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Use of Nicarbazin, Salinomycin and Zinc Oxide as Alternative Molting Methods for Commercial Laying Hens

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ABSTRACT

An experiment was carried out to evaluate the performance, egg quality and morphometry of the reproductive tract, liver, pancreas and tongue of laying hens submitted to different molting methods. Two hundred and eighty eight 72-week-old Isa brown layers were distributed according to a completely randomized design with six treatments (molting methods) and six replicates of eight birds each. Layers were fed diets containing 3000 ppm zinc oxide, 60 ppm or 120 ppm nicarbazin, 30 ppm or 60 ppm salinomycin, or were submitted to feed fasting. Data were submitted to analysis of variance and means were compared by the test of Tukey at 5% probability level. Molting methods alternative to feed fasting were effective to induce molting in layer and provided good performance results in the second laying cycle.

INTRODUCTION

Modern layer strains have high genetic potential for egg production and may produce eggs for more than one laying cycle by inducing molting. Forced molting is defined as a set of management practices to promote stress, inducing birds to stop producing eggs and the partial molting of the feathers (Roland & Brake, 1982). The objective of forced molting is to rest the reproductive system of layers for some time in order to recover their production capacity and to increase the longevity of layers for further 25 to 30 weeks, as well as to improve egg quality, reducing losses caused by poor eggshell quality (Berry, 2003).

Alternative molting techniques, except for the supply of zinc-rich diets, have shown to be as effective to discontinue egg production as feed restriction. Layers fed diets with nicarbazin reduce egg production and egg weight (Chapman, 1994), as nicarbazin may prevent ovule maturation (Baker *et al*., 1957). High doses of ionophore antibiotics, such as salinomycin, may cause severe cell function and morphological disorders in poultry (Novilla, 1992), and anorexia is one of the most frequent clinical signs (Schweitzer *et al*., 1984).

Similarly to mammals, poultry also have taste buds (Kare *et al*., 1976) and are capable of differentiating the flavor of different chemical solutions, although behavioral effects are not evident (Denbow, 1985). Changes in diet composition, such as drug inclusion and excessive mineral content, could result in changes in the taste buds, and therefore directly influence poultry intake activities (Denbow, 1985).

The objective of the present study was to evaluate molting methods alternative to feed fasting on the performance, egg quality, and morphometrics of the tongue, reproductive tract, liver, and pancreas of commercial layers.

MATERIALS AND METHODS

The experimental period included the phases of molting, resting, and second laying cycle (four periods of 28 days each). Hens were selected according to body weight and egg production. In the trial, 288 commercial Isa Brown layers with 72 weeks of age were distributed, according to a completely randomized experimental design into six treatments with six replicates of eight birds each, totaling 36 experimental units.

Feeds supplied during the experimental periods were formulated according to Rostagno (2011), as shown in Table 1. The molting diet contained 16.29% crude protein, 0.44% calcium, 0.275% available phosphorus, and 0.02% sodium.

The following treatments were applied for 14 days to induce molting: feed fasting and the dietary inclusion of 3000 ppm zinc oxide, 60 or 120 ppm nicarbazin, or 30 or 60 ppm salinomycin.

Table 1 – Ingredients and nutritional composition of the experimental diets.

Ingredients (%)	Molting diet	Resting diet	Lay diet
Corn grain	70.47	77.17	66.06
Soybean meal (45%)	19.50	11.70	21.80
Wheat midds	8.00	8.00	-
Limestone	0.35	1.40	9.20
Dicalcium phosphate	0.90	1.11	1.01
Mineral and vitamin supplement*	0.20	0.20	0.20
Salt	0.01	0.40	0.50
DL-methionine	0.15	0.02	0.16
Soybean oil	-	-	1.07
Variable portion**	0.42	-	-
Total	100	100	100
Nutritional composition			
Metabolizable energy (kcal/kg)	2.868	2.910	2.850
Crude protein (%)	16.29	13.31	15.60
Calcium (%)	0.440	0.870	3.850
Available phosphorus (%)	0.275	0.310	0.280
Total phosphorus (%)	0.529	0.543	0.483
Sodium (%)	0.020	0.170	0.210
Total lysine (%)	0.762	0.562	0.764
Digestible lysine (%)	0.684	0.499	0.694
Total methionine (%)	0.406	0.240	0.415
Digestible methionine (%)	0.377	0.216	0.389
Total methionine + cystine (%)	0.688	0.486	0.677
Digestible methionine + cystine (%)	0.622	0.430	0.620
Total threonine (%)	0.613	0.495	0.599
Digestible threonine (%)	0.526	0.425	0.523
Total tryptophan (%)	0.186	0.143	0.181
Digestible tryptophan (%)	0.164	0.125	0.162

