kg, with the help of a digital scale with a precision of 1.0 g, laying cessation (LC), measured in days interval start molting-weight loss 25 to 30% ($IILW_{25-30}$, days) mortality (Mo)%, molt- return range, to second lay cycle (IMR_{SLC}) in days, weight loss post-treatment (WL_{PT}) in g, body weight at laying restart (BW_{LR}) in days, feed intake during molting (FI_{DM}) in g, post-molting feed intake (FI_{PM}) in kg, body weight at restarting ovarian activity (BW_{ROA}) in kg, second cycle egg production (EP_{SC}) in units, egg weight (EW) in g and laying restart (LR) in days. Regarding the restart of ovarian activity, it was determined indirectly and it is the time (days) that the hen takes to ovoposita, its first post-molting egg. The difference with respect to the laying restart is that this last indicator is when the hen already has a constant production in its egg production.

Statistical analysis

Prior to data analysis, the normality of the distribution and the homogeneity of the variance were determined for the residuals; PROC UNIVARIATE (SAS Inst. Inc., Cary, NC, USA) was used for this (Guido, 2009). The test used to determine normality was the Shapiro-Wilks test (Flores *et al.,*2019); while the Bartlett test was used to determine homogeneity (Arsham and Lovric, 2011). A transformation was carried out (Gutiérrez and de la Vara,

2008) of the BWL and WL_{PT} variables to obtain homogeneity of variance, this under the following formula: $Y' = log_{10}(Y)$.

The data were analyzed by ANDEVA, through repeated measurements using the mixed procedures (MIXED) of the SAS (Littell *et al.,* 1998), with bird nested within FS as source of random variation and FS, evaluation day and interaction FS*day as fixed variation sources. The regression coefficients (PROC REG; SAS) were estimated for the live weight loss of the hen according to the day of evaluation, these being cubic or quadratic according to the distribution of values. Each regression equation was derived and equaled to zero, to determine the critical points (Sánchez, 2012). Differences between groups were obtained using the least squares means (LSmeans) methodology.

To determine the energy balance of the hens, the prediction equation established by the National Research Council (NRC, 1994) was used to estimate the metabolizable energy (ME) requirements:

$$ME_i = (173 - 1.97T)P^{0.75} + 5.5\Delta P + 2.07P.EW;$$

Where: *ME*=metabolizable energy, *T*=ambient temperature, °C; *P*=weight of the bird, kg; ΔP = weight gain, g; and *EW*=egg weight, g.

The ME consumption of hens was calculated from the ME contribution of the food consumed day^{-1} and the energy balance corresponded to the difference between energy intake and energy demand, a value obtained from the previously described equation. The values in the tables and figures are presented as least squares means ± SEM.

RESULTS

An effect of FS was found on food consumption, laying cessation and body weight loss (P <0.001). The hens that presented the lowest feed intake were those that consumed AM only (P <0.05): 71.6, 66.6 and 20.6 g day^{-1} less with respect to the FS based on WB, AM/WB and CD, respectively (Table 2). Laying cessation was longer (P <0.05) in the hens that consumed WB (7.3 days); this with respect to the other FS evaluated (Table 2). The pre-initiation of ovarian reactivity body weight loss was lower (2.9% less) in the hens that consumed AM and WB, with respect to the hens that were added AM/WB or CD (P <0.05) (Table 2).

Table 2. Productive performance of the hen during the period of feed restriction according to the diet

	Diet				
	AM	WB	AM/WB	CD	SEM
Initial live weight, kg	1.926	1.961	1.947	1.953	0.100
Feed intake, g	9.4[a]	81.0[b]	75.7[c]	30.0[d]	1.313
Laying cessation, d	3.0[a]	7.3[b]	4.4[c]	4.1[c]	0.033
Final live weight, kg	1.409[a]	1.459[a]	1.381[b]	1.385[b]	0.100
Weight loss, %	26.6[a]	25.2[a]	29.7[b]	29.4[b]	0.513

AM= alfalfa meal; WB= wheat bran; CD= control diet; SEM= standard error of the mean
[a, b, c, d] Different literals indicate statistical difference (P <0.05) within row

According to the distribution of the values for the body weight loss percentage of hens, it was found that hens that consumed the AM-based diet and hens that consumed CD; the regression estimators for such variable presented a grade four polynomial distribution (Table 3); while for hens that consumed WB and AM/WB presented polynomial distribution of grade three (Table 3).

