Effect of alliums on total lipid in plasma and tissues of Japanese quails
(Coturnix coturnix japonica) with respect to age and sex

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Received 27 November 2003; revised 4 October 2004

Dietary onion and garlic caused an increase in the level of liver and plasma total lipids in Coturnix coturnix japonica. This increase could be due to the effect of an increased feed intake, bile production, digestion and absorption that in turn caused an increased utilisation of dietary fat, increased transfer of dietary lipids to the liver and/or due to increased lipogenesis as such. However, there was no increase in the muscle lipid content. This effect in the muscle could be due to inhibitory effect of onion or garlic on lipoprotein lipase activity. Further, the changes in the tissue total lipid level in the control group due to change in age and sex were also observed.

Keywords: Alliums, Age, Japanese quails, Sex, Total lipid

IPC Code: Int. CI 7 A61P

Accumulation of lipid in tissues is a risk factor for onset of heart diseases. In view of the high cost and difficulty involved in overcoming these diseases, non-pharmacological dietary agents in mammals have been found to be effective and cheap. At the same time, consumption of quail meat and egg has been increasing, both of which are comparatively rich in lipid. Further, information on physiological norms of tissues is essential for understanding the pathological entity and not all norms have been established for Japanese quail (Coturnix coturnix japonica) except for an earlier report. In view of these, effects of age, sex and intake of onion (Allium cepa Linn.) and garlic (Allium sativum Linn.) on tissue total lipids have been studied in Japanese quails (Coturnix coturnix japonica)

Materials and Methods

Healthy, 3 week old Coturnix coturnix japonica chicks (210) of either sex were selected for the study. On the first day of the experiment, 30 birds (15 females and 15 males) were slaughtered by decapitation. The remaining 180 were distributed equally under three groups (T0, T1 and T2) with equal numbers of either sex and as uniformly as possible with respect to body weight. T0 was the control group on basal diet. T1 was the treatment group fed on onion supplemented diet for 6 weeks and T2 was the treatment group fed on garlic supplemented diet for 6 weeks. The conditions for rearing of birds and feeding of the experimental diet were similar to those as mentioned earlier.

Thirty birds (15 male and 15 female) from each of the groups were slaughtered at the end of 3 weeks. The eggs laid during the last 3 days were collected separately from all three groups (T0, T1 and T2). The remaining quails were also sacrificed at the end of the experiment (6 weeks) for collection of liver, plasma and muscle samples. All the samples collected were labelled and stored at −70°C until analysed for total lipid content in each of them. Pooled yolk samples of eggs laid during the last 3 days of the experiment from the respective groups were used for the assay of total lipid content in egg yolk.

Lipid from fixed volume/weight of each biological sample was extracted and the total lipid determined gravimetrically.

Statistical analysis as per ANOVA of the data was done to compare the three groups at 6 and 9 weeks of age in both sexes, between different ages within a sex
in both T1- and T2-group and between female and male birds of similar age in the T1- and T2-group. If the differences among the three groups appeared non-significant, a comparison of the results of the pooled T0, T1 and T2-groups was done. The respective pooled groups were obtained as per the statistical procedure. The results for total lipid in the yolk were also subjected to statistical analysis to know the difference between treatments, if any.

In the control group (3 week old T0-birds, 6 week old T0-birds and 9 week old birds T0-birds), statistical analysis was done to compare values at different ages within the same sex and between sexes within the same age group. The significance for all the comparisons was estimated at $P \leq 0.01$.

**Results and Discussion**

The results are presented in Table 1. Analysis of the results revealed significant variations in the total lipid content of liver due to dietary supplementation with onion and garlic. Onion and garlic are referred to as stimulants causing an increase in digestion and absorption of food along with bile production. It is also reported that there is an increase in the food intake and fat retention when bile acid is increased in the diet. An increase in the quantity of fatty acid binding protein due to increased bile secretion has also been observed. On the other hand, sulphur containing amino acids viz., active ingredients of alliums such as the onion and garlic, when fed to rats did not reduce liver lipids. Curcumin, the active principle of turmeric was found to cause a non-significant increase in the lipid content of WLH birds.

