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The Effect of a Dietary Innovative Multi-Material on Sex Hormones and Molting Period of Canaries and Laying-Hens

Research Article

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ABSTRACT

Two experiments were conducted to determine the effect of offering a multi-material innovative (MMI) feed including: *Vitex agnus-castus*, *Thymus vulgaris*, *Lavandula angustifolia*, Marigold (*Calendula officinalis*) on curtails molting and sex hormone concentrations in canaries and laying hens. In the first study, a total of 120 female molted canaries were allotted in to 12 cages of 10 birds with 4 replicates for 135 d. Treatments were control (drinking water without MMI) and 1.25 g or 2.25 g MMI dissolved in 1 L of drinking water. In the second study, a total of 72 molted laying hens were allotted to 24 cages of 3 birds with 8 replicates fed with similar treatments of the first study for 21 d. Results showed that at 45, 90, and 135 d, a linear and quadratic decrease (L and Q: P≤0.006) in plasma prolactin and a linear increase (L: P≤0.037) in plasma progestron and estrogen concentrations of canaries as MMI levels increased. On d 21, the plasma concentrations of prolactin (L and Q: P=0.001), progesterone (L: P=0.042), and estrogen (Q: P=0.036) increased in laying hens along with the increasing of MMI levels. Also, the feather rejuvenation of canaries and laying hens was decreased (L and Q: P≤0.018) with increasing MMI levels. The egg production increased in the first (L: P=0.045), second, and third (L and Q: P≤0.005) weeks with no pronounced trends in feed intake and drinking water of laying hens during the experiment. The use of MMI in the drinking water of canaries increased sex hormones production and shortened molting duration. Also, the innovative dietary additive has stimulating effects on egg production of laying hens without impressed feed or water consumption.

KEY WORDS canary, herbal additive, layer hens, molting.

INTRODUCTION

Hormones have major metabolic roles in reproductive function (e.g. egg production) and molting progress of poultry (Herremans *et al*. 1988; Hoshino *et al*. 1988; Dicerman and Bahr, 1988; Peebles *et al*. 1994; Renden *et al*. 1994), especially hormones upon the hypothalamo-hypophyseal-ovarian axis. Reproductive function in the female is controlled primarily by the interaction of the ovarian sex steroids progesterone and estradiol. The preovulatory rise of plasma progesterone is directly correlated to the ovulation of mature follicles (Johnson, 1984) and precedes and stimulates luteinizing hormone rise. A positive feedback mechanism between progesterone and luteinizing hormone for ovulation induction resulted in positive correlations between circulating levels of progesterone and egg production in layers (Tanabe *et al*. 1983). The importance of progesterone in controlling ovulation has been previously reported in chickens (Kappauf and Van Tienhoven, 1972) and turkeys (Mashaly *et al*. 1979). In mallard ducks, the cessation

of egg laying was associated with low circulating levels of both progesterone and estradiol (Bluhm *et al*. 1983). In turkey, both non-laying and laying hens with low egg production also have low levels of circulating progesterone (Mashaly *et al*. 1979). Thus, the modulation of endocrine factors may lead to the improvement of egg productivity in poultry. Kang *et al*. (2001) reported that high egg production is associated with higher weights of ovary and follicles and higher progesterone confirmed the positive relationship of progesterone expression with egg production. Several studies were conducted to reduce the secretion of prolactin through active and / or passive immunization in bantam hens to prevent development of broodiness (Sharp *et al*. 1989). These studies were targeted through dopamine system because of dopamine inhibits prolactin secretion via hypothalamus. In contrast of mammals, the biological effect of inhibition of prolactin in avian species is not well established (Reddy *et al*. 2006). Molting in avian species is defined as the periodic shedding and replacement of feathers (Berry, 2003).

Poultry normally go through periodic molts and do not have reproduction during molting. Molting naturally can be problematic for the producer because it is erratic and does not have economic benefits (Oguike *et al*. 2005) as well as results in severe stress, lowered immunity and increased mortality rate in birds (Holt, 2003). The canary (*Serinus canarius*) can be considered as a domesticated species of the order Passeriformes. People like this specious very much. Most canaries will stop singing and loose plumage when they begin to drop their feathers, which lead to economical losses.

On the other hand, natural molting in laying hens generally takes four months (North and Bell, 1990), which raises economic concerns as the hens continue to be fed during non-productive times (McDaniel and Aske, 2000). Innovative molting techniques should be safe, animal friendly, minimize bird stress, and duration of molting. Therefore, this study was designed to investigate the effect of offering a multi-material innovative on molting and sex hormone concentrations in canaries along with egg production of laying hens.