*Supplied per kg feed: 8,000 IU vitamin A, 1,800 IU vitamin D3, 12 mg vitamin E, 2 mg vitamin K3, 1 mg vitamin B1, 4 mg vitamin B2, 1 mg vitamin B6, 10 mcg vitamin B12, 0.40 mg folic acid, 0.04 mg biotin, 28 mg niacin, 11 mg calcium pantothenate, 6 mg Cu, 0.10 mg Co, 1 mg I, 50 mg Fe, 65 mg Mn, 45 mg Zn, 0.21 mg Se, 500 mg choline chloride 50%, 1400 mg methionine, 125 mg antioxidant.

Variable portion** The variable portion consisted of finely-ground rice hulls and/or zinc oxide and/or each of the anticoccidial agents.

During molting, the following performance parameters were evaluated: days until laying ceased, feed intake, egg production, body weight lost between 7 and 14 days.

During the resting period, feed intake, egg production, days to return to lay (50 – 60% egg production), days to lay of the first and the tenth egg, body weight recovery, feed conversion ratio (kg feed/dozen eggs), and livability were evaluated.

During the second laying cycle, the following performance parameters were evaluated at the end of each period: feed intake (feed offer minus feed residue), egg production (average number of eggs laid per experimental unit), feed conversion ratio (kg of feed per kg of eggs and per dozen eggs produced), average egg weight (total egg weight/number of eggs laid per experimental unit), and egg mass (egg production % x average egg weight). The following egg quality parameters were evaluated: Haugh units, calculated as 100 log (h + 7.57 - 1.7 $W^{0.37}$), where H = albumen height in mm, W = egg weight in g; eggshell percentage and thickness, and egg specific gravity. Egg specific gravity was determined immersing the eggs in graded NaCl solutions (1.065 to 1.100 g/cm^3, in 0.005 gradients).

Birds were sacrificed at the end of the molting period and of the resting period. Hens were sacrificed by neck dislocation after feed fasting for eight hours. Birds were previously fasted for eight hours and weighed immediately before sacrifice to allow subsequent calculation of ovary, oviduct, liver, and pancreas weights relative to body weight.

The tongue was removed from caudal region of the mouth cavity, immersed in formalin at 10% and submitted to morphometric evaluation, which included taste bud counting and measuring their area and diameter in histological slides. Tongue samples were fixed in Bouin solution (saturated solution of picric acid, acetic acid, and formalin) for 24 hours at room temperature. Samples were than rinsed in water to remove excessive fixing solution, immersed in ethanol at 70%, processed according to light microscopy routine methods with the inclusion in histosec. Samples were dehydrated in graded series of ethanol (80%, 90%, 95%, and absolute ethanol (3x), cleared in alcohol xylol solution (1:1) and xylol (3x), embedded in paraffin (3x), and included with histosec (40 minutes for each solution. Semi-serial cross sections (6µm) were cut per sample, which were then deparaffinized and stained with hematoxylin-eosin (HE), and again dehydrated and cleared for finally mounting the slides with Entellan®.

Taste bud number, area, and diameter were determined at 40x magnification using a light microscope (Olympus®) , which was coupled to a digital image analyzing system (Leica, Digital Image Processing And Analysis Software For Professional Microscopy- Qwin V3) together with Optimus 4.0 software program.

The obtained data were submitted to analysis of variance using the General Linear Model (GLM) procedure of SAS statistical package (SAS Institute, 2002). Means were compared by the test of Tukey at 5% probability level.

RESULTS AND DISCUSSION

The applied treatments significantly influenced the evaluated performance parameters (Table 2).

The feed intake of the hens fed the diet containing 3000 zinc oxide was significantly lower compared to the other treatments ($p < 0.05$). This is consistent with the results of Shippee *et al*. (1979), who observed that layers fed 1% zinc as zinc oxide or zinc acetate presented feed intake of 22 and 16g/bird , respectively, during the second week of the induced molting period, when hens also presented 0% egg production. This may due to reduced appetite (Bar *et al*. 2003) or palatability (Fok, 1989). It was also reported that feed intake may be reduced because zinc cation (Zn_2^+) induces follicle atresia, interrupting egg production (Johnson & Brake, 1992). Therefore, it is possible that the efficiency of the treatment with the diet with high zinc levels is directly related with feed intake.

