

Safety Evaluation of Caper (*Capparis ovata* Desf.) in Lohmann Roosters

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Abstract: The purpose of this study was to determine whether oral use of caper (*Capparis ovata* Desf.) is safe in Lohmann roosters. About 24 week-old roosters were randomly assigned to control and caper treatment groups (8 per group) and fed a standard diet (14% crude protein and 3000 kcal kg⁻¹ metabolizable energy). Roosters in the control group received 10 mL of tap water, whereas roosters in the treatment group received 1 g of caper per 1 kg of BW suspended in 10 mL of tap water. The experiment lasted for 39 days and treatments were given by oral gavages. Roosters were weighed at the end of caper treatments and blood was taken from the vena brachialis. Organ weights were recorded after sacrifices. No negative effect of caper treatment was observed in hemoglobin, hematocrit, plasma protein, red blood cell count, white blood cell count, biochemical parameters, BW, liver, kidney, spleen, pancreas, gizzard or heart weights. In addition, no apparent changes in these organs were detected by gross post mortem and histopathological examination to suggest toxic effects of oral use of caper for 39 days. Interestingly, caper treatment increased the thrombocyte levels in caper treated roosters. In conclusion, the results suggest that use of caper is safe in roosters.

Key words: Rooster, hematological parameters, biochemical parameters, liver enzymes, histopathology

INTRODUCTION

Caper (*Capparis* genus in the *Capparaceae* family) is a plant of tropical/subtropical and arid areas. Capers, grown wildly in various regions of the world were profited for several purposes since ancient times. Certain species and varieties of capers have been cultivated in especially Mediterranean regions and have become an important economic plant in Italy and Spain for last three decades. From ancient times, the floral buttons, fruits and roots of *C. spinosa* (capers) was employed as a flavoring in cooking and are also used in traditional medicine for their aphrodisiac, diuretic, antihypertensive, poultice and tonic properties (Baytop, 1984). Moreover, various parts of caper plant can be used as cosmetic. Capers are also used in different areas for landscape element, control of erosion or animal feeding (Ozcan, 1999). Thus, capers can be consumed for long time periods in human and animals.

Fruits and flower buds of capers can be considered as a part of a healthy diet since it contains high amounts of vitamins (vitamin A, E and C), minerals (phosphorus, iron, copper, magnesium), fatty acids (linoleic, oleic, linolenic and palmitic acids) and proteins (Ozcan, 1999). As a result, caper is consumed widely in Turkey and most

of the Mediterranean country. However, there is no study was previously performed concerning consumption of caper. Although, the use of caper may have positive effects on blood and liver parameters, it may not be safe on the same parameters when used over a longer time periods. Thus, the purpose of this study was to determine the effects of caper (*Capparis ovata* Desf.) on blood parameters, some liver enzymes and organs of Lohmann roosters.

MATERIALS AND METHODS

Animals and diets: This study was approved by the ethics committee of the Suleyman Demirel University. About 24 week-old roosters (2.2±0.2 kg) were used in the experiment. Roosters were housed individually in wire cages (50×50×50 cm) and held 4 weeks for adaptation prior to the experiment. All roosters were determined to be healthy by observation during the adaptation period and by individual physical examination. The photoperiod was 14 h of light: 10 h of darkness (lights on at 5 am and 24±4°C). Feed and water were provided *ad libitum*. The roosters were fed a standard commercial diet (14% crude protein and 3000 kcal kg⁻¹ metabolizable energy).

Plant material: Specimens of (*Capparis ovata*) CO flower buds were collected from the Budur region of Turkey. The aqueous extracts of pickled CO were prepared daily, just before administration. Pickled CO rinsed twice to remove excess salt. Then, rinsed CO was chopped in a blender for 15 sec for treatments.

Experiment and sampling: Roosters were randomly assigned to control and caper treatment groups (8 per group). The roosters in the treatment group received 1 g of chopped capers per 1 kg of BW suspended in 10 mL of tap water whereas the roosters in C received 10 mL of tap water. The experiment lasted for 39 days and treatments were given by oral gavage. The dose of 1 g kg⁻¹ was used according to the traditional phytotherapy.

Body weights were recorded weekly. At the end of the experiment, venous blood samples from each roosters were collected from the brachial vein. All of the blood samples were collected between 9 and 10 am within 4 min of restraint. Samples were prepared as serum and plasma and stored at 20°C until assayed for selected clinical chemistries. For hematological determinations, an additional 1 mL blood sample was placed into an EDTA tube. The day of the blood sample collection, the roosters were euthanized and the abdominal cavity of each rooster was opened. The liver, kidney, lung and heart were excised. The weights of the organs were recorded.

Total erythrocyte, leukocyte, percent leukocyte and thrombocyte were counted with haemocytometers using Natt-Herrick solution. Plasma protein values were measured by a refractometer (Atago, SPR-N, Japan). Hemoglobin concentrations were estimated by the cyanmethemoglobin method after centrifugation to remove erythrocyte nuclei.

