

## Evaluation of *Yucca schidigera* extracts supplementation on immunological response to Newcastle vaccination antioxidant enzymes, cholesterol levels and egg quality in laying hens

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### Abstract

The aim of this study was to evaluate the effect of *Yucca schidigera* extracts (YSE) on performance, egg quality, blood profile, antioxidant status and immune response to Newcastle disease vaccination in chickens. A total of 144 commercial Shaver laying hens aged 45 weeks-old were assigned to 6 dietary treatments and were supplemented with 0.5 or 1 ml /L of YSE until 49 weeks of age. The results obtained in this experiment showed there were no significant differences in live body weight, feed consumption, feed efficiency or egg production due to YSE supplementation while egg weight and egg mass significantly increased with YSE supplementation. Also there were increase ( $P < 0.05$ ) in yolk percent and yolk-to-albumen ratio and decrease in albumen per-cent compared with the non-supplemented group. Serum constituents (total cholesterol, LDL-cholesterol, albumin, immunoglobulin (IgG) and (IgM)), Zn-superoxide dismutase (SOD1), reduced glutathione peroxidase (GSH-Px) ) and egg cholesterol were significantly ( $P < 0.05$ ) influenced by YSE supplementation, while total protein, triglycerides, HDL-cholesterol, malondialdehyde (MDA) were nonsignificantly ( $P < 0.05$ ) influenced. The antibody titer against Newcastle disease virus was significantly higher in the YSE Supplementation groups. In addition, 0.5 ml/L treatment mainly acted on immunity and anti-oxidation whereas 1 ml/L treatment mainly improved egg weight and egg mass. In conclusion, YSE can be used as a feed additive due to its capability to improve performance, immune response and antioxidative function in layers.

**Keywords:** *Yucca schidigera*, layers, egg quality, antioxidant, immunity, new castle

### Introduction

*Yucca schidigera* (YS), a plant native to south-western United States and northern Mexico, is regarded highly for its pharmaceutical values due to the presence of steroidal saponins and polyphenols (Cheeke *et al.* 2006; Patel, 2012). Their surfactant properties may reduce the surface tension around cell membranes and this may aid nutrient absorption (Ryan and Quinn, 1999). Saponins could improve animal performance, increase antioxidant capacity, anti-tumor, reduce cholesterol, improve immunity and other useful biological functions (Gumus and MiK, 2012).

*Yucca* supplementation in diet is effective in improving egg production, egg mass and shell thickness in laying hens (Alagawany *et al.*, 2016). However, other researches noted that YSE to the layers did not affect egg production, albumin and yolk index, shape index, Haugh unit and shell thickness but reduced egg's specific gravity and number of cracked eggs (Ayasan *et al.*, 2005, Gurbuz *et al.*, 2011, Guclu, 2003 and Kutlu *et al.*, 2001). Egg yolk cholesterol and triglycerides were significantly reduced by dietary saponin supplementation (Afrose *et al.*, 2010, Yu *et al.*, 2011, Deng *et al.*, 2012, and Fan *et al.*, 2018). In recent decades, increasing attention has been

paid to new natural agents with lipid-reducing activity (**Wu *et al.*, 2009**). Saponin and bile acids interaction in the gut leads to formation of large mixed micelles which promotes increased cholesterol excretion (**Oakenfull, 1986**) and finally results in reduction of serum cholesterol level. The hypocholesterolemic activity of saponins is also due to delaying of intestinal absorption of dietary fat by inhibiting pancreatic lipase activity (**Han *et al.*, 2000**). Also, **Gaurav (2015)** and **Chaudhary (2017)** reported that serum total cholesterol level was significantly decreased and the HDL-cholesterol was significantly increased following supplementation of saponin rich feed additives.

Oxidation is a chemical reaction produces free radicals that damage the cells and manifest as adverse biological effects. Superoxide dismutase (SOD1), glutathione peroxidase (GSH-Px) and catalase (CAT) are the main antioxidant enzymes in the body, contributing to the antioxidant activity. Oxidative stress can cause diseases such as cystic fibrosis and pulmonary hypertension syndrome in poultry, resulting in chicken meat with unpleasant odors and loss in flavor, texture, consistency, appearance, and nutritional value (**Iqbal *et al.*, 2001**; **Fellenberg and Speisky, 2006**). Some studies have showed the potential of *Yucca* as a source of antioxidants (**Sobia *et al.*, 2013**, **Sun *et al.*, 2018**). Saponin promoted antioxidant activity in the body to scavenge free radicals and prevent the action of lipid peroxidation (**Shi *et al.*, 2014**). Resveratrol and Yuccaols which possess biological functions were identified in YS besides steroidal saponins (**Patel, 2012**). Resveratrol is well known to be an effective scavenger of hydroxyl, superoxide radicals. It also protects cell from lipid peroxidation in membranes and DNA damage caused by reactive oxygen species (ROS) (**Leonard *et al.*, 2003**). Phenolic constituents such as Yuccaols in YS which structurally related to resveratrol, also possess radical scavenging activity (**Piacente *et al.*, 2004**, **Patel, 2012**). YSE supplementation improved SOD and reduced glutathione (GSH) level, and reduced MDA concentration in serum of laying hens (**Alagawany *et al.*, 2016**).