The concentration of total liver lipid in the 6 and 9 week old T1-female birds was found to be similar to that of T0-female birds of similar age (Table 1). This indicates that either onion has no effect on the tissue treatment.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>Female birds</th>
<th>Male birds</th>
<th>Pooled sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (g%)</td>
<td>3 Week old</td>
<td>6 Week old</td>
<td>9 Week old</td>
<td>3 Week old</td>
</tr>
<tr>
<td>T0</td>
<td>4.74 ± 8.11$^{a}$</td>
<td>14.31 ± 0.26$^{c}$</td>
<td>10.08 ± 0.25$^{b}$</td>
<td>3.86 ± 0.06$^{a}$</td>
</tr>
<tr>
<td>T1</td>
<td>---</td>
<td>14.60 ± 0.26$^{b}$</td>
<td>10.17 ± 0.13$^{c}$</td>
<td>---</td>
</tr>
<tr>
<td>T2</td>
<td>---</td>
<td>15.15 ± 0.26$^{b}$</td>
<td>10.91 ± 0.26$^{b}$</td>
<td>---</td>
</tr>
<tr>
<td>Plasma (mg %)</td>
<td>3 Week old</td>
<td>6 Week old</td>
<td>9 Week old</td>
<td>3 Week old</td>
</tr>
<tr>
<td>T0</td>
<td>631.23 ± 10.44$^{a}$</td>
<td>804.78 ± 16.21$^{b}$</td>
<td>918.52 ± 9.35$^{c}$</td>
<td>450.71 ± 17.46$^{a}$</td>
</tr>
<tr>
<td>T1</td>
<td>---</td>
<td>834.73 ± 14.81$^{a}$</td>
<td>965.89 ± 8.24$^{b}$</td>
<td>---</td>
</tr>
<tr>
<td>T2</td>
<td>---</td>
<td>947.87 ± 14.67$^{b}$</td>
<td>1022.24 ± 10.12$^{c}$</td>
<td>---</td>
</tr>
<tr>
<td>Muscle (mg %)</td>
<td>3 Week old</td>
<td>6 Week old</td>
<td>9 Week old</td>
<td>3 Week old</td>
</tr>
<tr>
<td>T0</td>
<td>849.37 ± 12.36$^{a}$</td>
<td>1355.52 ± 0.65$^{b}$</td>
<td>2328.26 ± 53.42$^{c}$</td>
<td>739.73 ± 15.33$^{a}$</td>
</tr>
<tr>
<td>T1</td>
<td>---</td>
<td>1473.96 ± 12.66</td>
<td>2396.17 ± 60.22</td>
<td>---</td>
</tr>
<tr>
<td>T2</td>
<td>---</td>
<td>1628.59 ± 11.58</td>
<td>2719.17 ± 133.27</td>
<td>---</td>
</tr>
<tr>
<td>Yolk (g %)</td>
<td>T0</td>
<td>---</td>
<td>30.71 ± 1.14</td>
<td>---</td>
</tr>
<tr>
<td>T1</td>
<td>---</td>
<td>30.60 ± 1.15</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>T2</td>
<td>---</td>
<td>30.42 ± 1.23</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

1: Superscript bearing different capital letter within a row and sex differs significantly.
2: Superscript bearing different number within a row between similar age of different sex differs significantly.
3: Superscript bearing different small letter within a column differs significantly.

Table 1—Total lipid content of liver, plasma, muscle and egg yolk in Japanese quail

[Values are mean ± SE from 15 birds in each group]

Pooled: Refers to mean value of treatment irrespective of age and sex.
concentration of liver lipids or that the dose of onion incorporated in the diet is insufficient to cause any such effect. The liver lipid content in the 6 and 9 week old T2-female birds was significantly higher than that of T0-female birds of similar age, which could be due to the effect of garlic causing increased transfer of dietary lipids to the liver and/or due to an increased lipogenesis in liver with garlic diet. The liver lipid content in the 6 week old T1 and T2 male birds were found to be similar to that of 6 week old T0-female birds. The reason could be that the metabolic turnover of lipids may be very high at this age of the male birds. This condition may have changed for these birds at higher age and their liver lipid level increased when onion or garlic supplementation was continued for another 3 weeks. The increased liver lipid in the 9 week old T1 and T2-female birds could also be due to the effect of an increased feed intake, bile production, digestion and absorption which in turn may have increased the transfer of dietary lipids to the liver and/or increased lipogenesis as such. Above all as the allium products are good wormicides, this may also have helped the birds for a better utilisation of their diet.

