

Full Length Research Paper

Effects of progesterone injection on performance, plasma hormones and ovarian morphology of *ad libitum* and restricted fed broiler breeder hens

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An experiment was conducted to evaluate responses of feed-satiated and feed-restricted breeder hens to daily injection of progesterone (P_4). A total of 64 Cobb 500 hens were fed either restricted or *ad libitum* from 27 to 38 wk of age. Fourteen laying hens from each group were selected to conduct P_4 injection assay. Half of birds in each group ($n = 7$) were injected daily by 2.5 mg P_4 /kg BW and remaining birds were used as control. The P_4 was injected subcutaneously, at the base of the neck daily (at 0900 h) for 21 d. Settable and abnormal eggs were recorded daily. Blood samples were taken just before initiation of injections, 10-d and 20-d after initiation of injection. Plasma samples were analyzed for glucose, cholesterol, triacylglycerol (TAG), P_4 , estradiol (E_2), testosterone, T_3 and T_4 concentration. Settable egg production declined following P_4 injection, whereas total egg production (including soft shell egg) remain at high levels in injected birds during the first week after initiation of P_4 injection. Progesterone injection in feed-satiated and feed-restricted birds resulted in ovary regression; the ovary of these birds had no hierarchical follicle. Progesterone injection increased incidence of holding hard-shelled eggs in the uterus. Plasma E_2 concentrations were affected both by feeding pattern and P_4 injection. Progesterone injection depressed plasma E_2 concentration in both *ad libitum* and restricted fed hens. Hens with free access to feed had significantly lower plasma E_2 levels compared to restricted fed hens. Our results revealed that whereas injection of P_4 induced frequent ovulation early in the injection period in both feed-satiated and feed-restricted breeder hens; however this higher ovulation rate did not result in more settable egg production.

Key words: Progesterone, *ad libitum*, broiler breeder.

INTRODUCTION

Progesterone is the major hormone secreted by granulosa cells of large mature hierarchical follicles (F1) in birds. Progesterone acts at the level of the ovary and at the hypothalamus to stimulate the LH surge for ovulation. The progesterone production by F1 follicle peaks 6 to 8 h prior to ovulation (Johnson and van rinhoven, 1980) and triggers gonadotropin-releasing hormone (GnRH) release by the hypothalamus. This in turn is followed by an increase in LH and FSH release from the anterior pituitary. The LH stimulates an even greater output of progesterone by the granulosa cells of the F1 follicle (Etches,

1990), completing the positive feedback loop producing the LH peak 4 to 6 h prior to ovulation (Johnson et al., 1985). It has been shown that chronic or acute injection of P_4 may affect bird in different manner. Administration of an acute dose of exogenous P_4 has been shown to induce premature ovulation of a mature follicle at a specific time during ovulatory cycles in normal laying chicken hens (Nakada et al., 1994). Single injection of an acute dose of P_4 in laying hens during the preovulatory open period has been shown to have positive effect on inducing a preovulatory LH surge and ovulation (Wilson and Sharp, 1975, 1976; Johnson et al., 1985).

However, chronic injection of P_4 has been shown to increase baseline concentrations of P_4 and result in arrested laying and disrupted distribution of hierarchical follicles in turkeys (Liu et al., 2001b; Bacon and Liu,

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2004). High baseline concentrations of P₄ in arrested hens might negatively feedback on the ability of the hypothalamus to secrete surges of GnRH and subsequently surges of LH, or on the ability of the pituitary to respond to surges of GnRH secretion if they occur (Liu et al., 2001b; Bacon and Liu, 2004; Liu and Bacon, 2005).

Feed satiated hens have been shown to have more large yellow follicles than their restricted fed counterparts, so the objective of this study was to evaluate the effects of injection of a high dose of P₄ to simulate the essential P₄ surge required for establishment of LH surge in feed-satiated and feed-restricted broiler breeder hens during post peak production period (when ovulation rates are decreased) on egg production, ovulation rate and ovarian morphology. We also measured plasma glucose, TAG, cholesterol, P₄, E₂, testosterone, T₃ and T₄ concentration to know how P₄ injection affects these metabolites and hormones and consequently the bird's performance.

