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# Clinical Laboratory Serum Values in Rabbits Fed Diets Containing Black Cumin Seed

[1]Nabiela M. El Bagir, [1]Imtithal T.O. Farah, [2]Ahmed Alhaidary,
[2]Hasab E. Mohamed and [2]Anton C. Beynen
[1]Department of Biochemistry, Faculty of Veterinary Medicine,
University of Khartoum, Khartoum North, Sudan
[2]Department of Animal Production, College of Food and Agricultural Sciences,
King Saud University, Riyadh, Kingdom of Saudi Arabia

**Abstract:** Ingestion of black cumin seeds has wide variety of biological effects, implying that different processes in the body are influenced simultaneously. To assess to what extent clinical laboratory serum values are affected, rabbits were fed diets containing different levels of whole black cumin seed and serum was collected at various intervals. The base diet consisted of 60% lucerne and 40% sorghum. To formulate the experimental diets either 10, 15 or 20% of the base diet was replaced by black cumin seed. Body-weight gain was increased by the diets with 10 or 15% black cumin but not by the diet with 20%. Dietary black cumin seed raised serum concentrations of total protein, albumin and globulin but the diet with 20% produced lower values than did the 10 and 15% inclusion levels. Black cumin feeding increased serum urea and creatinine and lowered uric acid concentrations. Serum glucose, total lipid and cholesterol concentrations were lowered by consumption of black cumin. Black cumin seed in the diet did not affect the serum activities of alkaline phosphatase and glutamate pyruvate transaminase. Serum sodium and potassium were not influenced by black cumin but serum calcium and phosphate concentrations were increased. The major finding in this study with rabbits is that the highest dietary level of 20% versus either 10 or 15% black cumin seed lowered serum protein concentrations and diminished weight gain.

**Key words:** Rabbits, blood chemistry, protein, lipids, black cumin, concentrtion

## INTRODUCTION

*Nigella sativa* is a plant of the Ranunculacae family that produces seeds commonly known as black cumin. In traditional medicine, the oil component of the seeds is used to treat various diseases. Different types of research indeed substantiate a wide biological activity of black cumin oil, the active principle being thymoquinone (Ragheb *et al*., 2009). There is evidence that the oil fraction of black cumin seeds has anti-carcinogenic (Swamy and Tan, 2000), anti-inflammatory (Mutabagani and El-Mahdy, 1997; Ragheb *et al*., 2009), anti-microbial (Landa *et al*., 2009) and anti-parasite (Akhtar and Riffat, 1991) effects.

The wide variety of effects induced by the ingestion of black-cumin oil implies that different processes in the body are influenced simultaneously. It is likely that clinical laboratory values are affected as well. It is important to know the impact of black cumin on clinical laboratory values because this facilitates the interpretation of blood measurements in subjects using black-cumin oil as a drug. However, the information available in international journals is limited. In humans, the ingestion of black-cumin seeds lowered serum concentrations of uric acid and glucose but raised serum creatinine (Bamosa *et al*., 1997).

A hypoglycemic effect was also found in rabbits after intraperitoneal administration of volatile oil isolated from black-cumin seeds (Al-Hader *et al*., 1993). Oral administration of black-cumin oil reduced serum triacylglycerols and cholesterol concentrations in rats (El-Dakhakhny *et al*., 2000).

There is an incomplete picture of the effect of black cumin on the various clinical laboratory serum values. This prompted us to undertake the present study that shows the influence of dietary black cumin seed on a large number of clinical laboratory serum values in rabbits. To evaluate the effects of dose, if any, the rabbits were fed diets containing different levels of whole black cumin seed.

**Corresponding Author:** Nabiela M. El Bagir, Department of Biochemistry, Faculty of Veterinary Medicine, University of Khartoum, P.O. Box 32, Khartoum North, Sudan

## MATERIALS AND METHODS

**Animals and diets:** About 16 male rabbits, aged 3-4 months and weighing about 1.10 kg were used. They were housed in ground pens located in a shed with adequate ventilation and lighting. During the adaptation period of 2 weeks, they were fed lucerne and crushed sorghum. The lucerne was harvested in the afternoon, stored in the animal house and fed the next morning. Feed left-overs were measured and feed intakes were calculated. Based on the feed intakes, it was decided that each animal would eat at least 150 g lucerne and 100 g sorghum $day^{-1}$.