MATERIALS AND METHODS

Experimental design

The Animal Ethics Committee of the Agricultural Research Center of Qom, Qom-Iran approved the experiments. The experiment was carried out in the company of Simorgh in Esfahan (Esfahan-Iran). The MMI was the mixture of plant hydro-alcoholic extracts including; *Vitex agnus-castus*, *Thymus vulgaris*, *Lavandula angustifolia*, Marigold (*Calendula officinalis)*.

Experiment 1

A total of 120 female canaries (Munich-initial body weight=29±1 g) in last egg production season were housed in an environmentally controlled room. Canaries were assigned to 12 wire cages (50×40×40 cm). There were 3 dietary treatments, 4 replicates and 10 birds each replicate. The experiment started when the birds began to drop their feathers, which lead to economic losses. The cages were equipped with 2 water cups and 2 feeding troughs. Canaries were exposed to 16 hours daily photoperiod at 23 ˚C. The diets were formulated according to experienced canaries' producers and were based on cotton seed and millet (75:25%) and last for 135 d. Treatments were including: control [(C) water without MMI] and 1.25 g or 2.25 g MMI dissolved in 1 L of drinking water.

Experiment 2

In total, a total of 72 (30 weeks age) Bowens laying hens (with initial body weight=1195 g) were used in a house equipped with cages (50×45×45 cm). They exposed to 16 hours daily photoperiod at 18 ˚C. The experiment was started when the laying hens were dropped in egg production phase and had low egg production (about 5 to 7% flock). The laying hens with lower than 20% egg production were selected for the experiment. Birds distributed among 24 cages so that 3 hens were allotted to each cage. Treatments were similar to the experiment 1 and the experiment was lasted for 21 d. Laying hens were fed with basal diets formulated according to the Bowens strain guideline.

Sampling and data collection

Two representative canaries from each cage were randomly selected and 0.5 mL of blood from the brachial vein at 3 phases was collected (45, 90, and 135 d after the commence of experiment). In addition, two representative laying hens from each cage were randomly selected on d 21 after the commence of experiment. Blood samples were collected and transferred to vial tubes containing sodium heparin. The tubes were centrifuged (5000×*g* for 20 min at room temperature) and the supernatant (plasma) was removed. The samples were stored at -20 ˚C. They were assayed for estrogen and progesterone using ELISA by specific kits (DRG®, USA) and prolactin using gamma-counter by specific kits (Kavoushyar® Co. Tehran, Iran). Feed intake (FI), drinking water (DW) and the number of egg production were recorded for laying hens.

Statistical analysis

Data from both trials was analyzed in a completely randomized design using the GLM procedure of the SAS software (SAS, 2008).

All data were analyzed for normal distribution using the NORMAL option of the UNIVARIATE procedure. Pre-planned contrasts were carried out for investigate MMI effects. The P-value of ≤ 0.05 was deemed as significant.

RESULTS AND DISCUSSION

The plasma prolactin concentrations of canaries decreased (L and Q: P≤0.006) with increasing MMI levels at 45, 90, and 135 d of the experiment (Table 1).

Table 1 Ingredinets and chemical composition of the experimental

Ingredients (%)	Amount (%)
Corn grain	55.07
Soybean meal	27.45
Wheat barn	0.2
Oil	3.0
Oyster shell	9.37
Dicalcium phosphate	1.61
Common salt	0.31
DL-methionine	0.22
L-lysine HCL	0.03
L-threonine	0.05
Vitamin and mineral premix[1]	0.50
Enzyme	0.050
Vitamin A	0.1
Vitamin B	0.1
KCO_3	0.05
$NaHCO_3$	0.1
Calculated contents	
Metabolizable energy (kcal/kg)	2780
Crude protein %	16.5
Calcium %	4.02
Available phosphorus %	0.43
Sodium %	0.17
Lysine %	1.12
Methionine %	0.38
Met + Cys %	0.65
Threonine %	0.62

[1] Vitamin and mineral premix supplied per kilogram of diet: vitamin A: 22500 IU; vitamin D_3: 5000 IU; vitamin E: 45 IU; vitamin K_3: 5 mg; B_{12}: 0.04 mg; Choline: 625 mg; Mn: 74400 mg; Fe: 75000 mg; Zn: 64.675 mg; Cu: 6000 mg and Se: 200 mg.

