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Increased iNOS Gene Expression in the Granulosa Layer of F1 Follicle of Over-Fed and Under-Fed Broiler Breeder Hens

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■ Keywords

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ABSTRACT

To clarify the effects of high (20 and 40% more than normal) and low (20% less than normal) daily feed allowance on egg and body parameters, ovarian morphology and plasma glucose, cholesterol, triacylglycerol, leptin-like hormone, nitrite/nitrate and gene expression of inducible nitric oxide synthase (iNOS) in the granulosa layer of F1 follicle, broiler breeder hens (30-week-old) were fed for 30 days. Egg and body parameters significantly changed between treatments ($p<0.05$). Effect of different level of feed intake on ovarian morphology parameters was significant ($p<0.05$), except for white follicles. After 30 days of experiment, plasma nitrite/nitrate (as a index of plasma nitric oxide) and leptin-like hormone increased in FI+20% and FI+40% groups as compared to controls ($p<0.05$). Plasma level of leptin-like hormone also significantly ($p<0.05$) increased in FI+40% group. The relative amount of iNOS mRNA expression in the granulosa layer of F1 follicles was significantly higher in FI-20% and FI+40% groups than in control group only after 4 weeks of experiment. The amount of these elevations in the FI-20% and FI+40% groups were 32.4% and 60.9% respectively. It was concluded that iNOS gene is normally expressed in follicular granulosa cells of F1 follicle of broiler breeder hens 2-4 hours before ovulation. However, over- and underfeeding of hens increased iNOS expression in F1 follicle, which may be one of the atresia-inducing factors in hierarchical follicles as shown by the significant ($p<0.05$) increase of shrunken follicles in the ovary of over- and under-fed broiler breeder hens after 30 days of feeding.

INTRODUCTION

Broiler breeder hens are prone to several metabolic disorders and reproductive anomalies, including a high incidence of skeletal deformities and metabolic diseases, such as fatty liver (Griffin & Goddard 1994). In females, the capacity of rapid early growth coupled with free access to feed leads to enhanced adult fatness (Hocking et al., 2002) and poor reproductive performance (Heck et al., 2004; Renema & Robinson 2004). These undesirable outcomes appear to arise from increases in feed intake that occurred concomitantly with genetic selection for rapid early growth (Barbato 1994; Renema & Robinson 2004). In adulthood, the persistence of increased voluntary feed intake results in actual feed intakes in excess of the requirements for optimal adult health and performance, as indicated by reduced livability and increased incidence of metabolic diseases (Robinson et al., 1993, Griffin & Goddard 1994). Hens fed ad libitum exhibit a dramatic drop in egg production at an early age and consequently, produce fewer eggs overall (Yu et al., 1992a, b). Chen et al. (2006) showed that apoptosis-induced atresia occurred in the hierarchical follicles of over-fed broiler breeder hens. A large body of evidence suggests that nitric oxide (NO), either delivered by NO

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donors or generated by L-arginine metabolism, initiates apoptotic cell death (Bernhard et al., 1999). Nitric oxide (NO) is a diffusible gas that has been considered as a signaling molecule in a variety of cellular functions, including vasodilation, neurotransmission, macrophage toxicity, and myocardial functions (Bredt & Snyder 1994; Hassanpour et al., 2009). It has recently been shown that nitric oxide levels and inducible nitric oxide synthase (iNOS) mRNA are higher in chicken F1 follicle as compared to F2 follicle, suggesting the involvement of NO in ovulation (Sundaresan et al., 2007). Furthermore, it was demonstrated that destruction of the germinal disk region of the most mature follicle (F1) caused follicular atresia (Yoshimura et al., 1994). It seems that changes in inducible nitric oxide levels in granulosa cells may have a role in the most mature follicule (F1) atresia of broiler breeder hens. However, nitric oxide changes in over-fed broiler breeder hens has yet to be established. The objective of the present study was to determine whether differences in adiposity (caused by changing feed intake level) could change plasma levels of nitric oxide and iNOS mRNA expression in granulosa layer of F1 follicle in broiler breeder hens.