After the adaptation period, the rabbits were divided into four groups consisting of four animals each so that weight distributions in the groups were similar. The groups were fed the diets shown in Table 1. The diets consisted of lucerne and crushed sorghum without or with black cumin seeds. The seeds were mixed with the sorghum. Each rabbit received a fixed amount of 250 g of feed per day and had free access to water. The experimental period lasted 6 weeks.

**Chemical methods:** Blood samples were taken at the beginning of the experiment and then weekly. Samples were collected by incision of the marginal ear vein into glass tubes without anti-coagulant. The blood was allowed to clot at room temperature and serum was collected by low speed centrifugation. Serum was kept frozen at -20°C until analyses.

Total protein in serum was analyzed colorimetrically using the Biuret reagent. Serum albumin was determined with the use of bromocrysol green. Serum globulin was quantified as the difference between total protein and albumin.

Table 1: Ingredient and calculated macronutrient composition of the experimental diets

| | Dietary black cumin% | | | |
|---|---|---|---|---|
| Ingridients | 0 | 10 | 15 | 20 |
| **Ingredient (g/1000 g)** | | | | |
| Lucerne | 600.0 | 540.0 | 510.0 | 480.0 |
| Sorghum | 400.0 | 360.0 | 340.0 | 320.0 |
| Black cumin seed | 0.0 | 100.0 | 150.0 | 200.0 |
| **Macronutrient (g/100 g)** | | | | |
| Crude protein | 7.0 | 8.4 | 9.1 | 9.8 |
| Crude fat | 1.5 | 4.9 | 6.6 | 8.3 |
| Crude fiber | 6.0 | 6.0 | 5.9 | 5.9 |
| Ash | 2.8 | 2.9 | 2.9 | 3.0 |
| Moisture | 47.1 | 43.0 | 40.9 | 38.8 |
| Carbohydrates (NFE) | 35.6 | 34.8 | 34.6 | 34.2 |

The composition of lucerne, sorghum and black cumin seed, respectively was taken to be as follows (g/100 g product): crude protein, 5.4, 9.3, 21.3; crude fat, 0.6, 2.9, 35.5; crude fiber, 8.4, 2.4, 5.5; ash, 3.6, 1.5, 3.8; moisture, 70.0, 12.8, 5.5

Serum urea was analyzed on the basis of its reaction with diethyl monoxime and thiosemicarbazide. Uric acid was determined enzymatically with the use of uricase and peroxidase. Creatinine in serum was determined colorimetrically using picric acid. The reaction with ortho toluidine was used to measure glucose in serum. Total lipids were determined with the ortho-phospho-vanillin reagent. With the use of the Libermann-Burchard reagent serum total cholesterol was measured.

Alkaline phosphatase activity in serum was determined colorimetrically with p-nitrophenyl phosphate as substrate. The activity of glutamate pyruvate transaminase was assessed by the 2-4 dinitrophenyl procedure. Serum sodium and potassium concentrations were measured with the use of a flame photometric method. Serum calcium was analyzed by atomic absorption spectrophotometry with the use of a lanthanum-containing diluent.

**Data analysis:** The data are presented as group means at the beginning of the experiment (Initial) and as within-group averages for the 6 blood samples taken during the experiment (treatment). For each clinical laboratory value the overall SEM is given. Statistically significant differences between group means for the initial and treatment values were identified with the use of Duncan's multiple range test. The level of statistical significance was pre-set at $p<0.05$.

## RESULTS AND DISCUSSION

Table 1 shows the calculated composition of the experimental diets. The control diet contained 7.0% crude protein, 1.5% crude fat and 47.1% moisture. Increasing inclusion levels of black cumin were associated with higher protein and fat levels and lower moisture levels in the diet. The rabbits fully consumed the feed supplied each day. At the end of the experiment, group-mean body weights were 1.31, 1.45, 1.56 and 1.34 kg for the control group and the groups fed increasing amounts of black cumin, respectively.

The feeding of black cumin seed significantly raised serum concentrations of total protein, albumin and globulin (Table 2). The 20% level of black cumin consistently produced lower serum protein concentrations than did the 10 and 15% inclusion levels.

