



## Role of *Berberis lycium* in Reducing Serum Cholesterol in Broilers

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**ABSTRACT :** This study was planned to investigate the role of *Berberis lycium* in reducing serum cholesterol in broilers. Six experimental rations designated as A, B, C, D, E and F having 0, 0.5, 1.0, 1.5, 2.0 and 2.5% *Berberis lycium* were fed to 240 broiler chicks, randomly distributed into 24 replicates, so as to have 4 replicates per group and 10 chicks per replicate. The experiment lasted for 35 days. Average serum total cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL) were used as criteria of response. Average total serum cholesterol per chick was 129.33, 120.50, 116.50, 113.00, 101.67 and 114.00 mg/dl for group A, B, C, D, E and F respectively. Total serum cholesterol showed decreasing trend with the increasing level of *Berberis lycium* upto 2% ( $p < 0.05$ ). Mean serum triglyceride level per chick was 60.00, 58.17, 58.00, 55.33, 50.17 and 48.50 mg/dl for group A, B, C, D, E and F respectively. Mean serum triglyceride showed decreasing trend with the increasing level of *Berberis lycium* ( $p < 0.05$ ). Serum triglyceride was significantly lower in group F than other groups. Mean HDL per chick for the six experimental groups A, B, C, D, E and F was 52.08, 53.42, 60.42, 62.25, 62.92 and 54.50 mg/dl respectively. HDL showed increasing trend with the increase in the level of *Berberis lycium* upto 2%. The average serum LDL per chick was 65.25, 55.45, 44.48, 39.68, 28.72 and 49.80 mg/dl for group A, B, C, D, E and F respectively. LDL also showed decreasing trend with the increase in the level of *Berberis lycium* upto 2% ( $p < 0.05$ ). It was concluded that *Berberis lycium* added to feed at the rate of 2.0% can be used in broiler feed for reducing serum total cholesterol, triglyceride and LDL and increasing HDL. (**Key Words :** *Berberis Lyceum*, Cholesterol, Broiler)

### INTRODUCTION

Cholesterol is normally found in the body in cell walls and membranes, vitamin D, hormones, and fat-digesting enzyme. It takes only a small amount of cholesterol in the body to meet these needs. Excess cholesterol can get deposited in the arteries, including the coronary arteries, leading to atherosclerosis, or hardening of the arteries. Atherosclerosis leads to heart attack and stroke. Cholesterol is divided into LDL ("bad" cholesterol) in which 46% of the molecule is cholesterol, which carries cholesterol in the blood and can get deposited onto the walls of blood vessels, causing atherosclerotic plaques. HDL ("good" cholesterol) which includes 20% as cholesterol, helps clear the blood of cholesterol, and may even remove cholesterol from atherosclerotic blood vessels. High levels of total blood cholesterol are associated with the incidence of coronary heart disease. Since about a quarter of the saturated fatty acids in the diet are supplied by meat fat, this has increased the consumption of poultry and fish at the expense of red

meat (Carol and Merrily, 1984).

Cholesterol levels can be lowered through dietary changes or by prescribing drugs. Different herbs and natural products such as Chinese red-yeast-rice are highly effective in lowering cholesterol levels.

*Berberis lycium*, one of the plant species being abundantly available, is extensively used for the treatment of several human diseases under local practices in Pakistan (Khan, 2001). *Berberis lycium* contains berberine, berbamine, chinabine, karakoramine, palmatine, balauchistanamine, gilgitine, jhelumine, punjabine, sindamine, chinabine acetic acid, maleic acid, ascorbic acid (Khare, 2004). The plant contains major alkaloid berberine (Khosla, 1992), which is an isoquinoline alkaloid. This is usually taken from root or root bark of the *Berberis lycium*, and other *Berberis* species abundantly available in local forests. Both clinical trials and animal research have indicated that berberine administration prevented ischemia-induced ventricular tachyarrhythmia, stimulated cardiac contractility, and lowered peripheral vascular resistance and blood pressure (Chun et al., 1978; Marin-Neto et al., 1988). *Berberis lycium* has also been reported to reduce cholesterol, control sugar in diabetic patients and improve immune performance of the body (Saeed, 1976; Birdsall and Kelly,

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1997). In broiler feeding *Berberis lycium* at a level of 2% of total feed, has been reported to improve weight gain, feed efficiency and reduce mortality (Chand, 2005). The breast weight, dressing meat and gizzard weight were slightly increased.