Table 3. Regression estimators for live weight loss (%) according to pelecha day

Diet	*β0*	*β1*	*β2*	*β3*	*β4*	R^2	P < value
AM	-7.0122	-2.6416	0.1083	-0.0013	0.000005	0.74	<.0001
WB	-4.1437	-1.4325	0.0284	-0.00013	--	0.75	<.0001
AM/WB	-5.8978	-1.8328	0.0410	-0.00021	--	0.67	<.0001
CD	12.1803	-4.2995	0.1402	-0.0015	0.000005	0.70	<.0001

AM=alfalfa meal; WB=wheat bran; CD=control diet. R^2= coefficient of determination

According to the regression equations for weight loss FS^{-1} (Table 3), when derived and equaled to zero, the critical point was found (X; Day, Y; Weight loss). Regarding this, the hens of the FS based on AM, according to the critical point of the regression equation, were the ones that presented the fastest loss of ideal live weight (25 to 30%), so that the reactivity begins ovarian; which was at 16.9 days with a weight loss of 26.6% (Figure 1); while the WB-based FS hens were the ones that took the longest (32.4 days) to reach the required live weight loss, which was 25.2%. Hens that consumed AM/WB and hens that consumed CD presented the required weight loss in a range of 22.9 to 28.7 days (Figure 1).

Regarding the energy balance (kcal day^{-1}), the effect of FS and FS interaction per day (P <0.001) was found. The hens that presented the lowest (P <0.05) average pre-initiation of ovarian reactivation energy balance were those that consumed AM only (-247 kcal day^{-1}); this with respect to the hens that consumed WB (-184 kcal day^{-1}), AM/WB (-143 kcal day^{-1}) and CD (-192 kcal day^{-1}) (Figure 2). According to the diet per day interaction for energy balance, the hens that consumed WB or AM/WB are the ones that presented energy imbalance for the longest time; since not even during the ovarian reactivation phase (phase of lower energy demand and normalization of the diet supply) did they present a positive energy balance, as was the case of the hens that received AM alone or CD (Figure 2).

