



EFFECT OF THREE DIFFERENT PHOTOPERIOD SCHEDULES
ON SERUM LEPTIN AND LIPID PROFILE, ABDOMINAL FAT
PAD ADIPOSITY AND TRIGLYCERIDE CONTENT
IN BROILER CHICKENS

N. MOSLEH¹, S. NAZIFI² & F. GHANADZADEGAN³

¹Avian Disease Research Centre, ²Department of Clinical Studies, ³Graduate;
School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Summary

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This study was aimed at evaluating the effect of three different light regimens on serum leptin, lipid profile, abdominal fat pad triglyceride content and adiposity of broiler chickens. For this purpose, 60 one-day-old broiler chickens (Cobb 500) were distributed in three light-proof controlled rooms (20 chicks per room). All birds were reared under continuous light until 1 week of age. Then the chicks were treated as follows: 1. Continuous lighting (CL) programme (23L:1D), 2. Non intermittent restricted lighting programme (NIL) (6L:18D from day 7 to 28, 23L:1D from day 29 to 42) and 3. Intermittent lighting programme (IL) (1L:3D cycles). At day 42 of age, sera were collected from fasted chicks of each group and serum leptin levels and lipid profile were assayed. Then, abdominal fat weight and triglyceride content were evaluated. Feed intake, body weight and feed conversion ratio (FCR) were determined at the end of the experiment. Body weight of chickens reared under IL was slightly higher than other groups, but there was no significant difference among groups. Use of IL and NIL lighting schedules improved FCR. IL and NIL lighting programmes significantly reduced abdominal fat percentage in comparison with CL programme ($P<0.05$). Serum leptin levels were significantly higher in CL group in comparison with other groups ($P<0.05$). Blood leptin levels were positively correlated with abdominal fat pad size or adiposity in all groups. Serum triglyceride, cholesterol, lipoproteins (HDL, LDL, VLDL) and abdominal fat pad triglyceride content of birds under different photoperiod schedules did not differ significantly ($P>0.05$). In conclusion, use of IL and NIL programmes can enhance production efficiency and decrease adiposity and serum leptin level with no appreciable effect on abdominal fat pad triglyceride and serum lipid profile in broiler chickens.

Key words: abdominal fat pad adiposity, broiler chicken, leptin, photoperiod schedules

INTRODUCTION

In broilers, genetic selection for rapid growth rate has led to several undesirable

traits, including higher incidence of excessive body fat and metabolic diseases re-

sulting in low performance with high mortality. Control and prediction of fatness in broiler chickens are of high economic interest since most fat deposits are discarded during evisceration of carcass or processing of the meat, which leads to lower meat yields. Excessive abdominal fat does not just reduce carcass yield and feed efficiency which represents economical loss to the producers, but also cause rejection of the meat by the consumers since they prefer leaner meat (Lippens, 2003; Jennen, 2004) and difficulties in further processing (Tumova & Teimouri, 2010). These deficiencies have evoked increased interest in developing management techniques that will maximise productivity while minimising associated problems, especially reducing body fat deposition in broilers (Olanrewaju *et al.*, 2006).

Different techniques have been shown to reduce fat deposition in broiler chickens. Selection of live broiler chicken for reduced body fat deposition in the long term strategy or for improved feed conversion ratio (FCR) as well as feed restriction and increased dietary protein to energy ratio have been shown to be useful in reducing fat deposition. Environmental factors such as ambient temperature, housing system and lighting regimens can also affect fat deposition in broiler chickens (Tumova & Teimouri, 2010). Lightening programmes have received considerable attention as a management tool to improve broiler productivity and health. Effect of different light regimens on performance and disease control of broilers has been investigated by several researchers (Quarles *et al.*, 1974; Buckland *et al.*, 1975; Mahmud *et al.*, 2011). In 2005, Rahimi *et al.* demonstrated that intermittent lighting programme can reduce abdominal fat percentage in broiler chickens, which is simi-

lar to results that have been reported by previous researchers (Buyse *et al.*, 1996). Oyediji & Atteh (2005) reported that reducing photoperiod to 6 h per day could be used as a tool for reducing abdominal fat.

