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Salix safsaf or milk thistle as a natural plant source and its effect on the productive performance in late-phase laying hens

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Abstract
A total of 180 aged hens (58 weeks of age) were divided into 5 treatment groups of 36 each to evaluate the effect of *Salix safsaf* (SS) or Milk Thistle (MTH). Groups 2 and 3 were fed the basal diet supplemented with 1.5 or 3 g SS/kg, respectively. Groups 4 and 5 were given the basal diet plus 1.5 or 3 g MTH/kg, respectively. Group 1 was used as a control group. The experimental period lasted two months. SS or MTH in hen diets resulted in a substantial (p<.001) rise in EP and EM percentages compared to the control group; Furthermore, the large dosage of MTH alone significantly increased EW. Although SS or MTH had no effect on daily FI, FCR values improved significantly. The high levels of both SS and MTH increased eggshell weight (p<.001) compared to the control group. The egg albumin weight (percent) decreased somewhat after SS or MTH treatment, however the egg yolk weight increased dramatically (p<.001). Blood total cholesterol and LDL cholesterol concentrations were dramatically reduced (p<.001) in the SS or MTH groups, while HDL cholesterol was significantly elevated (p<.001). Blood total antioxidant capacity and concentration in the SS and MTH groups were significantly higher (p<.001) than in the control group. Adding SS or MTH to the hen diet increased blood estradiol-17b hormone secretion (p<.001) compared to the control group.

Keywords: Egg hens, *Salix safsaf*, milk thistle, egg production, blood constitutes

Introduction
Ageing is linked to reproductive failure in domestic fowls. At the end of the laying cycle, egg production rate and shell quality decreased, resulting in significant economic losses. This decline is linked to a drop in all levels of the hypothalamic-pituitary-gonadal axis (Johnson 2000) [20]. Furthermore, the end of the laying cycle coincides with a number of physiological changes, including a decrease in oestrogen output (Williams *et al*. 2005; Miao *et al*. 2019) [49, 28]. This will obviously impair the synthesis and transport of the liver's oestrogen-regulated yolk precursor, affecting the reproduction performance of older breeder chickens (Liu *et al*. 2018) [26]. Additionally, lower productivity in older breeder hens is due to ovarian senescence (Devine *et al*. 2012; Luderer 2014) [8, 27].
The Egyptian willow (*Salix safsaf*) is a tiny tree that has been cultivated in Egypt since prehistoric times. It is most commonly seen in moist areas, such as beside the waterways. For thousands of years, white willow, also known as salicin willow, has been used for its health advantages (Saller *et al*., 2008) [37]. Its long, slender, and pliant branches, leaves, seeds, and other plant parts were employed in medicine. Papyrus seeds are recommended in the Hearst medical manual for chilling veins and cooling a bone after it has been set (Lise Manniche, 1989) [25]. Willow fodder has a total N and ME content of around 26.3 g and 10.5 MJ per kilogramme DM, respectively (ThiMui *et al*., 2005) [47]. Willow is fairly digested and extremely pleasant to livestock, and it contains minerals such as calcium, magnesium, potassium, and zinc (Guevara-Escobar, 1999) [16]. Willow species produce low molecular weight phenolic glycosides such salicin (35 g/kg DM) and/or condensed tannin (CT, 38 g/kg DM) (Pitta *et al*., 2007) [31]. The principal salicylates identified in white willow are salicin and salicortin (*Salix safsaf*). They are converted by intestinal flora to saligenin Julkunen-Tiitto and Meier (1992) [21], absorbed into the bloodstream, and processed by the liver to salicylic acid; they are mostly excreted via renal excretion (Bissettet, 1994 and Fotsch *et al*., 1989) [5, 14].
Milk thistle (MT, *Silybum marianum*), also known as "wild Artichoke" in Egypt, is a weed that grows as a weed in poor terrain behind canals or drains and as a weed in some crops.

