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Biochemical, Immunological and Histopathological Alterations in Quail Fed on *Xanthophyllomyces dendrohous*

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Abstract

In the recent years, red yeast (RY) *Xanthophyllomyces dendrohous* have been used for color enhancing, pharmaceutical, and food industries. In search for a new substance which can be used as feed additives to reduced morbidity and mortality due to clinical diseases, the present study was carried out examine the biochemical, immunological and histopathological changes in quail exposed to *Xanthophyllomyces dendrohous*. A total of 360 quail chicks were allocated into 6 groups (60 chicks each) each group were divided into 3 replicates (20 chicks each). The first one served as control (without additives). The second, third, fourth and fifth groups were provided with 4, 8, 12 and 16g RY/kg diet respectively. Whereas, the sixth group exposed to 16g active dry yeast (ADY)/kg diet. Experimental period lasted for 28 days. Liver, renal function tests, lipid profile, total immunoglobulin and total antioxidant capacity were assessed in the serum. Furthermore, histopathological alterations of liver and kidney were investigated. Results revealed that, administration of RY affect all parameters measured in the current study in a concentration dependent manner. This could be attributed to the difference in concentration and profile of carotenoids in the tested product. High inclusion levels of RY in quail diet were seen to elevate within the normal range serum AST, urea, total cholesterol, and triglycerides. No significant differences were found between ADY administrative groups and control in most tested parameters. Furthermore, RY and ADY administration did not show any pathological alteration in the liver and renal tissues. In conclusion, RY in low doses can be safely used in quail diets, but the high doses are not recommended.

Keywords: *Quail, Biochemical, Immunological, Histopathological, Xanthophyllomyces dendrohous.*

Introduction

Poultry industry is one of the fastest growing parts of the agricultural economy and largest animal agriculture sectors. In the past 100 years, it has evolved from backyard household production to sophisticated commercial production units (Bolton, 2015). To complement the existing bird species, poultry industry continues to evolve with the addition of new bird species, such as the Japanese quail which produces high quality dietary protein for human consumption (Khosravi *et al*., 2016). Quail are easily adapting to various rearing conditions, resistant to various diseases and reach sexual maturity at 6 weeks of age wherefore the quail breeding is economically viable (Randall and Bolla, 2008). Quail require to high quality dietary protein

due to the nature of their digestive system. Therefore, the most important challenge in the long-term sustainability of quail production remains the cost of dietary protein (Wickramasuriya *et al*., 2015; Rezaeipour *et al*., 2016).

Antibiotics have been used in past decades to enhance poultry growth performance, health and control pathogens. It can have harmful impact such as the increases of microbial resistance to antibiotics and residues in chicken meat products which might be deleterious to consumers (Diarra *et al*., 2007; and Koc *et al*., 2010). In recent years, numerous studies have examined yeast products as alternatives to antibiotics in poultry diets (Bonos *et al*., 2010; Vahdatpour *et al*., 2011; Aydin and Aydin, 2012; Mousa *et al*., 2014).

Red yeast (*Xanthophyllomyces dendrorhous*) belonging to the Basidiomycetes phylum; naturally produced carotenoid astaxanthin which gives its pigmentation (Schmidt *et al*., 2011). Astaxanthin ($C_{40}H_{52}O_4$), belonging to the family of xanthophyll and is an orange‑red carotenoid (Wang *et al*., 2008), found in marine animals (Johnson *et al*., 1980; Bjerkeng *et al*., 1990). The red color is due to the conjugated double bonds at the center of the compound (Higuera-Ciapara *et al*., 2006). It has both lipophilic and hydrophilic properties and contains conjugated double bonds, hydroxyl and keto groups. It is a fat-soluble compound and because of its anomalous structure it is a potent antioxidant molecule due to the presence of hydroxyl and keto moieties on each ionone ring (Kim *et al*., 2005; Hussein *et al*., 2006; Liu and Osawa, 2007; Sandesh *et al*., 2008). It has some essential biological functions, including enhancing immune response (Jyonouchi *et al*., 1993) and it was shown to significantly effect immune function in several *in vitro* and *in vivo* studies (Rao *et al*., 2010; Rao *et al*., 2013 a and b). It is important for animal feeding, pharmaceuticals, cosmetics and the food industry (Palagyi *et al*., 2001). In marketable production, to enhance the yellowness of poultry products pigments are usually added into poultry feeds (Perez-Vendrell *et al*., 2001).