The diets containing black cumin significantly increased serum urea concentrations but there was no dose response effect (Table 3). Serum uric acid concentrations were lowered by the feeding the diets containing 15 or 25% black cumin seed but the diet with 10% seed had no effect (Table 3). Serum creatinine was

Table 2: Response of serum proteins to dietary black cumin in rabbits

| | Dietary black cumin% | | | | |
|---|---|---|---|---|---|
| Parameters | 0 | 10 | 15 | 20 | SEM |
| **Total protein (g/100 mL)** | | | | | |
| Initial | 5.76$^{a}$ | 5.90$^{a}$ | 6.07$^{a}$ | 6.23$^{a}$ | 0.10 |
| Treatment | 6.21$^{a}$ | 7.59$^{b}$ | 7.89$^{c}$ | 7.42$^{b}$ | - |
| **Albumin (g/100 mL)** | | | | | |
| Initial | 3.10$^{a}$ | 3.23$^{a}$ | 3.20$^{a,b}$ | 3.31$^{b}$ | 0.03 |
| Treatment | 3.29$^{a}$ | 4.11$^{b}$ | 4.13$^{b}$ | 3.97$^{c}$ | - |
| **Total globulin** | | | | | |
| Initial | 2.66$^{a}$ | 2.67$^{a}$ | 2.87$^{a}$ | 2.92$^{b}$ | 0.08 |
| Treatment | 2.92$^{a}$ | 3.48$^{b}$ | 3.76$^{c}$ | 3.45$^{b}$ | - |

Means in the same row not sharing the same superscript letter are significantly different

Table 3: Response of serum metabolites to dietary black cumin in rabbits

| | Dietary black cumin% | | | | |
|---|---|---|---|---|---|
| Parameters | 0 | 10 | 15 | 20 | SEM |
| **Urea (mg/100 mL)** | | | | | |
| Initial | 55.93$^{a,b}$ | 53.20$^{a}$ | 56.37$^{b}$ | 57.30$^{a,b}$ | 0.66 |
| Treatment | 48.46$^{a}$ | 54.15$^{b}$ | 53.70$^{b}$ | 55.01$^{b}$ | - |
| **Uric acid (mg/100 mL)** | | | | | |
| Initial | 1.73$^{a}$ | 1.87$^{a}$ | 1.97$^{b}$ | 1.97$^{b}$ | 0.04 |
| Treatment | 1.90$^{a}$ | 1.99$^{a}$ | 1.74$^{b}$ | 1.78$^{b}$ | - |
| **Creatinine (mg/100 mL)** | | | | | |
| Initial | 0.64$^{a}$ | 0.73$^{b}$ | 0.80$^{c}$ | 0.81$^{c}$ | 0.03 |
| Treatment | 0.59$^{a}$ | 0.71$^{b}$ | 0.79$^{c}$ | 0.78$^{c}$ | - |
| **Glucose (mg/100 mL)** | | | | | |
| Initial | 103.70$^{a}$ | 101.20$^{a}$ | 101.10$^{a}$ | 93.80$^{a}$ | 2.43 |
| Treatment | 98.30$^{a}$ | 77.00$^{b}$ | 72.70$^{b}$ | 88.80$^{c}$ | - |
| **Total lipids (mg/100 mL)** | | | | | |
| Initial | 446.70$^{a}$ | 443.30$^{a}$ | 446.70$^{a}$ | 443.30$^{a}$ | 4.16 |
| Treatment | 436.70$^{a}$ | 305.50$^{b}$ | 303.30$^{b}$ | 336.10$^{c}$ | - |
| **Total cholesterol (mg/100 mL)** | | | | | |
| Initial | 105.00$^{a}$ | 97.70$^{a}$ | 107.60$^{a}$ | 106.00$^{a}$ | 1.45 |
| Treatment | 110.90$^{a}$ | 71.30$^{b}$ | 74.10$^{b}$ | 70.20$^{b}$ | - |

Means in the same row not sharing the same superscript letter are significantly different

elevated by black cumin feeding but there was no dose-effect relationship (Table 3). Serum glucose concentrations were significantly lowered by consumption of the diets containing black cumin (Table 3).

The glucose lowering was smallest for the diet with the highest amount of black cumin. Serum concentrations of total lipids and cholesterol were decreased after feeding the diets with black cumin but there was no dose effect (Table 3).