Silymarin has recently been shown to have a galactogogic effect in women (Carotenuto and Di Pierro 2005; Zuppa *et al*., 2010) [6, 53], and some research has proven that milk thistle enhances lactation in cows (Tedesco *et al*., 2004) [46]. Hadolin *et al*. (2001) [17] discovered that the seed of MT includes betaine, tri-methyl glycine, and a considerable amount of oil, all of which play a key part in the extract's anti-inflammatory and anti-hepatitis activities. Potkanski *et al*. (2001) [32] found that replacing 20% of a concentrate cow's diet with endosperm from milk thistle (*Silybum marianum* L.) induced an increase in milk fat content when compared to a control. According to Wu *et al*. (2009) [51], MT has anti-oxidant properties. According to Hamed *et al*. (2016) [18], MT seed has around 392.1 mg/100g total polyphenols and 174.7 mg/100g antioxidant activity (equivalent to ascorbic acid).
The purpose of this research is to look at the photochemical effects of *Salix safsaf* and milk thistle on reproductive and productive performance, egg quality, plasma hormone profiles, and antioxidant status in aged laying hens.

Materials and Methods

Experimental design

To investigate the effect of some natural plant products (*Salix safsaf* or milk thistle on enhances hens' reproductive and productive performance, a total of 180 aged hens at the end of egg production curve (58 weeks of age) from Matrouh strain (Egyptian local strain) with initial body weight ranged between (1649 – 1692.6 g) were used during the spring period. The experiment was conducted at Borg EL-Arab Poultry Research Station (Alexandria), Animal Production Research Institute, Agricultural Research Centre.
These procedures suggest minimal stress to the animal to ensure the rights and welfare by eliminating harm or suffering to animals according to official decrees of the Ministry of Agriculture in Egypt regarding animal welfare (Decree No. 27 (1967) that enforces the humanity treatment of animals generally).
Birds have been fed a diet that contains 2745 kcal/ kg ME and 16.2% crude protein. Feed and water were provided *ad-libtum* throughout the experimental period. Hens were randomly assigned to 5 treatments of 3 replicates each (12 hens each) in floor pens under a 16-h light: 8-h dark lighting schedule. Group 1 was serving as a control group that fed the basal diet, whereas, Groups 2 and 3 were fed the basal diet supplemented with 1.5 or 3 g *Salix safsaf*/kg basal diet (SS1.5 and SS3), respectively. While, groups 4 and 5 were fed the basal diet supplemented with 1.5 or 3 g Milk thistle/kg basal diet (MTH1.5 and MTH3), respectively. The experiment period lasted for two months. *Salix safsaf* and Milk thistle were prepared in powder shape before adding them to the basal diet.

Data collected

Daily egg production (EP) as (%), egg weight (EW) as (g), and egg mass (EM) as (g of egg/hen/day) were recorded for each replicate in each treatment group. As well, Daily feed intake (FI) (g/hen/day) and feed conversion ratio (FCR) (g feed/g egg) were recorded. 15 eggs from each treatment (5 eggs from each replicate) were randomly chosen and used to estimate eggshell, albumin, and egg yolk weights as a percent- age of egg weight. As well, eggshell thickness (mm) was measured by a micrometer after the inner egg- shell membrane was removed, as well, Yolk color intensity was measured based on the standard color of the yolk using a Roche yolk color phone with a range of score from 1 to 15 light yellow to the dark yellow.
At the end of the experimental period, 5 hens from each treatment were randomly chosen and sacrificed then the ovary (without the ovary yolk follicle) and the oviduct was removed and weighted to the nearest 0.1 g and recorded as a percentage of final body weight. Ovary yolk follicles were separated and divided, dependent on size to large yolk follicles (LYF > 10 mm diameter) and small yolk follicles (SYF > 5—10 mm diameter), as well, ovary large white follicles
(LWF ¼ 3—5 mm diameter) were counted (Renema *et al*. 1995) [34].
At the end of the experimental period, blood samples of each treatment were collected from birds during scarifying to obtain plasma or serum; blood samples were collected at 8 Am from hens who had already laid eggs to obtain blood samples at the same stage of the egg production cycle. Fresh blood samples were taken after collection to deter- mine blood pictures, including, the red blood cell count (RBCs). White blood cells count (WBCs), haemoglobin (Hb, g/dL), and haematocrit (PCV). Heparin was used as an anticoagulant but a part of the sample was withheld to obtain serum. Plasma or serum was obtained by centrifugation the blood at 3000 rpm for 20 min and stored at —20 ^{0}C for biochemical analysis. Plasma total protein (TP) concentration (g/DL), plasma Total cholesterol (Tc) concentration (mg/dL), high-density lipoproteins (HDL) cholesterol and low-density lipoproteins (LDL) cholesterol concentrations as (mg/dL), total antioxidant capacity (TAC mmol/L) were measured. Serum samples were used to determine the concentrations of oestradiol 17-b hormone (E_2) (the Estradiol ELISA Test Kit has a sensitivity of 6.5 pg/ml) by Immunoassay Technique Elisa Kits (Fortress Diagnostics Ltd, Antrim, UK, 2021).