Astaxanthin has great importance in food, feed, and pharmaceutical applications. The antioxidant properties of astaxanthin are used against oxidative injury caused by several diseases. It has been shown to impact on several diseases including cancers, hypertension, diabetes, cardiovascular, gastrointestinal, liver, neurodegenerative, and skin diseases (Rao *et al*., 2014). Therefore, the target of this work is to evaluate the effect of different levels of *Xanthophyllomyces dendrohous* in quail diets on some biochemical and immunological parameters, in addition to histological examination.

Materials and Methods

This study was carried out at the farm of Animal and Poultry Production, Faculty of Agriculture, Minia University. It was designed to study the effect of red yeast *Xanthophyllomyces dendrorhous* or active dry yeast (ADY) on biochemical, immunological and histological alterations of quail chicks. The chemical analyses were carried out at laboratories of Animal and Poultry Production De-

partment, Faculty of Agriculture, Minia University.

Red yeast isolate and treatment

Xanthophyllomyces dendrorhous (formerly Phaffia) strain: NRRL Y-17269 [VKM Y-2268] supplied by American Type Culture Collection (ATCC) Manassas, VA 20108 USA. Active dry yeast: *Saccharomyces cerevisiae*. Diet was treated with *Xanthophyllomyces dendrorhous* inoculants containing a minimum of 3×10^7 viable cells mL^{-1}.

Experimental design.

A total number of 360 unsexed Japanese quail chicks, at two weeks old, randomly distributed into 6 experimental groups of 60 birds each. Each group contains three replicates of 20 birds each. The birds were housed in an open house in cleaned and fumigated battery cages (1 x 0.6 x 0.4 meter as length, width and height). Feed and water were offered *ad-libitum* during the experimental periods (2-6 weeks of age).

The basal diet contained adequate levels of nutrients for growing quail chicks (25 %CP and 2945 ME/Kg diet) as recommended by the National Research Council, NRC, (1994). Birds of all experimental groups were fed on a commercial basal diet supplemented with or without Red yeast or active dry yeast as follows:-

1-Basal diet, 2-Basal diet supplemented with 4g Red Yeast/kg diet, 3. Basal diet supplemented with 8g Red Yeast/kg diet, 4. Basal diet supplemented with 12 g Red Yeast/kg diet, 5. Basal diet supplemented with 16 g Red Yeast/kg diet, 6. Basal diet supplemented with 16 g ADY/kg diet.

Liver Function Tests (LFTs)

Estimation of liver functions by measuring, the activities of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glucose using diagnostic kits (Vitro, Germany). Also, total protein, albumin, globulin and albumin globulin ratio were determined using commercial kits (Bio-Med, Egypt), where, alanine and aspartate aminotransferases were determined based on the colorimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (Reitman and Frankel, 1957), total protein was determined by the Biuret method (Peters, 1968), albumin by the bromocresol green method (Doumas *et al*., 1971). All analytical testes were done using T80 UV Spectrophotometer UK. Serum globulin concentration and albumin /globulin (A/G) ratio were calculated using the following equations: - Globulin (g/dl) = Total proteins (g/dl) – Albumin (g/dl)

Renal Function Tests (RFTs)

Serum levels of urea, uric acid and creatinine were determined and expressed as mg/dL, using commercially Bio-Med reagent kits Egypt according to (Tietz, 1986 and Tietz and Saunders, 1990).

Lipid profile

Serum triglycerides were determined according to (Stein, 1987) using reagent kits purchased from biodiagonstic chemical company (Egypt). Serum cholesterol was determined according to (Ellefson and Caraway, 1976) using reagent kits purchased from spectrum chemical company (Egypt).

Total antioxidant capacity (TAC)

Total Antioxidant Capacity (TAC) was measured using suitable commercial kits according to guidelines and recommendation of (Trachootham *et al.,* 2008).

Turbidity test for estimation of total immunoglobulins level (Total Ig).

Serum total immunoglobulins level was carried out according to (Mcewan *et al.,* 1970 and Pfeiffer *et al.,* 1977). This technique is a salting out procedure dependent on the biochemical properties of immunoglobulin in relation to characteristics of ZnSO4.

