Long-term induced hyperthyroidism in breeder hens: Effects on blood plasma biochemical attributes, indicators of oxidative stress, and markers of liver function

F. Saemia,⁎, A. Zare Shahneh, M. Zhandi, S. Kargar, R. Keshavarz, A. Akhlaghi

a Department of Animal Science, University College of Agriculture and Natural Resources, University of Tehran, 4111, 31587-77871, Karaj, Iran

b Department of Animal Science, College of Agriculture, Shiraz University, Shiraz 71441-65186, Iran

ARTICLE INFO

Keywords: Ascites Egg production Liver Peroxidation Shell thickness Thyroid hormone

ABSTRACT

A 4-week-long administration of extra thyroxine in broiler breeder hens was suggested to reduce the cold-induced ascites in their offspring. In the present study the hypothesis was tested to disclose the plausible adverse effects of long-term maternal hyperthyroidism (100 days) on blood plasma biochemical attributes, indicators of oxidative stress, and markers of liver function in the exposed breeder hens. Also, the association between egg production rate and egg shell strength with alkaline phosphatase activity of plasma were tested.

Seventy 47-week-old Cobb 500 breeder hens were randomly allotted to two treatment groups as control (CON) and hyperthyroid (HYPER), each consisting of five replicates of seven birds. Thyroxine (T4; 0.3 mg/bird/d) was orally administered to the HYPER group for 100 consecutive days; whereas the CON group received the drinking water only. Blood sampling was done every 5 week from 47 to 64 week of age for quantification of plasma T4 and T3, and biochemical attributes as well as indicators of oxidative stress. Results indicated that plasma concentration of T4 was greater for the HYPER birds (P < 0.05); however, that of T3 was not different between the experimental groups. Total cholesterol, HDL, LDL, and VLDL, and triglyceride concentrations were not affected by T4 administration. Induced hyperthyroidism had no apparent effect on in vitro plasma lipoperoxidation indices, including CDmax, CDauc, and CD lag phase; whereas, CD oxidation rate and MDA were greater in HYPER group as compared with their CON counterparts. The effect of oral T4 on plasma aspartate aminotransferase and alanine aminotransferase was not significant although the plasma level alkaline phosphatase was higher in HYPER birds (P > 0.05). No significant treatment effect was observed on egg production. Hens belonging to HYPER group recorded a higher egg shell thickness than those of the CON birds, although the correlation coefficient of egg shell thickness with plasma concentration of alkaline phosphatase was not significant. Overall, apart from the changes in parameters evaluated, the values were within their biological ranges. Therefore, it might be stated that the long term administration of T4 did not adversely affect the biochemical characteristics of broiler breeder hens. Future studies are needed to make a final decision on use of this treatment to reduce the ascites incidence in offspring.

1. Introduction

The ascites is the metabolic disorder that is a serious cause of loss to the broiler industry in many countries, because of the high rate of mortality and decreased weight gain (Julian, 1993). Several methods have been suggested to decrease ascites such as genetic selection (Pavlidis et al., 2007) and feed restriction (Ozkan et al., 2006). Moreover it was reported 4-wk-long maternal hyperthyroidism caused decrease in cold-induced ascites incidence in their broiler chickens, which could ascribe to the effect of thyroid hormones on improved efficiency of O2 and CO2 exchange (Akhlaghi et al., 2012).

Thyroid hormones influence the overall energy expenditure and enhance basal metabolic rate by affecting on mitochondria (Lin et al., 2006; Rey et al., 2013). Hyperthyroidism is associated with accelerated oxidative metabolism and decreased lipid and lipoprotein plasma content (Costantini et al., 1998). Considerable lipid peroxidation, as characterized by a higher low density lipoprotein (LDL) content in lipid peroxides, a lower lag phase, and a higher oxidation rate was reported.

⁎ Corresponding author.

E-mail addresses: fatemeh.saemi@ut.ac.ir (F. Saemi), azareh@ut.ac.ir (A.Z. Shahneh), mzhandi@ut.ac.ir (M. Zhandi), skargar@shirazu.ac.ir (S. Kargar), reziakeshavarz@yahoo.com (R. Keshavarz), aakhlaghia@shirazu.ac.ir (A. Akhlaghi).

