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

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Summary


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 mor-
tality. Control and prediction of fatness in
broiler chickens are of high economic
interest since most fat deposits are dis-
carded 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 economi-
cal 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 manage-
ment techniques that will maximise pro-
ductivity 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 chi-
kens. Selection of live broiler chicken for
reduced body fat deposition in the long
term strategy or for improved feed con-
version ratio (FCR) as well as feed restric-
tion and increased dietary protein to en-
ergy ratio have been shown to be useful in
reducing fat deposition. Environmental
factors such as ambient temperature, hous-
ing system and lighting regimes 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 (Quar-
les 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 per-
centage in broiler chickens, which is simi-
lar to results that have been reported by
previous researchers (Buyse et al., 1996).
Oyedeji & Atteh (2005) reported that re-
ducing 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 ex-
penditure, 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 be-
tween blood leptin levels and fat pad size
has been demonstrated in rodents and hu-
mans (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 investi-
gated in layers and broilers (Cassy et al.,
2004), and it has been shown that exoge-
nous leptin induces different responses in
food intake in chicks for instance intrave-
nous 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 intracere-
broventricular 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 bet-
ter understand the role of leptin on lipid
metabolism in chickens, we designed an
experiment to compare the effect of three
different photoperiod regimens on adipo-
sity and its correlation with serum leptin

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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>
<thead>
<tr>
<th>Table 1. Composition of the diet during starter, grower and finisher periods of the experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients %</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Corn</td>
</tr>
<tr>
<td>Soy bean meal 44</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
</tr>
<tr>
<td>Calcium carbonate</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Sunflower oil</td>
</tr>
<tr>
<td>DL-methionine</td>
</tr>
<tr>
<td>L-lysine</td>
</tr>
<tr>
<td>Vitamin premix a</td>
</tr>
<tr>
<td>Mineral premix b</td>
</tr>
</tbody>
</table>

Calculated nutrient composition

<table>
<thead>
<tr>
<th>Metabolizable energy (kcal/kg)</th>
<th>2990</th>
<th>3090</th>
<th>3185</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td>21</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.99</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>Available phosphorus (%)</td>
<td>0.5</td>
<td>0.48</td>
<td>0.45</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.2</td>
<td>1.1</td>
<td>1.08</td>
</tr>
<tr>
<td>Methionine+cysteine (%)</td>
<td>0.89</td>
<td>0.84</td>
<td>0.81</td>
</tr>
</tbody>
</table>

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 K2 2,000 mg; vitamin B6 3,000 mg; vitamin B12 1,000 mg; vitamin B1 1,800 mg; biotin, 100 mg; vitamin B2 1,600 mg; vitamin B3 10,000 mg; vitamin B5 10,000 mg; vitamin B6 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.
Effect of three different photoperiod schedules on serum leptin and lipid profile, abdominal fat pad...

birds of each group. Serum was immediately separated and stored at –20 °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 °C for determination of triglyceride content.

**Leptin determination**

Serum leptin concentration was measured by enzyme-linked immuno sorbent 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×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×triglyceride (LDL=total cholesterol–HDL cholesterol–0.2×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) +MgCl₂·6H₂O+ 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 ± 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-
references 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

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**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).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
</tr>
<tr>
<td>Group 1 (CL)</td>
<td>2333±199&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 2 (NIL)</td>
<td>2363±136&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 3 (IL)</td>
<td>2441±108&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different superscript letters denote significant differences (P<0.05) in each column.
Effect of three different photoperiod schedules on serum leptin and lipid profile, abdominal fat pad size and body weight in broiler chickens.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>TG</th>
<th>CHOL</th>
<th>HDL-C</th>
<th>VLDL-C</th>
<th>LDL-C</th>
<th>Abdominal fat pad TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (CL)</td>
<td>TG</td>
<td>0.29±0.06</td>
<td>4.05±0.61</td>
<td>1.19±0.27</td>
<td>0.058±0.01</td>
<td>3.23±0.46</td>
<td>3.24±0.71</td>
</tr>
<tr>
<td></td>
<td>CHOL</td>
<td>0.06</td>
<td>0.61</td>
<td>0.27</td>
<td>0.01</td>
<td>0.46</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>HDL-C</td>
<td>0.23</td>
<td>4.85±0.31</td>
<td>1.24±0.23</td>
<td>0.046±0.01</td>
<td>3.34±0.51</td>
<td>2.80±0.53</td>
</tr>
<tr>
<td></td>
<td>VLDL-C</td>
<td>0.05</td>
<td>0.31</td>
<td>0.23</td>
<td>0.01</td>
<td>0.51</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>LDL-C</td>
<td>0.32</td>
<td>4.25±0.44</td>
<td>1.20±0.25</td>
<td>0.064±0.01</td>
<td>3.26±0.58</td>
<td>3.18±0.32</td>
</tr>
<tr>
<td></td>
<td>Abdominal fat pad</td>
<td>0.08</td>
<td>0.44</td>
<td>0.25</td>
<td>0.01</td>
<td>0.58</td>
<td>0.32</td>
</tr>
</tbody>
</table>

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 Oyedeji & 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 (Oyedeji & 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 Oyedeji & 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
Effect of three different photoperiod schedules on serum leptin and lipid profile, abdominal fat pad

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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