Hatchability performance

During the last five days of each month, fertile eggs from each treatment were collected daily and stored in the controlled room till the time of incubation. During the incubation period, eggs of all treatments were received standard temperature (37.5 ^{0}C) and relative humidity (52%) for 18 days. During the last 3 days, eggs were incubated at 36.5 ^{0}C and 65% relative humidity. All hatching chicks were counted and weighed within 45 min after hatch. Hatchability was calculated as a percentage of fertile eggs. The un-hatched eggs were broken out and the infertile eggs were counted to calculate the percentage of fertility.

$$\text{Fertility ð\%Þ ¼} \frac{\text{Fertile eggs}}{\text{Total eggs set}} \times 100$$

$$\text{Hatchability ð\%Þ ¼} \frac{\text{Hatching chicks}}{\text{Number of fertile eggs}} \times 100$$

Statistical analysis

The data were tabulated and statistically analysed, where appropriate, by using the one-way ANOVA of SAS (SAS Institute 2009) [39]. The significance of the effects was tested at levels $p \leq .05$ (ω) and $p \leq .01$ (ωω) and compared using the Turkey test (SAS Institute 2009) [39].
The statistical model used to be as follows:

*Y*ij ¼ l þ *Ti* þ *eij*

Where: *Y*ij ¼ the dependent variable, lþ the overall mean, *Ti*¼ the effect of treatments, and *eij*¼ the random error.