Layers fed 60 ppm nicarbazin produced more eggs than those submitted to feed fasting. This result is different from the findings of Park *et al*. (2004), who did not observe any differences in egg production among different induced-molting methods (fasting and dietary inclusion of zinc acetate or zinc propionate).

Layers submitted to feed fasting during the induced-molting period presented higher weight loss on day 7 compared with those fed 60 ppm nicarbazin, but not with those submitted to the other treatments. On day 14 of induced molting, hens in the feed fasting treatment lost the most body weight during this period and those fed 60 ppm nicarbazin and 3000 ppm zinc oxide lost the least body weight. Body weight loss contributes for molting as it affects reproduction and body fat reserves (Park *et al*. 2004). According to Alodan & Mashaly (1999), the regression of the reproductive tract is proportional to body weight loss, which rejuvenates the reproductive tract and reduces body fat, thereby enhancing tissue efficiency.

The fed-fasted hen required less days to cease egg laying compared with those fed the 30 ppm zinc diet, but were not different from those submitted to the other treatments. According to North & Bell (1990) and Bell (2003), the sooner the hens cease laying eggs, the sooner they will start producing eggs during the resting period, and the peak of egg production in the second cycle occurs sooner.

During the resting period, feed intake significantly increased ($p<0.05$) when hens were fed 3000 ppm zinc during the molting period relative to those fed 60 ppm nicarbazin, but was not different compared with the other treatments. This is probably due to the low feed intake of the hens fed 3000 ppm zinc oxide during the molting period. On the other hand, Hassanabadi & Kermanshahi (2007) did not find any reduction in the feed intake of layers fed 20,000 ppm zinc oxide during the molting period.

Table 2 – Average performance parameters obtained during the molting period.

Treatments	Feed intake (g/hen/d)	Egg production (%)	Body weight loss Day 7 (%)	Body weight loss Day 14 (%)	Days to lay cessation
Zinc oxide 3000 ppm	38.18b	15.71ab	16.74ab	18.40b	6.16ab
Nicarbazin 60 ppm	62.39a	20.46a	11.68b	11.68c	6.33ab
Nicarbazin 120 ppm	61.90a	15.90ab	18.49ab	13.44bc	6.00ab
Salinomycin 30 ppm	58.94a	19.50ab	19.62ab	12.81bc	7.50a
Salinomycin 60 ppm	59.60a	18.23ab	26.73ab	14.52bc	6.33ab
Feed fasting	-	14.17b	30.34a	28.93a	4.00b
F value	63.08*	3.64*	4.71*	20.39*	3.11*
CV[1] (%)	5.58	18.13	21.57	21.04	26.11

*Significant at 5% probability level. [a,b] Means followed by different letters are statistically different. [1] Coefficient of variation.

Layers fed 60 and 120 ppm nicarbazin needed fewer days to lay the 1st and 10th egg during the resting period, suggesting a direct relationship between the beginning of lay in the resting period and body weight loss during molting. This was also observed by Sgavioli *et al*. (2011), who obtained lower body weight loss and shorter interval to lay the first egg during the resting period in layers fed alfalfa to induce molting.

Table 3 – Average performance parameters obtained during the resting period.

Treatments	Feed intake (g/hen/d)	Egg production (%/hen/d)	Return to lay (days)	1st egg laid (days)	10th egg laid (days)	Feed conversion ratio (kg/dz)	Livability (%)
Zinc oxide 3000 ppm	103.80a	31.42	9.16	8.16ab	12.66ab	4.22	88.19
Nicarbazin 60 ppm	91.00b	22.83	7.00	2.66c	8.66c	4.31	91.66
Nicarbazin 120 ppm	99.61ab	30.93	7.66	4.16c	8.5c	3.71	91.36
Salinomycin 30 ppm	95.72ab	25.90	13.66	5.00bc	10.83bc	3.44	91.36
Salinomycin 60 ppm	94.17ab	23.05	11.33	5.33bc	10.33bc	5.16	91.36
Feed fasting	97.55ab	25.74	16.00	10.66a	15.33a	4.84	93.75
F value	2.48*	1.28ns	2.41 ns	15.62*	17.42*	1.73ns	0.31ns
CV[1] (%)	7.13	30.40	51.66	30.07	13.48	26.34	8.58

ns = not significant *Significant at 5% probability level. [a,b] Means followed by different letters are statistically different. [1] Coefficient of variation.