https://doi.org/10.1016/j.livsci.2018.10.009
Received 17 February 2018; Received in revised form 15 October 2018; Accepted 24 October 2018
for hyperthyroidism (Costantini et al., 1998). The acceleration of increased ROS production in hyperthyroidism state in the mitochondria is a side effect of the elevated level of electron carriers, that hyperthyroid tissues enhance their metabolic capacity (Venditti and Di Meo, 2006) and the enhanced mitochondrial free radical production, thereby affecting the antioxidant defense system (Costantini et al., 1998). Susceptibility of plasma lipids to lipoperoxidation was determined by quantifying the in vitro conjugated dienes (CD) generation induced by the addition of copper ions. This method is applicable because it represents the balance between pro and antioxidant agent in blood (Kargar et al., 2015). Thyroid hormones regulate the hepatic role as well as liver metabolizes the thyroid hormones and modulate their endocrine function (Malik and Hodgson, 2002). There are the complex associations between the thyroid gland and the liver in health and disease, the disorders of these two organs would influence each other (Malik and Hodgson, 2002).

An association between the egg production and blood plasma alkaline phosphatase activity has been reported in the hens (Gutowska et al., 1943). Good producers making eggs with good shell strength; have a higher alkaline phosphatase activity compared with the poor producers with feeble egg shells (Gutowska et al., 1943).

In the present study the question was raised as to what extent the long-term hyperthyroidism might affect the blood attributes and antioxidant status and markers of liver function in broiler breeder hens. Virtually, probable adverse effects on blood plasma biochemical attributes, indicators of oxidative stress, markers of liver function, and egg production would limit the use of this treatment to diminish the ascites incidence in progeny chicks.

2. Materials and methods

2.1. Birds and experimental treatments

All procedures in the current study were approved by the Animal Care and Welfare Committee of Department of Animal Science, College of Agriculture, Shiraz University (Shiraz, Iran). A total of seventy 47-week-old Cobb 500 breeder hens were individually caged and randomly allotted to two treatment groups (5 replicates of 7 hens each), including control (CON) and hyperthyroid (HYPER). Thyroxine (T4) (Iran Hormone Pharmaceutical Company, Tehran) was orally administered to all birds (Kargar et al., 2015). Basal (CDBaseline) and maximum (CDMax) absorbance at 245 nm and the net area under the curve of plasma CD (CDAUC) were computed by the trapezoid method and treated as the response variable (Schnitzer et al., 1998). Basal (CDBaseline) and maximum (CDMax) absorbance at 245 nm and the net area under the curve of plasma CD (CDAUC) were computed by the trapezoid method and treated as the final results (Gobert et al., 2009; Karçar et al., 2015). The concentration of plasma malondialdehyde (MDA), which is a degradation product of lipid peroxidation, was also determined by thiobarbituric acid reacting substances method, in which the absorbance of a colored complex that is formed from the reaction of MDA with 2-thiobarbituric acid in acid environment is measured at 532 nm using a spectrophotometer (UV 2100, Unico Co., China; Schnitzer et al., 1998). Basal (CDBaseline) and maximum (CDMax) absorbance at 245 nm and the net area under the curve of plasma CD (CDAUC) were computed by the trapezoid method and treated as the final results (Gobert et al., 2009; Karçar et al., 2015). The concentration of plasma malondialdehyde (MDA), which is a degradation product of lipid peroxidation, was also determined by thiobarbituric acid reacting substances method, in which the absorbance of a colored complex that is formed from the reaction of MDA with 2-thiobarbituric acid in acid environment is measured at 532 nm using a spectrophotometer (UV 2100, Unico Co., China) (Placer et al., 1966). Besides, the plasma concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (Pars Azmoo Co., Tehran, Iran) were determined using spectrochemical analysis (Butler and Laqua, 1995).

Egg production was recorded on a weekly basis and either egg shell thickness was evaluated from 47 to 64 wk of age.