Histopathological examination

Samples from liver and kidney were taken from all chick's groups after slaughtered. They were fixed in 10 % neutral buffer formalin, embedded in paraffin, sectioned at 3 microns and stained with hematoxylin and eosin stain (H&E stain). Then they were examined by light microscopy (Freida, 1990).

Statistical Analysis

Data were statistically analyzed, employing one-way analysis of variance (ANOVA) and Tukey multiple comparison tests, using the GraphPad Prism® 7 software (version 7.04). The results of the comparison between the control and the treatments were considered statistically significant with 95% confidence interval ($P < 0.05$).

Results

Liver function tests:

Serum AST activity of quail exposed to different concentrations of RY for 28 days significantly increased by 9.56, 18.14 and 12.06% at a concentration of 4, 8, 12g RY/kg diet respectively compared to the control serum chicks (Table 1). In addition, opposite trend was observed in ALT activity. RY induced statistically significant ($p < 0.05$) decrease in ALT activity by 9.15 and 10.09% at a concentration of 12 and 16g RY/kg diet respectively compared with the control group (Table 1). While, ADY treatment (16g/kg diet) maintained AST and ALT activities at levels similar to the control group (Table 1). Administration of RY elevated glucose level (mg/dl) in a concentration dependent manner by 25.09, 19.54, 17.37% at a concentration of 4, 12, 16g RY/kg diet respectively (Table 1). Also, ADY exposure significantly ($p<0.0001$) raised glucose level (mg/dl) by 41.27% relative to control group.

Table 1. Average values of liver function tests in quail administered various doses of red yeast (RY) and active dry yeast (ADY).

Treatments	AST	ALT	Glucose (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A / G ratio
control	48.83 ± 0.20	23.18 ± 0.006	79.44 ± 0.15	5.10 ±0.18	3.00 ± 0.15	2.09 ± 0.33	1.43 ± 0.41
RY (4 g/kg)	53.50 ± 2.64*	22.17 ± 0.87	99.37 ± 4.79***	4.72 ± 0.10	3.02 ± 0.18	1.70 ± 0.17	1.83± 0.67
RY (8 g/kg)	57.69 ± 2.35***	23.78 ± 0.68	70.40 ± 0.67	4.36 ± 0.29	3.24 ± 0.11	1.12 ±0.22**	2.96 ± 0.21**
RY (12g/kg)	54.72 ± 0.56**	21.06 ± 0.15*	94.96 ± 0.64**	4.74 ± 0.23	3.06 ± 0.15	1.68 ± 0.35	1.83 ± 0.54
RY (16 g/kg)	47.67 ± 0.81	20.84 ± 0.93*	93.24 ± 2.16**	4.83 ± 0.41	3.12 ± 0.22	1.70 ± 0.19	1.90 ± 0.06
ADY (16 g/kg)	51.17 ± 1.63	22.51 ± 0.88	112.62 ± 7.88****	5.00 ± 0.31	3.15 ± 0.10	1.84 ± 0.29	1.74 ± 0.30

Values are presented as mean ± SD, One-way analysis of variance (ANOVA), and Tukey multiple comparison tests showed statistically significant differences in relation to the control: (*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

The results of total proteins and their fractions in serum of all treated groups are presented in Table 1, which indicated that, globulin level (g/dl) was significantly ($p < 0.01$) decreased by the administration of RY by 46.41% at a concentration of 8g RY/kg diet compared to control group. Likewise, A/G ratio was significantly ($p < 0.01$) increased by the administration of RY by 106.99% at a concentration of 8g RY/kg diet compared to the control group. While ADY treatment maintained total protein and all their fractions similar to the control group (Table 1). No significant ($P < 0.001$) differences were observed in albumin (g/dl) levels between control and other treatments (Table 1).

Renal function tests:

Urea, uric acid and creatinine levels (mg/dl) of all treated groups are indicated in Figure 1A, B and C, RY promoted a significant ($P < 0.05$) increase in urea level of serum chicks by 5.23 % at a concentration of 12g RY/kg diet compared to the control chicks group. In addition, ADY treatment-maintained urea level closed to untreated group (Figure 1A). Moreover, it was found that, no significant differences were observed in uric acid and creatinine (mg/dl) concentrations between control and other treatments (Figure 1 B, C).