MATERIALS AND METHODS

Birds and management

A total of 64 Cobb 500 broiler breeder pullets with similar body weight (2050 ± 40 g) were selected from a commercial flock (20 week of age) and moved to individual cages (0.6 × 0.4; 0.24 m²/bird) to conduct the research. Soft plastic wires were placed at cage floor to minimize bird's foot damages. Each cages had an individual feeder and a nipple waterer. Birds did not have access to each others feed. Pullets were fed restricted amounts of feed to provide a standard BW according to the Cobb Breeder Management Guide (Vantress, 2005) until 27 wk of age. Birds fed a pre-lay diet from 21 wk of age to first egg production and a broiler breeder layer diet thereafter (2700 kcal AMEn and 15.20% crude protein). Photostimulation occurred at 22 wk of age by providing 16 h of light (lights on at 0700 h) and this photoperiod was maintained until experiment termination. At 27 wk of age (50% egg production), hens were selected on the basis of their egg production and BW and assigned to two feeding regimens from 27 to 38 wk of age: i) *ad libitum* feeding and ii) restricted feeding. At 38 wk of age, 14 laying hen (hens produced at least one egg during last 10 d) from *ad libitum* and restricted fed hens were selected to conducted progesterone injection assay. At this time, hens from *ad libitum* group were 500 g heavier than their restricted counterparts. Half of birds in each group (n = 7) were injected daily by 2.5 mg P₄/kg BW and remaining birds were used as control. The P₄ injections were injected subcutaneously, at the base of the neck, daily (at 0900 h) for 21 d. The P₄ vials were diluted by canola oil and 0.15 ml/ kg BW were injected each time.

Laying performance

Eggs were manually collected 2 times per day. Hen-day egg production was calculated weekly from daily egg counts and the numbers of abnormally double-yolked and soft shell egg were also recorded daily. Ovulation rate was calculated as (normal eggs + soft shell eggs + 2×double yolk eggs).

Necropsy and tissue collection

At the end of experimental period, 4 hens per treatment were

slaughtered for necropsy. Liver, abdominal fat pad and ovary were collected at necropsy. Weight of liver, ovary and abdominal fat pad were divided by (BW/100) to estimate their fractional contribution. Ovaries were weighed (after removing hierarchical follicles) and follicles were classified into 3 groups: hierarchical follicles (large yellow follicles, > 8 mm), small yellow follicles (2 to 8 mm) and large white follicles (2 to 5 mm) according to the system devised by Gilbert et al. (1983).

Blood sampling

To evaluate plasma hormones and metabolites, first bleeding was carried out before initiation of injections and two subsequent bleedings were repeated every 10 d. Blood samples were obtained just before P₄ injection. Blood samples were collected in EDTA coated tubes from the brachial vein of all hens per group. Blood samples were immediately centrifuged at 2000 × g for 15 min to collect blood plasma. Plasma samples were stored at -20°C until assayed for glucose, TAG, cholesterol, as well as T₃, T₄, progesterone, estradiol and testosterone.

Plasma metabolites and hormones

Plasma glucose, total triacylglycerol and cholesterol (CHO) were determined enzymatically using an automated analyzer (Hitachi 902, Japan). Specific radioimmunoassay was used to determine plasma hormone concentrations. All samples were analyzed within 1 assay in order to avoid inter-assay variations. Plasma P₄ and E₂ were determined by commercial RIA kits (ICN, Biochemichals, Cleveland, OH) with intra-assay CV of 6.46 and 7.15, respectively. The use of the P₄ and E₂ kits for chicken has previously validated by Onagbesan et al. (2006). Plasma testosterone was determined by using RIA kit (DRG, Germany) according to manufacturer's procedure with an intraassay CV of 6.39. Total T₃ and T₄ concentration were determined using commercial RIA kits (DRG, Germany). The intra-assay CV for T₃ and T₄ were 3.58 and 4.62, respectively.

Statistical analysis

The experimental birds were housed in individual cages. The experimental design was completely randomized with factorial arrangement using individual broiler breeder hens as experimental unit. There were 2 method of feed allocation with or without P₄ injection. Performance data as well as plasma metabolites and hormones data were analyzed as repeated measures using Proc Mixed of SAS software (SAS Institute, Cary, NC). Differences between means were evaluated using least square means procedure. Data obtained in necropsy were analyzed using GLM procedure of SAS software and means were separated by Duncan multiple range test. Significant differences were considered to be P < 0.05.