2.2. Blood sampling and analyses

Blood samples were drawn from the wing vein of the birds, using a 5 mL syringe in EDTA-coated tubes every 5 week from week 47 to 64 week of age. The samples were centrifuged (1800 x g for 12 min), using a rotating centrifuge (International Equipment Co., Needham Heights, MA) and plasmas were separated, and each sample was divided into 3 aliquots, and stored at −20 °C until analyzed for T3 and T4 assays, using ELISA reader (Anthos2020, Biochrom Co, England) and ELISA kits (Padtan Elm, Iran) validated for chickens (Akhlagli et al., 2012). The intra- and inter-assay coefficients of variation were reported 12.6 and 13.2 for T3 and 13.2 for T4, respectively. The concentrations of plasma total cholesterol, high- (HDL), low- (LDL), and very low- (VLDL) density lipoprotein cholesterol as well as triglyceride contents were also quantified in the plasma samples (Pars Azmoo Co., Tehran, Iran) using spectrochemical analysis (Butler and Laqua, 1995). Susceptibility of plasma lipids to lipoperoxidation was evaluated by measuring the in vitro conjugated dienes (CD) generation induced by the addition of copper ions (Kargar et al., 2015). Copper chloride (10 mM) was added to each plasma sample, and absorbance of UV light was continuously monitored at 245 nm for 90 min (at 5-min intervals) using a UV-VIS recording spectrophotometer (UV 2100, Unico Co., China; Schnitzer et al., 1998). Basal (CDBaseline) and maximum (CDMax) absorbance at 245 nm and the net area under the curve of plasma CD (CDAUC) were computed by the trapezoid method and treated as the final results (Gobert et al., 2009; Karçar et al., 2015). The concentration of plasma malondialdehyde (MDA), which is a degradation product of lipid peroxidation, was also determined by thiobarbituric acid reacting substances method, in which the absorbance of a colored complex that is formed from the reaction of MDA with 2-thiobarbituric acid in acid environment is measured at 532 nm using a spectrophotometer (UV 2100, Unico Co., China) (Placer et al., 1966). Besides, the plasma concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (Pars Azmoo Co., Tehran, Iran) were determined using spectrochemical analysis (Butler and Laqua, 1995).

2.3. Statistical analysis

The experiment was carried out as a completely randomized design. The data were tested for normality, and transformations of data were used when appropriate. Data were subjected to the procedure GLM, but repeated measure data were analyzed by the procedure MIXED (Institute, 2004). The Pearson correlation coefficients were estimated using the correlation procedure (Institute, 2004). Body weight was included in the model as a covariate for analysis of variance. The means were compared by the least squares means and the level of significance was set at P ≤ 0.05. The following model was used to ascertain the effect of long-term induced hyperthyroidism on blood plasma biochemical attributes, indicators of oxidative stress, markers of liver function, egg production, and egg shell thickness which considered as dependent variables (Yijk):

\[ Y_{ijk} = \mu + T_{i} + D_{j} + L_{k} + T_{i} \times L_{k} + e_{ijk} \]

Where, \( Y_{ijk} \) = the response at time \( k \) on animal \( j \) in treatment group \( i \),
3. Results

The results of this research indicated that hyperthyroidism resulted in an increase in plasma concentration of T₄ with no effect on plasma T₃ concentration (Table 2). Hyperthyroidism had no effect on total cholesterol, HDL, LDL, VLDL and triglyceride concentration, whereas the effect of week on total cholesterol and LDL concentration were significant (Table 2). There were decreasing trends in total cholesterol and LDL concentration from week 47.

The sensitivity of plasma lipids to lipoperoxidation was determined by monitoring the in vitro CD generation produced by copper salts. As shown in Table 3, CD_{baseline}, CD_{AUC} and CD lag phase were not affected by hyperthyroidism, but the effect of treatment on CD_{baseline}, CD oxidation rate, and MDA were significant. Hyperthyroidism increased CD oxidation rate and MDA in breeder hens' plasma; however, CD_{baseline} was higher in the CON group. Moreover, the interaction effect of treatment and experimental weeks on CD_{baseline} and MDA tended to be significant (Figs. 1 and 2). Treatment effects on enzymatic parameters are presented in Table 3. Aspartate aminotransferase and alanine aminotransferase concentration were not different between two experimental groups. The results showed alkaline phosphatase concentration was increased in the HYPER hens as compared with the CON groups (22.1 versus 19.9 U/L; P = 0.004).

No significant differences were observed in egg production; however, the week and treatment and week interaction effect were significant on egg production (Table 2). As illustrated in Fig. 3 the egg production was decreased within each time period from 53 - 63 week of age in HYPER groups compared to the counterparts. Hyperthyroidism affected the egg shell thickness (P = 0.005; Table 2). The HYPER hens had greater shell thickness as compared with the CON group. Also a significant effect of week on egg shell thickness was observed, where from week 47 through 64, the egg shell thickness fluctuated. In this study the correlation coefficients of egg production and egg shell thickness with plasma concentration of alkaline phosphatase were not significant. Long-term hyperthyroidism elevated alkaline phosphatase concentration and egg shell thickness (Table 4); although, the correlation coefficient of egg shell thickness with alkaline phosphatase concentration was not significant.