Percent hematocrit values were estimated by microhematocrit methods after centrifugation of blood-filled capillary tubes. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentrations (MCHC) were calculated from the values of total erythrocyte, percent hematocrit and hemoglobin (Schalm and Jain, 1986). Percent leukocyte distribution was determined from the blood smears made from the EDTA sample that were stained with May Grunwald-Giemsa stain. One hundred cells were counted and classified.

In vitro enzymatic colorimetric method was used for the determination of glucose (Glucose GOD-PAP, Biolabo Sa, Maizy, France), triglyceride (TR-F400 CH, Chema Diagnostica, Via Pellegrini Jesi, Italy) and cholesterol (CT-F400CH, Chema Diagnostica, Via Pellegrini Jesi, Italy) in serum. Alanine aminotransferase (ALT; kinetic enzymatic method without pyridoxal activation), serum

Alkaline Phosphatase (ALP; optimized calorimetric method) and Serum Aspartate Aminotransferase (AST; kinetic enzymatic method without pyridoxal activation) concentrations were measured by auto analyzer (The Roche Modular PP Auto Analyser, Roche Diagnostics, Mannheim, Germany).

Histopathology: After sacrifice, left kidney, left lung, heart and a 1 cm wide strip of the liver was removed and placed in buffered formalin for histological examinations. All tissue samples were routinely processed into paraffin; 5 µm thick sections were stained with Hematoxylin and Eosin (H and E). The slides were coded and examined in a single-blind fashion by a pathologist.

Statistical analyses: Results are presented as mean±SE. The data were analyzed by Proc t-test procedure. All statistical analyses were carried out using SAS statistical package. The minimum level of significance was set at p<0.05.

RESULTS AND DISCUSSION

Even though caper is commonly used to cure a variety of diseases, there is no study in the literature available about traditional use of capers' safety. Thus, the current study was conducted to assess safety evaluation of caper consumption. Overall, the data indicated no treatment related clinical problems. Survival rates, body weight changes and postmortem observations were similar between the treatments. The roosters in different groups appeared healthy and showed no unusual behavior during the entire experimental period. The changes in body weight gains were also similar and there was no remarkable change in body weight between control and treated groups. The results of the current study suggested that the caper intake did not negatively affect the utilization of nutrients nor the metabolism of the birds.

The blood parameters are presented in Table 1. The hematological parameters evaluated were within the normal values reported for roosters (Spano *et al.*, 1987). All hematological parameters except for thrombocyte counts, showed no significant differences between the treatment groups. The heterophil/lymphocyte ratio (~0.5) and plasma protein levels were also similar between the treatments. Absence of the dietary effect on the blood hematological parameters indicated that caper supplementation did not result in hemotoxicity in the roosters. Plasma protein concentrations are directly related to protein intake and quality (Bock, 1989). Furthermore, the heterophil: lymphocyte ratio is a strong

Table 1: Effects of caper treatments on hematological parameters of Lohman roosters after oral gavages for 39 days (mean±standard error)

| Parameters | Treatments | | p-value |
|--|-------------|-------------|---------|
| | Control | Caper | |
| Hemoglobin (g dL ⁻¹) | 15.6±0.58 | 15.2±0.35 | 0.520 |
| Hematocrit (%) | 38.3±1.13 | 37.1±1.21 | 0.460 |
| Plasma Protein (g dL ⁻¹) | 5.3±0.21 | 5.6±0.39 | 0.470 |
| RBC ¹ (10 ⁶ /μL) | 2.97±0.14 | 3.18±0.11 | 0.250 |
| MCV ² | 130.9±9.48 | 117.8±9.31 | 0.140 |
| MCH ³ | 53.2±2.8 | 48.2±2.6 | 0.140 |
| MCHC ⁴ | 40.7±1.19 | 41.1±1.42 | 0.740 |
| Thrombocyte (10 ⁵ /μL) | 0.244±0.04 | 0.377±0.05 | 0.004 |
| WBC ⁵ (10 ³ /μL) | 27.1±0.98 | 25.6±0.89 | 0.290 |
| Heterophil (%) | 25.5±1.81 | 26.9±1.93 | 0.610 |
| Lymphocyte (%) | 45.8±1.27 | 46.7±0.74 | 0.530 |
| H:L ratio ⁶ | 0.568±0.057 | 0.579±0.045 | 0.870 |

¹RBC: Red Blood Cells; ²MCV: Mean Corpuscular Volume; ³MCH: Mean Corpuscular Hemoglobin; ⁴MCHC: Mean Corpuscular Hemoglobin Concentration; ⁵WBC: White Blood Cells

Table 2: Effects of caper treatments on hematological and biochemical parameters of Lohman roosters after oral gavages for 39 days (mean±standard error)

| Parameters | Treatments | | p-value |
|-------------------|-------------|------------|---------|
| | Control | Caper | |
| Total cholesterol | 186.6±6.91 | 188.8±6.8 | 0.77 |
| Triglyceride | 80.07±3.09 | 81.12±4.16 | 0.84 |
| Glucose | 217.2±6.3 | 223.03±5.1 | 0.48 |
| ALT ¹ | 23.91±1.75 | 24.65±1.68 | 0.76 |
| AST ² | 250.3±14.9 | 260.9±14.7 | 0.62 |
| ALP ³ | 349.11±15.4 | 351.8±14.8 | 0.89 |

¹ALT: Alanine amino transferase; ²AST: Aspartate amino transferase; ³ALP: Alkaline Phosphatase

indicator of stress (Islam *et al.*, 2004) and a ratio of about 0.5 was suggested to an optimal stress in chickens (Gross and Siegel, 1983). Thus, these results also suggest that the caper treatment did not appear to impede hematopoiesis, feed intake or metabolism of the roosters.

