



# Effect of olive leaves on ascites incidence, hematological parameters and growth performance in broilers reared under standard and cold temperature conditions

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## ABSTRACT

The effects of dietary olive leaves supplementation on ascites indices, hematological parameters, and broiler performance were separately assessed under standard (based on Arian strain management guide) and cold temperature conditions (induced ascites) with same experimental diets. For the standard temperature conditions, 400 day-old male broilers were divided into four experimental groups: control group, and three groups with olive leaf supplementation at 5, 10, or 15 g/kg diet (oleuropein content, 72.63 mg/g). The experiments were performed in four replicates of 25 birds per pen. The same grouping with another 400 birds was used for the cold temperature conditions. Growth performance, physiological and biochemical parameters, and ascites indices (right ventricle [RV] and total ventricle [TV] weight and RV/TV) were evaluated. Under both temperature conditions, growth parameters were similar among all groups. Ascites-related mortality, systolic blood pressure, packed cell volume, alanine aminotransferase, erythrocyte osmotic fragility, red blood cell count, and triiodothyronine level decreased linearly with increasing olive leaf supplementation under both conditions ( $P < 0.05$ ). Lactate dehydrogenase and alkaline phosphatase activities showed the same, although non-linear trend ( $P < 0.05$ ), and thyroxine levels showed a linear increasing trend ( $P < 0.001$ ) under both conditions. Increasing olive leaf supplementation was associated with a linear decrease in RV/TV under the standard temperature condition and a linear decrease in RV, TV, and RV/TV under cold stress ( $P < 0.001$ ). It concluded that dietary olive leaf supplementation at a dose of 10 g/kg has an anti-hypertensive effect and decreases ascites incidence without impairing broiler performance under standard and cold ambient temperatures.

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**Abbreviations:** ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EOF, erythrocyte osmotic fragility; FCR, feed conversion ratio; Hb, hemoglobin; LDH, lactate dehydrogenase; PCV, packed cell volume; RBC, red blood cell; RV, right ventricle weight; SBP, systolic blood pressure; T<sub>3</sub>, triiodothyronine; T<sub>4</sub>, thyroxine; TV, total ventricle weight.

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## 1. Introduction

Modern commercial broiler chickens are genetically selected for high growth rate, feed efficiency, and meat yield, therefore, have high metabolic rates (Julian, 1993; Luger et al., 2002; Baghbanzadeh and Decuyper, 2008; Hassanzadeh, 2010). With a feed consumption of approximately 5 kg, male broilers can achieve a live weight of 3 kg at 42 days of age (Leeson, 2007). However, the increased metabolic rates make broilers highly susceptible to metabolic disorders (Druyan et al., 2008), and the cardiovascular and musculoskeletal systems are the most commonly affected (Julian, 2005). Ascites syndrome is the most prevalent cardiovascular-related metabolic disorder (Hassanzadeh, 2010). Ascites can cause a mortality of up to 8% in broiler flocks (20–30% in heavy flocks) (Pakdel et al., 2002); moreover, it occurs at the end of the growing period, which is very important in terms of economic losses (Pakdel et al., 2002; Druyan et al., 2008; Hassanzadeh, 2009). The pathogenesis of ascites involves an imbalance between oxygen requirement and oxygen supply, which results in hypoxemia (Baghbanzadeh and Decuyper, 2008; Hassanzadeh, 2009, 2010). Hypoxemia increases pulmonary arterial pressure and therefore, causes enlargement of the right ventricle (RV) (Julian, 1993; Wideman et al., 2010).

The incidence of ascites is influenced by environmental and genetic factors (Gupta, 2011). Environmental factors such as nutrition (feed intake, energy, protein, sodium, toxins, density), water quality, lighting programs, altitude, diseases, house environment (ventilation, dust, CO<sub>2</sub>, NH<sub>3</sub>, heat and cold) affected the incidence of ascites (Julian, 2000; Baghbanzadeh and Decuyper, 2008; Gupta, 2011). The genotype of the broiler has a profound influence on its susceptibility to ascites. A study of Azizian et al. (2013) illustrated that broilers from various stocks differing in growth rate and feed efficiency differ in their susceptibility to ascites.

