ASSOCIATION OF WATER BASED INFUSION OF ALLIUM SATIVUM AND WITHANIA SOMNIFERA WITH SERUM LIPID PROFILE OF BROILER CHICKS

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ABSTRACT

A study was conducted to explore the effects of different levels of water based infusion of Allium sativum (A. sativum) and Withania somnifera (W. somnifera) mixture in 1:6 respectively on lipid profile of broiler chicks. One hundred and twenty day-old broiler chicks were distributed into four groups; A, B, C and D each having three replicates (10 chicks replicate-1). The chicks were reared in pens in an open sided house for 35 days. Chicks in group B, C and D received water based infusion of A. sativum and W. somnifera at rate of 5, 7.5 and 10 ml/L of drinking water, respectively, while group A served as control. Water based infusion of A. sativum and W. somnifera at the rate of 10 ml/L in broiler chicks reduced total cholesterol, triglycerides, low density lipoproteins while increased high density lipoproteins level. It is concluded that water based infusion of A. sativum and W. somnifera in 1:6 at rate of 10 ml/L of drinking water improved the lipid profile of broiler chicks.

Keywords: Broiler, Allium sativum, Withania somnifera, serum cholesterol.

INTRODUCTION

There is greater concern regarding the use of medicinal plants in Improving immunity and growth rate because of easy availability, low cost, good antimicrobial action, reduced disease risks and diversified functions (Lewis et al., 2003; Charis, 2000). In traditional medical system herbal medicines have been used for thousands of years and have great contribution to maintain human health (Rahman et al., 2011). Herbs have several pharmacological properties like anti-inflammatory (Udupa et al., 1994), immune-modulant (Mushtaq and Durrani, 2007) and anthelmentic activity (Iqbal et al., 2006). The use of herbal medicine is becoming more and more popular as the herbal preparations have no or less side effects (Rajasekaran et al., 2001).

Garlic (Allium Sativum Linn.) is a common spicy flavoring agent used since ancient times. Due to its characteristic flavor and medicinal properties, Allium Sativum (A. sativum) has been cultivated round the globe (Zargari et al., 1997). A. sativum contains more than 200 chemical compounds. Some of its more important ones include volatile oil with sulphur containing compounds (alllicin, allliin and ajoene) and enzymes (alliinase, peroxidase and myrosinase). Allicin gives garlic its antibiotic properties and is responsible for its strong odor (Andrew, 1990). A. sativum is used to treat the symptoms of acne and it can manage high cholesterol levels (Qureshi et al., 1983). Its paste in the diets of laying hens reduced serum and yolk cholesterol concentrations (Chowdhury et al., 2002). It has been investigated that addition of garlic powder to poultry diet increased egg weight and decreased egg yolk cholesterol concentration (mg/g yolk), serum triglyceride and cholesterol concentrations without adverse effect on the performance and egg weight (Yalcin et al., 2006).

Withania somnifera is a popular Indian medicinal plant, which is well documented for curing human diseases. W. somnifera is also known as ashwagandha, ginseng, and winter cherry. It has been an important herb in the ayurvedic and indigenous medical system for over 3000 years. Numerous studies indicated that W. somnifera possesses antioxidant, antitumor, anti-stress, anti-inflammatory, immuno-modulatory, hematopoetic, anti-ageing, anxiolytic, anti-depressive rejuvenating properties and also influences various neurotransmitter receptors in the central nervous system (Pattipati et al., 2003). The constituents of W. somnifera are the steroidal alkaloids and steroidal lactones which are withanolides (Elskakka et al., 1990; Mishra et al., 2000) with the main active chemical constituent Withaferin A which is a phytosteroid (Lavi, 1965). Other constituents include saponins containing an additional acyl
group (sitoindoside VII and VIII). Experimental animals treated with W. somnifera showed significant increase in hemoglobin concentration, red blood cell count, white blood cell count, platelets count and body weight as compared to control (Ziauddin et al., 1996). It has hypoglycemic, diuretic, hypocholesterolemic and immune-modulatory properties (Andallu and Radhika, 2000; Das et al., 2001; Gautam et al., 2004; Mushtaq and Durrani, 2007).