Results and Discussion

Productive performance

Table 1 shows data from (SS) and (MTH) treatments for daily feed intake (FI) as (g/h/d), egg production (EP) as (percent), egg weight (EW) as (g), egg mass (EM) as (g of egg/hen/day), and feed conversion ratio (FCR) as (g feed/ g egg). It is worth noting that all experimental groups started with relatively identical initial LBWs ranging from 1649 – 1692.6 g, and all hens had an egg production rate of around 73%. (with insignificant differences). The data showed no significant effects of (SS) or (MTH) at any of the studied levels on FI compared to the control group, as hens in the treated groups consumed similar amounts of feed. The data demonstrated no significant effects of (SS) or (MTH) on FI in any of the tested levels as compared to the control group, as the hens in the treatment groups consumed comparable amounts of feed. Despite the fact that the inclusion (SS) or (MTH) in hens' meals had no influence on FI, the FCR was improved; hence, the FCR values decreased gradually and, significantly, and this effect was dependent on the level of (SS) or (MTH). Furthermore, found that including *Boswellia serrata* and Salix Alba in the diet of Leghorn chickens dramatically boosted egg production during the first 5 weeks of laying, with hens in the treated group producing more eggs than those in the control group. In conflict to this result, (Sugito *et al* (2015) [43] state that the administration of *Salix tetrasperma* and its combination (tumaric and neem) had no significant (P>0.05) effect on egg production, food intake, and feed conversion ratio. The content of bioactive compounds in SS and MTH contains several compounds such as tannins, saponins, flavonoids, triterpenes, phenol compounds, and sterols (Pitta *et al*., 2007; Kahkonen *et al*. 1999; Khayyal *et al*. 2005; El-Wakil *et al*. 2015) [31, 22, 24, 11]. The tannin and saponin compounds have been known to be antinutrients, (Tadele 2015) [45]. Antinutrient content was not suspected to interfere with the absorption of nutrients. It could also be that salix extract contains bioactive compounds that have stomachic and adaptogenic activities so that the activity of feed consumption was not disrupted. Also, the phytochemical screening for Milk thistle aerial parts (leaves, barks and fruits) showed that the total polyphenols content and antioxidant activity are 188.5 mg/100g and 320 mg/100g, respectively, (Abdalla *et al* 2018). EP and EM results of hens' meals containing (SS) or (MTH) indicated a highly significant ($p \leq .001$) increase compared to the control group, and this impact was (SS) or (MTH) level-dependent. Furthermore, the (SS3) group resulted in an increase in EP and EM levels compared to (MTH1.5) group. Both (SS) and (MTH) groups at any examined level increased average EW compared to the control group, and this increase was significant only at the high dose (MTH) group. Reduced production has been attributed to the low nutrient utilization or due to the presence of antinutritional factors that affect feed utilization or it may be attributed to the decreased energy utilization from SS and MTH-containing diets (Basyony *et al* 2018 and Jabali *et al*. 2017) [4, 19]. In different method, Faryadi *et al* (2021) [12] confirmed that when Lohman selected leghorn (LSL) laying hens were fed diets containing three types of silymarin (purified extract from the seeds of the milk thistle) as (powder, nano-silymarin, and lecithin-silymarin), egg production, egg weight, and egg mass rose, while the feed conversion ratio dropped ($p<.05$).As well as, (Šťastník *et al* 2017) [42] when added (MTH7) in laying diet. Also, (2017) found in their study with hens decrease in feed intake during the whole experiment with the inclusion (MTH30) group. Feed conversion ratio was significantly reduced in this group, too. The broiler chicken feed mixture contained (MTH15) seeds significantly reduced feed conversion ratio (Zahid and Durrani, 2007) [52]. In the same experiment provide by (Hashemi Jabali *et al*. (2017) [19] the inclusion of (MTH3) seed meal significantly increased egg production and due to this egg weight was significantly increased, as well. This finding confirmed the results of (Schiavone *et al*. (2007) [40].

Egg quality characteristics

Table 2 shows the effects of (SS) and (MTH) on certain egg performance indicators (eggshell weight, eggshell thickness, albumin weight, and egg yolk weight). The high level in both tested groups increased eggshell weight more than the control group, and this impact was significant ($p \leq .001$), whereas lower level had no influence on eggshell weight. Although the insertion of a low level of SS and MTH in the hens' meals had no influence on eggshell weight, eggshell thickness (mm) was raised significantly ($p \leq .05$) in all tested groups compared to the control, and this effect was level-dependent. The egg albumin weight (percentage) of the SS or MTH groups was somewhat lower than the control group, but the egg yolk weight was significantly higher ($p \leq .001$). Similarly, the yolk color score of groups treated with SS and MTH was considerably higher ($p \leq .001$) than the control. (Sugito *et al* (2015) [43] found that administration of *Salix tetrasperma* or its combination with with extract of turmeric and neem did not affect yolk color, albumen height, egg weight, and HU value, but significantly ($P<0.01$) increased eggshell thickness. One of the clear results in the present study was that dietary SS or MTH to aged hens resulted in an improving eggshell weight and eggshell thickness. So, (Radwan *et al*. (2008) [33] said that several bioactive compounds of plant extract may improve micro environment condition in the chicken's uterus, so that egg mineralization may occur more perfectly and the eggshell was thicker. This assumption agrees with the results (Šťastník *et al* (2017) [42] who mentioned that dietary MTH in laying hen's diet caused a higher Haugh unit, a higher millimeter height of the egg and thinner eggshell in the group receiving 7% of the seed cakes were found. Besides, dietary phytoestrogens improved eggshell quality in Hyline brown laying hens (Ni *et al*. 2007; Gu *et al*. 2013) [30, 15] and quails (Sahin *et al*. 2007) [35] during the late laying period. According to (Saleh *et al*. (2019) [36] the improvements in eggshell quality characteristics were probably related to the increasing serum calcium concentration that associated with phytoestrogens treatment. From another point of view, (Wistedt *et al*. (2012) [50] declared that phytoestrogens are involved in regulating the carbonic anhydrase activity via oestrogen receptor-α and oestrogen receptor-β located in the shell gland. Thus, it might be speculated that aged laying hens might be more sensitive to phytoestrogen supplementation and increased calcium absorption and utilization, which finally leads to enhancing eggshell quality characteristics (Saleh *et al*. 2019) [36]. Our results showed that the increasing eggshell thickness in the SS and MTH low-level groups compared to the control group despite the eggshell weight was not differ may be due to the way the active components in the tested materials affect calcium deposition and the formation of cones during the formation of the eggshell in the oviduct.