Another useful effect of YSE is to reduce ammonia concentration and fecal odors (**Cheeke, 2000**). Ammonia, a bacterial breakdown product of uric acid is the most noxious gas in poultry houses. Poor management practices and wet litter are the predisposing factors, favoring the continual release of ammonia from the litter. YSE has been reported to reduce atmospheric ammonia in poultry farms by inhibiting urease enzyme activity (**Ayasan *et al.*, 2005**, **Piacente *et al.*, 2005** and **Ayasan, 2013**).

Dietary supplementation of YSE improved immunity in layers (**Alagawany *et al.*, 2016**). The effectiveness of YSE towards NDV has been attributed to the presence of saponin components. Saponins are capable of stimulating immune system and thereby enhancing resistance to the diseases (**Cheeke, 2001**). **Gurbuz *et al.* (2011)** observed higher antibody titers of NDV with the combination of YSE and yeast cell walls in layer hens. Also in broiler chickens, **Sahoo *et al.* (2015)** and **Sun *et al.* (2018)** reported that antibody titers against Newcastle disease virus was significantly higher in the YSE treated group after ND vaccination.

### Materials and Methods

***Yucca schidigera* extract:** YSE is a component of a commercial product (Ultratural Plus<sup>®</sup>, Santufo Corporation, Mississauga, Canada). Each 1 liter contains: YSE: 200 mg (saponin 10 gm), Seaweed extract: 1%, Enzymes, Mannan oligosaccharides 10 gm, citric acid (98%) 10 gm, sodium benzoate 5 gm and purified water up to 1 liter.

### Experimental design:

A total of 144 commercial Shaver brown laying hens aged 43 weeks were supplied from a local layer farm and randomly divided into six dietary treatment groups. A completely randomized design was used, with six replications of four hens each; four birds were housed per (50×50×45 cm) wire pen with individual feed-troughs with a common water-trough, and the wire cages were placed in a clean and open-sided house. Room temperature was kept at 21°C, and the light program consisted of 16 h light daily throughout the experiment. Before the experiment, the birds were fed with a bal-

anced basic diet for two weeks to allow them to adapt most and not affect the rate of egg production. Diets were formulated to meet nutrients recommendation of Shaver management guide which met or exceeded the NRC (1994) recommendations. The duration of experimental period was 4 weeks, from 45 to 49 weeks old. Treatments were as follows:

**Group 1 (G1):** Non vaccinated and non-treated.

**Group 2 (G2):** vaccinated using LaSota (Ornipest<sup>®</sup>, Bioveta, Komenského-Czech Republic) by eye drop and non-treated.

**Group 3 (G3):** vaccinated as G2 and treated with YSE 0.5 ml/L for three successive days and repeated after two weeks for another three days.

**Group 4 (G4):** Non vaccinated treated with YSE 0.5 ml/L for three successive days and repeated after two weeks for another three days.

**Group 5 (G5):** vaccinated as G2 and G3 and treated with YSE 1 ml/L for three successive days and repeated after two weeks for another three days.

**Group 6 (G6):** Non vaccinated treated with YSE 1 ml/L for three successive days and repeated after two weeks for another three days.

#### **Egg production and egg quality criteria:**

Eggs from each replicate were collected and weighed at the same time every day to calculate hen-day egg production, egg weight and egg mass. Feed consumption was recorded daily and calculated as g per day per bird. The value of feed efficiency was calculated as g feed per g egg. Egg components weekly were determined using four eggs from each replicate. Eggs were weighed, and then egg length and width were determined before breaking. The egg was carefully broken on a glass plate (35×25 cm) to measure both external and internal egg quality characteristics. Yolk was separated from albumen, and egg shell was cleaned of any adhering albumen. Albumen weight was calculated by subtracting yolk weight and shell weight from the whole egg weight. Egg shape indices were calculated as the ratio of egg width to the length (Awosanya *et al.*, 1998). Four eggs were collected randomly from each replicate every 15 days and the yolk Cholesterol levels were analyzed. Yolk samples were made to saponification and detection using

high-performance liquid chromatography (Zhang *et al.*, 1999).