Keeping in view the important uses of A. sativum and W. somnifera, this study was conducted to evaluate the effects of water based extract of these plants on lipid profile of broiler chicks.

MATERIALS AND METHODS

The present study was conducted to explore the potential of water based infusion of A. sativum and W. somnifera mixture in 1:6 on lipid profile of broiler chicks at the Poultry Farm of The University of Agriculture, Peshawar, Pakistan.

Experimental Design

One hundred and twenty day-old broiler chicks were randomly distributed into four groups; A, B, C, and D. Each group was replicated three times and each replicate had 10 chicks. The chicks were reared under similar environmental conditions on floor. The basal diet was formulated according to standard protocol (Table 1). The experiment lasted for 35 days and relevant data were recorded.

Table 1. Composition of the basal diet (%).

<table>
<thead>
<tr>
<th>Ingredient and composition</th>
<th>STARTER</th>
<th>FINISHER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow corn</td>
<td>63.8</td>
<td>72.2</td>
</tr>
<tr>
<td>Soy bean meal (44% CP)</td>
<td>28</td>
<td>21.5</td>
</tr>
<tr>
<td>Fish meal (72%CP)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Vitamin and Minerals* (%)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>DL Methionine</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Metabolizing Energy (Kcal/kg)</td>
<td>2921</td>
<td>2994</td>
</tr>
<tr>
<td>Crude Protein %</td>
<td>21.4</td>
<td>18.1</td>
</tr>
<tr>
<td>Lysine %</td>
<td>1.19</td>
<td>0.93</td>
</tr>
<tr>
<td>Methionine %</td>
<td>0.55</td>
<td>0.33</td>
</tr>
<tr>
<td>Methionine and Cysteine %</td>
<td>0.89</td>
<td>0.62</td>
</tr>
<tr>
<td>Calcium %</td>
<td>1.09</td>
<td>1.08</td>
</tr>
</tbody>
</table>

*Provided per kg of diet: Mn 80 mg; Zn 60 mg; Fe 60 mg; Cu 5 mg; Co 0.2 mg; I 1 mg; Se 0.15 mg; choline chloride 200 mg; vitamin A (retinol) 12000 IU; vitamin D3 (cholecalciferol) 2400 IU; vitamin E (DL-a-tocopherol) 50 IU; vitamin K (menadione) 4 mg; vitamin B1 (thiamine) 3 mg; vitamin B2 (riboflavin) 6 mg; vitamin B5 (pantothenic acid) 25 mg; vitamin B6 (pyridoxine) 5 mg; vitamin B12 (cyanocobalamin) 0.03 mg; folic acid 1 mg.

Preparation and Administration of Water based Infusion

The extract mixture (A. sativum and W. somnifera) in ratio of 1:6 was prepared according to the procedure described by Leila, et al. (1977). The water based extract of the medicinal plants was given at the rate of 5, 7.5 and 10 ml/L of drinking water to group B, C, and D, respectively, while group A served as control.

Lipid Profile Determination

For lipid profile determination, 5 ml blood sample was taken from randomly six birds of each group at the end of experiment. Blood was centrifuged at 4000 rpm for 10 minutes. Serum was separated and refrigerated till further analysis.

For total cholesterol enzymatic calorimetric method was followed as described by Allain et al. (1974) using Chemistry Analyzer (Micro Lab 200 Merck) and Elitech Kit. While triglycerides were determined by enzymatic calorimetric method (Werner et al., 1981) using already mentioned analyzer and kit. For HDL determination standard procedure was adopted (Lopes-Virella et al., 1977). LDL cholesterol was calculated by the following formula:

LDL cholesterol (mg/dL) = Total cholesterol - (TGR/5) + HDL cholesterol
Statistical Analysis

The data recorded for all the parameters, were statistically analyzed by the standard procedure of Analysis of Variance (ANOVA), using Completely Randomized Design as described by Steel and Torrie (1981). The statistical package SAS (1998) was used to perform the analysis.

RESULTS AND DISCUSSION

Total Cholesterol

Significant (P<0.05) lower total cholesterol value was observed in group C and D as compared to control (Table 2). Maximum decrease in total cholesterol level was observed in broiler chicks that received highest dose level (10 ml/L) of plant extract.