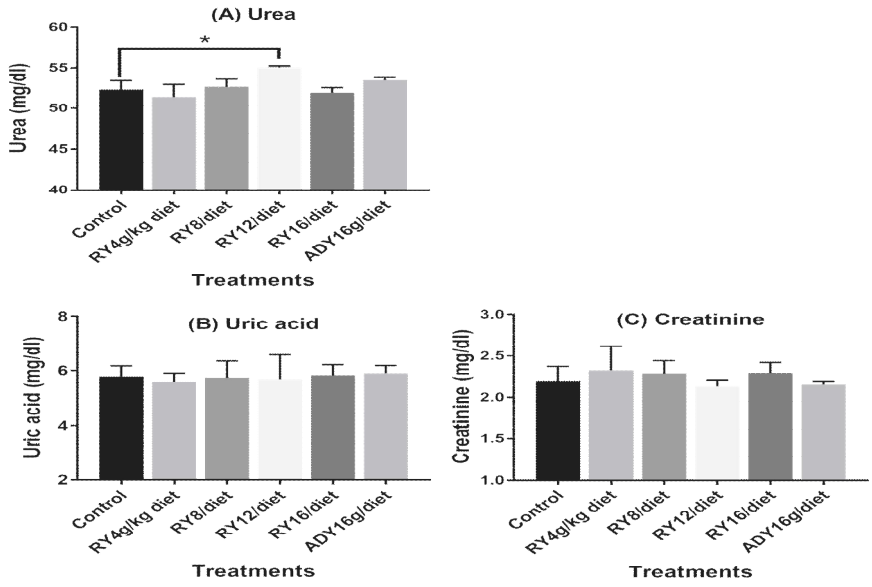

Figure (1): Mean values of urea mg/dl (A), uric acid mg/dl (B) and creatinine mg/dl (C) in serum of quail administered various doses of red yeast (RY) and active dry yeast (ADY) in diet. One-way ANOVA and Tukey multiple comparison tests showed statistically significant differences in relation to the control: (*$P < 0.05$).

Lipid profile:

For lipid profile parameters measured immediately after slaughter, experimental diets significant effects ($P < 0.001$) on serum triglycerides (mg/dl). Highly significant increases in its levels were observed at a concentration of 8, 12 and 16g RY/kg diet by 11.77, 11.00 and 8.63% respectively relative to control (Figure 2A). Also, ADY treatment raised

triglycerides level by 9.17%. Furthermore, RY (8, 12, 16 g/kg diet) promoted a very high significant (P < 0.0001) increase in cholesterol level (mg/dl) by 19.87, 15.79 and 12.45% at a concentration of 8, 12 and 16g RY/kg diet respectively (Figure 2B).

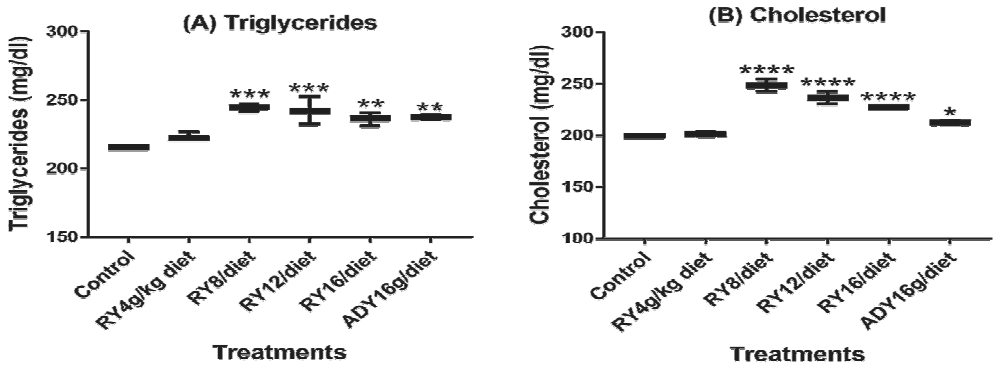

Figure (2): Mean values of triglycerides mg/dl (A) and cholesterol mg/dl (B) in serum of quail administered various doses of red yeast (RY) and active dry yeast (ADY). One-way ANOVA and Tukey multiple comparison tests showed statistically significant differences in relation to the control: (*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

Serum total antioxidant capacity (TAC) and total immunoglobulin(Total Ig) levels

Measurement of TAC level may reflect clinical severity of sepsis. The results of TAC analyses are presented in (Figure 3A). A high significant increase in TAC (P < 0.0001) was observed in treated birds at a concentration of 4, 8 and 16g RY/kg diet by 63.64, 72.42 and 55.56% respectively relative to control birds. In response to an immunogen, plasma cells produce immunoglobulins which are glycoprotein molecules and which function as antibodies. The effects of RY administration on the total Ig level are shown in (Figure 3B). The concentration of total Ig significantly (P < 0.001) decreased by the administration of RY at a concentration of 4, 8 and 12 g/kg in a concentration depended manner by 21.10, 53.36 and 16.41 relative to the control group respectively. No significant effects of ADY on total Ig was detected between control and the treatment group.