Increasing egg yolk weight due to SS and MTH treatments may be attributed to their phytoestrogen compounds that have a mimic oestrogen-like action, which stimulates the liver to synthesize the yolk protein and yolk fat thus increasing the transmission activity of these components of the liver to the ovarian follicles. Similar results were demonstrated by (Šťastník *et al* (2017) [42] they mentioned that treating aged hens with 7% MTH resulted in increasing eggshell quality characteristics and egg yolk weight. On the other site, showed that administration of S. Tetrasperma or its combination did not affect yolk color, albumen height, egg weight, and HU value, but significantly ($P<0.05$) increased eggshell thickness. In addition, the yolk color score of groups treated with SS or MTH was increased significantly than the control and this effect may be due to increase yolk lipid content that contains feed pigments. In accordance to (Šťastník *et al*. (2019) dietary supplementation of MTH in aged hens had a significant positive effect on yolk color, whereas, it was significantly increased of treated groups than the control.

The information on ovary, oviduct, and ovarian yolk follicles is summarized in (Table 3). The studied material had no effect on ovary weight, but there was a significant rise ($p\leq.001$) in oviduct weight when compared to the control group. Furthermore, chickens treated by (SS) and (MTH) at either of the examined dosages had a significantly higher number of large yolk follicles (LYF) ($p\leq.05$), small yolk follicles (SYF) ($p\leq.001$), and LYF weight ($p\leq.001$) than the control group. However, there was no effect of adding (SS) or (MTH) to the hen ratio of ovary relative weight (percentage) or the amount of huge white follicles (LWF). Due to the phytoestrogen effect of SS and MTH that resulted in boosting the blood oestradiol level in the SS or MTH treated hens groups (Table 3), there was a significant increase ($p<.001$) in oviduct weight compared to the control group, where, oviduct weight and length were subjected to oestradiol stimulation, whereas, there is a positive correlation between the oviduct activity and blood oestrogen levels. As well, treated hens with SS or MTH at any of the studied levels resulted in a significant increase in the number of large yolk follicle (LYF) ($p<.05$), small yolk follicle (SYF) ($p<.001$), and LYFs weight ($p<.001$) than the control group and this increasing may be due to the phytoestrogen and endogenous oestradiol participated in stimulation the synthesis and transmission of protein and lipids from the liver to ovary yolk follicles. On the other hand, there was no effect due to adding SS or MTH in a hen ratio of ovary relative weight (%) and the number of large white follicles (LWF). Our results were in agreement with the results of (Abou-Shehema *et al*. (2016) [1] observed that feeding silymarin (5 g/kg) enhanced the number of more developed ovulatory follicles, which was associated with higher oestrogen and progesterone concentrations in the blood and suppression of steroidogenesis in laying hens. Additionally, (Dai *et al*. (2021) [7], who revealed that dietary hawthorn-leaves flavonoids supplementation could effectively maintain the number of primordial follicles in aged breeder hen’s ovary comparison with the control group. Therefore, they suggest that hawthorn-leaf flavonoids could improve the reproductive performance of the aged breeder hens by relieving ovarian oxidative stress and maintaining the number of primordial follicles.