4. Discussion

Treatment with exogenous T₄ resulted in an enhancement of plasma T₄ levels in HYPER group compared to those of the CON group; however, the effect of treatment on plasma T₃ levels was not significant. It can be ascribed that the excess exogenous T₄ might quickly changed to

### Table 2
Blood plasma thyroid hormones and selected biochemical attributes in Cobb 500 breeder hens orally administered with extra thyroxine (LS mean ± SE).a

<table>
<thead>
<tr>
<th>Trait</th>
<th>CON</th>
<th>HYPER</th>
<th>P-value</th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₃ (ng/mL)</td>
<td>1.59 ± 0.26</td>
<td>1.64 ± 0.24</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>T₄ (ng/mL)</td>
<td>10.24 ± 0.36 b</td>
<td>27.08 ± 0.89 a</td>
<td>0.003</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>T₃ - T₄</td>
<td>0.152 ± 0.007 a</td>
<td>0.059 ± 0.007 b</td>
<td>0.002</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>139.1 ± 1.94</td>
<td>138.5 ± 1.94</td>
<td>NS</td>
<td>0.008</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>70.38 ± 1.22</td>
<td>70.55 ± 1.22</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>51.76 ± 2.12</td>
<td>52.44 ± 2.12</td>
<td>NS</td>
<td>0.04</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>16.96 ± 2.63</td>
<td>15.51 ± 2.86</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>81.49 ± 2.58</td>
<td>81.35 ± 2.58</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS: Non-significant (P > 0.05).

* Thyroxine (T₄) was orally administered to the HYPER group (0.3 mg/bird/d) and the CON group received the drinking water only. Blood sampling was done every 5 week from 47 to 64 week of age (wk 47-64; n = 35 hens/treatment group).

### Table 3
Blood plasma biochemical attributes and serum enzymes in Cobb 500 breeder hens orally administered with extra thyroxine (LS mean ± SE).a

<table>
<thead>
<tr>
<th>Trait</th>
<th>CON</th>
<th>HYPER</th>
<th>P-value</th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD_{baseline} (mol/mL)²</td>
<td>17.1 ± 1.10 *</td>
<td>13.6 ± 1.10 b</td>
<td>0.02</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>CD_{max} (mol/mL)³</td>
<td>22.4 ± 1.56</td>
<td>18.7 ± 1.56</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>CD_{AUC} (mol/mL × 90 min)⁸</td>
<td>40.6 ± 3.56</td>
<td>49.9 ± 3.56</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>CD lag phase (min)⁵</td>
<td>12.2 ± 1.10</td>
<td>13.4 ± 1.10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>CD oxidation rate</td>
<td>0.01 ± 0.002 ²</td>
<td>0.02 ± 0.002 a</td>
<td>0.03</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>MDA (mol/mL)⁴</td>
<td>0.52 ± 0.005 ²</td>
<td>0.58 ± 0.006 a</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Enzymatic parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L)²</td>
<td>151.7 ± 2.79</td>
<td>150.1 ± 2.79</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)⁴</td>
<td>14.6 ± 0.36</td>
<td>14.7 ± 0.36</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>ALP (U/L)³</td>
<td>19.9 ± 0.54 b</td>
<td>22.1 ± 0.54 *</td>
<td>0.004</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

MDA = Malondialdehyde; CD = Conjugated dienes; AUC = Area under the curve; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase.

Basal and maximum absorbance at 245 nm and the net area under the curve of plasma CD were computed by the trapezoid method and treated as the final results. NS: Non-significant (P > 0.05).

* Thyroxine (T₄) was orally administered to the HYPER group (0.3 mg/bird/d) and the CON group received the drinking water only. Blood sampling was done every 5 week from 47 to 64 week of age (wk 47-64; n = 35 hens/treatment group).
Lipid peroxidation is a marker of oxidative stress in hyperthyroid tissues because polyunsaturated fatty acids are particularly susceptible to ROS effects and by-products of lipid peroxidation (Venditti and Di Meo, 2006). Nevertheless in this research hyperthyroidism had no effect on total cholesterol, HDL, LDL, VLDL and triglyceride concentration. In hyperthyroid state higher lipid peroxidation, which is associated with higher low density lipoprotein content in lipid peroxides and lower LDL concentration (Costantini et al., 1998). The results of this study showed that LDL concentration was not influenced by

the reverse-T3 which is inactive form (Decuypere et al., 1987).

Table 4

<table>
<thead>
<tr>
<th>Trait</th>
<th>CON</th>
<th>HYPER</th>
<th>F-value</th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg production (%)</td>
<td>47.04 ± 2.13</td>
<td>46.90 ± 2.13</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Egg shell thickness (mm)</td>
<td>0.34 ± 0.003</td>
<td>0.36 ± 0.003</td>
<td>0.005</td>
<td>0.018</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS: Non-significant (P > 0.05).