MATERIAL AND METHODS

Birds

One hundred and ninety-two commercially reared Arbor Acres Plus Fast Feathering broiler breeder hens (30 weeks old) were used for this study. Hens were fed according to Arbor Acres Plus nutritional recommendations. Hens were fed a soybean and corn based breeder layer mash diet that supplied 11.5 MJ of metabolizable energy (ME)/kg and 155g/kg crude protein of diet. Diet composition was calculated from published values for feed ingredients (NRC, 1994). Feed was offered between 0700 and 0730 h within a 15L:9D photoperiod, in which lights were turned on at 0600 h. Hens had free access to water throughout the experiment.

Management of laying performance

Egg production and feed intake were daily recorded. Hens were allocated to four different levels of feed intake (FI): Control (163 g/day), FI-20% (20% less than control feed intake, i.e. 130.4 g/day), FI+20% (20% more than control feed intake, i.e. 195.6 g/day) and FI+40% (40% more than control feed intake, i.e., 228.2 g/day) with six replicates of eight hens each for 30 days. Eggs were collected from each group at 24-hour intervals. Whole eggs were weighed on an electronic scales. Egg shells were broken with a sharp knife and the yolk was separated and weighed, taking care to ensure that no albumin remains adhered to yolk, with the aid of tissue paper.

Tissue and blood sampling

At the end of 15th and 30th days of experiment (period 1 and 2, respectively), 24 birds from each group were anesthetized and then killed 2-4 h before expected oviposition, based upon the individual bird's sequence of laying. In order to estimate approximate time of oviposition in each bird, we used broiler breeder trap nests (two traps per each group) and egg oviposition of all hens from 0600 h to 2100 h was continuously checked by two well-trained staff. Approximately 75% of the eggs were laid between 1500 h. and 1700 h. One day before the hens were sacrificed, blood samples from each bird were collected into anticoagulant tubes, and then were centrifuged at 5000× g to collect blood plasma. Plasma samples were stored at -20 °C until assaying for fasting plasma glucose, triacylglycerol (TAG), cholesterol, and leptin-like protein. In addition, immediately before hens were anesthetized, blood samples were collected into anticoagulant tubes for plasma NO measurement. Prior to killing, the presence of a hard-shelled egg in the shell gland was confirmed by cloacal examination. Liver, ovary, oviduct, and abdominal fat pad weights were measured. Ovarian follicles were separated from the ovary and measured in order to identify the largest (F1) in each ovary, based on hierarchical follicles weight (Cassy et al., 2004). The granulosa layer of F1 follicle was separated (Gilbert et al., 1977), immediately frozen in liquid nitrogen, and stored at -80°C for subsequent analysis. The follicles were classified into three 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). Shrunken follicles containing dark yellowish to brown yolk contents surrounded by loose connective tissue were classified as atretic follicles (Chen et al., 2006).

RNA extraction of granulosa layer of F1 follicle

Single-step, acid guanidinium thiocyanate/phenol/chloroform extraction method was used for total RNA extraction of granulosa cells (Chomczynski & Sacchi, 2006). Briefly, 100 mg of the homogenized granulosa layer were placed in a denaturing solution containing 4M guanidinium thiocyanate. The homogenate was then sequentially mixed with 2M sodium acetate (pH 4), water-saturated phenol, and chloroform/isoamyl alcohol (49:1). The resulting mixture was centrifuged,

yielding an upper aqueous phase containing total RNA. Following 100% isopropanol precipitation, the RNA pellet was redissolved in denaturing solution, reprecipitated with isopropanol, and washed with 75% ethanol. The RNA samples were resuspended in DEPC-treated water. RNA amount and quality were determined by spectrophotometry. Only RNA of sufficient purity, with an absorbance ratio (A260/280) higher than 1.9, was considered for cDNA synthesis, and analyzed by electrophoresis on 1.5% agarose gel stained with 0.5 mg/ml ethidium bromide.