RESULTS

Performance data

Progesterone injection significantly affected settable egg production. Progesterone injected bird produced less settable egg compared to non-injected birds both in restricted and *ad libitum* fed groups (Figure 1). Drop in egg production continued through experimental period and reached to zero, 3 wk after P₄ injections. Egg production

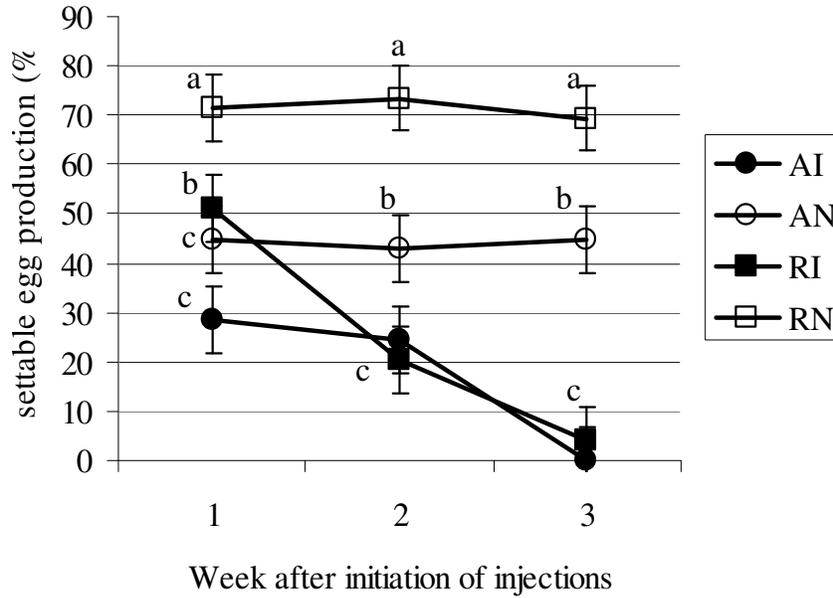


Figure 1. Settable egg production in *ad libitum* fed with (AI) or without (AN) P₄ injection and in restricted fed hens with (RI) or without (RN) P₄ injection. Data are means ±SEM. a-c Data points with different letters are significantly different at the age of the hens (P < 0.05).

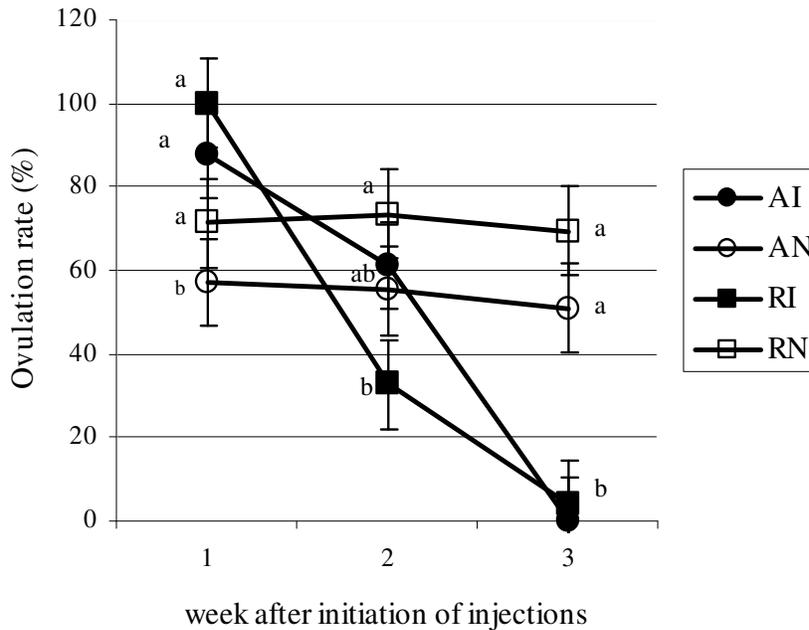


Figure 2. Ovulation rate (%) in *ad libitum* fed with (AI) or without (AN) P₄ injection and in restricted fed hens with (RI) or without (RN) P₄ injection. Data are means ±SEM. a-b Data points with different letters are significantly different at the age of the hens (P < 0.05).

in *ad libitum* non-injected birds was significantly lower than their restricted fed counterparts.