#### **Blood sampling and biochemical analysis:**

Blood samples were randomly collected from three birds per each treatment from wing vein into sterilized tubes that closed with rubber stoppers. Samples were let to coagulate and centrifuged at 3500 rpm for 15 min to obtain serum, and the serum samples were kept in Eppendorf tubes at -20 °C until analyzed. The following serum biochemical parameters, total protein (g/dl), albumin (g/dl), triglyceride (mg/dl), total cholesterol (mg/dl), low-density lipoprotein (LDL) (mg/dl), high density lipoprotein (HDL) (mg/dl), immunoglobulins G (IgG), M (IgM), and A (IgA) levels were estimated in serum using commercial bio-diagnostic kits provided from Bio-diagnostic Company (Dokki, Giza, Egypt) and a spectrophotometer (Shimadzu, Japan) (Akiba *et al.*, 1982). For antioxidant parameters, serum samples were subjected to the measurement of Zn-superoxide dismutase (SOD1) activity and levels of reduced glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) by spectrophotometer (Shimadzu, Japan). The activity of SOD1 was measured by the xanthine oxidase method, which monitors the inhibition of reduction of nitro blue tetrazolium by the sample (Winterbourn *et al.*, 1975). The level of GSH-Px was determined according to Beutler *et al.* (1963). The concentration of MDA was analyzed by the spectrophotometer (Jensen *et al.*, 1997).

#### **HI Test:**

Antibody titers against ND Vaccine was measured by haemagglutination-inhibition test according to Jahanian (2009), and results were expressed as log<sub>2</sub> of the reciprocal of the last dilution. The used antigen was

#### **Statistical analysis**

Data in the tables are presented as arithmetic means and standard error of means (SEM). The data were analyzed by SPSS 11.00 software for Windows (SPSS, Chicago, IL, USA). The differences between groups were determined by one-way ANOVA test. Duncan's multiple-range tests were performed. Linear and quadratic effects were also tested. The level of sta-

tistical significance was set at  $p < 0.05$ .

### Results

The effect of YSE supplementation on the performance of laying hens during the experimental period is shown in table,1. There were no significant differences ( $P < 0.05$ ) in final body weight (FBW), feed conversion ratio (FCR) and hen housed average egg produced due to YSE treatments throughout the experimental period,. While egg weight and egg mass in the YSE supplemented groups were significantly ( $P < 0.05$ ) increased compared with control group. Also the higher dose of YSE (1 ml / L) resulted in higher rate significantly ( $P < 0.05$ ) and non-significantly ( $P > 0.05$ ) than the lower dose 0.5 ml /L regarding egg weight and egg mass respectively.

Addition of YSE to laying hen significantly increased the yolk percent and yolk-to-albumen ratio and decreased the albumen percent ( $P < 0.05$ ) compared with the non-supplemented groups. Egg shell percentage and shape index were not significantly affected ( $P > 0.05$ ) by YSE treatments (table, 2).

Lipid profile showed that total cholesterol and LDL-cholesterol concentrations were significantly reduced ( $P < 0.05$ ) by addition of YSE compared with control group, while there were no significant differences ( $P < 0.05$ ) in HDL-cholesterol and triglyceride concentrations between treated and control groups. There were differences ( $P < 0.05$ ) in yolk cholesterol content among treatment treated and control group. At 49 week groups supplemented with 1ml/L shows higher significant reduction in yolk cholesterol level than groups supplemented with 0.5ml/L of YSE (table 3).

There were no significant differences ( $P > 0.05$ ) were detected in the concentrations of serum total protein and serum albumin, among treated and control groups. Serum IgM and IgG levels were significantly higher ( $P < 0.05$ ) in treated groups compared to the control. IgM level in groups vaccinated and treated (G3 & G5) tended to be higher ( $P < 0.05$ ) than the treated non vaccinated groups (G4 & G6) . The SOD1 and GSH-Px levels were upgraded in treated group than the control ( $P < 0.05$ ). Groups supple-

mented with 0.5 ml of YSE treatment showed significant increase ( $P < 0.05$ ) in GSH-Px levels than groups supplemented with 1ml of YSE treatment. There were no significant differences in the concentration of MDA among all the treated groups and control (table 4).

The result of HI test showed that, antibody titers against ND were significantly increased ( $P < 0.05$ ) in groups vaccinated and supplemented with YSE compared (G3 & G5) at 7, 14, 21 and 28 days post vaccination and in vaccinated non-treated G2 and than in non-vaccinated (G1) and non-vaccinated and supplemented with YSE (G4 & G6). At 14, 21 and 28 days post vaccination there were significant increase ( $P < 0.05$ ) in antibody titers against ND in group vaccinated and supplemented with 0.5 ml YSE (G3) than in group vaccinated and supplemented with 1ml YSE (table, 5).