Semiquantitative reverse-transcription PCR

The extracted RNA was reverse-transcribed to cDNA in a 20-ml volume containing 1 mg of extracted RNA, 200 ng random hexamer 0.5mM dNTP. This mixture was heated to 65.°C for 5 min, and 40 u of RNase inhibitor, RT buffer (50mM Tris-HCl, 75mM KCl, 3mM $MgCl_2$), 10mM DTT and 200 u M-MLV reverse transcriptase (Fermentase, Karlsruhe, Germany) were added. This mixture was incubated for 10 min at 25°C, followed by 50 min at 37°C. The prepared cDNAs were heated at 75°C for 15 min to denature MMLV reverse transcriptase and then stored at -20°C.

The following primer sequences for the PCR reactions were included: iNOS primers, sense: 5'-AGGCCAAACATCCTGGAGGTC-3', antisense: 5'-TCATAGAGACGCTGCTGCCAG-3' and β-actin primers, sense: 5'-ACTGGATTTCGAGCAGGAGAT-3', antisense: 5'-TTAGAAGCATTTGCGGTGGACAA-3' (used as a housekeeping gene) (Teshfam et al., 2006). Normalization of the samples was accomplished using RT-PCR for the housekeeping gene β-actin to control the efficacy of RNA extraction, integrity, and amount of iNOS mRNA present in the samples. PCR reaction conditions were optimized for each of the primer pairs to obtain a linear relationship between input RNA and final PCR product. PCR was performed in a total volume of 25 ml, containing 5mM Tris-HCl; 10mM NaCl; 0.01mM EDTA; 1mM $MgCl_2$; 0.1mM of each dNTP; 0.1 mM of each primer; 2 ml cDNA; and 2.5 u Taq polymerase (Fermentase, Germany).

The PCR program for iNOS and β-actin consisted of 10 min at 94°C, 35 cycles (iNOS) and 28 cycles (β-actin) of amplification (50 s at 94°C, 60 s at 63°C and 60 s at 72°C). An aliquot of each reaction mixture was submitted to electrophoresis in 2% agarose gel and stained with 0.5 mg/ml ethidium bromide. Band density was determined using Photo-Capt V.99 Image software, and relative densities were expressed as iNOS/β-actin density.

Plasma glucose, cholesterol, TAG, nitrite/nitrate and Leptin-like hormone analyses

Plasma glucose level was measured by the glucose oxidase method, using an automated analyzer (Glucose, Colorimetric Enzymatic, Parsazmun, Tehran, Iran). Total cholesterol and TAG plasma concentrations were calculated respectively by "Cholestrol, Enzymatic Photometric, Parsazmun, Tehran, Iran" and "Triglycerides, Colorimetric Enzymatic, Parsazmun, Tehran, Iran" Kits, according to the manufacturer's recommendations. Plasma nitrite/nitrate concentration, as an index of nitric oxide production, was measured by a colorimetric assay kit (Nitric Oxide Assay Kit, Shinjuku, Tokyo, Japan). Plasma level of leptin-like hormone was determined by a multi-species leptin-like hormone RIA kit (DRG Research Inc, Germany), according to the recommendations of the manufacturer.

Statistical analysis

All data from the study were analyzed as a completely randomized design. Comparisons were made between control and treatment (T) groups using one-way ANOVA (Duncan's multiple range tests) (SAS Institute, 1996), with $p<0.05$ accepted as significant. All results are expressed as mean ± SEM.

RESULTS

Egg and body parameters

Egg and body parameters of hens are shown in Table 1. High feed intakes significantly ($p<0.05$) increased body weight and liver weight in the two higher feed intake groups, while low feed intake (i.e. FI-20% group) reduced these parameters as compared to the controls. There were also significant reduction of abdominal fat weight in FI-20% group during the two experimental periods, and increased in other groups (only during the second experimental period) as compared to the control group. After 15 experimental days (period 1), egg production was only significantly reduced in the FI+40% group ($p<0.05$); however, after 30 experimental days (period 2), egg production significantly decreased ($p<0.05$) in all treated groups as compared to the control. Average egg and egg yolk weights were higher in the FI+40% group and lower in the FI-20% group as compared to control only in period 2 ($p<0.05$).