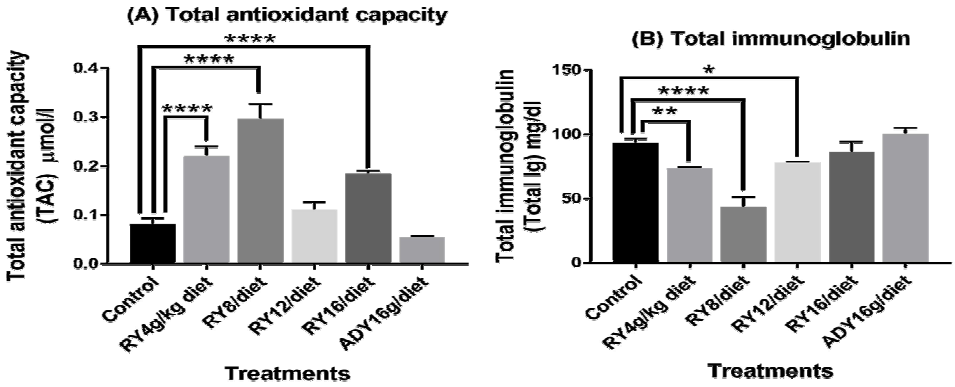

Figure (3): Mean values of total antioxidant capacity (TAC) (A) and total immunoglobulin (Total Ig) mg/dl (B) in serum of quail administered various doses of red yeast (RY) and active dry yeast (ADY) One-way ANOVA and Tukey multiple comparison tests showed statistically significant differences in relation to the control: (*P < 0.05, **P < 0.01, and ****P < 0.0001).

Histopathological alterations

The basic structure of liver sections, stained with H&E stain showed numerous hepatic lobules. The central vein is located in the middle of the lobule. The hepatocytes are polygonal in shape with granulated, eosinophilic cytoplasm and centrally located nuclei with one or two nucleoli and delicate strands of chromatin. Also, Kupffer cells appeared between the hepatocytes as spindle-shaped cells (Fig. 4 A, B, C, D, E and F).