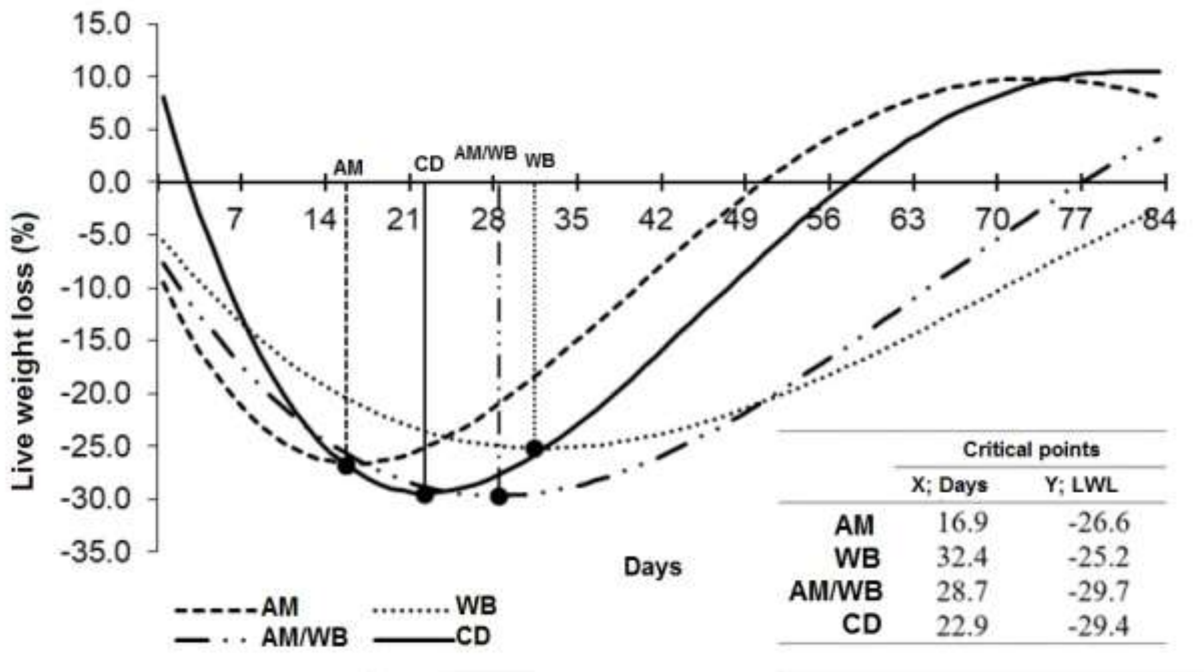

Figure 1. Prediction curves and critical points for live weight loss (%) of hens according to diet and day. AM = alfalfa meal; WB=wheat bran; CD=control diet

According to the effect of FS (P <0.001) for the weight recovered during the ovarian reactivation phase, the hens that presented the lowest recovery of live weight were those that consumed WB only (15.5%), with respect to the other FS (Table 4). The ovarian reactivation of the hens that consumed WB alone or AM/WB was shorter (range from 11.6 to 12.3 days), with respect to the hens that consumed only AM or CD (P <0.05) (Figure 2 and Table 4. According to the beginning of laying, the AM -based FS hens were the ones that presented it the fastest (35.7 days); while in the other FS the restart of laying was at: 44.7, 40.3 and 40.2 days for WB, AM/WB and CD, respectively (Figure 2).

No effect of FS was found on egg weight and production (P> 0.05). Egg weight ranged from 63.7 to 67.5 g; while the estimated production during a period of forty days was 25.6 average eggs (Table 4). However, the hens that consumed AM only, showed a trend in the increase of eggs produced; 6.0 more average eggs with respect to the other diets evaluated (Table 4).