Significant increase and decrease in ovary weight were observed in FI+40% and FI-20% groups, respectively, in the two experimental periods as compared to controls. Oviduct weight was reduced in all groups, but which was only significant different in

FI+40% (only in period 2) and FI-20% (both periods) groups as compared to the controls.

Ovarian morphology

Morphologic parameters of the ovarian organs are shown in Table 2. In the first experimental period, the number of large yellow follicles was significantly ($p<0.05$) lower in FI-20% group and higher in FI+40% group, whereas it was higher in both FI+20% and FI+40% groups in the second experimental period as compared to the controls. There were also a decrease in the number of postovulatory follicles and an increase in the ratio of atretic follicles/ovary in FI+40% group as compared to the controls ($p<0.05$).

During the second experimental period, the number of large yellow follicles was significantly ($p<0.05$) higher than in the controls in FI+20% and FI+40% groups, while the number of small yellow follicles was lower in FI-20% and FI+40% groups. The number of postovulatory follicles also declined in all treated groups. The ratio of atretic follicles/ovary during this period increased only in FI-20% and FI+40% groups ($p<0.05$). The number of white follicles was not significantly different among control and treated groups.

Plasma glucose, cholesterol, TAG, nitrite/nitrate and leptin-like hormone

Plasma levels of glucose, nitrite/nitrate, TAG, cholesterol, and leptin-like hormone in broiler breeder hens fed with four levels of feed intake are shown in Table 3. In the first experimental period, plasma glucose levels significantly ($p<0.05$) increased only in FI+40% hens. Plasma level of leptin-like hormone presented a significant ($p<0.05$) increase in the FI+40% group. Other measured parameters did not show significant variations among treatment groups during this period.

In the second experimental period, plasma levels of nitrite/nitrate, TAG, leptin-like hormone increased in FI+20% and FI+40% groups as compared to control hens ($p<0.05$). Plasma levels of glucose and cholesterol were also significantly ($p<0.05$) higher in FI+40% group than in the controls, while cholesterol level of the FI-20% group was lower ($p<0.05$) during. at this period.

iNOS mRNA expression in granulosa layer of F1 follicles

The expression of iNOS gene was studied using semi-quantitative RT-PCR in the granulosa layer of F1 follicles in broilers breeder hens at different levels of feed intakes during two periods (after 15 and 30 days). Reverse transcription- PCR results are shown in Figure 1. The expression of β-actin, as housekeeping gene, and of iNOS gene were detected in all samples of the granulosa layer of F1 follicles in control and treated groups.

Table 1 - Egg and body parameters in the broiler breeder hens fed at different levels of feed intake.

Parameters	Period 1				Period 2			
	Control	FI-20%	FI+20%	FI+40%	Control	FI-20%	FI+20%	FI+40%
Egg production (%)	83.1a±0.52	81.6a±0.84	83.0a±1.00	77.2b±1.20	81.6a±1.05	78.0b±0.96	76.3b±0.87	70.1c±1.95
Egg weight (g)	56.7ab±0.35	55.7b±0.65	56.9ab±0.32	57.3a±0.34	58.2b±1.86	54.8c±0.43	59.0ab±0.34	60.1a±0.32
Yolk weight (g)	15.3ab±0.31	14.6b±0.35	15.5ab±0.31	15.7a±0.33	16.1b±0.25	14.4c±0.15	17.2ab±0.26	17.9a±0.27
Body weight (g)	3416c±44.0	3181d±45.5	3553b±50.2	3730a±48.0	3514c±47.5	3107d±50.0	3692b±46.1	4071a±51.0
Liver weight (g)	59.1c±2.0	49.9d±1.9	68.4b±3.3	87.7a±5.7	60.6c±2.6	49.1d±1.6	80.3b±2.7	97.0a±6.4
Abdominal fat Weight (g)	79.8b±7.8	56.9c±6.6	91.9a±7.3	96.0a±6.0	90.5c±7.0	55.2c±7.1	109.4ab±6.0	129.0a±7.2
Ovary weight (g)	68.3b±2.7	57.9c±3.7	72.3b±2.8	84.7a±4.5	74.3b±2.1	54.4c±1.7	78.1ab±1.8	81.7a±3.0
Oviduct weight (g)	62.9a±1.3	55.2b±1.3	61.1a±1.6	62.0a±1.5	62.8a±1.1	47.8b±1.4	56.3a±1.1	50.7b±1.6