Some blood constituents and 17-b estradiol level

Table 4 shows that adding SS or MTH to hens' diets enhanced blood oestradiol-17b hormone secretion, with blood oestradiol-17b hormone concentrations increasing considerably ($p\leq.001$) in the SS and MTH groups compared to the control group and this increase was level dependent. (Furthermore, Nakari (2005) [29] and (Noppe *et al*. (2008) reported that in plant extract there was phytosterol compound which may serve as steroid hormones. (Sugito (2007) [44] reported that S. tetrasperma plant contained steroid compound reaching 10.08%, while (ElShazly *et al*. (2012) [10] has identified several phytosterols in Salix plant, namely: beta-sitosterol acetate, friedelin, 3beta-friedelinol, beta-amyrin, beta-sitosterol, betasitosterol-O-glucoside, palmitic acid, catechol, and tremulacin.

When compared to the control group, there was a steady and significant drop ($p\leq.001$) in blood cholesterol (Chol) and the dangerous part of cholesterol (LDL) concentrations in the treatment groups, and this reduction was level-dependent. Feeding chickens SS or MTH, on the other hand, resulted in a steady and significant increase in the beneficial part of cholesterol (HDL) content ($p\leq.001$) compared to the control group. According to our findings, both SS and MTH at any examined dose led in a significant reduction ($p\leq.001$) in blood total Chol and LDL concentrations, while there was a steady and significant increase in the beneficial part of HDL concentration ($p\leq.001$) compared to the control group. The flavonoides chemicals in the studied materials may be responsible for the decrease in total Chol and LDL concentrations. (Saracila *et al*. (2018) [38] observed multiple advantages of feeding broilers 1% willow bark extract: all plasma energy profile markers (glycemia, cholesterol, and triglycerides) were considerable ($P<0.05$) lower compared to the control group. The lower total cholesterol content may represent the hypocholesterolemic features of the dietary willow bark extract (1 percent), which has a significant impact on the health of the broilers. In line with these findings, (Karimi *et al*. (2015) [23] demonstrated in a study on normolipidemic rabbits that aromatic water from *Salix aegyptiaca* reduced total cholesterol levels to levels comparable to simvastatin. According to (Jabali *et al* (2017) [19], dietary inclusion of 15 g/kg milk thistle meal resulted in a significant ($p<.01$) reduction in plasma triglyceride concentration, but plasma cholesterol and HDL concentration were not affected by dietary energy levels; however, dietary administration of 60 g/kg milk thistle meal was able to reduce and increase serum cholesterol and HDL concentrations ($p<.05$). Blood total protein concentrations in the 3 g SS and 3 g MTH groups looked to be slightly lower compared to the other SS and MTH treated groups and the control group, but this difference was not statistically significant. As compared to the control group, blood total antioxidant capacity (TAC) concentration increased significantly ($p\leq.001$) in the SS and MTH groups, with a preference for the group fed with 3 g MTH/kg diet. This results supported by, Asghar and Masood (2008) who proved that silymarin can be utilized as a dietary natural antioxidant supplement to prevent free radical-related illnesses. Silymarin is thought to enter the nucleus and operate on RNA polymerase enzymes and rRNA transcription, resulting in enhanced ribosomal formation. This, in turn, accelerates protein and DNA synthesis (Sonnenbichler and

Zetl, 1986) [41], which improves the cytoplasmic biosynthetic apparatus, resulting in an increase in the synthesis rate of both structural and functional proteins. The measuring of TAC as an antioxidant barometer was significantly improved for Gimmizah cockerels fed on 4 types of diet (Basle-diet; Basle-diet including vitamin E (150 mg VE /kg diet) as an immunomodulator nutrient; 0.5 g commercial silymarin (SLM) /kg diet and basal diet supplemented with 12.5 and 25g of fine grind aerial parts of milk thistle plant (MTh) /kg diet as a natural source of SLM (represented 0.5 and 1g SLM /kg diet, respectively). the best improvement for TAC was 19.7% for the group supplied with MTh compared with control group and VE or SLM, (Abdalla *et al*. 2018).