During the second laying cycle, performance was significant influenced ($p < 0.05$) by the treatments (Table 4).

Layers fed 30 ppm salinomycin laid significantly lighter eggs compared to those submitted to feed fasting. However, egg weight was not different among treatments with zinc oxide, nicarbazin at both concentrations, and 60 ppm salinomycin. This result may be explained by the greater body weight loss of those hens during molting, which directly affects reproductive tract rejuvenation and fat reserve loss (Alodan & Mashaly, 1999).

There was no influence of treatments on the other evaluated parameters ($p>0.05$).

Although fed-fasted layers laid heavier eggs during the second laying cycle (Table 4), no changes in eggshell quality were observed.

Table 4 – Average performance parameters of layers during the second laying cycle.

Treatments	Feed intake (g/hen/d)	Egg production (%)	Feed conversion ratio (kg/dz)	Feed conversion ratio (kg/kg)	Egg weight (g)	Egg mass (g/day)
Zinc oxide 3000 ppm	88.81	54.17	2.02	2.71	63.89ab	36.19
Nicarbazin 60 ppm	89.56	54.14	2.03	2.70	64.19ab	36.20
Nicarbazin 120 ppm	92.03	53.50	2.03	2.61	66.09ab	34.50
Salinomycin 30 ppm	93.17	54.10	2.02	2.99	62.52b	33.85
Salinomycin 60 ppm	90.45	59.67	1.91	2.74	64.95ab	36.15
Feed fasting	101.44	61.71	2.01	2.47	67.95a	38.47
F value	0.82ns	0.26 NS	0.83 NS	1.92 NS	4.75*	0.61 NS
CV[1](%)	10.1	9.04	6.90	7.81	2.17	9.95

ns = not significant *Significant at 5% probability level. [a,b] Means followed by different letters are statistically different. [1] Coefficient of variation.

The different methods applied to induce molting did not affect internal and external egg quality parameters evaluated during their second laying cycle (Table 5). McCormick & Cunningham (1987) also did not report any effect of treatments on egg quality.

Table 5 – Average egg quality parameters determined during the second laying cycle.

Treatments	Haugh units	Eggshell (%)	Eggshell thickness (mm)	Egg specific gravity (g/cm³)
Zinc oxide 3000 ppm	94.81	9.70	0.392	1.088
Nicarbazin 60 ppm	94.36	9.66	0.394	1.087
Nicarbazin 120 ppm	94.78	9.84	0.401	1.088
Salinomycin 30 ppm	94.77	9.65	0.390	1.088
Salinomycin 60ppm	95.83	9.52	0.393	1.087
Feed fasting	96.17	9.59	0.401	1.089
F value	0.26 ^{ns}	0.28 ^{ns}	0.37 ^{ns}	0.61 ^{ns}
CV[1](%)	2.52	3.79	2.89	0.16

ns = not significant. [1] Coefficient of variation.

Layers submitted to induced molting by feed fasting and dietary inclusions of 3000ppm zinc oxide and 30ppm salinomycin presented lower ovary weight relative to body weight, indicating more faster ovary regression when compared with the other treatments. According to Brake (1993), ovary weight loss is simultaneous to body weight loss, and this process is directly linked to the recovery of the hen's reproductive tract during the molting period. Berry (2003) also reported that the ovary completely regressed when layers lost 25% of their body weight. In the present study, although layers fed zinc oxide and 30ppm salinomycin lost less weight during molting, they showed similar ovary regression at the end of molting period, indicating that the lack of correlation between body weight loss during molting and ovary regression.

The applied treatments significantly affected organ relative weights, except for oviduct weight, as evaluated at the end of the molting period (Table 6).

Table 6 – Average relative organ weights of layers sacrificed at the end of the molting period.