Ovulation rate in hens from different treatment are presented in Figure 2. Although egg production declined

following P₄ injection, however ovulation rate remain at high levels in injected birds in the first week after initiation of P₄ injection. *Ad libitum* fed hens without P₄ injection had significantly lower ovulation rate compared to birds

from remaining treatment. Whereas ovulation rate in injected birds was numerically higher than non-injected birds in the first week after initiation of P₄ injection, a dramatic decline occurred during the second and third wk post P₄ injections. The decline in ovulation rate in injected birds was more severe in restricted birds compared to *ad libitum* fed hens.

The number of soft shell eggs in P₄ injected birds was significantly higher than their non-injected counterparts in the first week post injections; however along with decline in ovulation rate in the later weeks, numbers of soft shell eggs tend to decrease (Table 1).

Hens with P₄ injection produced more soft shell eggs compared to non-injected hens (Table 1). *Ad libitum* fed birds also produced more (P < 0.05) double yolk eggs than restricted fed hens (Table 1).

Carcass and ovary data

Abdominal fat pad fractional weight in *ad libitum* fed birds were significantly higher than restricted fed birds (Table 1); furthermore, feed-satiated hens had significantly heavier liver compared to feed-restricted birds. However P₄ injection had no significant effect on abdominal fat and liver fractional weight (Table 1).

Ovary fractional weight in hens with P₄ injection both in feed-satiated and feed-restricted birds were significantly lower than non-injected birds (Table 1).

Progesterone injection in feed-satiated and feed-restricted birds resulted in ovary regression; the ovary of these birds had no hierarchical follicle, SYF and also LWF (Table 1). The numbers of LYF in *ad libitum* fed hens were significantly higher than feed-restricted birds. Progesterone injection increased incidence of holding hard-shelled eggs in the uterus. Three hens from *ad libitum* and 2 hens from restricted fed birds (42 and 28% of necropsied birds, respectively) hold a hard-shelled egg in their uterus (data not shown).

Hormones and metabolites

Plasma glucose, TAG and CHO concentrations of hens from different treatments are presented in Table 2. *Ad libitum* fed hens had significantly (P < 0.0001) higher plasma glucose, TAG and CHO levels than their restricted fed counterparts. However, P₄ injection did not have any significant effect on these metabolites.

Progesterone injection significantly (P < 0.0001) reduced plasma P₄ concentration both in *ad libitum* or restricted fed birds 10 and 20 d after initiation of P₄ injection (Figure 3). Plasma P₄ levels were not different between feed-satiated and feed-restricted birds.

Plasma E₂ concentrations were affected both by feeding pattern and P₄ injection (Figure 4). Progesterone injection depressed (P < 0.0001) plasma E₂ concentration

in both *ad libitum* and restricted fed hens. Hens with free access to feed had significantly lower (P < 0.0001) plasma E₂ levels compared to restricted fed hens.

Plasma testosterone concentrations of hens from different treatments are presented in figure 5. *Ad libitum* fed birds had significantly higher (P < 0.0001) plasma testosterone levels compared to their restricted fed counterparts. Hens with P₄ injection had significantly lower (P < 0.001) plasma testosterone concentrations than non-injected birds.

Ad libitum fed birds had significantly higher (P < 0.0001) plasma T₃ (Figure 6) and lower (P < 0.0001) plasma T₄ concentration (Figure 7) than their restricted fed counterparts. Progesterone injection did not change plasma T₃ or T₄ concentration neither in *ad libitum* fed hens nor in restricted fed birds.

DISCUSSION

Progesterone acts on ovary and hypothalamus to stimulate the LH surge for ovulation. The rise in progesterone concentration stimulates a rise in LH concentration, which in turn stimulates a further rise in progesterone. This positive feedback loop is what causes the progesterone and ultimately LH surge (Johnson et al., 1985).

The current study was designed to examine the effects of a simulated progesterone surge (by injection of a high dose of P₄) on broiler breeder hens performance, ovary morphology, plasma metabolites and hormones concentrations in either feed-satiated or feed-restricted hens. Although P₄ injection resulted in increased ovulation rate during the first week post injections, however, settable egg number decreased along with initiation of injections because of high number of soft shell and double yolk egg production. During the second and third wk after injections both ovulation rate and egg production decreased in feed-satiated or feed-restricted birds. Increased ovulation rate during first week after injection may be due to simultaneity of P₄ injection with open period of LH. High dose of P₄ acts as a progesterone surge and drives follicles to ovulation. Inability of oviduct to direct all ovulated follicles resulted in higher incidence of soft shell and double yolk eggs. Liu and Bacon (2005) observed higher incidence of soft shell egg after P₄ injections in broiler breeder. Similar to results obtained in the current study, egg production decreased after P₄ injection in turkey (Bacon and Liu, 2004), broiler breeder (Liu and Bacon, 2005) and in Japanese quail (Tell et al., 1999; Liu and Bacon, 2004). Egg production ceased 3 wk after injecting daily P₄. Necropsy data shown a regressed ovary with no LYF, SYF or LWF in all hens with progesterone injection. Taken together, these data suggest that P₄ treatment has a strong negative effect on egg production rate and hierarchical follicular maintenance and development. Liu and Bacon (2005) came to similar conclusion.