On the other hand, abdominal fat pad size is influenced by nutritional, metabolic and hormonal factors (Murray *et al.*, 2012). One of the metabolic hormones which seem to play an important role in the regulation of food intake, energy expenditure, lipid metabolism and body weight is leptin (Friedman, 2002). Leptin is produced mainly by adipose tissue (Klok *et al.*, 2007). The effect of leptin on reducing food intake and correlation between blood leptin levels and fat pad size has been demonstrated in rodents and humans (Maffei *et al.* 1995; Brunner *et al.*, 1997; Flynn & Plata-Salaman, 1999). Leptin is expressed in the liver as well as in the adipose tissue of chickens (Taouis *et al.*, 1998). The acute effect of leptin on regulation of food intake has been investigated in layers and broilers (Cassy *et al.*, 2004), and it has been shown that exogenous leptin induces different responses in food intake in chicks for instance intravenous or intraperitoneal injection of the chicken or ovine leptins lowered the food intake of starved 9-day-old broiler or 5-week-old layer male chickens by 11–34% (Dridi *et al.*, 2000) whereas intracerebroventricular administration of mouse leptin does not reduce food intake in the chicken (Bungo *et al.*, 1999). As far as we know, the effect of photoperiod on leptin level and abdominal fat pad status in broilers has not been clarified yet. To better understand the role of leptin on lipid metabolism in chickens, we designed an experiment to compare the effect of three different photoperiod regimens on adiposity and its correlation with serum leptin

levels in broiler chicks. Moreover, lipid profile, feed intake, body weight and FCR were measured at the end of the experiment. Since adipocytes are one of the important targets for this hormone and the major place for lipids, especially triglycerides storage (Fruhbeck & Salvador, 2000), abdominal fat pad triglyceride content was also determined.

MATERIALS AND METHODS

Experimental design and sampling

Sixty one-day old broiler chickens of the Cobb 500 strain (both genders) were randomly divided into 3 groups and kept in three light-proof controlled rooms (20 chicks per room). All chicks were fed a

standard diet and allowed free access to water and food throughout the experiment. Nutrient composition of the diet (starter, grower, finisher) is presented in Table 1.

All birds were reared on continuous light until 1 week of age. Then the chicks were treated as follows: group 1: continuous lighting (CL) programme (23L: 1D); group 2: non intermittent restricted lighting programme (NIL) (6L: 18D from day 7 to 28, 23L: 1D from day 29 to 42) and group 3: intermittent lighting programme (IL) (1L: 3D cycles). At the end of the experimental period (42 day of age), food intake per group was recorded.

Birds were deprived of feed 12 h before being weighed and bled. Blood was collected from the wing vein of fasted

Table 1. Composition of the diet during starter, grower and finisher periods of the experiment

Ingredients %	Starter (0–10 days of age)	Grower (11–22 days of age)	Finisher (23–42 days of age)
Corn	57.4	64.4	68.5
Soy bean meal 44	36.5	29.7	26
Dicalcium phosphate	2.15	2	1.9
Calcium carbonate	0.9	0.85	0.8
Sodium chloride	0.25	0.2	0.2
Sunflower oil	2	2	1.67
DL-methionine	0.2	0.2	0.23
L-lysine	0.1	0.15	0.2
Vitamin premix ^a	0.25	0.25	0.25
Mineral premix ^b	0.25	0.25	0.25
Calculated nutrient composition			
Metabolizable energy (kcal/kg)	2990	3090	3185
Crude protein (%)	21	19	18
Calcium (%)	0.99	0.91	0.89
Available phosphorus (%)	0.5	0.48	0.45
Lysine (%)	1.2	1.1	1.08
Methionine+cysteine (%)	0.89	0.84	0.81

^aThe vitamins supplied per 2.5-kg premix: vitamin A 9,500,000 IU; vitamin D3 2,000,000 IU; vitamin E 18,000 IU; vitamin K₃ 2,000 mg; vitamin B₆ 3,000 mg; vitamin B₉ 1,000 mg; vitamin B₁₂ 15 mg; vitamin B₁ 1,800 mg; biotin, 100 mg; vitamin B₂ 6,600 mg; vitamin B₃ 10,000 mg; vitamin B₅ 30,000 mg; choline chloride 250,000 mg. ^bThe mineral supplied per 2.5-kg premix: Mn 100,000 mg; I 1,000 mg; Fe 50,000 mg; Se 200 mg; Zn 100,000 mg; Cu 10,000 mg.

birds of each group. Serum was immediately separated and stored at $-20\text{ }^{\circ}\text{C}$ until use. Then, chicks of each group were humanely slaughtered and abdominal fat pad including fat surrounding gizzard, bursa of Fabricius, cloaca, and adjacent muscles was removed and weighed individually. Furthermore, collected samples were stored at $-70\text{ }^{\circ}\text{C}$ for determination of triglyceride content.