Numerous nutritional and management programs have been devised to alleviate ascites and economic losses (Baghbanzadeh and Decuyper, 2008; Rajani et al., 2011). The basis of these strategies is to control growth rate without impairing overall growth performance. Nonetheless, most ascites-control programs limit growth rate and maximum profitability. Strategies that will successfully alleviate ascites while supporting optimum growth rate of broilers are critical.

Since hypertension seems to be involved in the development of ascites, any factor that reduces blood pressure, especially pulmonary vascular pressure, may help control ascites (Wideman, 2000, 2001; Wideman et al., 2010). Olive leaves (*Olea europaea*) are a major, copious by-product of olive tree cultivation and olive mills. Approximately 100 g leaves are present for every kilogram of olives used in the oil industry. Olive tree pruning produces many olive leaves (~25 kg/tree) (Bouaziz et al., 2008; Leonardi et al., 2008; Paiva Martins et al., 2009). Empirical, experimental, and clinical studies have documented that olive leaves have the potential to reduce blood pressure in rats (Al-Qarawi et al., 2002; Khayyal et al., 2002) and humans (Somova et al., 2003; Omar, 2010), but their effect on blood pressure in broiler chickens remains unknown. In fact, it is documented that the olive leaf extract reduced the blood pressure and cholesterol of plasma in rats (Perrinjaquet-Moccetti et al., 2008) and monounsaturated fatty acids available in olive leaf such as oleic acid reduced lipids of plasma and prevent incidence of cardiovascular disease (Huang et al., 2010).

Olive leaves contain lignocellulosic compounds, which can be detrimental to the environment. However, the use of these leaves as livestock feed is important economically, ecologically and dates back many years (Martin Garcia et al., 2003; Molina Alcaide and Yanez Ruiz, 2008; Abo Omar et al., 2012). Oleuropein and its derivatives (e.g. hydroxytyrosol) are important components of olive leaves (Bouaziz et al., 2008). Oleuropein is the major phenolic compound in olive leaves (Lee et al., 2009) and has anti-hypertensive activity (Al-Qarawi et al., 2002; Bouaziz et al., 2008; Omar, 2010), which may mean that olive leaves can be used to control ascites. Moreover, because these leaves are abundant, their use as a feed ingredient is economically feasible (Molina Alcaide and Yanez Ruiz, 2008; Carcel et al., 2010).

Based on the available literature, no studies have investigated the effect of olive leaves on ascites and growth performance in broiler chickens. Therefore, the present study aimed to assess the effects of olive leaves on ascites incidence, systolic blood pressure, hematological parameters, and growth performance in broilers reared under standard and cold-temperature conditions.

## 2. Materials and methods

### 2.1. Olive leaves

Fresh olive leaves (*O. europaea* L., Yellow variety) were obtained from an olive farm (Fadak, Ghom, Iran), shadow-dried at room temperature, and finely ground (Mill, Gallenkamp size 8 in., UK). The chemical composition of the olive leaves for dry matter (DM), crude protein (CP), ether extract (EE), starch, ash, calcium, phosphorus, and other minerals (K, Na, Cl, Mg, Fe, Mn, Cu, and Zn) were determined by Association of Official Analytical Chemists (AOAC) methods with codes 934.01, 976.05, 920.39, 920.40, 942.05, 935.13, 965.17, and 968.08, respectively (AOAC, 1990).

The apparatus used for DM, CP, EE, ash and minerals were Oven T6760 Heraeus, Germany; Kjeltac Auto Analyser 1030 Tecator, Sweden; Soxtec system HT 1046 Tecator, Sweden; Electric furnace M110 Heraeus, Germany; and Atomic Absorption Spectrophotometer GBC 2000, Australia, respectively.

The oleuropein concentration in the olive leaves was measured by high performance liquid chromatography (HPLC) (Well Chromm 2000, Knauer, Berlin, Germany) using a vertex column [Eurospher 100-5 C18 (250 mm × 4 mm; dp = 3 µm), Knauer, Germany]. Separation was carried out at room temperature with UV-vis detector (Smartline UV Detector K2500, Knauer,

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