The plasma prolactin concentrations reduced (P<0.001) in canaries fed with MMI than control group at 45, 90 and 135 d of the experiment. The plasma progestron and estrogen concentrations of canaries increased linearly (L: P≤0.037) with increasing MMI levels at 45, 90 and 135 d of the experiment. The canaries that received MMI had greater (P=0.003) plasma progestron concentrations than control group at 135 d of the experiment. In addition, the plasma estrogen concentrations in canaries that received by MMI was greater (P≤0.039) than those of control group at 45, 90 and 135 d of the experiment. On d 21, the plasma concentrations of measured hormones [prolactin (L and Q: P=0.001), progesterone (L: P=0.042) and estrogen (Q: P=0.036)] increased in laying hens with increasing MMI levels.

The laying hen that received MMI had greater (P≤0.03) plasma prolactin, progesterone and estrogen concentrations than the control group. The feather dropped and rejuvenation time of canaries and laying hens decreased (L and Q: P≤0.018) with increasing MMI levels (Table 2). Similar responses were observed in canaries and laying hens that received MMI) than those received the control diet (P≤0.001). The number of egg produced increased in the first (L: P=0.045), second and third weeks (L and Q: P≤0.005) after the beginnings of experiment (Table 3). These parameters were greater (P<0.001) in laying hens fed with MMI rather those fed with the control in second and third weeks. No trends were observed in FI and DW of laying hen subjected to treatments during the experiment.

Data of the plasma sex steroid hormones changes during molting in laying hens are so limit and almost there is no information in canaries. Prolactin is the mainly peptide at hypothalamic level. The elevated levels of prolactin in birds play a negative role on reproductive performance. This is further concurred when dopamine stimulates the prolactin secretion from anterior pituitary gland (Youngren *et al.* 1998). The egg production in birds declines with increasing the concentration of prolactin (Table 4). It seems that the offering MMI containing plant extracts to canaries led to change the reproductive system of the females from low functional sate to a high functional state. Action of plant extracts attributed to lowering prolactin concentration and low levels of prolactin, in turn, increases progesterone levels by inhibiting enzymes, which catabolizes progesterone, that observed for canaries and laying hens in the present study. On the other hand, progesterone is the only steroid produced by the ovulating follicle (Decuypere *et al.* 1993). The effects of progesterone on avian reproductive behavior are to a large extent unknown and often contradictory among studies (Blas *et al.* 2010). The peak of progesterone levels are attained in females during incubation suggests that progesterone could be involved in the expression of parental behaviors. This is supported by recent studies showing that non-breeding females have lower circulating progesterone levels compared to breeding females during the incubation period, but not at other times of the cycle (Blas and Hiraldo, 2010). The plasma concentrations of progesterone vary with age (Joyner *et al.* 1987).

The progesterone levels of 4.29, 3.80 and 1.64 ng/mL were recorded for pullets, old layers and old non-laying hens, respectively (Joyner *et al.* 1987). Increasing levels of the plasma progesterone also was found in the current study with aging, which can imply that ovulating follicle of canaries was activated and they are ready for reproduction. However, molt induction by reduction in day length and starvation precipitate significant decrease in sex steroids and gonadotropins.

Table 2 The plasma hormonal concentrations (ng/mL) of canaries (1.5, 3 and 4.5 months after the beginnings of trial) and laying hen at d 21 post-trial period subjected to treatments

Item	C	T1	T2	SEM	P-value		
					Linear	Quadratic	Contrast
Canaries							
Prolactin							
1.5	323.2	256.4	219.3	13.1	< 0.001	0.006	< 0.001
3	406.7	299.4	239.4	21.0	< 0.001	0.001	< 0.001
4.5	353.1	326.3	246.6	13.7	< 0.001	< 0.001	< 0.001
Progesterone							
1.5	2.8	3.1	3.8	0.192	0.031	0.569	0.087
3	3.1	3.3	4.1	0.200	0.037	0.420	0.125
4.5	3.4	4.0	4.9	0.214	0.001	0.610	0.003
Estrogen							
1.5	269.3	283.4	329.8	10.21	0.008	0.326	0.039
3	343.5	363.2	443.8	15.01	0.001	0.112	0.007
4.5	319.7	360.8	449.1	19.11	0.001	0.342	0.006
Laying hens							
Prolactin	2.23	2.55	2.51	0.05	0.001	0.001	< 0.001
Progesterone	1.27	0.20	0.47	0.20	0.042	0.073	0.017
Estrogen	697.33	556.00	435.33	47.71	0.136	0.036	0.030

C: control [drinking water without multi-material innovative] and T1 and T2: 1.25 g or 2.25 g dissolved multi-material innovative in 1 L of drinking water.
SEM: standard error of the means.
Contrast: C *vs.* T1/T2.