The present project was designed to study the effect of *Berberis lycium* on serum total, HDL and LDL cholesterol and triglyceride in broilers.

## MATERIALS AND METHODS

In order to investigate the effect of *Berberis lycium* on serum total cholesterol, triglyceride, High density lipoprotein (HDL) and Low density lipoprotein (LDL) in broilers, a study was conducted at the Poultry Farm of N.W.F.P. Agricultural University Peshawar.

### Experimental design

The experiment was conducted in completely randomized design (CRD). A total of 240 broiler chicks were obtained from the local commercial market and were divided into six groups A, B, C, D, E and F. The experiment lasted for 35 days. Each group was further divided into four replicates with ten chicks per replicate. The birds were raised in individual pens on conventional deep litter system, in an open sided house. All the pens were located in the same house and each pen was provided with a feeder and drinker. The birds were vaccinated against Newcastle Disease (ND) and Infectious Bursal Disease (IBD).

### Addition of *Berberis lycium* to feed

The root bark of *Berberis lycium* was collected from district Swat. After drying it was ground with the help of electric grinder. The powder was added to commercial broiler starter and finisher feed at the rate of 0, 0.5, 1.0, 1.5, 2.0 and 2.5% for group A, B, C, D, E and F respectively.

### Collection of blood samples and biochemical analysis

At the end of the experiment, three birds were randomly selected from each replicate and blood samples were collected for biochemical analysis. Blood samples were transferred to sterilized centrifuge tubes and were allowed for clotting at room temperature. The blood samples were centrifuged for 10 minutes in a centrifuge at 4000 rpm for serum separation. Serum samples were stored in freezer at 0°C for later analysis of total cholesterol, triglycerides, high- and low-density lipoproteins (HDL and LDL). These tests were done in the Biochemistry Laboratory of Pakistan Medical Research Council, Peshawar using Chemistry Analyzer (Micro Lab 200 Merck).

Cholesterol levels were determined by enzymatic colorimetric method of Allain et al. (1974) using Chemistry analyzer (Micro Lab 200 Merck) and an Elitech kit

(Meditek Instrument, Peshawar, Pakistan). Triglyceride levels were determined by the enzymatic colorimetric method of Werner et al. (1981) using the same analyzer. Chylomicrons, VLDL (very low-density lipoproteins), and LDL (low-density lipoproteins) were precipitated by adding phosphotungstic and magnesium ions to the sample. For this purpose one part of sample and three parts of precipitant were used. Centrifugation left only the HDL (high-density lipoproteins) in the supernatant; their cholesterol content was determined using the procedure described by Lopes-Virella et al. (1977).

LDL Cholesterol was calculated by the following formula:

$$\text{LDL Cholesterol (mg/dl)} \\ = \text{Total cholesterol} - \frac{\text{TGL}}{5} - \text{HDL cholesterol}$$

Economics of the various rations were calculated on the basis of prevailing prices in the market. The parameters studied were feed cost per chick and gross return per chick at various feeding levels of *Berberis lycium*.

The data were statistically analyzed with the standard procedures of analysis of variance (ANOVA), using Completely Randomized Design. Means were compared for significance of differences by least significance differences (LSD) as suggested by Steel and Torrie (1981).

To establish the association between levels of *Berberis lycium* in the feed with serum total cholesterol, triglyceride, HDL and LDL in broilers, the regression model of Wonnacott and Wonnacott (1985) was used.

Pearson's correlations between serum total cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL) were worked out using the following formula

$$r_{x,y} = \frac{\text{Cov}_{(x,y)}}{\delta_x \delta_y}$$

The statistical package (SAS, 1998) was used to perform the above analysis on computer.