Ad libitum fed broiler breeders are known to have more

Table 1. Effect of P₄ injection in restricted fed and *ad libitum* fed hens on carcass, ovary and egg parameters.

Treatment		Abdominal fat weight (%)	Liver weight(%)	Ovary weight(%)	LYF per ovary	SYF per ovary	LWF per ovary	Double yolk egg (%)	Soft shell egg (%)
Main effect									
Feeding pattern	<i>Ad libitum</i>	2.47 ^a	2.08 ^a	1.03	4.75 ^a	5.12	11.50	5.10 ^a	10.88 ^a
	restricted	1.16 ^b	1.45 ^b	0.97	2.25 ^b	6.25	15.25	1.36 ^b	5.10 ^b
P ₄ injection	injected	1.68	1.78	0.45 ^b	0.00 ^b	0.00 ^b	0.00 ^b	4.76 ^a	14.28 ^a
	Non injected	1.68	1.75	1.55 ^a	7.00 ^a	11.37 ^a	26.75 ^a	1.70 ^b	1.70 ^b
Feeding pattern	P₄ injection								
<i>Ad libitum</i>	injected	2.56 ^a	2.11 ^a	0.35 ^b	0.00 ^c	0.00 ^b	0.00 ^b	6.80 ^a	18.36 ^a
<i>Ad libitum</i>	Non injected	2.39 ^a	2.06 ^a	1.71 ^a	9.50 ^a	10.25 ^a	23.00 ^a	3.40 ^b	3.40 ^b
Restricted	injected	1.34 ^b	1.40 ^b	0.55 ^b	0.00 ^c	0.00 ^b	0.00 ^b	3.40 ^b	13.60 ^a
Restricted	Non injected	0.98 ^b	1.50 ^b	1.40 ^a	4.50 ^b	12.50 ^a	30.00 ^a	0.00 ^b	0.00 ^b
Pooled SEM		0.25	0.09	0.12	0.54	1.32	4.09	2.45	3.14

Table 2. Glucose, cholesterol and triacylglycerol concentration in restricted fed or *ad libitum* fed hens with or without P₄ injection.

Treatment		Glucose (mg/dl)			Cholesterol (mg/dl)			Triacylglycerol (mg/dl)		
		before Initiation of injections	10d after Initiation of injections	20 d after Initiation of injections	before Initiation of injections	10d after Initiation of injections	20 d after Initiation of injections	before Initiation of injections	10d after Initiation of injections	20 d after Initiation of injections
<i>Ad libitum</i>	Injected	296.14 ^a	295.28 ^a	293.00 ^a	172.96 ^a	183.74 ^a	187.31 ^a	1753.31 ^a	1742.47 ^a	1736.64 ^a
<i>Ad libitum</i>	Non injected	298.22 ^a	296.44 ^a	298.64 ^a	174.62 ^a	187.16 ^a	184.60 ^a	1772.53 ^a	1715.86 ^a	1786.29 ^a
Restricted	Injected	217.78 ^b	217.62 ^b	217.59 ^b	114.76 ^b	123.47 ^b	121.42 ^b	1341.61 ^b	1309.44 ^b	1305.09 ^b
Restricted	Non injected	218.60 ^b	219.39 ^b	216.61 ^b	115.83 ^b	117.85 ^b	123.14 ^b	1330.88 ^b	1305.09 ^b	1246.80 ^b
Pooled SEM		7.69	7.53	7.68	0.24	0.26	0.25	41.95	41.37	49.16

hierarchical follicles than their restricted fed counterparts (Yu et al., 1992; Chen et al., 2006; Sun et al., 2006). It seems that higher ovulation rate in *ad libitum* fed hens with P₄ injection compared to restricted fed birds may be due to more LYF number in their ovary.