**Table (1).** Effects of YSE supplementation on productive performance traits of laying hens from 45 to 49 weeks of age

Parameters	Experimental chicken groups					
	G1	G2	G3	G4	G5	G6
<b>Egg weight (g)</b>						
45 weeks	61.33±0.088 <sup>a</sup>	61.27±0.119 <sup>a</sup>	61.56±0.170 <sup>a</sup>	61.35±0.115 <sup>a</sup>	61.35±0.104 <sup>a</sup>	61.36±0.133 <sup>a</sup>
47 weeks	61.98±0.133 <sup>c</sup>	61.93±0.088 <sup>c</sup>	62.77±0.318 <sup>b</sup>	63.15±0.132 <sup>b</sup>	63.88±0.106 <sup>a</sup>	63.63±0.088 <sup>a</sup>
49 weeks	62.23±0.058 <sup>c</sup>	62.28±0.105 <sup>c</sup>	63.33±0.209 <sup>b</sup>	63.50 ±0.104 <sup>b</sup>	63.96 ±0.212 <sup>a</sup>	63.87±0.153 <sup>a</sup>
<b>Egg mass (g)</b>						
45 weeks	55.85±0.21 <sup>a</sup>	55.67±0.305 <sup>a</sup>	65.15±0.3 11 <sup>a</sup>	56.07±0.123 <sup>a</sup>	55.09±0.214 <sup>a</sup>	65.02±0.123 <sup>a</sup>
47 weeks	56.60±0.258 <sup>c</sup>	56.72±0.214 <sup>c</sup>	57.53±0.220 <sup>cb</sup>	57.67±0.388 <sup>ab</sup>	57.71±0.332 <sup>ab</sup>	57.61±0.208 <sup>a</sup>
49 weeks	56.28±0.370 <sup>b</sup>	56.47±0.565 <sup>b</sup>	58.38±0.021 <sup>a</sup>	57.96±0.235 <sup>a</sup>	58.31±0.335 <sup>a</sup>	58.35±0.322 <sup>a</sup>
<b>Hen housed average</b>						
45 weeks	91.07±0.343 <sup>a</sup>	90.87±0.526 <sup>a</sup>	91.27±0.523 <sup>a</sup>	91.47±0.200 <sup>a</sup>	91.07±0.344 <sup>a</sup>	91.07 ±0.344 <sup>a</sup>
47 weeks	90.99±0.415 <sup>a</sup>	91.07±343 <sup>a</sup>	91.07±0.687 <sup>a</sup>	91.27±0.399 <sup>a</sup>	90.67±0526 <sup>a</sup>	91.65±0.326 <sup>a</sup>
49 weeks	90.48±0.344 <sup>b</sup>	90.68±532 <sup>ab</sup>	92.04±0.191 <sup>a</sup>	91.62±0343 <sup>ab</sup>	91.27±0.523 <sup>ab</sup>	91.45±0.508 <sup>ab</sup>
<b>Daily feed intake (g)</b>						
45 weeks	106±0.577 <sup>a</sup>	106.67±0.882 <sup>a</sup>	107±1.453 <sup>a</sup>	106.67±1.453 <sup>a</sup>	107.±1.155 <sup>a</sup>	107±1.212 <sup>a</sup>
47 weeks	105.67±0.882 <sup>a</sup>	106.33±0.882 <sup>a</sup>	106.33±0.882 <sup>a</sup>	106.67±0.882 <sup>a</sup>	107.33±1.202 <sup>a</sup>	106.33±0.882 <sup>a</sup>
49 weeks	106.33±1.202 <sup>a</sup>	105.67±0882 <sup>a</sup>	105.67±1.201 <sup>a</sup>	104.67±1.453 <sup>a</sup>	105.33±0.882 <sup>a</sup>	105±0.577 <sup>a</sup>
<b>Feed conversion ratio (g feed/g egg)</b>						
45 weeks	1.73±008 <sup>a</sup>	1.74±0.015 <sup>a</sup>	1.77±0.01 <sup>a</sup>	1.74±0.233 <sup>a</sup>	1.74±0.020 <sup>a</sup>	1.75±0.18 <sup>a</sup>
47 weeks	1.7±0.015 <sup>a</sup>	1.71±0.012 <sup>a</sup>	1.69±0.012 <sup>a</sup>	1.70±0.015 <sup>a</sup>	1.68±0.022 <sup>a</sup>	1.67±0.015 <sup>a</sup>
49 week	1.7±0.015 <sup>a</sup>	1.7±0.012 <sup>a</sup>	1.66±0.019 <sup>a</sup>	1.68±0.015 <sup>a</sup>	1.65±0.015 <sup>a</sup>	1.66±0.008 <sup>a</sup>
<b>body weight (g)</b>						
Initial (45 weeks)	1662±1.202 <sup>a</sup>	1661.5±0.577 <sup>a</sup>	1665±0.882 <sup>a</sup>	1664±0.577 <sup>a</sup>	1662±1.202 <sup>a</sup>	1662±0.577 <sup>a</sup>
Final (49 weeks)	1885±1.202 <sup>a</sup>	1784±0.860 <sup>a</sup>	1788±0.880 <sup>a</sup>	1785±1.520 <sup>a</sup>	1787±0.845 <sup>a</sup>	1789±0.202 <sup>a</sup>