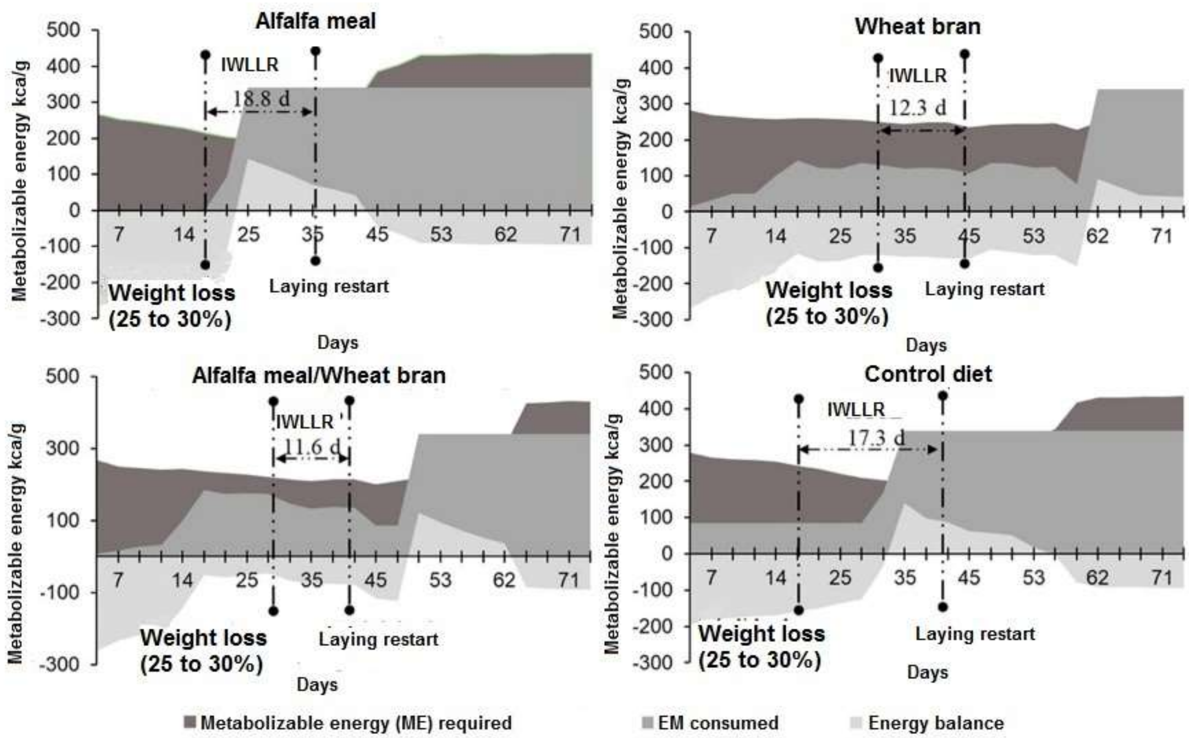

Figure 2. Required and consumed metabolizable energy and energy balance of hens according to diet and day. IWLLR = ideal weight loss-laying restart interval

DISCUSSION

The results of this study support previous research on the effects of high-fiber, low-calorie diets on induced shedding and productive behavior in laying hens (Petek and Alpay, 2008). It has been established (Bell, 2003) that the induction of molting by fasting and the molting method by rapid induction through the implementation of low-calorie diets, can be equally effective in promoting the molting process; This can be observed in weight loss and laying cessation during the molting stage (table 2 and figure 1); where the AM-based FS was the one that had the best results, it caused the molt with weight loss and rapid interruption of the laying (Petek and Alpay, 2008). It is important to emphasize that the laying cessation was in a range between 3.0 to 7.3 days after starting the implementation of the diets; since the faster this process is the hen will be subjected to less stress, which improves the health of the birds in the long term; therefore, according to this guideline, the induced molt regimen can promote the health and well-being of the hens (McCowan *et al.,* 2015).

Table 4. Productive performance of hens' post-restart of laying diet, according to the diet established in the period of food restriction

	Diet				
	AM	WB	AM/WB	CD	SEM
Live weight at CDR, kg	1.563[a]	1.651[a]	1.561[a]	1.629[a]	0.139
Live weight at ROA, kg	1.926[a]	1.953[b]	1.947[b]	1.990[c]	0.139
Live weight at laying restart, %	18.9[a]	15.5[b]	19.1[a]	18.2[a]	0.498
Laying restart, días	18.8[b]	12.3 [a]	11.6 [a]	17.3 [b]	0.517
Egg weight, g	66.6	67.5	65.5	63.7	3.012
Egg production [&], units	30.1	26.2	22.5	23.6	2.540

AM = alfalfa meal; WB = wheat bran; CD = control diet, SEM = standard error of the mean, RCD = restart control diet; ROA= restart of ovarian activity; [&]Estimated production over a period of forty days. [a, b, c] Different literals indicate statistical difference ($P < 0.05$) within row.

When analyzing molting in the health-production context according to specialists in the reproductive physiology of birds, the arguments issued by organizations that promote animal welfare lose their justification, due in the first instance to the fact that molting is a physiological process and, in the second instance, the induced molt accelerates the stress period of the birds. In addition to this, if one starts from epistemological processes of stress, the absence of stress is death (Selye, 1973); In addition, there are 2 types of stress, allostatic "good stress" and pantostatic "bad stress". On the basis of these investigations, it is difficult to support the assumption that hens are in danger during induced molting; since molting is an inherent physiological process of the species and production patterns, or the physiology of the hen changes through adaptive phases in its life continuously, regardless of how they are housed or what management practices are imposed (Koelkebeck and Anderson, 2007).

It has been shown that zero percent egg production can be obtained by shedding chickens on any abstinence diet, compared to total fasting; however, regardless of this, post-molt performance is similar in hens fed a molt diet based on corn: soybean meal (47: 47%), or corn: wheat (23: 71%), compared to fasting for 10 days (Scheideler and Beck, 2002). This behavior was not presented in this research, since the hens were not subjected to fasting and they did show cessation of laying (Table 2). Regarding what was reported by Woodward *et al.* (2005), possibly when implementing conventional diets the hens did not cease laying for two reasons: 1) the inputs implemented in the diets are high in protein and energy, which could keep the hens in a state of energy comfort for a longer time and, 2) by presenting energy comfort, the birds do not experience acute stress; therefore, the neuroendocrine centers that induce total ovarian shedding and restoration are not stimulated.