^{a,b,c} - Means with the different supercripts in the same row (within the same period) are significantly different ($p<0.05$);Period 1, after 15 days of experiment; Period 2, after 30 days of experiment; FI-20%, group with feed intake of 130.4 g/day; FI+20%, group with feed intake of 195.6 g/day; FI+40%, group with feed intake of 228.2 g/day.

Table 2 - Ovarian morphology of broiler breeder hens fed at different levels of feed intake.

Parameters	Period 1				Period 2			
	Control	FI-20%	FI+20%	FI+40%	Control	FI-20%	FI+20%	FI+40%
Large yellow follicle	6.3^{bc}±0.26	6.1^{c}±0.26	6.8^{ab}±0.23	7.4^{a}±0.20	7.3^{b}±0.27	6.5^{b}±0.23	8.5^{a}±0.28	8.8^{a}±0.47
Small yellow follicle	16.1^{abb}±0.62	14.9^{b}±0.57	17.4^{a}±0.81	18.0^{a}±0.58	16.9^{a}±0.46	14.2^{b}±0.60	15.6^{ab}±0.64	12.0^{c}±0.67
White follicle	25.4^{a}±0.82	24.3^{a}±0.97	23.8^{a}±0.65	25.6^{a}±0.98	23.4^{a}±0.74	23.5^{a}±1.09	22.1^{a}±1.03	21.8^{a}±0.82
Postovulatory follicle	9.2^{a}±0.37	8.4^{a}±0.33	7.9^{ab}±0.26	7.0^{b}±0.22	8.4^{a}±0.26	7.3^{b}±0.30	7.5^{b}±0.36	5.4^{c}±0.32
Atretic yellow follicles/ovary	0.0^{b}	0.7^{ab}±0.23	0.4^{ab}±0.19	0.8^{a}±0.30	0.0^{b}	$1.5b^{a}$±0.28	0.7^{b}±0.25	1.9^{a}±0.35

^{a,b,c} - Means with the different supercripts in the same row (within the same period) are significantly different ($p<0.05$);Period 1, after 15 days of experiment; Period 2, after 30 days of experiment; FI-20%, group with feed intake of 130.4 g/day; FI+20%, group with feed intake of 195.6 g/day; FI+40%, group with feed intake of 228.2 g/day.

Table 3 - Plasma levels of nitrite/nitrate, TAG, leptin, glucose, cholesterol, and estradiol in broiler breeder hens fed at different levels of feed intake.

Parameters	Period 1				Period 2			
	Control	FI-20%	FI+20%	FI+40%	Control	FI-20%	FI+20%	FI+40%
Glucose (mg/dl)	157.4b±3.0	162.2b±2.8	165.6ab±4.1	180.6a±4.5	173.8b±4.7	164.2b±3.0	185.7b±4.8	219.5a±5.0
Nitrite/Nitrate(µM)	35.7a±3.8	36.8a±4.6	38.4a±5.0	40.1a±5.0	32.8c±2.3	44.3b±3.8	39.9bc±3.4	59.1a±3.2
TAG (mg/dl)	487.3a±15.2	450.6a±21.1	488.5a±13.3	484.1a±11.4	573.8c±21.3	464.2c±19.5	885.7b±27.8	1023.5a±30.1
Cholestrol(mg/dl)	146.7a±4.8	148.7a±7.1	157.7a±5.9	156.5a±6.0	142.3b±5.2	116.9c±4.4	143.3b±5.4	173.1a±6.9
Leptin (ng/dl)	0.69b±0.06	0.63b±0.05	0.74ab±0.08	0.86a±0.08	0.63c±0.05	0.51c±0.06	0.79b±0.07	1.20a±0.09

a,b,c - Means with the different supercripts in the same row (within the same period) are significantly different (p<0.05); Period 1, after 15 days of experiment; Period 2, after 30 days of experiment; FI-20%, group with feed intake of 130.4 g/day; FI+20%, group with feed intake of 195.6 g/day; FI+40%, group with feed intake of 228.2 g/day.