* Thyroxine (T4) was orally administered to the HYPER group (0.3 mg/bird/d) and the CON group received the drinking water only. Egg production was recorded on a weekly basis and either egg shell thickness was evaluated from 47 to 64 wk of age (wk 47–64; n = 35 hens/treatment group).
induced hyperthyroidism, whilst, Costantini et al. (1998) showed thyroid hormones affect on LDL oxidation. They approved that LDL oxidation is obviously higher in hyperthyroidism than in hypothyroidism or control subjects.

In the current study CD baseline in the HYPER hens was considerably lower than that of the CON group. CD Max, CD LCD, and CD lag phase were not significant in this experiment. In contrast to this study Costantini et al. (1998) found the shorter lag phase in hyperthyroid patients. In the current trial the oxidation rate was increased in HYPER hens, consistent with the finding of Costantini et al. (1998) who found hyperthyroidism was associated with a higher oxidation rate than the control group. The results of this study showed the effect of hyperthyroidism on plasma MDA concentration was significant between two experimental groups. Hens administered with thyroxine showed an increase in MDA concentration compared with the CON counterparts. As reported by Venditti and Di Meo (2006) in rat liver, T3 induced hyperthyroidism which was correlated with altered lipid peroxidation markers including increase of MDA levels. Apart from the changes in oxidation rate and MDA concentration, the values were within their biological ranges.

In the present study T4 treatment increased plasma ALP concentration, while no significant effects were observed in plasma AST and ALT concentration. However, Malik and Hodgson (2002) observed an increase in the AST and ALT in hyperthyroid subjects. Alanine aminotransferase, is a cytosolic enzyme that represents liver injuries, the elevation of ALT was seen in hyperthyroid cats as in humans (Archer and Taylor, 1996). Serum alkaline phosphatase in 64% of hyperthyroid patients was increased (Malik and Hodgson, 2002). Banovac and Koren (2000) demonstrated that T3 motivates the liberation of membrane-bound fragment of ALP in osteoblastic cells. Therefore, it can be assumed that in this experiment hyperthyroidism caused the enhancement of the ALP. These alterations have been related to enhanced bone metabolisms that are ascribed to the direct effects of thyroxine on bone cells (Archer and Taylor, 1996).

In the present study there were no significant differences between treatments for the weekly egg production. Furthermore, the correlation coefficient between egg production and plasma concentration of alkaline phosphatase was not significant. This observation is in agreement with Auchinachie and Emile (1934) who stated that the egg production of the hens was not associated with their blood plasma alkaline phosphatase level, although alkaline phosphatase activity is an important agent in the prediction of the productivity of the hens (Gutowska et al., 1943). Good producers have higher plasma alkaline phosphatase and the blood inorganic phosphorus which at the time of egg formation is elevated, although in this experiment the egg production was not affected by treatment in spite of higher ALP activity in HYPER hens.

In the current research it has also been shown that T3 administration caused a marked increase in the egg shell thickness. It can be stated that increased shell thickness in hypertrophic hens of the present study can be attributed to higher ALP activity, although statically analysis showed they were not correlated with each other. Both alkaline phosphatase and carbonic anhydrate activities are thought to affect the shell calcification mechanism (Snapir and Perek, 1970). Thyroxine and T3 directly trigger osteoblastic bone resorption; moreover, they stimulate osteoblastic activity in trabecular and cortical bone (Archer and Taylor, 1996).

5. Conclusions

Overall, the current study revealed that long-term hyperthyroidism had no adverse effects on plasma lipid peroxidation. However, some In vitro plasma lipoperoxidation indices such as CD oxidation rate and MDA tended to elevate in hyperthyroid state; although the values were within their biological ranges. Thyroxine administration had no effects on liver enzymes except for the alkaline phosphatase, higher value recorded for HYPER group that was within its biological range. Also the treatment increased egg shell thickness, nevertheless the egg production was not affected. Therefore, it might be concluded that the long term administration of T4 did not adversely affect the blood plasma biochemical attributes, indicators of oxidative stress, markers of liver function in broiler breeder hens, and egg production. Further studies are needed to suggest this treatment for reducing the incidence of ascites in progeny chicks.

Conflict of interest

All authors declare no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgment

The authors wish to express their appreciation to the Research Council of University and the academic members of the Animal Science Department for their technical assistance.

References