\*Values are expressed as means ± standard error of the mean.

a, b, c Means with different superscripts, within row, are significantly differ ( $P < 0.05$ ).

**Table (2).** Effects of YSE supplementation on egg quality criteria of laying hens

Parameters (at 49 weeks)	Experimental chicken groups					
	G1	G2	G3	G4	G5	G6
<b>Albumen %</b>	66.30±196 <sup>a</sup>	66.20±062 <sup>a</sup>	63.43 ±233 <sup>b</sup>	63.50±0.173 <sup>b</sup>	63.45 ±0.202 <sup>b</sup>	63.56±0.010 <sup>b</sup>
<b>Yolk%</b>	24.42±0.102 <sup>b</sup>	24. 47±0.008 <sup>b</sup>	26.90±0.028 <sup>a</sup>	26.87±0.049 <sup>a</sup>	26.85 ±0.060 <sup>a</sup>	26.75 ±0.068 <sup>a</sup>
<b>Yolk/albumen %</b>	0.37±0.012 <sup>b</sup>	0.38±0.011 <sup>b</sup>	0.43±0.005 <sup>a</sup>	0.43±0.005 <sup>a</sup>	0.45±0.010 <sup>a</sup>	0.43±0.010 <sup>a</sup>
<b>Shell%</b>	9.67 ±0.015 <sup>a</sup>	9.68±0.017 <sup>a</sup>	9.73±0.0152 <sup>a</sup>	9.72±0.115 <sup>a</sup>	9.74±0.0112 <sup>a</sup>	9.70 ±0.012 <sup>a</sup>
<b>Shape index</b>	78.33±0.152 <sup>a</sup>	78.39±0.088 <sup>a</sup>	78.36±0.135 <sup>a</sup>	78.32±0.080 <sup>a</sup>	78.36±0.151 <sup>a</sup>	78.36±0.115 <sup>a</sup>

\*Values are expressed as means ± standard error of the mean.

a, b, c Means with different superscripts, within row, are significantly differ ( $P < 0.05$ ).



**Table (3).** Effects of YSE supplementation on serum lipid profile, immunoglobulins and egg yolk cholesterol level of laying hens