Decrease blood protein concentration of high levels of SS and MTH groups may be correlated to increase the egg production rate of these treated groups, where stimulation occurred in the synthesis and transmission of yolk protein from the liver to ovary yolk follicles and the rate of egg albumin secretion were increased from oviduct glands. (Saracila *et al*. (2018) [38] found that giving 1 percent white willow bark extract to layer hens had no effect on serum total protein and globulin, serum albumin, or A/G ratio when compared to the control group. While, Adding Milk Thistle (*Silybum marianum*) and Vitamin E resulted in a significant increase in total protein, albumin and globulin, (Aljugaifi *et al* 2018) [2].

Blood Hb content, RBCs count, and PCV (Table 5) were not modified in groups treated with Safsaf or Milk thistle compared to the control group, however WBCs count was increased in Safsaf and Milk thistle treated groups and this increase was significant ($p \leq .001$) with the high amount of Safsaf and Milk thistle. When compared to the control group, diets containing vitamin E (150 mg VE /kg diet), 0.5 g commercial silymarin (SLM) /kg diet, and 12.5 and 25g milk thistle plant (MTh) /kg diet resulted in significantly higher red blood cell counts (RBCs) and haemoglobin (Hb), blood white cell counts (WBCs), packed cell volume (PCV), lymphocyte, phagocytic activity (PA), and Furthermore, MTh supplementation significantly reduced the heterophil percentage and the heterophil to lymphocyte ratio (H/L ratio) compared to the control group, but it had no effect on the monocyte and eosinophil percentages, (Abdalla *et al* 2018).

There was a significant effect due to adding SS or MTH to hens-diet on the percentage of egg fertile and hatchability of fertile egg. Additionally, feeding hens with high SS levels or low and high levels of MTH enhanced the proportion of fertilized eggs and hatching significantly ($p \leq .027$ and .024) compared to the control, with a preference for the group treated with 1.5 and 3 g MTH/kg feed.

Table 1: Productivity of aged laying hens treated with various amounts of *Salix safsaf* (SS) or Milk thistle (MTH).

Items	SS			MTH		SEM	P value
	Control	SS1.5	SS3	MTH1.5	MTH 3		
Initial body weight (g)	1692.6	1649	1674	1660.6	1680	45.7	0.95
Feed intake (g/hen/day)	128.67	128	126.33	127.33	130	0.7	0.62
Feed conversion ratio (g feed/g egg)	4.19^{a}	3.72^{b}	3.42^{cd}	3.48^{c}	3.24^{d}	0.03	0.001
Egg production %	67.67^{d}	71.47^{c}	76.74^{b}	75.32^{b}	78.46^{a}	0.07	0.001
Egg weight (g)	55.42^{b}	58.15^{ab}	57.45^{ab}	58.21^{ab}	60.73^{a}	2.7	0.04
Egg mass (g of egg/hen/day)	31.23^{d}	34.90^{c}	37.34^{b}	37.07^{b}	40.52^{a}	0.11	0.001

abc,dMeans in the same row having different superscripts are significantly different ($p \leq .05$).

Table 2: Egg quality characteristics of aged laying hens treated with varying amounts of *Salix safsaf* (SS) or Milk thistle (MTH).