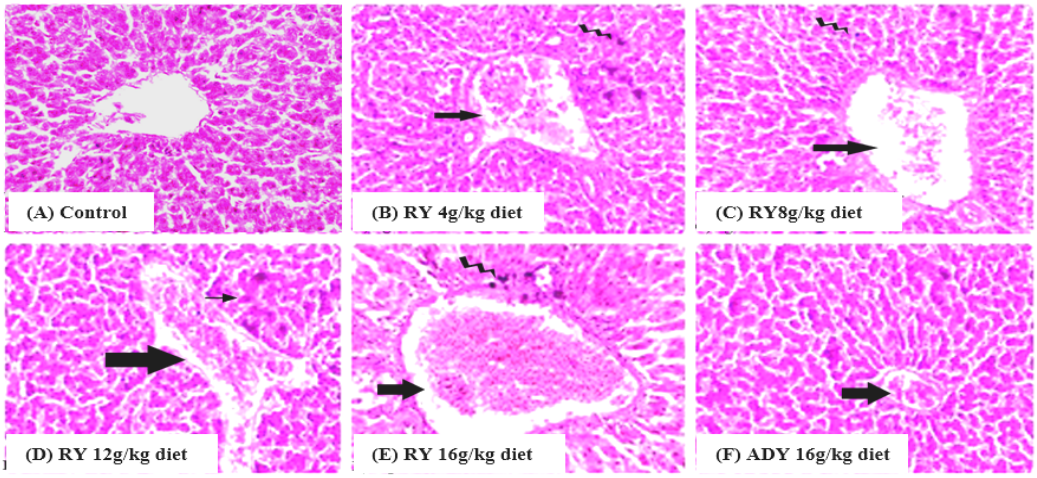

Figure (4): Histopathological examination of quails liver following the exposure of various concentrations of **red yeast (RY) and active dry yeast (ADY) in diet. (H&E 40×).** H&E = Hematoxylin and eosin. (A): Control chicks liver showing normal hepatic structure, (B): Histological section of liver from a red yeast treated quail chicks (4g/kg diet) showing middle congestion in blood vessel (thick arrow), and pyknotic nuclei (zigzag arrow), (C): Histological section of liver from a red yeast treated quail chicks (8g/kg diet) showing mild congestion in blood vessel (thick arrow) and pyknotic nuclei (zigzag arrow), (D): Histological section of liver from a red yeast treated quail chicks (12g/kg diet) showing strong contraction in the central vein associated with congestion in blood vessel (thick arrow) and fibroblast cells (thin arrow), (E): Histological section of liver from a red yeast

treated quail chicks (16g/kg diet) showing dilatation in the central veins associated with congestion in blood vessel (thick arrow) and pyknotic nuclei (zigzag arrow), (F): Histological section of liver from an active dry yeast treated quail chicks (16g/kg diet) showing mild congestion in blood vessel (thick arrow) and normal hepatic structure.

The kidney consists of an outer cortex and an inner medulla. The outer cortex of control and treated quail kidney's, contains the renal corpuscles which appear as large spherical structure and renal tubules (proximal and distal convoluted tubules). Each renal corpuscle is surrounded by the Bowman's capsule composed of simple squamous epithelial cells. It encloses the urinary space and the capillary tuft of the glomerulus which consists of blood capillaries. (Fig. 5 A, B, C, D, E and F).

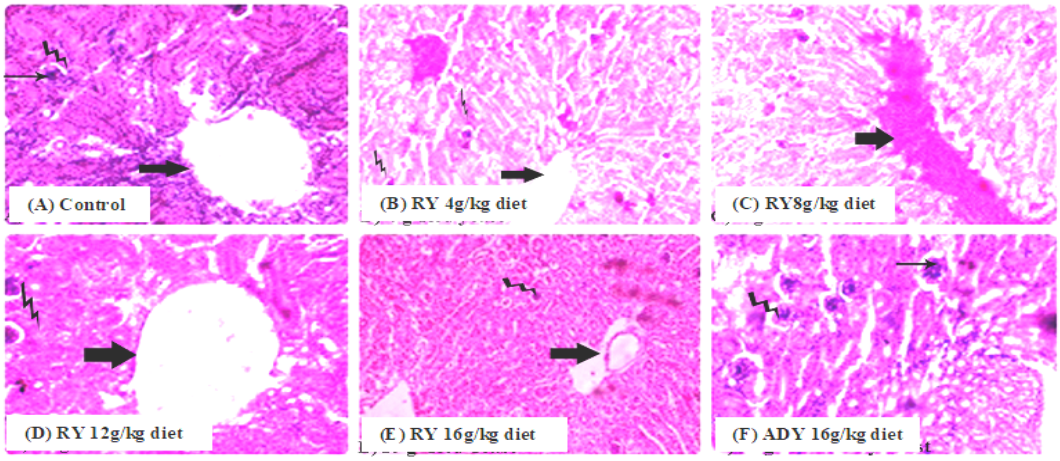

Figure (5): Histopathological examination of quails kidney following the exposure of various concentrations of red yeast (RY) and active dry yeast (ADY) in diet. (H&E 40×). H&E = Hematoxylin and eosin. (A): Control chicks kidney showing normal glomerulus (thin arrow), clear central vein (thick arrow) and normal bowman's capsule (zigzag arrow), (B): Histological section of a kidney from a red yeast treated quail chicks (4g/kg diet) showing clear blood vessel (thick arrow) and normal bowman's capsule (zigzag arrow) with dilated bowman's space, (C): Histological section of a kidney from a red yeast treated quail chicks (8g/kg diet) showing congestion in blood vessel (thick arrow), (D): Histological section of a kidney from a red yeast treated quail chicks (12g/kg diet) showing clear blood vessel (thick arrow) and normal bowman's capsule (zigzag arrow) with dilated bowman's space, (E): Histological section of a kidney from a red yeast treated quail chicks (16g/kg diet) showing clear blood vessel (thick arrow) and normal bowman's capsule (zigzag arrow) with dilated bowman's space, (F): Histological section of a kidney from an active dry yeast treated quail chicks (16g/kg diet) showing normal glomerulus (thin arrows) and bowman's capsule (zigzag arrow)