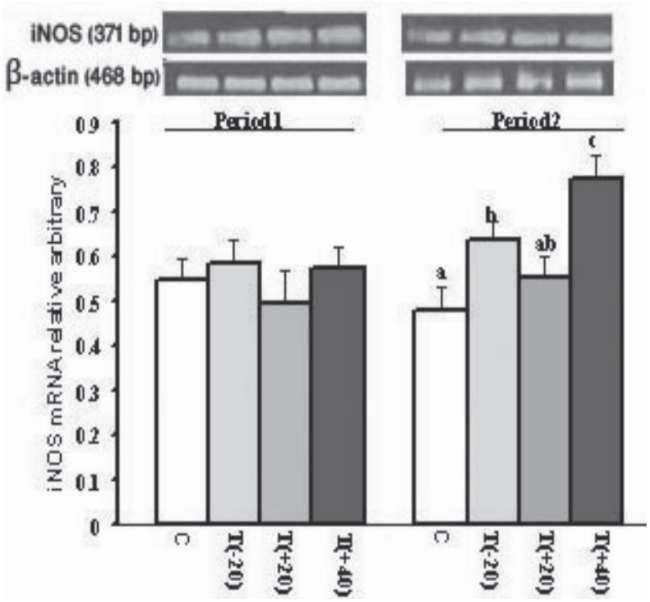

Figure 1 - Above: Gel electrophoresis samples of semi-quantitative RT-PCR for determination of iNOS mRNA levels in the granulosa cells of F_1 follicles in control and treatment groups during two experimental periods; ß-actin was as housekeeping gene. Below: Comparison of the relative density of iNOS/β-actin PCR products in the granulosa cells of F_1 follicles between control and treatment groups at two periods. Values are expressed means ± SE. ^{a,b,c} - Means with the different supercripts in the same row (within the same period) are significantly different (p<0.05);Period 1, after 15 days of experiment; Period 2, after 30 days of experiment; FI-20%, group with feed intake of 130.4 g/day; FI+20%, group with feed intake of 195.6 g/day; FI+40%, group with feed intake of 228.2 g/day.

The relative amount of iNOS mRNA expression in the granulosa layer of F1 follicles was significantly (p< 0.05) higher in FI-20% and FI+40% groups than in control hens only in second experimental period, with increases of. 32.4% and 60.9% for the FI-20% and FI+40%groups,respectively (Figure 1).

DISCUSSION

This study was designed to investigate the levels of iNOS gene expression in the granulosa layer of broiler breeder hens offered different levels of feed. Plasma leptin-like hormone, TAG, and glucose levels were the most important indexes to determine reproductive abnormalities, which are caused by increased feed consumption (Chen et al., 2006). The results of this study showed that obesity-associated disorders are commonly observed in broiler breeder hens fed an excess of 40% of their defined feed requirements. In the present study, the lower egg production pf FI+20% and FI+40% groups is consistent with earlier studies (Hocking et al., 1987, 1989; Yu et al., 1992a,b). The higher egg yolk weight (Table 1), coupled with reduced egg production, may be the result of prolonged follicle retention within the hierarchy of feed-satiated hens, which allows more plasma yolk-targeted very low density lipoprotein to be internalized (Chen et al., 2006). Likewise, prolonged retention of follicles within the hierarchy would increase exposure of all follicular cell types to altered metabolic and hormonal signals. Such a situation would increase the likelihood of granulosa cell dysfunction and induction of follicle atresia, as by the development of follicular atresia in hens in FI+40% and FI+20% groups during the second period of measurements (Table 1).