With respect to the diets implemented in this research, being hypocaloric and hyperfiberic, they stimulate acute stress (allostasis), which is reflected in a rapid decrease in body weight (figure 1), due to the fact that the calories consumed do not satisfy nutritional requirements; therefore, the birds enter a negative energy balance (Figure 2); since fiber in monogastrics is not digested by gastrointestinal enzymes, this modifies the absorption of bile salts, cholesterol and glucose (Molist *et al.,* 2009). The modification in the absorption of these components stimulates body catabolism and weight loss, an aspect observed in birds that consumed AM mainly (Figures 1 and 2). In addition to this, the consumption of fibrous diets in monogastrics reduces the digestibility coefficient of organic matter (Rentería *et al.,* 2008), which indicates that this strategy promotes mechanical satiety in the animal without exceeding the intake of metabolizable energy. Therefore, this strategy would comply with the animal welfare standards, since the birds would not present chronic stressful periods (pantostatic), due to the benefits that fiber has in relation to gastric filling.

According to the acute decrease in body weight in the hens that consumed CD, it was due to the contribution (30 g day^{-1}) of food, in order not to provide total fasting; such contribution does not satisfy the nutritional requirements; therefore, weight and energy balance were negative (Figures 1 and 2). Regardless that this feeding protocol was successful in inducing molt and did not affect subsequent productivity, it lacks the properties of fiber; therefore, the birds were exposed to starvation periods. Based on Webster (2003) theory, the least effective FS were those containing WB. According to Jasso (2012) if the ideal weight loss (25 to 30%) does not occur in a period of less than 15 days and there is greater stress in the bird, due to the fact that cortisol is synthesized for a longer period of time (pantostasis), with respect to whether total fasting or in this case AM-based FS is applied, protocols that induce acute stress. These protocols promote a temporary increase in cortisol a few days after the first phase of the molt (weight loss), later; Because all energy expenditure is derived from fat metabolism; corticosterone levels will be low during the second phase of molting (ovarian reactivation) (Webster, 2003). This can be observed indirectly in the energy balance presented by the birds that consumed WB (Figure 2), birds that presented a lower energy balance; This can be attributed to the properties of WB that can be fermented in the gastrointestinal tract and produce short chain fatty acids that can participate in the maintenance of the animal (Berruezo *et al.,* 2011), which limits the molting process of sharp way.

It has been reported (Davis *et al.,* 2000), that molting is a stress in the hen that causes the laying cessation and a significant loss in body weight. However, stress is what initiates the molt both in the commercial environment and in the natural state. The benefits that the molt causes are evident, it improves the quality of the meat up to 9%; reproductive efficiency and egg quality (Anderson, 2002), which translates into better health and vitality compared to hens that have never molted. This response was observed in the present investigation, since the restart of laying activity, egg weight and production were not affected by the diets implemented.

Finally, there are some limitations that must be taken into account when interpreting the results of this study. The hormonal behavior of the hypothalamic-pituitary-adrenal gland axis was not evaluated; However, even with such limitations, this research provides valuable information for specialists in the area and poultry producers on the importance of the implementation of hypocaloric and hyper-fibrous diets, as a strategy to induce molt in laying hens in a successful way; since the implementation of these diets does not propitiate pantostatic stress to the animals, which is a limitation that these systems have before the new laws of animal welfare; Likewise, this type of diet reduces the time for ovarian reactivation and laying restart.

CONCLUSION

The implementation of diets based on alfalfa meal to induce molting in laying hens offers advantages over conventional fasting-based molting methods, because it accelerates the loss of body weight by promoting a lower energy balance, which is It is reflected in a shorter ovarian reactivation time; this without altering the post-molt productivity of the hen.

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