Discussion

X. dendrorhous produce various carotenoids, including β-carotene, astaxanthin, canthaxanthin, and zeaxanthin (Alvarez *et al.,* 2006). Canthaxanthin is a xanthophyll with orange-red pigment and antioxidant properties which led to its widely used in poultry farming providing the characteristic color to chicken skin and egg yolk (Zhang *et al*., 2011). In the biosynthesis of astaxanthin β-carotene used as an intermediary molecule (Ojima *et al*., 2006). Due to its strong antioxidant properties and provitamin A activity, astaxanthin is widely used in the food, feed, cosmetic, and pharmaceutical industries (Green and Fascetti, 2016). It is used as a feed additive in chicken, quail farming and egg production (Higuera-Ciapara *et al*.,2006; Breithaupt, 2008). It is well known that, inclusion ADY in poultry diets maintains normal intestinal microflora, alters metabolism by increasing digestive enzyme activity, improves digestion, and stimulates the immune system (Kabir, 2009). Although not many studies have been carried out with red yeast and quail, some positive results have been observed in the growth performance in broiler chickens (Perenlel *et al*., 2014). In this trend, this study was carried out investigate to what extent can *X. dendrorhous* be safely used in the diet of quail chicks. Furthermore, ADY was used in this study as one of the most common yeast used in the poultry diets to compare its effects with *X. dendrorhous.*

Determination of some serum biochemical parameters of quail is essential to evaluate the effectiveness of diets on different functions of the liver that is, hepatocellular integrity (transaminases), stores and produces sugar (glucose level) and protein synthesis (total proteins and their fractions). In this study, the various inclusion levels of RY in diet were seen to elevate AST and reduce ALT activities within the normal range (AST: 23.85 – 90.70 IU/L, ALT: 10.17-38.51 IU/L), which could be a result of higher amount of carotenoid levels produced by *X. dendrorhous* in the diet (Alvarez *et al*., 2006).

It is well known that, due to the dysfunction of pancreatic β-cells and tissue damage, oxidative stress induced by hyperglycemia. The oxidative stress caused by hyperglycemia in pancreatic β-cells could reduce by astaxanthin which produces by *X. dendrorhous* and astaxanthin also improve serum glucose levels **(**Uchiyama *et al*., 2002). This has been confirmed in the present study by increasing in the serum concentrations of glucose (mg/dl) (less than normal range 182.51-194.51 mg/dl) (Table 1) after RY and ADY administration respectively.

The clinical investigations of renal functions such as serum urea, creatinine and uric acid level are important to identify the renal dysfunctions (Yadav *et al*., 2014). In the current investigation, it was found that, no significant alterations were observed in serum uric acid and creatinine levels after RY and ADY administration respectively. The end product of amino acid catabolism, produced by liver is urea. An increase in blood urea may be associated with kidney disease (Mitchell and Kline, 2006). In this study serum urea con-

centration (mg/dl) was raised within the normal range from 52.32 to 55.06 (mg/dl) after administration with RY (12g/kg diet). This confirmed that, a higher concentration does of RY in quail's diet is not recommended.

The hyperlypidimic action has been noticed on the levels of total cholesterol and triglycerides in serum in some groups supplemented with RY and ADY. In the present study, dietary supplementation at concentrations 8, 12 and 16g /kg diet of RY and 16g /kg diet of ADY in quail chickens was found to cause a significant ($p<0.001$) increase in the mean values of total cholesterol and triglycerides as compared to control birds. This could be attributed to the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6-phosphatase dehydrogenase (Queshi *et al*., 1983).

Red yeast produces many pigments which have antioxidant activities in particular astaxanthin. It provides protection against free radical damage to preserve immune-system defenses (Ambati *et al*., 2014). In mouse model it showed higher immuno-modulating effects (Jyonouchi *et al*., 1991). After dietary supplementation of astaxanthin decreased humoral immune response was reported (Jyonouchi *et al*., 1991, 1994). The results of the current study are in accordance with the previous studies which revealed that, dietary supplementation of RY was found to cause a very highly significant ($P < 0.0001$) increase in TAC at a concentration of 4, 8 and 16g RY/kg diet and decrease humoral immune response by decreasing the total Ig level at a concentration of 4, 8 and 12g RY/kg. The variation in response to inclusion of RY could be attributed to the difference in concentration and profile of carotenoids.