Parameters		Experimental chicken groups					
		G1	G2	G3	G4	G5	G6
Serum	total protein (g/dl) 47 weeks 49 weeks	4.5±0.005 <sup>a</sup> 4.53±0.02 <sup>a</sup>	4.52±0.005 <sup>a</sup> 4.50±0.00 <sup>a</sup>	4.53±0.173 <sup>a</sup> 4.55±0.020 <sup>a</sup>	4.51±0.011 <sup>a</sup> 4.50±0.00 <sup>a</sup>	4.55±0.011 <sup>a</sup> 4.55±0.01 <sup>a</sup>	4.54±0.00 <sup>a</sup> 4.53±0.023 <sup>a</sup>
	Albumin (g/dl) 47 weeks 49 weeks	2.34±0.035 <sup>b</sup> 2.36±0.017 <sup>b</sup>	2.32 ±0.020 <sup>b</sup> 2.33±0.013 <sup>b</sup>	2.49±0.036 <sup>a</sup> 2.76±0.012 <sup>a</sup>	2.47±0.01 <sup>a</sup> 2.73±0.010 <sup>a</sup>	2.46±0.015 <sup>a</sup> 2.75±0.017 <sup>a</sup>	2.47±0.010 <sup>a</sup> 2.74 ±0.006 <sup>a</sup>
	Triglyceride (mg/dl) 47 weeks 49 weeks	179±0.578 <sup>a</sup> 170.7±0.333 <sup>a</sup>	181±0.077 <sup>ab</sup> 171±0.540 <sup>a</sup>	175.3±1.202 <sup>ab</sup> 169±0.577 <sup>a</sup>	174.7±1.764 <sup>a</sup> 168±0.557 <sup>a</sup>	175±1.155 <sup>a</sup> 170±0.576 <sup>a</sup>	176.3±1.764 <sup>a</sup> 168±0.607 <sup>a</sup>
	Total Cholesterol (mg/dl) 47 weeks 49 weeks	165.2±1.155 <sup>b</sup> 160±1.150 <sup>b</sup>	163.7±0.882 <sup>b</sup> 160.7±0.667 <sup>b</sup>	138.7±0.923 <sup>a</sup> 130.3±1.453 <sup>a</sup>	141.7±445 <sup>a</sup> 131.3±1.202 <sup>a</sup>	144.3±0.880 <sup>a</sup> 134.3±1.202 <sup>a</sup>	142±1.110 <sup>a</sup> 131.7±1.230 <sup>a</sup>
	HDL (mg/dl) 47 weeks 49 weeks	85.67±0.882 <sup>a</sup> 80.33±0.761 <sup>a</sup>	84±1.528 <sup>a</sup> 80.33±0.87 <sup>a</sup>	85±0.882 <sup>a</sup> 82±0.577 <sup>a</sup>	88±0.577 <sup>a</sup> 82.67±0.667 <sup>a</sup>	85.2±1.115 <sup>a</sup> 80.67±0.667 <sup>a</sup>	86±1.764 <sup>a</sup> 81.67±1.455 <sup>a</sup>
	LDL (mg/dl) 47 weeks 49 weeks	62±0.576 <sup>b</sup> 49.67±0.880 <sup>b</sup>	62.33±1.764 <sup>b</sup> 50.33±1.202 <sup>b</sup>	52.33±0.867 <sup>a</sup> 41±0.563 <sup>a</sup>	51.33±0861 <sup>a</sup> 43±0.577 <sup>a</sup>	53.67±0.333 <sup>a</sup> 44±0.577 <sup>a</sup>	54±1.155 <sup>a</sup> 44±0.882 <sup>a</sup>
	IgG (mg/dl) 47 weeks 49 weeks	1.26±0.015 <sup>c</sup> 1.19±0.007 <sup>c</sup>	1.23±0.00 <sup>c</sup> 1.22±0.011 <sup>c</sup>	1.89±0.008 <sup>a</sup> 1.75±0.018 <sup>a</sup>	1.83±0.001 <sup>b</sup> 1.67±0.007 <sup>b</sup>	1.87±0.009 <sup>a</sup> 1.72±0.012 <sup>a</sup>	1.80±0.008 <sup>b</sup> 1.64±0.008 <sup>b</sup>
	IgM(mg/dl) 47 weeks 49 weeks	12.34±0.058 <sup>b</sup> 12.01±0.10 <sup>b</sup>	12.21±0.02 <sup>b</sup> 12.06±0.070 <sup>b</sup>	15.71±0.067 <sup>a</sup> 14.89±0.035 <sup>a</sup>	15.63±0.006 <sup>a</sup> 14.75±0.008 <sup>a</sup>	15.69±0.023 <sup>a</sup> 14.84±0.012 <sup>a</sup>	15.6±0.012 <sup>a</sup> 15.70±0.026 <sup>a</sup>
	Yolk cholesterol (mg/gm) 47 weeks 49 weeks	13.9±0.020 <sup>b</sup> 12.34±0.008 <sup>a</sup>	13.14±0.014 <sup>b</sup> 12.34±0.008 <sup>a</sup>	10.84±0.046 <sup>a</sup> 9.22±0.012 <sup>b</sup>	10.80±0.014 <sup>a</sup> 9.26±0.00 <sup>b</sup>	10.79±0.015 <sup>a</sup> 8.88±0.026 <sup>c</sup>	10.76±0.015 <sup>a</sup> 8.84±0.017 <sup>c</sup>

\*Values are expressed as means ± standard error of the mean.  
a, b, c Means with different superscripts, within row, are significantly differ ( $P < 0.05$ ).