## RESULTS AND DISCUSSION

Findings pertaining to serum total cholesterol, triglycerides, high- and low-density lipoproteins (HDL and LDL) are presented under various sections as follows:

### Total serum cholesterol

Average total serum cholesterol per chick was 129.33, 120.50, 116.50, 113.00, 101.67 and 114.00 mg/dl for group

**Table 1.** Lipids profile of broiler chicks fed on different levels of *Berberis lycium*

| Parameters                | Group               |                      |                      |                      |                      |                      | F value | P value | LSD value |
|---------------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|---------|---------|-----------|
|                           | A<br>(0% B lycium)  | B<br>(0.5% B lycium) | C<br>(1.0% B lycium) | D<br>(1.5% B lycium) | E<br>(2.0% B lycium) | F<br>(2.5% B lycium) |         |         |           |
| TGR (mg/dl)               | 60.00 <sup>a</sup>  | 58.17 <sup>ab</sup>  | 58.00 <sup>ab</sup>  | 55.33 <sup>abc</sup> | 50.17 <sup>bc</sup>  | 48.50 <sup>c</sup>   | 2.021   | 0.0634  | 8.80      |
| Total cholesterol (mg/dl) | 129.33 <sup>a</sup> | 120.50 <sup>ab</sup> | 116.50 <sup>ab</sup> | 113.00 <sup>bc</sup> | 101.67 <sup>c</sup>  | 114.00 <sup>bc</sup> | 3.29    | 0.0103  | 14.21     |
| HDL (mg/dl)               | 52.08 <sup>a</sup>  | 53.42 <sup>a</sup>   | 60.42 <sup>a</sup>   | 62.25 <sup>a</sup>   | 62.92 <sup>a</sup>   | 54.50 <sup>a</sup>   | 1.02    | 0.4151  | 13.45     |
| LDL (mg/dl)               | 65.25 <sup>a</sup>  | 55.45 <sup>ab</sup>  | 44.48 <sup>bc</sup>  | 39.68 <sup>cd</sup>  | 38.72 <sup>d</sup>   | 49.80 <sup>bc</sup>  | 0.59    | 0.0001  | 13.97     |

TGR = Triglyceride, HDL = High density lipoprotein, LDL = Low density lipoprotein.

Means in the same row bearing different superscripts are significantly different ( $p < 0.05$ ).

B.lycium = *Berberis lycium*.

**Table 2.** Prediction of reduction in serum total cholesterol in broilers from percent *Berberis lycium* in the feed

| Response variable (X0) = Reduction in the serum total cholesterol |        |        |
|---|--------|--------|
| Regressor:<br>X1 = Percent of <i>Berberis lycium</i> in the feed  |        |        |
| Estimates   | b0     | b1     |
| Parameter estimate  | 125.60 | -7.81  |
| ±SE   | 3.69   | 2.44   |
| t   | 27.40  | -3.27  |
| p   | 0.0001 | 0.0021 |

R<sup>2</sup> (adjusted) = 11.53%.

A, B, C, D, E and F respectively (Table 1). The total serum cholesterol data when subjected to analysis of variance revealed significant differences among the groups. Minimum total serum cholesterol was recorded in group E (101.67 mg/dl) as compared to the control (120.40 mg/dl). There was a decreasing trend in total serum cholesterol in response to increasing level of *Berberis lycium* upto 2.0%. However, total serum cholesterol was increased when the level of *Berberis lycium* was increased to 2.5% in group F. Level of *Berberis lycium* was found significantly and negatively associated with serum total cholesterol ( $b = -7.81 \pm 2.44$ ; Table 2). The findings suggested that one percent increase in the level of *Berberis lycium* resulted a decrease of 7.81 mg/dl in serum total cholesterol. Total cholesterol had a significant ( $p < 0.05$ ) positive correlation with LDL cholesterol (Table 5). This indicated that the level of serum LDL increased as serum total cholesterol increased. As evident from the observations recorded in Table 1, addition of *Berberis lycium* at 2.0% resulted in minimum total serum cholesterol (101.67 mg/dl).