Histologically, administration of high dose RY (12g/kg diet) caused mild liver and kidney injuries including white blood cells infiltrations with fibroblast cells in the liver and congested blood vesicles in the kidney. ADY administration did not show any pathological alteration in the liver tissue, glomerulus and renal tubules.

In summary, RY can be safely used as a feed supplement in quail to enhance their antioxidant status and immune response. In addition, dietary supplementation with RY at high levels in quail diet cause minor changes in some blood biochemical parameters including liver and renal function testes. Also, high dose of RY administration caused slight liver and kidney damage whereas, ADY did not show any pathological alteration in the liver and renal tissues. Future studies may be recommended to investigate the possibility of RY cultural filtrate inclusion as a supplement in the drinking water during periods of stress. Conclusively, RY can be used to improve the quality of quail chickens. However, high doses (more than 8g/kg diet) are not recommended.

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التغيرات البيوكيميائية والمناعية والهستوباثولوجيه في السمان المغذى على الخميرة الحمراء

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الملخص

استخدمت الخميرة الحمراء في السنوات الأخيرة لتعزيز اللون وفي الصناعات الصيدلانية بالإضافة الي الصناعات الغذائية. بحثا عن مادة جديدة يمكن استخدامها كأحد الإضافات الغذائية في علائق الدواجن لتقليل نسبه انتشار الامراض ونسبة النفوق نتيجة للإصابة بالأمراض. أجريت هذه الدراسة لفحص التغيرات البيوكيميائية والمناعية والهستوباثولوجية في طيور السمان التي تم تعريضها للخميرة الحمراء. تم تقسيم ٣٦٠ كتكوت من طيور السمان عند عمر أسبوعين عشوائيا الي ٦ مجموعات (٦٠ كتكوت لكل مجموعة) وتم تقسيم كل مجموعه الي ٣ مكررات (٢٠ كتكوت لكل مكررة). استخدمت المجموعة الاولي كمجموعة ضابطة. أما المجموعة الثانية والثالثة والرابعة بالإضافة الي المجموعة الخامسة فقد تمت معاملتهم بالخميرة الحمراء بتركيزات ٤، ٨، ١٢، ١٦ جم/ كجم عليقة على التوالي. بينما تم تعريض المجموعة السادسة للخميرة الجافة النشطة بتركيز ١٦ جم / كجم عليقة. ولقد استمرت فترة اجراء التجربة لمدة ٢٨ يوم. وتم تقدير وظائف الكبد والكلي، مستوي الدهون، الاجسام المضادة الكلية بالإضافة الي القدرة الكلية المضادة للأكسدة في سيرم دم الطيور التي استخدمت في التجربة. بالإضافة الي ما سبق فقد تم فحص التغيرات النسيجية التي حدثت في كل من الكبد والكلي. ولقد أظهرت نتائج هذه الدراسة أن المعاملة بالخميرة الحمراء تؤثر على جميع القياسات التي تم تقديرها من خلال هذه الدراسة بطريقة تعتمد على التركيز. وقد يرجع السبب في ذلك الي الاختلاف في نوع وكمية الكاروتينيدات الموجودة في كل تركيز من تركيزات الخميرة الحمراء المختبرة. ولقد وجد ان إدراج تركيزات عالية من الخميرة الحمراء في علائق السمان قد أدي الي ارتفاع مستوي انزيم AST، اليوريا، الكوليسترول الكلي بالإضافة الي الجلسريدات الثلاثية ضمن المعدل الطبيعي، الا انه لم يظهر أي اختلافات معنوية بين المجموعة المعاملة بالخميرة الجافة النشطة والمجموعة الضابطة في معظم القياسات التي تم تقديرها. علاوة على ذلك فلم تظهر المعاملة بكل من الخميرة الحمراء والخميرة الجافة النشطة أي تغيرات مرضية في انسجة كل من الكبد والكلي. وختاما يمكن ان نخلص الي انه يمكن بأمان استخدام الخميرة الحمراء بجرعات منخفضة في علائق السمان الا انه لا ينصح باستخدام الجرعات العالية.