Progesterone injection significantly lowered E₂ concentration both in feed-satiated and feed-restricted hens. Plasma concentrations of E₂ are

increased after follicle stimulating hormone (FSH) injection in laying hens late in the reproductive period (Palmer and Bahr, 1992), suggesting that the secretion of FSH is also decreased after P₄ injection. Liu and Bacon (2005) reported a decline in LH concentration after P₄ injection. Taken together, these data suggest P₄ injection may have negative effect on secretion of GnRH. High concentrations of E₂ are required to sensitize the

hypothalamic pituitary axis to the positive feedback effects of progesterone (Wilson and Sharp, 1976) to stimulate vitellogenin formation in the liver (Redshaw and Follett, 1972), to regulate calcium metabolism (Etches, 1987), to stimulate and maintain a functional oviduct and to maintain secondary sexual characteristic. The lower concentration of E₂ may thus lead to regression of the oviduct and impaired follicles development after

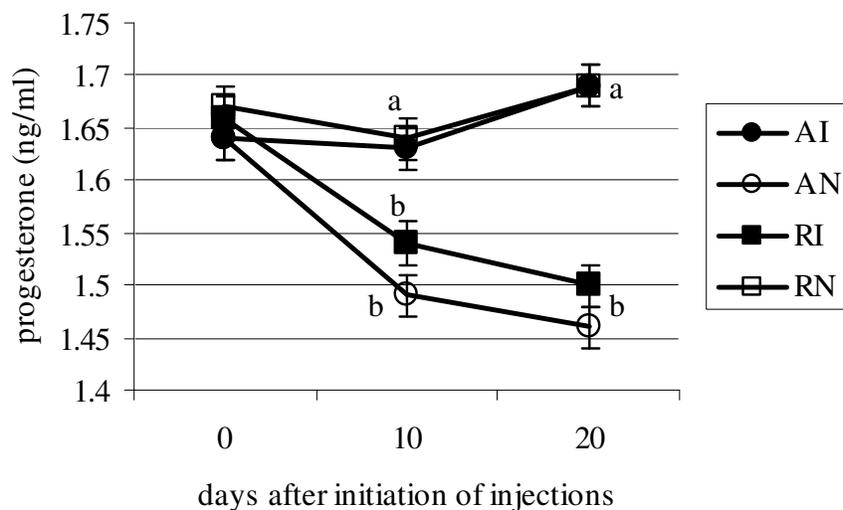


Figure 3. Progesterone concentration in *ad libitum* fed with (AI) or without (AN) P₄ injection and in restricted fed hens with (RI) or without (RN) P₄ injection. Data are means \pm SEM. a-b Data points with different letters are significantly different at the age of the hens (P < 0.05).

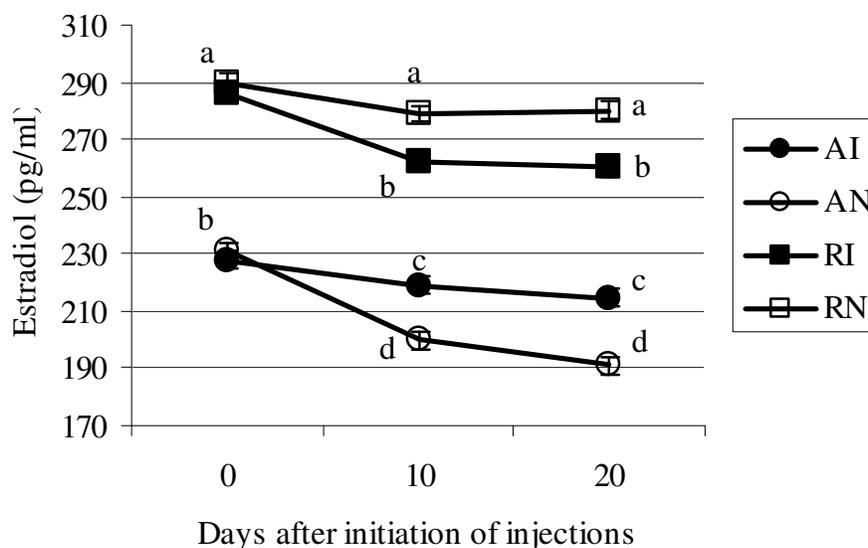


Figure 4. Estradiol concentration in *ad libitum* fed with (AI) or without (AN) P₄ injection and in restricted fed hens with (RI) or without (RN) P₄ injection. Data are means \pm SEM. a-c Data points with different letters are significantly different at the age of the hens (P < 0.05).