*Berberis* species have been reported to have hypotensive properties. Berbamine, an alkaloid isolated from *Berberis lycium*, produced a significant decrease in the mean arterial blood pressure and the amplitude of cardiac contractions in cats in 5 and 10 mg/kg doses. Doses ranging from 0.125 to 1 mg of berbamine significantly reduced the amplitude of contractions of the isolated rabbit heart. The perfusate

collected from the rat hindquarter preparations after injection of 5 mg doses of berbamine produced a marked contraction of the isolated guinea pig ileum preparation which was antagonized by dimetane maleate. On the basis of these observations it was suggested that berbamine produces its hypotensive effects due to its direct depressant action on the myocardium and release of histamine from the tissues (Khan et al., 1969). Administration of *Berberis vulgaris* fruit extract (0.05-1 mg/100 mg body weight of rats) significantly reduced the mean arterial blood pressure and heart rate in anaesthetized normotensive and desoxycorticosteron acetate-induced hypertensive rats in dose dependent manner (Fatehi et al., 2005).

Medicinal plants have decreased total serum cholesterol in humans and animals. Roots of winter cherry (*winthania somnifera*) significantly decreased blood serum cholesterol in humans (Andallu et al., 2000). *Allium victorialis* decreased serum total cholesterol in rabbits and mouse (Kim et al., 2000). Ginger (*zingiber officinale* R.) reduced serum and tissue cholesterol (Murugaiah et al., 1999). *Ocimum sanctum* powder supplementation reduced serum total cholesterol (Rai et al., 1997). *Nigella sativa* L. seeds significantly lowered serum cholesterol (Hassanin and Hassan, 1996). Chinese medicinal preparation (Hokoei-to) reduced total cholesterol (Yokozawa et al., 1996). *Avena sativa* reduces total serum cholesterol in rabbits (Juzwiak et al., 1994).

### Triglycerides

Mean serum triglyceride level per chick for the six experimental groups A, B, C, D, E and F having 0, 0.5, 1.0, 1.5, 2.0 and 2.5% *Berberis lycium* was 60.00, 58.17, 58.00, 55.33, 50.17 and 48.50 mg/dl respectively (Table 1). Minimum serum triglyceride level was recorded in group F (48.50 mg/dl) as compared to the control (60.00 mg/dl). There was a decreasing trend in serum triglyceride level in response to increasing level of *Berberis lycium*. Level of *Berberis lycium* was found significantly and negatively associated with serum triglyceride ( $b = -4.81 \pm 1.47$ ; Table 3).

**Table 3.** Prediction of reduction in serum triglyceride in broilers from percent *Berberis lycium* in the feed

| Response variable (X0) = Reduction in the serum triglyceride |        |        |
|--|--------|--------|
| Regressor:   |        |        |
| X1 = Percent of <i>Berberis lycium</i> in the feed           |        |        |
| Estimates  | b0     | b1     |
| Parameter estimate   | 61.04  | -4.81  |
| ±SE  | 2.23   | 1.47   |
| t  | 27.40  | -3.27  |
| p  | 0.0001 | 0.0017 |

R2 (adjusted) = 12.00%

**Table 4.** Prediction of reduction in serum LDL cholesterol in broilers from percent *Berberis lycium* in the feed

| Response variable (X0) = Reduction in the serum LDL cholesterol |        |        |
|---|--------|--------|
| Regressor:  |        |        |
| X1 = Percent of <i>Berberis lycium</i> in the feed              |        |        |
| Estimates   | b0     | b1     |
| Parameter estimate  | 58.82  | -9.27  |
| ±SE   | 3.91   | 2.58   |
| t   | 15.04  | -3.59  |
| p   | 0.0001 | 0.0006 |

R2 (adjusted) = 14.33%

The findings suggested that one percent increase in the level of *Berberis lycium* resulted a decrease of 4.81 mg/dl in serum triglyceride. As evident from the observations recorded in Table 1, addition of *Berberis lycium* at 2.5% resulted in minimum serum triglyceride level (48.50 mg/dl).