The dietary treatments did not affect the serum activities of alkaline phosphatase and glutamate pyruvate transaminase (Table 4). The diets with 15 or 20% black cumin significantly increased serum phosphate concentrations (Table 4). Serum concentrations of sodium were unaffected by consumption of black cumin seeds (Table 4). The diet containing 15% black cumin significantly increased serum potassium but the diets with 10 or 20% had no influence (Table 4). Serum calcium concentrations were raised by black cumin feeding with a trend towards dose dependency (Table 4).

Table 4: Response of serum organ-function indicators to dietary black cumin in rabbits

| | Dietary black cumin% | | | | |
|---|---|---|---|---|---|
| Parameters | 0 | 10 | 15 | 20 | SEM |
| **Alkaline phosphatase (U L$^{-1}$)** | | | | | |
| Initial | 100.10$^{a}$ | 113.30$^{b}$ | 125.40$^{c}$ | 123.40$^{c}$ | 7.24 |
| Treatment | 109.60$^{a}$ | 110.00$^{a}$ | 103.40$^{a}$ | 104.10$^{a}$ | - |
| **Glutamate pyruvate transaminase (U L$^{-1}$)** | | | | | |
| Initial | 45.00$^{a}$ | 44.60$^{a}$ | 44.30$^{a}$ | 44.60$^{a}$ | 0.36 |
| Treatment | 45.90$^{a}$ | 44.90$^{a}$ | 45.20$^{a}$ | 44.80$^{a}$ | - |
| **Phosphate (mg/100 mL)** | | | | | |
| Initial | 4.10$^{a}$ | 3.67$^{b}$ | 3.33$^{c}$ | 3.23$^{c}$ | 0.03 |
| Treatment | 3.62$^{a}$ | 3.74$^{a}$ | 3.89$^{b}$ | 3.97$^{b}$ | - |
| **Sodium (mEq L$^{-1}$)** | | | | | |
| Initial | 63.90$^{a}$ | 59.80$^{a}$ | 59.60$^{a}$ | 60.80$^{a}$ | 0.54 |
| Treatment | 64.40$^{a}$ | 65.00$^{a}$ | 65.90$^{a}$ | 64.00$^{a}$ | - |
| **Potassium (mEq L$^{-1}$)** | | | | | |
| Initial | 0.93$^{a}$ | 0.93$^{a}$ | 0.97$^{a}$ | 1.10$^{b}$ | 0.03 |
| Treatment | 0.91$^{a}$ | 0.88$^{a}$ | 0.96$^{b}$ | 0.92$^{a}$ | - |
| **Calcium (mg/100 mL)** | | | | | |
| Initial | 10.50$^{a}$ | 9.80$^{b}$ | 10.00$^{b}$ | 10.20$^{a}$ | 0.06 |
| Treatment | 10.50$^{a}$ | 11.30$^{b}$ | 11.40$^{b}$ | 11.70$^{b}$ | - |

Means in the same row not sharing the same superscript letter are significantly different

To formulate the experimental diets, substantial amounts of black cumin seeds were added to the lucerne-sorghum base diet. This approach caused differences in the macronutrient compositions of the experimental diets. When compared with the control diet, the experimental diets with black cumin seed contained more protein and more fat. The control diet contained 13.2% protein in the dietary dry matter which causes growth limitation in young rabbits (De Blas *et al*., 1981). Thus, the increased weight gain seen in the rabbits fed the diets containing either 10 or 15% black cumin seed may be explained by the higher protein intakes. The diet containing 20% black cumin did not support enhanced growth. This diet contained 16.0% protein and 13.6% fat in the dietary dry matter. The fat component of black cumin seed is rich in linoleic acid (Babayan *et al*., 1978). Therefore, it was expected that the diet with 20% black cumin would stimulate growth in the rabbits (Alhaidary *et al*., 2010). Possibly, black cumin seeds contain anti-nutritional or toxic factors that impair nutrient utilization. It has been shown in mice that oral administration of methanol extracts of black cumin seed lowered body weight (Vahdati-Mashhadian *et al*., 2005).