Biochemical parameters investigated were not altered due to treatment. Serum total cholesterol, triglyceride and glucose concentration were not significantly changed between the groups (Table 2). Serum ALT, AST and ALP levels were within the normal ranges for the roosters (Spano *et al.*, 1987) and were not affected by feeding extracts. The liver is prone to the toxicity induced from chemical agents as it has an essential role in altering and clearance of these chemicals. A damage to the internal organs tend to cause a rise in serum metabolic indices. The activity of ALT and AST tend to increase when there is a damage in the liver. Thus ALT, AST and ALP are known to be sensitive markers of liver problems. AST is found in multiple organs such as the liver, heart, kidney, brain and skeletal muscle. Therefore, the liver, heart or muscle damage can cause an increase in serum AST concentrations (Boyd, 1983). Accordingly, a rise in ALP activities indicates non-specific tissue irritation because it increases due to cellular damage to numerous organs

Table 3: Effects of caper treatments on body and organ weights of Lohman roosters after oral gavages for 39 days (mean±standard error)

| Parameters | Treatments | | p-value |
|---|-------------|-------------|---------|
| | Control | Caper | |
| Body weight (g) | 2070.6±35.8 | 2022.8±56.4 | 0.49 |
| Organ weights (g) | | | |
| Liver | 25.8±0.88 | 26.5±1.63 | 0.71 |
| Left kidney | 5.12±0.24 | 5.14±0.24 | 0.96 |
| Pancreas | 3.83±0.13 | 3.91±0.21 | 0.77 |
| Heart | 9.65±0.60 | 9.45±0.52 | 0.80 |
| Spleen | 2.45±0.13 | 2.62±0.31 | 0.62 |
| Gizzard | 12.47±0.59 | 12.95±0.63 | 0.59 |
| Relative internal organ weights (g/100 g BW) | | | |
| Liver | 1.24±0.04 | 1.30±0.06 | 0.46 |
| Left kidney | 0.247±0.012 | 0.253±0.008 | 0.69 |
| Pancreas | 0.186±0.07 | 0.193±0.009 | 0.52 |
| Heart | 0.486±0.025 | 0.466±0.020 | 0.97 |
| Spleen | 0.118±0.007 | 0.128±0.013 | 0.52 |
| Gizzard | 0.600±0.021 | 0.641±0.031 | 0.29 |

(Bush, 1991; Ceron *et al.* 1995). Overall, no significant changes in these biochemical findings suggested that the metabolism of the animals was not adversely affected by caper consumption.

After sacrifice detailed postmortem examinations of the birds were performed. Necropsy findings showed no treatment related changes and pathological lesions in roosters. Moreover, no absolute increase in organ weights was apparent in the current study (Table 3). Histopathological parameters (liver, left kidney, spleen, heart) also were tested to evaluate whether the treatment resulted any organ toxicity. There were no microscopic changes to suggest any adverse effects of including caper to the diet.

Our search of the literature failed to reveal significant information regarding the safety evaluation of the traditional use of flower buds and fruits of the caper. The relevant published literature data involved studies conducted with the leaves and the roots of caper plant. A slight, anticonvulsant activity of oxindole isolated from the roots of *Capparis tomentosa* (*C. tomentosa*) was previously reported (Dekker *et al.*, 1987). Oral administration of dried leaves or stems of *C. tomentosa* caused inappetence, locomotoric disturbances, paresis of the hind limbs and recumbency, anemia and disturbed, kidney and liver functions in Nubian goats (Ahmed *et al.*, 1993). Pathological lesions were apparent in the gray matter of the spinal cord, the renal proximal convoluted and collecting tubules and the heart tissues. Moreover, dried leaves of *C. tomentosa* decreased serum total protein and calcium and increased glutamic oxalacetic transaminase, ammonia, sodium and potassium in desert sheep and Zebu calves (Ahmed *et al.*, 1981). The clinical signs of the poisoning, such as weakness of the hind limbs, staggering, flexion of the fetlock and the phalangeal joints and recumbancy were also apparent.

CONCLUSION

The finding of the current study suggested that the traditional use of the pickled *Capparis ovata* flower buds are safe and well tolerated for the 39 day study period and therefore can be used in Lohman roosters. Moreover, high thrombocyte count due to caper treatment should be evaluated further for use in diseases causing low thrombocyte counts.

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