Table 2. Effect of different levels of aqueous extract of A. sativum and W. somnifera on serum lipid profile of broiler chicks

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol(mg/dL)</th>
<th>Triglycerides(mg/dL)</th>
<th>HDL(mg/dL)</th>
<th>LDL(mg/dL)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>242.83a</td>
<td>146.83a</td>
<td>32.33c</td>
<td>120.66c</td>
<td>9.87</td>
</tr>
<tr>
<td>B</td>
<td>167.36b</td>
<td>127.33b</td>
<td>39.60b</td>
<td>69.33b</td>
<td>6.54</td>
</tr>
<tr>
<td>C</td>
<td>153.00b</td>
<td>144.33a</td>
<td>55.16b</td>
<td>56.16b</td>
<td>5.98</td>
</tr>
<tr>
<td>D</td>
<td>152.33c</td>
<td>092.56b</td>
<td>71.33c</td>
<td>38.26c</td>
<td>8.54</td>
</tr>
</tbody>
</table>

a-cDifferent superscript in a column differ significantly (P<0.05)
A: Control; B: 5 ml/L, C: 7.5 ml/L, D:10 ml/L aqueous extract

High level of cholesterol is responsible for heart disease (Brinton et al., 1991). Different medicinal plants and natural products are highly effective in decreasing cholesterol level. Our findings are closely related with the work of Andallu and Radhika (2000), who found that infusion of W. somnifera had significant effect on blood cholesterol level. The results of our findings are similar with Chowdhury et al. (2002), who worked on garlic paste in laying hens and observed significant reduction in egg yolk cholesterol and serum total cholesterol. The mechanism by which garlic reduces the plasma cholesterol concentration is not fully understood. Garlic reduces duodenal cell proliferation and thinner the epithelial thickness in chicks. Thinner intestinal epitheliums enhance nutrient absorption and reduce the metabolic demands of the gastrointestinal system (Masoud et al., 2006). Inhibition of cholesterol synthesis is thought to be a principal mechanism by which garlic lowers blood cholesterol, although other mechanisms may also be important. Some of the investigators are of the opinion that garlic depresses lipogenic and cholesterogenic activities of liver enzymes such as malic enzyme, fatty acid synthase, glucose-6-phosphate dehydrogenase and 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase (Khan et al., 2008). Result of our findings are similar to the findings of Nishant et al. (2006), who reported that W. somnifera significantly (P<0.05) lowered the cholesterol in hypercholesteremic male albino rats. Result of our findings is relevant to Andallu and Radhika (2000), who reported significant decrease in cholesterol in hyperlipidemic rats. Some authors are of the opinion that garlic’s trace minerals, such as tellurium, also inhibit hepatic cholesterol synthesis (Larner, 1995), but most attribute garlic’s antilipemic effects to di- allyl di-sulfide, a decomposition product of allicin (Newall et al., 1996; Adoga, 1987). The cholesterol lowering effect of W. somnifera could be due to elevated excretion of cholesterol and bile acids through fecal sterol excretion. It could also be attributed to higher phystosterol contents, which may lead to decrease in intestinal transit time for cholesterol and carbohydrate absorption from gut (Ebihara and Schneeeaman, 1989).

Triglycerides

Data regarding serum triglycerides level of broiler chicks treated with water based infusion of mixture of A. sativum and W. somnifera is presented in Table 2. Significant (P<0.05) differences were observed among the various treated groups. Lowest level of triglycerides was found in group that received highest dose level of infusion. The finding of our research is similar to Konjufca et al. (1997) and Hemalatha et al. (2004), who reported that garlic in broiler chicks reduced serum triglycerides. Present study also reinforces the findings of Anwar and Meki (2003) that garlic had a significant effect in reducing blood triglyceride levels in diabetic animal models. Result of our study is relevant to Andallu and Radhika (2000), who reported significant decrease in triglycerides in hyperlipidemic rats. Our findings are in agreement with Visavadiya and Narasimhacharya (2007), who added root powder of W. somnifera to the diet of hypercholesteremic male albino rats at rate of 0.75 and 1.5 g/rat/day and registered significant increase in plasma triglycerides levels.
The triacylglycerol-lowering effect of garlic, might be explained in part by its inhibitory action on fatty acid synthesis (Yeh and Liu, 2001). The triglyceride lowering effect of *W. somnifera* could be attributed to its higher phytosterol contents, which may lead to decrease in intestinal transit time for cholesterol and carbohydrate absorption from gut, that ultimately decreased hepatic lipogenesis and reduction in hepatic and plasma triglyceride concentrations (Mamo et al., 1991).