P₄ injection. Similar to our results, Liu and Bacon (2005) observed a depression in plasma E₂ along with regressed ovary after injection of P₄ in broiler breeder hens. Lower E₂ concentration of *ad libitum* fed hens compared to restricted fed hens observed in this research is in agreement with Onagbesan et al. (2006).

Plasma P₄ concentrations in hens with daily injection of P₄ decreased 10 and 20 d after initiation of injections. Injection of acute dose of P₄ will result in higher P₄ surge concentration which in turn lowers P₄ production through

negatively affecting LH and FSH production. Liu and Bacon (2005) concluded that higher P₄ surge concentration and lower LH baseline concentration may be associated with decline in egg production in P₄ injected breeder hens. Progesterone concentration decreased following P₄ injections in broiler breeder hens in the study of Liu and Bacon (2005).

Besides E₂ and P₄, progesterone injection lowered plasma testosterone levels both in feed-satiated and restricted fed hens. It seems that P₄ injection negatively

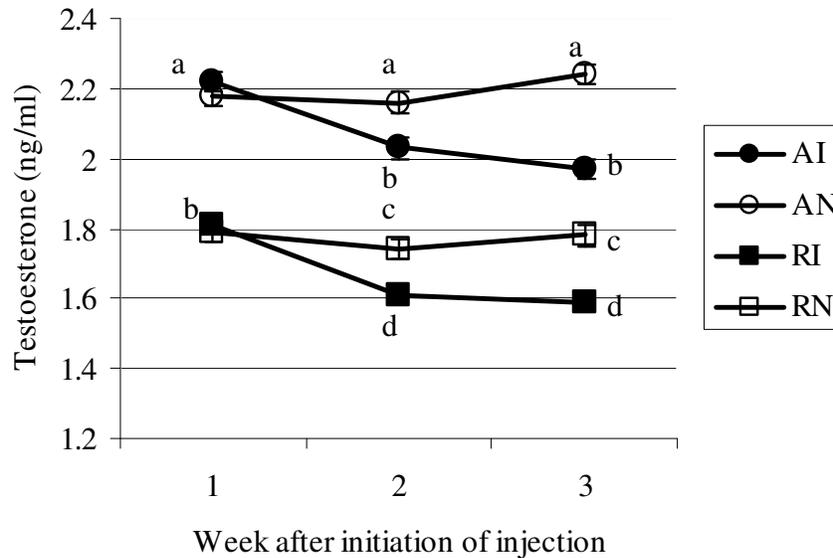


Figure 5. Testosterone concentration in *ad libitum* fed with (AI) or without (AN) P₄ injection and in restricted fed hens with (RI) or without (RN) P₄ injection. Data are means \pm SEM. a-d Data points with different letters are significantly different at the age of the hens (P < 0.05).