**Table (4).** Effects of YSE supplement on serum antioxidant enzymes

Parameters	Age	Experimental chicken groups					
		G1	G2	G3	G4	G5	G6
SOD1 (U/ml)	47 week	195.7±1.760 <sup>d</sup>	199±1.528 <sup>d</sup>	254.3±0.088 <sup>a</sup>	244±0.088 <sup>c</sup>	249.7±0.871 <sup>ac</sup>	244.3±0.880 <sup>bc</sup>
	49 week	208.3±1.330 <sup>d</sup>	205±0.557 <sup>d</sup>	274.3±1.202 <sup>a</sup>	271.3±0.882 <sup>ac</sup>	264.7±1.202 <sup>b</sup>	267.3±1.202 <sup>bc</sup>
GSH-Px (ng/ml)	47 week	8.78±0.008 <sup>c</sup>	8.74±0.017 <sup>c</sup>	11.65±0.009 <sup>a</sup>	11.60±0.012 <sup>a</sup>	11.35±0.012 <sup>b</sup>	11.41±0.008 <sup>b</sup>
	49 week	9.2±0.029 <sup>c</sup>	9.23±0.011 <sup>c</sup>	12.54±0.012 <sup>a</sup>	12.63±0.018 <sup>a</sup>	12.42±0.058 <sup>b</sup>	12.38±0.034 <sup>b</sup>
MDA (mmol/ml)	47 week	15.36±0.008 <sup>a</sup>	15.34±0.006 <sup>a</sup>	15.25±0.012 <sup>b</sup>	15.28±0.012 <sup>bc</sup>	15.32±0.011 <sup>ac</sup>	15.31±0.013 <sup>ac</sup>
	49 week	16.45±0.021 <sup>a</sup>	16.44±0.015 <sup>a</sup>	16.41±0.006 <sup>a</sup>	16.43±0.011 <sup>a</sup>	16.45±0.005 <sup>a</sup>	16.47±0.010 <sup>a</sup>

\*Values are expressed as means ± standard error of the mean.

a, b, c Means with different superscripts, within row, are significantly differ ( $P < 0.05$ ).

**Table (5).** Effects of YSE supplement on serum antibody titers against ND using a hemagglutination inhibition test (log2)

Days post vaccination	Experimental chicken groups					
	G1	G2	G3	G4	G5	G6
0	4.9±0.173	4.96±0.120	5±0.066	4.99±0.114	4.95±0.185	4.88±0.025
7	4.33±0.33 <sup>b</sup>	6±0.00 <sup>a</sup>	6.67±0.33 <sup>a</sup>	5±0.00 <sup>b</sup>	6.33±0.33 <sup>a</sup>	4.67±0.33 <sup>b</sup>
14	5±0.00 <sup>d</sup>	6.33±0.33 <sup>c</sup>	9±0.00 <sup>a</sup>	5.33±0.33 <sup>d</sup>	7±0.00 <sup>b</sup>	5±0.00 <sup>d</sup>
21	4±0.00 <sup>c</sup>	7±0.00 <sup>b</sup>	8.67±0.33 <sup>a</sup>	4.67±0.33 <sup>b</sup>	7.67±0.33 <sup>b</sup>	4.67±0.33 <sup>c</sup>
28	3.67±0.33 <sup>c</sup>	5±0.00 <sup>b</sup>	7.67±0.33 <sup>a</sup>	4.33±0.33 <sup>c</sup>	5.66±0.33 <sup>b</sup>	4±0.00 <sup>c</sup>

\*Values are expressed as means ± standard error of the mean.

a, b, c Means with different superscripts, within row, are significantly differ ( $P < 0.05$ ).

## Discussion

In the current study, the results indicated that the supplementation of YSE to laying hens give the best productive performance in the terms of Egg weight and Egg mass throughout the experimental period. The improvement in egg production parameters with yucca supplementation may be due to the provision of certain compounds that improve digestion, absorption, and utilization of nutrients in the digestive tract (Almuhanna *et al.*, 2011). Also, this improvement in egg weight and egg mass may be related to positive effects of steroid saponin present in yucca on nutrient absorption from the gastrointestinal tract. Moreover, it could be attributed to the biological activity of phenolic compounds such as resveratrol and yuccaols which are found in yucca plant causing greater feed efficiency and utilization, resulting in improved productive performance (Bozin *et al.*, 2006). These results were in accordance with Wang and Kim (2011) who

reported that supplementation of yucca extract exerted positive effects on egg weight and Alagawany *et al.* (2015a) who reported that yucca supplementation to the diets of layer hens improved egg mass. This result is contrary to that reported by Chepete *et al.* (2012) who showed that yucca in the layer diets did not affect mass of eggs produced of laying hens when compared with the un-supplemented group. *Yucca schidigera* Extract supplementation to the diet did not affect daily feed consumption, egg production rate and feed efficiency ( $P > 0.05$ ) of laying quails. Also body weight gain was not affected by addition of yucca ( $P > 0.05$ ). These results are consisted with previous experiment of Kutlu *et al.*, 2001 and Alagawany *et al.*, 2015a) determining the effects of different dietary yucca extract supplementation in laying hens.