Leptin determination

Serum leptin concentration was measured by enzyme-linked immunosorbent assay (ELISA), using a commercial kit (chicken leptin (lep) ELISA kit, Cusabio, China) according to manufacturer's protocol. The detection limit for leptin was 0.08 ng/mL . The intra- and inter-assay coefficients of variation were 8% and 10%, respectively, for the measurement.

Serum lipid and lipoprotein profile determination

The serum was analysed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (Abbel & Kendall, 1952; Burtis & Ashwood 1994), triglyceride by the enzymatic procedure of McGowan *et al.* (1983). Lipoproteins were isolated using a combination of precipitation and ultracentrifugation. HDL-cholesterol was measured using the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate non-HDL lipoproteins which were sedimented by centrifugation ($10,000\times g$ for 5 min). The residual cholesterol was then measured by the enzymatic method (Burtis & Ashwood, 1994). LDL-cholesterol was calculated as the difference between the total cholesterol measured in the precipitate and in the HDL fraction minus $0.2\times$ triglyceride

($\text{LDL}=\text{total cholesterol}-\text{HDL cholesterol}-0.2\times\text{TG}$). VLDL-cholesterol was estimated as one-fifth of the concentration of triglycerides (Friedewald *et al.*, 1972).

Determination of triglyceride content of abdominal fat pad

Lipid extraction was conducted using the method described by Rodriguez-Sureda & Peinado-Onsurbe (2005). To evaluate triglyceride content, the extracts were first dissolved in 6 mL of LPL buffer [Pipes (1,4 piperazinediethanesulfonic acid) + $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ + FFA-BSA (free fatty acids-bovine serum albumin)] with 0.1% sodium dodecyl sulfate solution. Triglycerides were measured immediately using enzymatic method after further processing as described by Rodriguez-Sureda & Peinado-Onsurbe (2005).

Statistical analysis

Variables are presented as mean values \pm standard deviation (SD). For comparison of different parameters the one way ANOVA test and Tukey's multiple comparison test were used. Association between serum leptin level and abdominal fat pad was investigated using Pearson's correlation coefficients, and only statistically significant correlations were reported. Data were analysed by SPSS software, version 11.5. A P-value less than 0.05 was considered as statistically significant.

RESULTS

Performance parameters

Although final body weight of chickens reared under IL was slightly higher compared to the other groups, there was no significant difference among groups ($P>0.05$). Moreover, no significant diffe-

Table 2. Body weight (mean±SD), feed intake (mean±SD), FCR, abdominal fat pad weight (mean±SD) and abdominal fat weight/body weight ratio (%) in chickens under different photoperiod schedules: group 1: continuous lighting (CL) programme, group 2: non intermittent restricted lighting programme (NIL) and group 3: intermittent lighting programme (IL).

Groups	Parameters				
	Body weight (g)	Feed intake (g)	FCR	Abdominal fat pad weight (g)	Abdominal fat weight/body weight
Group 1 (CL)	2333±199 ^a	4339±115 ^a	1.86	50.40±7.33 ^a	2.23±0.43 ^a
Group 2 (NIL)	2363±136 ^a	4111±150 ^a	1.74	41.92±7.69 ^b	1.80±0.34 ^b
Group 3 (IL)	2441±108 ^a	4344±184 ^a	1.78	39.25±8.61 ^b	1.64±0.43 ^b

Different superscript letters denote significant differences (P<0.05) in each column.

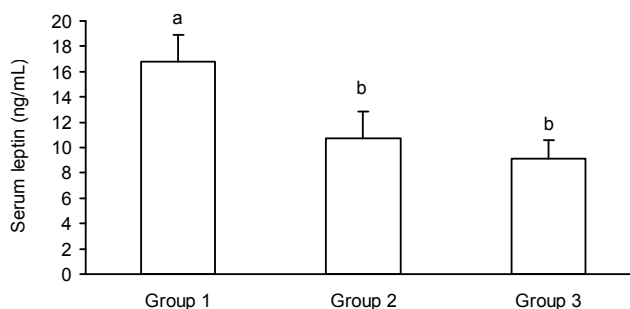


Fig. 1. Serum leptin levels (mean±SD) in chickens under different photoperiod schedules. Group 1: continuous lighting (CL) programme, group 2: non intermittent restricted lighting programme (NIL); group 3: intermittent lighting programme (IL). Different letters denote significant differences (P<0.05).

rences were observed in feed intake among groups (P>0.05). However, IL and NIL lighting schedules improved FCR (Table 2).