The liver lipid content in the T2-birds of either sexes at 6 and 9 week age was more than that in the T1-birds of either sexes for the same age groups. These results show an increased potency of garlic compared to onion in exhibiting its alliaceous effect on liver lipids.

Comparison of the values of liver lipid concentration based on age within a sex or based on sex within an age group indicates that duration of feeding and sex in T1 and T2-groups influence the effect of onion or garlic on liver lipid content.

In the control group of both sexes when three age periods were checked, the concentration of total lipid in the liver was lowest for the 3 week old female birds, thereafter it increased at the sixth week and then decreased at the ninth week. The liver lipid levels at all the three ages were significantly different. This increase in the 6 week old birds agrees with the earlier observations in quails of different experimental conditions.

Furuse et al. reported that 7 week old laying quails rapidly synthesize fatty acids and accumulate lipid in the liver. This phenomenon is due to the influence of oestrogen that stimulates hepatic lipid synthesis and also due to an increase in the activities of liver lipogenic enzymes such as ATP citrate lyase and malic enzyme just after the commencement of laying as compared to non-laying pullet and the WLH cockerel. A reduction in the rate of oestrogen secretion by the ovary once the laying has started may be the reason for the decrease in the liver lipid concentration in the 9 week old female birds.

In the control group, the liver lipid levels in the 3 week old and 6 week old male birds were found to be almost same indicating their poor pancreatic activity or bile secretion in younger ages. This could also be due to a steady state of lipogenesis occurring in the liver of male quails as observed by Saadoun and Leclercq in 2, 5 and 15 week old male chicks. A significant increase was observed in the 9 week old male birds as compared to 3 week old male birds, but the difference between 6 and 9 week old male birds was not significant. The increase in the 9 week old male birds could be due to the influence of androgens as well. The concentration of testosterone in commercial cockerels was observed to be consistent from 11 weeks of age. In the control group, the liver lipid content when compared between sexes at 3 weeks of age revealed no significant difference indicating little influence of sex hormones on hepatic lipogenesis at this age. The liver lipid content was more in the 6 and 9 week old female birds as compared to 6 and 9 week male birds respectively. This may be due to the influence of oestrogen causing increased lipogenesis via increased lipogenic enzyme activity.

Analysis of the results further revealed significant variations in the total plasma lipid content due to dietary supplementation with onion and garlic. The major site of lipid synthesis in the bird is the liver. The lipid synthesized in the liver is transported via circulation to extra hepatic tissues for energy purpose or for storage. The lipid in circulation is taken up by extra hepatic tissues only after being acted upon by lipoprotein lipase (LPL). In birds, antibiotics are found to increase the absorption of dietary nutrients and also to decrease LPL concentration in the peripheral circulation and the antibiotic/antibacterial effects of onion and garlic may have played a role here also.

An increase in plasma total lipid content in the T1 and T2-birds as compared to T0-birds could be due to a reduction in the peripheral LPL activity or due to an increased absorption of dietary nutrients as a result of the wormicidal effects of onion and garlic. The plasma lipids were found to be greater in the T2-birds...
than in the T1-birds indicating increased efficiency of garlic over that of onion.\textsuperscript{4,5}

Comparison of plasma total lipids between age groups for the same sex in all the groups as well as between sex of similar age in the T1 and T2-groups indicated that the duration of feeding, age and sex influenced the effects of onion or garlic on this parameter.

In the control group, the plasma total lipid level is found to increase with age in both sexes. Plasma lipid may vary with hepatic lipid content.\textsuperscript{12} However, in the 9 week old female birds though there was a decrease in the liver lipid content, the plasma lipid content was found to be increased. This is probably due to decreased clearance of lipids from plasma in the layers\textsuperscript{12,13,19}. This increase in the total lipid in the plasma could also be due to the very low density lipoprotein (VLDL) induced by oestrogen in the laying birds which is rich in triglyceride and phospholipids and less sensitive to LPL\textsuperscript{5} and also because the predominant component of lipid in the plasma is triglyceride and phospholipids. Thus indicating that these factors may have influenced the level of plasma lipids.

The plasma lipid content in the male birds of the control group was found to increase significantly with age. In the present study, though the liver lipid content was constant until 6 week of age, the increase in the plasma lipid content with age could be due to the abdominal fat which is greater in the male than in the female quails\textsuperscript{10} and it may be contributing to lipid requirements of the body. Hence during its transport it causes an increase in the plasma. It is also observed that there was some form of metabolic regulation preventing the development of true obesity in birds\textsuperscript{46}, hence the abdominal fat in the male birds could be under dynamic state so as to avoid obesity by causing an increase in the plasma lipids.