Table 3 The feather dropped and complete rejuvenation time in canaries and laying hens (day)

Item	C	T1	T21	SEM	P-value		
					Linear	Quadratic	Contrast
Canaries							
Dropped	40	38	19	2.84	< 0.001	0.003	0.001
Rejuvenation	124	110	77	5.99	< 0.001	0.001	< 0.001
Laying hens							
Dropped	35	21	15	2.59	< 0.001	0.018	< 0.001
Rejuvenation	130	88	81	6.59	< 0.001	< 0.001	< 0.001

C: control [drinking water without multi-material innovative] and T1 and T2: 1.25 g or 2.25 g dissolved multi-material innovative in 1 L of drinking water.
SEM: standard error of the means.
Contrast: C *vs.* T1/T2.

Table 4 The egg production (egg/hen) in the first, second, and third weeks of the trial as well as the average feed intake (g/d) and drinking water (mL/d) of laying hen subjected to treatments

Week	C	T1	T2	SEM	P-value		
					Linear	Quadratic	Contrast
First	2.71	3.50	4.75	0.41	0.045	0.791	0.084
Second	2.86	5.71	5.57	0.34	< 0.001	0.001	< 0.001
Third	3.57	7.43	7.00	0.45	< 0.001	0.005	< 0.001
Average feed intake	88.43	92.50	92.03	124	0.264	0.408	0.177
Water intake	179.00	181.75	180.50	0.55	0.870	0.802	0.790

C: control [drinking water without multi-material innovative] and T1 and T2: 1.25 g or 2.25 g dissolved multi-material innovative in 1 L of drinking water.
SEM: standard error of the means.
Contrast: C *vs.* T1/T2.

Tanabe *et al.* (1981) reported that plasma levels of progesterone and oestradiol in laying hens decreased during starvation with the plasma progesterone levels ranged between 0.51 and 0.72 ng/mL during starvation, in contrast with 1.38 ng/mL when fed *ad libitum*. In addition, it was reported that molting occurs when plasma concentrations of steroids and gonadotropins are low (Etches, 1996) and changes in the reproductive functions during forced-molting were associated with reduced levels of luteinizing hormone and sex steroids in laying hens (Decuypere and Verheyen, 1986; Jacquet *et al.* 1993), Humboltd penguins (*Spheniscus humboltdi*) (Otsuka *et al.* 1998), the emperor (*Aptenodytes forsteri*), adelie (*Pygoscelis adeliae*) penguins (Groscolas and Leloup, 1986), mallard ducks (Bluhm *et al.* 1983) and turkey (Mashaly *et al.* 1979). Declined progesterone during the molt was coincident with the cessation of egg production (Hoshino *et al.* 1988), which be explained by the absence of the large yellow yolk filled follicles in the ovaries. Thus, the rising levels of plasma progesterone by the use of MMI in DW could be attributed to the redevelopment and / or regeneration of the large yellow follicles in the ovary.

The use of MMI in DW for canaries increased sex steroids in the current study which indicated that MMI have the potential for shortening molting. An observation obtained in the present study (almost 21 d for canaries and 47 d for laying hens). On the other hand, *in vitro* studies describe dopaminergic effects of *Vitex agnus-castus*, yielding potent inhibition of prolactin in cultured pituitary cells. The flavonoid apigenin can be isolated from *Vitex agnus-castus* and has selective binding affinity for the β-estrogen receptor subtype (Wuttke *et al*. 2003). Additional *in vitro* studies provide evidence of prolactin inhibition with direct binding to dopamine receptors. These changes are because of low concentration of prolactin by the actions of plant extracts at ovarian level. Low concentration of the steroid hormones in birds fed with control diet seems because of interference of high prolactin concentration (Reddy *et al*. 2006), which can explain the lower egg production in birds fed with control diet. In agreement with the results of Ariana *et al*. (2011), the egg production of laying hens with MMI treatments increased in the present study. The increased egg production with the use of MMI might be related to their effects on stimulating bird digestive systems and feed efficacy. In this regards, the supplementation of broiler chicken diets with plant extracts results in growth promotion, nutrient digestibility enhancement, and improvement of feed efficacy (Papageorgiou *et al*. 2003; Yakhkeshi *et al*. 2012). It was reported that the plant extracts increased the production of digestive enzymes and the improved utilization of digestive products through enhanced liver functions (Langhout, 2000; Hertrampf, 2001; Williams and Losa, 2001).

CONCLUSION

The results indicated that the use of MMI in drinking water of canaries resulted in shortening molting duration (almost 30 d) associated with greater steroids hormones and lower prolactin production. It can provide a condition to return the birds on reproductive phase fast. The innovative plant additives have stimulating effects on egg production without impress feed or water consumption in laying hens.

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