Abdominal fat pad

The abdominal fat pad weight and abdominal fat weight/body weight ratio are reported in Table 2. Birds in CL group had significantly higher (P<0.05) values than other groups in both parameters. However, no significant differences were observed in these values between IL and NIL groups (P>0.05).

Serum leptin

Fig. 1 depicts circulating leptin concentrations after an overnight fast in each group at the end of the experiment. Serum leptin levels were significantly higher in CL group in comparison with other groups (P<0.05). No significant differences were observed in serum leptin concentration between IL and NIL groups (P>0.05).

Evaluation of the association between serum leptin level and abdominal fat pad showed that leptin concentrations were

Table 3. Serum lipids and lipoproteins profile (mmol/L) and abdominal fat pad triglyceride content (mmol/L) in chickens under different photoperiod schedules: group 1: continuous lighting (CL) programme, group 2: non intermittent restricted lighting programme (NIL) and group 3: intermittent lighting programme (IL). Data presented as mean±SD

Groups	Parameters					
	TG	CHOL	HDL-C	VLDL-C	LDL-C	Abdominal fat pad TG
Group 1 (CL)	0.29± 0.06	4.05± 0.61	1.19± 0.27	0.058± 0.01	3.23± 0.46	3.24± 0.71
Group 2 (NIL)	0.23± 0.05	4.85± 0.31	1.24± 0.23	0.046± 0.01	3.34± 0.51	2.80± 0.53
Group 3 (IL)	0.32± 0.08	4.25± 0.44	1.20± 0.25	0.064± 0.01	3.26± 0.58	3.18± 0.32

TG – triglycerides; CHOL – total cholesterol; HDL-C – high- density lipoproteins; VLDL-C – very low-density lipoproteins; LDL-C – low-density lipoproteins.

directly and significantly ($P < 0.05$) related to abdominal fat pad size or adiposity in all groups (CL group: $r = 0.873$, $P = 0.000$; NIL group: $r = 0.765$, $P = 0.016$; IL group: $r = 0.743$, $P = 0.02$).

Serum lipid profile and abdominal fat pad triglyceride content

The mean values of serum total cholesterol, triglyceride, HDL-c VLDL-c, LDL-c and abdominal fat pad triglyceride content are presented in Table 3. Serum triglyceride, cholesterol, lipoproteins (HDL, LDL, VLDL) and abdominal fat pad triglyceride content of birds under different photoperiod schedules were not significantly different ($P > 0.05$).

DISCUSSION

The present study aimed to evaluate the effects of three different photoperiod programmes on abdominal fat pad size and its correlation with serum leptin levels in broiler chickens. Abdominal fat pad triglyceride content and serum lipid profile were also investigated. Furthermore, feed

intake, body weight and FCR were measured at the end of the experiment.

Unexpectedly, reduction in feeding time for broilers under IL or NIL photoperiod did not significantly result in reduced feed intake which is inconsistent with results of Oyedeleji & Atteh (2005). Although final body weight was not significantly affected by different photoperiod regimens, in birds under IL lightening programme it was slightly higher than the other groups. Despite this, FCR in chickens under IL or NIL programmes was still comparable with or better than those of broilers exposed to CL regimen. This may be due to lower energy expenditure on physical activity in broilers exposed to reduced photoperiod programmes (Oyedeleji & Atteh, 2005). These results are in agreement with previous studies (Rahimi *et al.*, 2005; Mahmud *et al.*, 2011). Mahmud *et al.* (2011) reported that intermittent lighting system results in a significant increase in the average weight gain and better FCR in comparison with continuous lightening.

The data obtained from abdominal fat pad weighing indicated that IL and NIL

programmes caused lower percentage of abdominal fat in broiler chickens in comparison to CL regimen, which agreed with Rahimi *et al.* (2005) and Oyediji & Atteh (2005). This result confirms the positive effect of both IL and NIL programmes on reduction of abdominal fatness in broiler chickens with no appreciable effect on meat production which is very important from economic aspect and meat quality. Therefore, rearing broilers under reduced photoperiod programmes can be used as an effective technique or way to reduce fat deposition in broiler chickens industry.