Both clinical trials and animal research have indicated that berberine administration prevented ischemia-induced ventricular tachyarrhythmia, stimulated cardiac contractility, and lowered peripheral vascular resistance and blood pressure (Chun et al., 1978) (Marin-Neto et al., 1988).

Herbs and medicinal plants have an effect on serum triglycerides in humans and animals. Roots of winter cherry (*winthania somnifera*) significantly decreased blood serum triglycerides in humans (Andallu et al., 2000). *Allium victorialis* decreased serum total triglycerides in rabbits and mouse (Kim et al., 2000). Ginger (*zingiber officinale R.*) reduced serum and tissue triglycerides (Murugaiah et al., 1999). *Ocimum sanctum* powder supplementation reduced serum triglycerides (Rai et al., 1997). Curcuma xanthorrhiza reduced serum triglycerides (Yasni et al., 1993).

Effects of alpha-galactosidase (GAL) on broiler corn-soybean meal diet was investigated (Wang et al., 2005). On d 21, triglycerides level of broilers showed interaction between energy and enzyme levels ( $p < 0.05$ ).

#### High density lipoprotein (HDL) cholesterol

Mean HDL cholesterol per chick for the six experimental groups A, B, C, D, E and F having 0, 0.5, 1.0, 1.5, 2.0 and 2.5% *Berberis lycium* was 52.08, 53.42, 60.42, 62.25, 62.92 and 54.50 mg/dl respectively (Table 1). Though the differences in serum high density lipoprotein were

**Table 5.** Pearson's correlation between serum lipids in broiler chicks fed different levels of *Berberis lycium*

|                   | Total cholesterol | HDL   | TGR   | LDL    |
|-------------------|-------------------|-------|-------|--------|
| Total cholesterol | 1.00              | 0.313 | 0.162 | 0.663  |
| HDL               | 0.00              | 1.000 | 0.175 | 0.0001 |
| TGR               | -                 | 0.00  | 1.000 | 0.0001 |
| LDL               | -                 | -     | 0.00  | 1.000  |
|                   |                   |       |       | 0.00   |

HDL = High density lipoprotein, TGR = Triglyceride, LDL = Low density lipoprotein.

statically non significant yet apparent HDL was quite high in group E (62.92 mg/dl) as compared to the control (52.08 mg/dl). There was an increasing trend in serum HDL in response to increasing level of *Berberis lycium* upto 2.0%. However, serum HDL was drastically decreased when the level of *Berberis lycium* was increased to 2.5% in group F. HDL had significant ( $p < 0.05$ ) negative correlation with triglyceride and LDL cholesterol (Table 5). This indicated that the level of serum triglyceride and LDL decreased as serum HDL increased. As evident from the observations recorded in Table 1, addition of *Berberis lycium* at 2.0% resulted in maximum serum HDL (62.92 mg/dl).

Medicinal plants have increased HDL cholesterol in humans and animals. Ginger (*zingiber officinale R.*) increased HDL (Murugaiah et al., 1999). Chinese medicinal preparation (Hokoei-to) increased HDL cholesterol (Yokozawa et al., 1996). *Avena sativa* increased HDL cholesterol in rabbits (Juzwiak et al., 1994). Curcuma xanthorrhiza increased serum HDL cholesterol (Yasni et al., 1993).

Effect of Lacquer (*Rhus verniciflua*) Supplementation on Growth Performance, Nutrient Digestibility, Carcass Traits and Serum Profile of Broiler Chicken was studied (Lohakare et al., 2006). The serum cholesterol and HDL showed a linear decrease as the level of supplementation increased at 3 wk; at 5 wk serum cholesterol, HDL and triglyceride levels decreased significantly showing a positive linear effect of lacquer on fat metabolism.