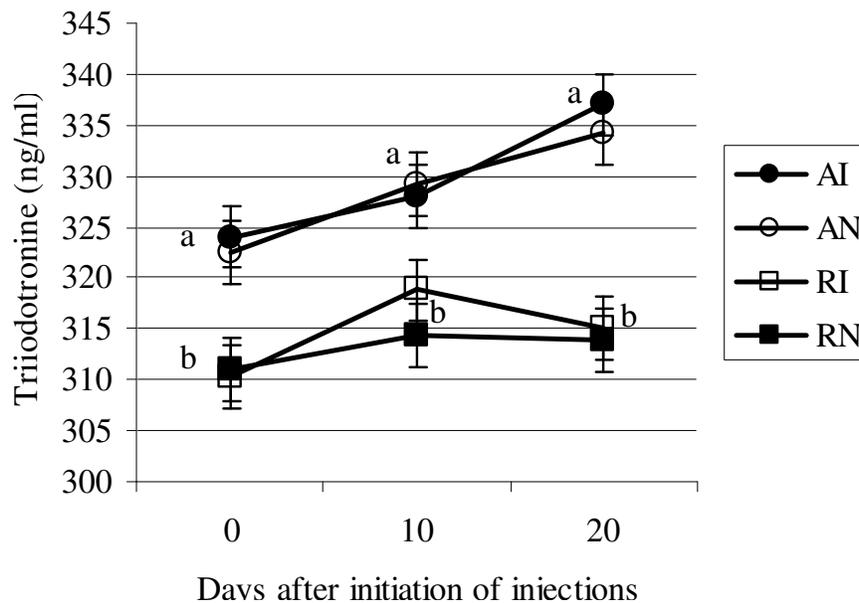


Figure 6. Plasma T₃ concentration in *ad libitum* fed with (AI) or without (AN) P₄ injection and in restricted fed hens with (RI) or without (RN) P₄ injection. Data are means \pm SEM. a-b Data points with different letters are significantly different at the age of the hens (P < 0.05).

affect ovarian steroidogenesis.

Whereas P₄ injection had no significant effect on plasma metabolites, feeding pattern significantly affected plasma glucose, TAG and CHO. *Ad libitum* fed hens had higher levels of plasma glucose, TAG and CHO compared to restricted fed birds. These results are consistent

with those reported by Chen et al. (2006) and Sun et al. (2006).

Progesterone injection increased incidence of holding a hard-shelled eggs in the uterus. Similar to results observed in our study, Liu and Bacon (2005) reported a high incidence of holding hard-shelled eggs in broiler

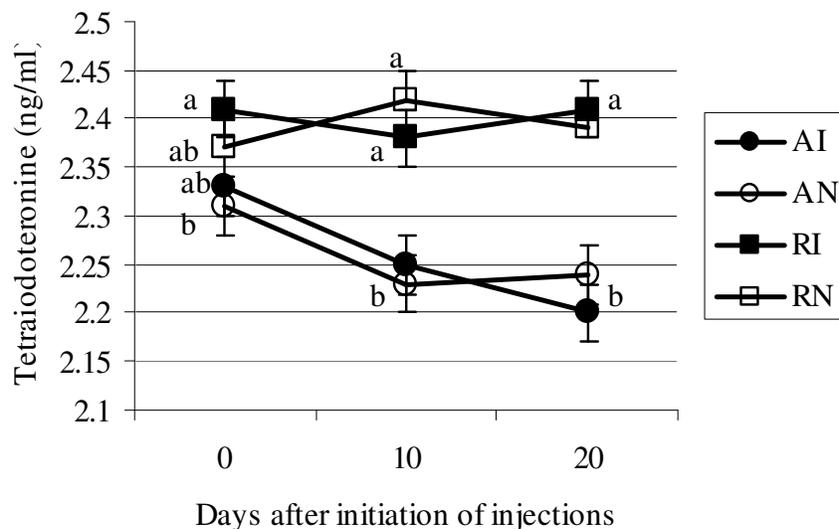


Figure 7. Plasma T₄ concentration in *ad libitum* fed with (AI) or without (AN) P₄ injection and in restricted fed hens with (RI) or without (RN) P₄ injection. Data are means ± SEM. a-b Data points with different letters are significantly different at the age of the hens (P<0.05).

breeder hen's uterus. Holding a hard-shelled egg in the uterus was also induced by exogenous P₄ in turkey (Bacon and Liu, 2004) and quail hens (Lin et al., 1993; Tell et al., 1999; Liu and Bacon, 2004), suggesting that P₄ may be associated with inhibition of muscular contraction of the uterus at oviposition in these species.

Progesterone injection had no significant effect on plasma T₃ and T₄ concentrations; however *ad libitum* fed hens had lower T₃ and higher T₄ level compared to restricted fed hens. Numerous studies suggest that feed restriction decrease T₃ and increase T₄ levels in broiler breeders (Darras et al., 1995; Bruggeman et al., 1997).

In conclusion, whereas injecting an high dose of P₄ once a day for 3 wk induced frequent ovulation early in the injection period in both feed-satiated and feed-restricted breeder hens; however this higher ovulation rate did not result in more settable egg production. Egg production decreased with P₄ injection and ceased at the termination of experiment. Decreased egg production in P₄ injected bird was associated with lower levels of sex steroid hormones (P₄, E₂ and testosterone) and regressed ovary. Progesterone injection increased numbers of hens holding a hard-shelled egg in their uterus. Progesterone injection had no significant effect on glucose homeostasis and lipid metabolism. Restricted fed and laying *ad libitum* fed breeder hens respond in a similar way to a high dose of P₄ injection.

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