Moreover, it has been shown that chickens under CL programme had significantly higher serum leptin levels than birds in IL and NIL groups. The obvious increase in serum leptin level in birds under CL programme could be described by larger abdominal fat pad size. Data give further evidence that abdominal fat pad either expressed as a percentage of body weight or as an absolute amount may be (or is) a major determinant of leptin concentrations in broiler chickens since we found a strong relationship between leptin levels and adiposity similarly to rodents and humans (Maffei *et al.*, 1995; Considine *et al.*, 1996).

Despite the higher adiposity and serum leptin level in broilers exposed to CL regimen in comparison with birds exposed to IL and NIL programmes, this elevated leptin signal does not induce the expected reduction in food intake in this group. Several studies have investigated the effect of leptin on feed intake in chickens (Bungo *et al.*, 1999; Denbow *et al.*, 2000; Dridi *et al.*, 2000). Leptin depresses food intake in various strains (slow versus fast growth) of chicken from 5 weeks of age after intraperitoneal or ICV treatment. ICV administration of recombinant human leptin to 2-day-old chicks was ineffective

(Denbow, 2000; Dridi *et al.*, 2000). Cassy *et al.* (2004) showed that intraperitoneal injection of recombinant chicken leptin reduced (38%) feed intake in 56-day-old layer chickens, more moderately reduced (15%) food intake in 9-day-old layer chicks and had no significant effect in 9-day-old broiler chicks. In the present study, although serum leptin level in IL and NIL groups was lower than that in the CL group, feed intake was approximately equal in all groups, so this study provides evidence that broiler chickens may be resistant to the effects of endogenous leptin or less sensitive (or responsive) to the inhibitory effect of leptin on feed intake, which is in agreement with Cassy *et al.* (2004). According to data obtained in the present study, it is unlikely that leptin has evolved to prevent food intake in broiler chickens because the elevated plasma leptin levels accompanied by increased adipose tissue mass did not reduce feed intake.

Despite this, control of feed intake and total body energy is an extremely complex area involving several possible mechanisms which have attempted to explain this (Ferket & Gernat, 2006). In addition, although adiposity is the most important factor in leptin expression and release (Trayhurn *et al.*, 1999), other factors may affect blood leptin level and leptin expression with no body weight association (Mooradian *et al.*, 2000; Zhao & Wu, 2005; Lee *et al.*, 2007).

The absence of significant relationship between different photoperiod schedules and serum lipid or lipoprotein profile demonstrates that different photoperiod programmes in broiler chickens do not contribute significantly to the variability of serum lipid or lipoproteins levels. Like mammals, many factors such as gender, age, nutrition, health status, endocrine

system (insulin, glucagon) and etc. can influence serum lipid profile in chickens (Yanaihara *et al.*, 1983). These factors may prevent any changes in serum lipid or lipoproteins levels in different conditions such as photoperiod programmes.

Triglyceride storage in adipose tissue depends on the availability of plasma lipid substrate originating from either the diet or lipogenesis in the liver since lipogenesis is very limited in adipose tissue (Saadoun & Leclercq, 1987; Hermier, 1997). Griffin *et al.* (1992) demonstrate that about 80–85% of the fatty acids that accumulate in the adipose tissue in broiler chickens are derived from plasma lipids. We found that abdominal fat pad triglycerides content as well as serum lipid or lipoprotein profile of broilers under different photoperiod programmes were not significantly different among groups.

In conclusion, use of IL and NIL programmes can enhance production efficiency, decrease adiposity and serum leptin level with no appreciable effect on abdominal fat pad triglyceride content and serum lipid profile in broiler chickens. Moreover, plasma leptin concentration of chickens under different photoperiod programmes is directly correlated to abdominal fat pad size which itself is influenced by photoperiod schedules.

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Correspondence:

Dr. S. Nazifi
Professor of Veterinary Clinical Pathology
Department of Clinical Studies,
School of Veterinary Medicine,
Shiraz University, Shiraz,
P.O. Box: 1731- 71345, Iran
Tel : +98-711-2286940
Fax : +98-711-2286950
e-mail: nazifi@shirazu.ac.ir