Treatments	Ovary (%)	Oviduct (%)	Liver (%)	Pancreas (%)
Zinc oxide 3000 ppm	0.35b	1.21	1.77ab	0.15ab
Nicarbazin 60 ppm	1.07ab	2.5	1.63ab	0.20ab
Nicarbazin 120 ppm	1.62a	2.32	2.15a	0.19ab
Salinomycin 30 ppm	0.38b	2.41	1.64ab	0.23a
Salinomycin 60 ppm	1.57a	2.72	1.87ab	0.20ab
Feed fasting	0.27b	1.33	1.36b	0.15b
F value	9.62*	3.00 ^{ns}	3.58*	3.21*
CV5 (%)	45.00	35.60	16.20	19.35

ns = not significant *Significant at 5% probability level. [a,b] Means followed by different letters are statistically different. [1] Coefficient of variation.

The lack of effect of induced-molting methods on relative weight of the oviduct ($p < 0.05$) was also observed by Araújo *et al*. (2007), who evaluated different molting methods (California method, diet with high zinc, low calcium or low sodium diets) and did not detect any influence of the treatments on this parameter.

On the other hand, Sgavioli *et al*. (2013) evaluated different molting methods (dietary inclusion of 90, 70, or 50% alfalfa, of 2800 ppm zinc oxide or feed fasting) and obtained higher oviduct weights when layers were fed diets including alfalfa relative to zinc oxide.

Layers submitted to feed fasting presented lower liver relative weight (1.36%) compared with those fed 120 ppm salinomycin, but it was not statistically different relative to the other treatments.

Pancreas relative weight was statistically lower in feed-fasted layers compared with those fed 30 ppm salinomycin.

The results of the present experiment are consistent with the finding of Domingues *et al*. (2012), who used 3000 ppm zinc oxide to induce molting, and obtained similar ovary, oviduct, liver, and pancreas weight values after molting.

After the period of resting, no differences ($p > 0.05$) were observed in the relative weight of the evaluated organs (Table 7).

Table 7 – Average relative organ weights of layers sacrificed at the end of the resting period.

Treatments	Ovary (%)	Oviduct (%)	Liver (%)	Pancreas (%)
Zinc oxide 3000 ppm	1.79	2.56	2.43	0.19
Nicarbazin 60 ppm	2.48	3.34	2.34	0.19
Nicarbazin 120 ppm	2.29	3.67	2.59	0.19
Salinomycin 30 ppm	1.91	3.18	2.51	0.17
Salinomycin 60 ppm	2.17	3.44	2.55	0.17
Fasting	1.97	3.02	2.41	0.18
F value	0.69 ^{ns}	1.34 ^{ns}	0.24 ^{ns}	0.37 ^{ns}
CV[1] (%)	28.47	18.99	15.36	17.80

ns = not significant. [1] Coefficient of variation.

The results indicate that, during resting, layers submitted to the different molting treatments presented similar organ recovery. Therefore, the effects observed during molting did not persist after resting.

When tongue morphometric parameters were evaluated, only taste bud numbers were significantly influenced by the treatments (Table 8).

Table 8 – Average values of tongue morphometric parameters evaluated after molting.

Treatment	Area (µm)	Diameter (µm²)	Taste bud number
Zinc oxide 3000 ppm	1716.7	51.99	5.35b
Nicarbazin 60 ppm	2231.9	48.43	4.90b
Nicarbazin 120 ppm	1716.7	45.95	5.60b
Salinomycin 30 ppm	2231.9	51.65	4.35b
Salinomycin 60 ppm	1972.1	48.65	5.10b
Fasting	820.07	31.48	8.00a
F value	18.01^{ns}	21.37^{ns}	5.02*
CV[1] (%)	37.84	19.39	44.38

ns = not significant *Significant at 5% probability level. [a,b] Means followed by different letters are statistically different. [1] Coefficient of variation.

Layers fed diets containing zinc oxide, nicarbazin and salinomycin presented less taste buds compared to those submitted to feed fasting during molting.

According to Gonzáles (2002), feed palatability is perceived by the taste buds, which act as sensors and may be affected by chemical substances. Shosberg *et al*. (1986) mentioned that those agents, due to their toxicity when added at high levels, may reduce the number of taste buds, and consequently, feed palatability.

CONCLUSIONS

Based on the results obtained in the present study, it was concluded that methods alternative to feed fasting were efficient to induce feather molting in layers. Relative to the main performance parameters, only the diet with 30 ppm salinomycin caused lower egg weight. Therefore, it may be inferred that methods other than feed fasting can be safely applied to induce molting in layers, promoting adequate performance in the second laying cycle.

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