**High-density Lipoproteins**

Significant (P<0.05) difference was observed in mean HDL level in group C and D as compared to control, while non-significant difference was recorded between group B and control (Table 2). The highest level of HDL level was recorded in the group that received 10 ml/L of the plants extract. HDL transports cholesterol from blood vessels and body tissues to the liver for re-utilization or excretion. Low level of HDL increases risk for heart diseases while its high level protects against cardiovascular diseases (Khan et al., 2008). Finding of our study is similar to Nishant et al. (2006) and Hemallatha et al. (2004), who worked on *W. somnifera* aqueous extract in rats and reported that the extract had significant effect on serum HDL level. Our findings are also similar to Konjufca et al. (1997), who observed significant higher level of HDL in broiler chicks while using garlic and copper paste. Similarly Choi et al. (2010) reported that garlic paste significantly increased the body weight because of high concentration of HDL in broiler chicks and is due to the faster metabolic rate of cholesterol in body. Our findings are in agreement with Visavadiya and Narasimhacharya (2007), who added root powder of *W. somnifera* to the diet of hypercholesteremic male albino rats at rate of 0.75 and 1.5 g/rat/day and registered significant increase in plasma HDL-cholesterol levels. *A. sativum* extract significantly decreases levels of cholesterol in plasma and liver by increasing the activity of plasma lecithin: cholesterol acyltransferase (LCAT), which plays a key role in lipoprotein metabolism. LCAT converts free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is then sequestered into the core of a lipoprotein particle, ultimately making the newly synthesized HDL. Therefore activation of LCAT lowers cholesterol levels and increases levels of HDL (Shrivastava et al., 2012).

**Low-density Lipoproteins**

Aqueous extract of mixture of *A. sativum* and *W. somnifera* significantly reduced LDL level of broiler chicks. Significantly (P<0.05) low level of LDL was observed in all treated groups as compared to control group (Table 2). Minimum LDL cholesterol level was observed in group that received 10 ml/L of plants extract. A high level of LDL reflects an increased risk of heart disease, as it leads to atherosclerosis while low level of LDL reflects a lower risk of heart disease. Our findings are similar to Andallu and Radhika (2000), who reported decreased LDL level by the application of *W. somnifera*. Our findings are similar to Roughani et al. (2005), who reported that oral administration of *W. somnifera* mixed pelleted food at the dose of 6.25% for 2 months, produced significant reduction in LDL cholesterol. Our results are also in collaboration with Qureshi et al. (2011), who reported that *W. somnifera* in dose level of 2 percent, caused a gradual and significant reduction in LDL cholesterol. Some reports indicate that continuous uses of medicinal plants infusion drastically reduce the serum LDL levels (Doggrell, 2005; Abidi et al., 2006; Khan et al., 2008). *A. sativum* extract significantly decreases levels of cholesterol in plasma and liver by increasing the activity of plasma lecithin: cholesterol acyltransferase (LCAT), which plays a key role in lipoprotein metabolism. The activation of LCAT lowers cholesterol levels and increases levels of HDL. The activation of post heparin lipolytic activity (PHLA) in plasma and hepatic lipoprotein lipase (LPL) is responsible for a significant decrease in levels of LDL and very low density lipoprotein (VLDL) (Shrivastava et al., 2012).

**CONCLUSIONS AND RECOMMENDATIONS**

It can be concluded from the present results that aqueous extract of *A. sativum* and *W. somnifera* at the level of 10ml/L may be used to improve the lipid profile in broiler chicks.

**REFERENCES**