The diets containing 10 or 15% black cumin seed raised the serum concentrations of total protein, albumin and globulins. However, when the amount of dietary black cumin was increased up to 20%, there was a fall of serum total protein, albumin and globulins but the values in the control group were not reached. The higher protein intakes associated with 10 or 15% black cumin seed in the diet may explain the observed increase in serum proteins. The lower serum protein concentrations in the rabbits fed

the diet with 20% black cumin might be explained by diminished protein synthesis. Serum urea levels were not increased by feeding the diet with 20% black cumin instead of either 15 or 10% indicating that protein degradation was not enhanced. On the other hand, serum creatinine concentration which is an index of muscle mass and muscle turnover was similar for the diets with 15 or 20% black cumin. This observation is at variance with the idea of diminished protein synthesis in the rabbits fed the diet with 20% black cumin instead of 15 or 10%.

Serum uric acid concentration was decreased by the feeding of black cumin seed whereas the concentration of creatinine was raised. This outcome agrees with earlier findings in humans (Bamosa *et al*., 1997). A decrease in serum uric acid could point at modification of purine catabolism whereas an increase in creatinine could mirror enhanced muscle turnover. Unfortunately, there are no literature data that provide clues to explain the observed effects on serum uric acid and creatinine.

This study with rabbits confirms that black cumin seed has hypoglycemic (Al-Hader *et al*., 1993) and hypolipidemic activity (El-Bagir *et al*., 2006; Kocyigit *et al*., 2009; Nader *et al*., 2010). There was no clear dose-response relationship indicating that the dietary inclusion level of 10% black cumin seed already had a maximum effect on the serum concentrations of glucose, total lipids and cholesterol. The glucose-lowering effect of black cumin seed may be explained by an insulin-like stimulation of glucose uptake by muscle and adipose tissue (Benhaddou-Andaloussi *et al*., 2010), stimulation of insulin release (Rchid *et al*., 2004) and inhibition of intestinal glucose absorption (Meddah *et al*., 2009). The hypocholesterolemic effect of black cumin seed may relate to upregulation of the low-density lipoprotein receptor and inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (Al-Naqeep *et al*., 2009).

The serum activities of alkaline phosphatase and glutamate pyruvate transaminase were not influenced by the intake of black cumin seed. This suggests that liver function was not adversely influenced by black cumin. There is evidence that black cumin seed may protect against liver dysfunction as caused by endotoxemia (Helal, 2010) which may relate to its anti-inflammatory and anti-oxidant activity (Ragheb *et al*., 2009).

Serum sodium concentrations were left unchanged by the feeding of black cumin and serum potassium concentrations were not systematically changed. This suggests that kidney function was not affected. In a rat model with doxorubicin-induced nephropathy, it has been shown that the active principle of black cumin seed, thymoquinone, diminished the severity of the nephrotic syndrome (Badary *et al*., 2000). The diets with 15 or 20% black cumin seed produced an increase in serum phosphate concentrations. This effect probably does not relate to kidney function but may be secondary to the extra intake of phosphorus with black cumin seeds. The phosphorus level in black cumin seeds has been reported to be 0.54% (Sultan *et al*., 2009). The addition of 20% black cumin seed to the diet increased the calculated dietary phosphorus level from 0.17-0.25%. Serum phosphate concentrations in rabbits are reflected by phosphorus intake (Ritskes-Hoitinga *et al*., 2004).

The diets containing black cumin seed raised serum calcium concentrations and there was a dose-dependent trend. In rabbits, serum calcium concentrations are determined by calcium intake (Chapin and Smith, 1967). The reported calcium content of black cumin is 0.57% (Sultan *et al*., 2009). This implies that increasing intakes of black cumin seed were associated with extra intake of calcium. The calculated calcium content of the control diet was 0.06% and that of the diet with 20% black cumin seed was 0.16%.

## CONCLUSION

This study with rabbits shows that clinical laboratory serum values are affected by high intakes of black cumin seed. It should be stressed that the addition of large amounts of black cumin seed to the diets was done by replacement of portions of the base diet so that there were multiple differences between the composition of the control and experimental diets. The present study indicates that the well-known lowering effects of dietary black cumin seeds on serum concentrations of glucose, total lipids and cholesterol also hold for rabbits. The highest inclusion level of 20% versus either 10 or 15% black cumin seed in the diet lowered serum protein concentrations and weight gain which may point at an adverse effect of black cumin seed at very high intakes.

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