#### Low density lipoprotein (LDL) cholesterol

Average serum LDL cholesterol per chick was 65.25, 55.45, 44.48, 39.68, 28.72 and 49.80 mg/dl for group A, B, C, D, E and F respectively (Table 1). The serum LDL data when subjected to analysis of variance revealed significant differences among the groups. Minimum serum LDL was recorded in group E (28.72 mg/dl) as compared to the control (65.72 mg/dl). There was a decreasing trend in serum LDL in response to increasing level of *Berberis lycium* upto 2.0%. However, serum LDL was increased when the level of *Berberis lycium* was increased to 2.5% in

**Table 6.** Economics of broiler chicks feed different levels of *Berberis lycium*

| Parameters                | Group                      |                              |                              |                              |                              |                              | F value | P value |
|---------------------------|----------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|---------|---------|
|                           | A<br>(0% <i>B.lycium</i> ) | B<br>(0.5% <i>B.lycium</i> ) | C<br>(1.0% <i>B.lycium</i> ) | D<br>(1.5% <i>B.lycium</i> ) | E<br>(2.0% <i>B.lycium</i> ) | F<br>(2.5% <i>B.lycium</i> ) |         |         |
| Feed cost /chick (Rs.)    | 37.9                       | 40.7                         | 39.5                         | 40.7                         | 39.7                         | 42.2                         | 1.42    | 0.2648  |
| Gross return /chick (Rs.) | 88.3                       | 89.2                         | 87.0                         | 88.6                         | 92.1                         | 87.0                         | 1.73    | 0.1792  |

*B.lycium* = *Berberis lycium*.

group F. Level of *Berberis lycium* was significantly and negatively associated with serum LDL cholesterol ( $b = -9.27 \pm 2.57$ ; Table 4). The findings suggested that one percent increase in the level of *Berberis lycium* resulted a decrease of 9.27 mg/dl in serum LDL cholesterol. As evident from the observations recorded in Table 1, addition of *Berberis lycium* at 2.0% resulted in minimum serum LDL (28.72 mg/dl).

Medicinal plants have an effect on low density lipoprotein in humans and animals. Roots of winter cherry (*winthania somnifera*) significantly decreased blood serum LDL and VLDL in humans (Andallu et al., 2000). *Allium victorialis* decreased serum LDL cholesterol in rabbits and mouse (Kim et al., 2000). Ginger (*zingiber officinale R.*) reduced serum and tissue LDL cholesterol (Murugaiah et al., 1999). *Ocimum sanctum* powder supplementation reduced serum LDL and VLDL cholesterol (Rai et al., 1997). Chinese medicinal preparation (Hokoei-to) reduced LDL cholesterol (Yokozawa et al., 1996).

Broiler performance is affected by various plant extracts. Cau et al. (2005) suggested that green tea Polyphenols (GTP) and Fructo-oligosaccharides (FOS) in semi-purified diets can decrease mortality and change the caecal colonic flora population. but GTP shows antibiotic-like effects of non-selectively decreasing all colonic flora and then metabolites, and FOS acts selectively by increasing profitable microflora and decreasing production of caecal microflora metabolites besides volatile fatty acids.

#### Economics of experimental rations

The average cost of feed per chick was Rs.37.9, 40.7, 39.5, 40.7, 39.7 and 42.2 for treatment A, B, C, D, E and F respectively (Table 6, US\$1 = Rs.61). Gross return per chick was Rs.88.3, 89.2, 87.04, 88.6, 92.1 and 87.0 for treatment A, B, C, D, E and F respectively. Maximum return of Rs.92.1 per chick was recorded in group E. As evident from the findings there was an increase of Rs.3.81 per chick, amounting to a significant amount of Rs.3,810 per 1,000 boilers in group E as compared to the control. The higher return in group E is due to the optimal level of *Berberis lycium* (2.0%) in the ration, resulting in efficient feed utilization.

Broiler industry is one of the major livestock resources of Pakistan with tremendous potentials for support to the

national economy (Quresh et al., 2002). Production of broiler meat with lower cholesterol would provide technical boost to this industry.

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