In the present study, an iNOS gene upregulation was observed in granulosa layer of F1 follicles in FI-20% and FI+40% groups. NO is synthesized by the ovary and it is hypothesized it plays a role in ovulation and luteolysis (Rosselli et al., 1998). Huang et al. (2005) found that leptin-like hormone induced NO production in human granulosa cells in dose- and time-dependent manner, and that NO may mediate the action of leptin-like hormone in human granulosa cells. It was demonstrated that leptin-like hormone could increase NO production in the pituitary and serum (Huang et al., 2005). In our study, plasma leptin-like hormone and nitric oxide concentration significantly increased in FI+40% hens in the second experimental period (p<0.05). Amongst the different isoforms of nitric oxide synthases, iNOS was reported to be involved in chicken reproductive regression (Anish et al., 2008). Locally-produced NO influences the regression of mammalian reproductive

tissues by regulating blood flow (Neuvians et al., 2004), steroidogenesis (Gobbetti et al., 1999), and cytokines (Torres and Forman 2000). The present study demonstrated the expression of iNOS mRNA in chicken granulosa layer of F1 follicle for the first time.

However, Sundaresun et al. (2007) suggested the involvement of nitric oxide in ovulation. Based on the present results, a basal level expression of iNOS mRNA was found in the F1 follicle of control birds, which may indicate that a basal level of nitric oxide is normal in the F1 follicle for its function. Furthermore, it was assumed that increased iNOS expression, along with plasma nitric oxide levels, in hens fed 40% higher and 20% lower than usual feed intake might be involved in follicle F1 regression. The higher levels of atretic follicles in birds in the FI+40% and FI-20% groups may be due to the continuously increased levels of tissue nitric oxide production. However, we did not measure caspase gene expression in granulosa layer of F1 follicles, but it has been suggested that local nitric oxide could cause ovarian caspase activity (Anish et al., 2008). Caspases are activated by nitric oxide during reproductive regression in mammals (Skarzynski et al., 2005). The accumulation of nitric oxide in cells leads to activation of caspases, resulting in apoptosis (Marriott et al., 2004). This hypothesis is supported by a previous study (Anish et al., 2008). Many studies have shown that nitric oxide has both cytotoxic and cytoprotective effects. The pro-apoptotic effects appear to be linked to the production of high concentrations of nitric oxide by the activity of iNOS. On the other hand, antiapoptotic effects are mainly mediated by low amounts of nitric oxide (Vega et al., 2000). It was confirmed that a basal level of nitric oxide at ovarian level is necessary for the growth and development of follicles (Mitchell et al., 2004). However, the up-regulation of iNOS in granulosa layer of F1 follicle, as found in the present study, probably had an adverse effect on the ovulatory process of F1 follicles, leading to their regression.

The number of postovulatory follicles (POF) can be used as an index of egg production, and the lower POF in the hens fed by higher levels of feed intake was associated with the lower egg production, as shown in the Table 1. Furthermore, as previously shown (Sundaresan et al., 2007), it appears that iNOS and its product (NO) play an important role in the POF regression via endocrine changes and may be responsible for the lower number of POF in the hens of the FI+40% group.

Increased plasma leptin-like hormone concentration in FI+20% and FI+40% hens in second experimental period were closely associated with the observed ovarian abnormalities (Table 1). Several studies conducted on theca and granulosa cells have shown that leptin-like hormone may have direct negative effects on ovarian steroidogenesis in various mammalian species (Spicer, 2003). Feed-satiated hens with abnormal ovaries had significantly greater plasma leptin-like hormone concentration (Chen et al., 2006). We observed ovarian abnormalities in hens fed below (FI-20%) and above (FI+%4) their suggested feed requirements. It seems that plasma concentrations of leptin-like hormone below or above a normal range may interfere in the breeders' reproductive capacity.

CONCLUSION

It was concluded that iNOS gene was normally expressed in follicular granulosa cells of broiler breeder hens. High and low feed intake increase iNOS expression in the granulosa layer of F1 follicle of broiler breeder hens. It appears iNOS via its product (NO) plays a role in follicular regression.

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