Our study revealed that ad-dition of YSE to laying hen resulted in a significant increase in yolk percent and yolk-to-albumen ratio and

decrease in albumen percent compared with the non-supplemented group. These results are contrary to that reported by **Chaudhary (2017)** who reported that supplementation with graded level of saponin did not manifest any significant difference in quality of egg lay. In addition, our study showed that shell thickness and shape index were not significantly affected by YSE supplementation which are in line with the results of **Ayasan et al., (2005); Gurbuz et al., (2011) and Alagawany et al., (2016)** observed that yucca supplementation to layer's diet had no effect on shape index and shell weight compared to non-supplemented groups. Biochemical blood parameters usually reflect the health of an animal. These parameters are vital indicators of the nutritional and physiological status of birds and animals (**Abd El-Hack and Alagawany, 2015**). Serum total protein was not altered due to YSE treatment. However, it significantly increased serum albumin levels. These results are in accordance with **kaya et al. (2003)**.

Lipid profile revealed that serum total cholesterol and low density lipoprotein cholesterol levels decreased significantly, while triglycerides and high density lipoprotein cholesterol contents was not affected by YSE treatments. **Yu et al. (2011)** reported that the saponins significantly reduced the serum total cholesterol and LDL cholesterol in broilers. Similarly, **Gaurav (2015) and Chaudhary (2017)** reported that serum total cholesterol level was significantly decreased following supplementation of saponin rich feed additives. The hypocholesterolemic activity of saponins is due to delaying of intestinal absorption of dietary fat by inhibiting pancreatic lipase activity (**Han et al., 2000**).

In this study the level of egg yolk cholesterol in the YSE supplemented groups were significantly reduced compared to control groups and interestingly the level of reduction was significantly increased after 2<sup>nd</sup> treatment (at 49 weeks) compared to 47 weeks, which indicates the importance of continuous use of YSE. These results are in agreement with the data of **Kutlu et al. (2001)** who reported that egg yolk cholesterol of laying hens fed yucca powder was found to be lower than control groups and feeding length (weeks) of supplemental yucca

had an effect on yolk cholesterol.

Comparing to the control group, supplementation of YSE exhibited a positive impact on IgM and IgG in all YSE treated groups. However, the vaccinated-treated groups (G3 and G5) had higher significant IgG compared to G1, G2, G4 and G6. These results in accordance with **Su et al. (2016) and Alagawany et al. (2016)** who reported that diets supplemented with YE increased, IgG, IgM level. There was significant difference in level of IgG between vaccinated and non-vaccinated groups.

The effect of YSE on antioxidant parameters including serum activity of SOD1 and concentrations of GSH-Px and MDA were studied. The serum SOD1 activity and GSH-Px concentration were significantly increased in YSE supplemented groups specially with the dose of 0.5 ml/L rather than 1 ml/L. On the contrary, the MDA concentrations were not affected with YSE addition in comparison with the control groups. These results partially similar to finding of (**Alagawany et al., 2015b**) who showed that the intake of herbs or their contents resulted in an increase in the activity of antioxidant enzymes such as SOD1 and GSH-Px and a decrease in MDA concentration. SOD1 is a metalloprotein enzyme that mainly contributes to the antioxidant defense system. Consequently, elevated levels of this enzyme may improve the steady state of the antioxidant system of poultry. The level of serum MDA is an indicator for evaluating antioxidant systems. It is suggested that the vital role of yucca as a natural antioxidant is attributed to the phenolic hydroxyl groups in this herb that serve as a hydrogen donor to the proxy radicals produced in the first stage of lipid oxidation, thus lowering and inhibiting the formation of hydroxyl peroxide (**Hashemipour et al., 2013; Alagawany et al., 2015b**).

The current result showed that, antibody titers against ND were significantly increased ( $P \leq 0.05$ ) in vaccinated non-treated (G2) and groups vaccinated and supplemented with YSE compared (G3 & G5) at 7 days than in non-vaccinated, non-treated (G1) and vaccinated and supplemented with YSE (G4 & G6). At 14, 21 and 28 days there were significant increase ( $P \leq 0.05$ ) in antibody titers against ND in group vaccinated and supplemented with



0.5ml/l YSE (G3) than in group vaccinated and supplemented with 1ml of YSE. These result for HI titer were similar to those of **Adaszyńska-Skwirzyńska & Szczerbińska (2017)** and **Ali *et al.* (2019)** who reported that feed additive containing saponin has the potential to stimulate the innate immune response and enhance antibody production against NDV.

In conclusion, the present results suggested that *Yucca schidigera* extract (YSE) supplementation in layer exhibited positive effects on growth performance in term of egg weight and egg mass, improved egg quality indices and reduced lipid content of eggs without any adverse effect on laying percentage, feed intake (FI) and feed conversion ratio (FCR). Also YSE has a significant reduction of serum total cholesterol, LDL and egg yolk cholesterol along with significant increase in antioxidant enzyme activity and finally, enhancement and augmentation of antibody titers against NDV.

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