In the control group, the concentration of lipid in the plasma of 3 week old female birds was significantly higher compared to the same age male birds probably because the former are superior in digesting the dietary lipids.\textsuperscript{13} It was also observed that the fatty acid binding protein increases by 50\% from 3 and 5 week of age in chickens.\textsuperscript{13} The fatty acid binding protein is responsible for the delivery of precursor molecules for the lipid synthesis from the intestinal mucosal cells to the site where synthesis is taking place. Hence an increased digestion of exogenous lipid leads to increased portomicrons reaching the liver from where they re-enter circulation as VLDL molecules. This increase in VLDL may be the contributory factor for the increased lipids in the plasma of 3 week old female birds.

In the control group, the plasma lipid concentration in the 6 and 9 week old female birds was found to be higher than the corresponding age group of male birds respectively. This could be due to the influence of oestrogen in the female birds\textsuperscript{16} and/or due to an increased hepatic lipogenic activity in the females.\textsuperscript{11} It has been also reported that with the approach of egg laying, both the overall weight and lipid content of the liver undergo dramatic increase as a result of extensive and interrelated hormonal activity,\textsuperscript{12} thus enhancing the concentration of plasma lipids in 6 and 9 week old female birds.

The influence of dietary onion and garlic on total lipid content in the muscle was found to be non-significant in both sex of both age groups. The situation of an increase in the liver or plasma lipids would invariably tend to give rise to obesity and increased lipid accumulation in the adipose tissue. However, no increase in the muscle lipid content was observed in the present study as a consequence of hyperlipidemia due to onion or garlic. This could be due to an inhibitory effect of onion and garlic on LPL activity\textsuperscript{17} or due to increased activity of the muscle itself.

Although, the influence of dietary onion or garlic on total lipid content in muscle was found to be non-significant in either sex of both age groups, the results of the pooled T2-group were significantly higher than that of the pooled T0 and T1-group. The levels in the pooled T0 and T1-groups were similar. The increase in the pooled T2-group could be due to insulin like activity of garlic\textsuperscript{5} stimulating lipogenesis in the muscle.

In the control group, the absence of any significant difference in the muscle lipid content of 3 and 6 week old male birds, but an increase observed in the 9 week old male birds could be due to increased lipogenic activity in the muscle of older male birds. The extra hepatic lipogenic activity when observed at 2, 5 and 15 week old male chickens was found to be markedly increased only at 15 week of age. This may be attributed to the influence of insulin and testosterone.\textsuperscript{14} It has been reported that the concentration of testosterone in plasma is steady from eleven weeks of age in commercial cockerels\textsuperscript{15}. The increased muscle lipid content in the 9 week old male quails could be in accordance with the
earlier findings\textsuperscript{14,15} as quails are known for their rapid growth and early maturity compared to other commercial birds. The increased lipid at the ninth week of age could also be due to testosterone influencing lipogenesis\textsuperscript{2,15,16} and for structural and storage purposes\textsuperscript{20}. In the control group, the muscle lipid content in both sexes of the 3 week old birds was similar indicating very little influence of gonadal hormones at this age. An increase in the muscle lipid in 6 week old female birds compared to 6 week old male birds could be due to the influence of oestrogon in the female birds on peripheral tissues\textsuperscript{11,16}. The muscle lipid content of the 9 week old female birds was similar to that of 9 week old male birds because of decreased release of oestrogon from the ovary, once the bird has started laying\textsuperscript{7}.

The total lipid content in the egg yolk of Japanese quail ranged from 36.89 to 37.56% of its weight when the birds were fed diets supplemented with palmitic, oleic and linoleic acid\textsuperscript{21}. This indicates that yolk is resisting dietary changes to influence its composition in the Japanese quails. In the present study also no change in the yolk lipid concentration was observed due to either onion or garlic supplementation in the feed. Similarly dietary supplementation with plant sterols which are hypocholesterolemic and dietary bile acid sequestrants such as colestipol and lovastatin were shown to be ineffective in altering lipid content of egg yolk in WLH birds\textsuperscript{22-26}. From the foregoing, it may be surmised that yolk lipid is unalterable to dietary treatments.

References