Items	SS			MTH		SEM	*P*- value
	Control	SS1.5	SS3	MTH1.5	MTH3		
Egg Shell weight (%)	14.42^{b}	14.14^{b}	15.43^{a}	14.29^{b}	15.14^{a}	0.28	0.95
Eggshell thickness (mm)	0.366^{c}	0.379^{b}	0.387^{ab}	0.380^{b}	0.390^{a}	0.23	0.62
Albumen weight (%)	55.77	54.88	53.17	54.29	52.05	3.98	0.001
Yolk weight (%)	32.81^{c}	33.98^{b}	34.40^{b}	34.42^{b}	35.831^{a}	0.21	0.001
Yolk color scores	6.68^{b}	7.55^{a}	7.68^{a}	7.68^{a}	7.55^{a}	0.253	0.04

abcMeans in the same row having different superscripts are significantly different ($p \leq .05$).

Table 3: The features of the ovary, oviduct, and follicles of aged laying hens treated with varying amounts of *Salix safsaf* (SS) or Milk thistle (MTH).

Items	SS			MTH		SEM	P value
	Control	SS1.5	SS3	MTH1.5	MTH3		
Ovary weight (%)	2.31	2.24	2.41	2.33	2.26	0.039	0.95
Oviduct weight (%)	2.26^{c}	2.56^{b}	2.72^{ab}	2.83^{a}	2.94^{a}	0.063	0.62
Number of LYF	1.77^{c}	2.43^{bc}	2.77^{b}	2.77^{b}	3.77^{a}	0.183	0.001
Number of SYF	8.17^{b}	9.83^{a}	10.17^{a}	10.17^{a}	10.5^{a}	0.36	0.001
Number of LWF	15.5	14.97	15.17	15.83	15.5	0.365	0.04
Total of LYF weight (g)	15.45^{b}	17.44^{a}	17.85^{a}	17.56^{a}	17.86^{a}	0.21	0.001

[abc]Means in the same row having different superscripts are significantly different ($p \leq .05$). LYF: large yolk follicles; LWF: large white follicles; SYF: small yolk follicles.

Table 4: Blood components and Oestradiol-17b hormone levels in aged laying hens given varied doses of *Salix safsaf* (SS) or Milk thistle (MTH).

Items	SS			MTH		SEM	P value
	Control	SS1.5	SS3	MTH1.5	MTH3		
Total protein g/dL	7.22	7.25	6.76	7.1	6.7	0.44	0.076
Total cholesterol mg/dL	153[a]	140[b]	123[c]	136[b]	123[c]	2.9	0.001
HDL cholesterol mg/dL	29.67[c]	36.67[b]	48[a]	38.67[b]	51[a]	1.38	0.001
LDL cholesterol mg/dL	86.67[a]	74.33[b]	62.33[c]	62.33[c]	61.67[c]	1.28	0.001
Oestradiol-17b pg/mL	221.50[d]	299.33[c]	356.33[b]	357.33[b]	435.33[a]	5.6	0.001
TAC mmol/L	1.11[c]	1.23[b]	1.23 [b]	1.25[b]	1.35[a]	0.004	0.001

[abc]Means in the same row having different superscripts are significantly different (*p* .05). TAC: Blood total antioxidant capacity.

Table 5: Blood haematology, fertility, and hatchability of age laying hens treated withering doses of *Salix safsaf* (SS) or Milk thistle (MTH).

Items	hSS			MTH		SEM	P value
	Control	SS1.5	SS3	MTH1.5	MTH3		
RBCs ($10^6/mm^3$)	2.54	2.53	2.71	2.58	2.74	0.11	0.287
Hb (g/c)	11.9	11.87	12.4	12.2	12.03	0.13	0.076
PCV %	32.3	31.97	32.53	32.4	33.17	0.12	0.957
Fertability %	88.01[b]	88.10[b]	89.92[a]	89.59[a]	90.76[a]	13.11	0.027
Hatchability of fertile egg %	84.6[b]	84.81[b]	85.49[a]	85.92[a]	85.93[a]	18.13	0.046

[abc]Means in the same row having different superscripts are significantly different (*p* .05). RBCs: red blood cells count; WBCs: white blood cells count.

Conclusion

Salix safsaf or milk thistle, a natural herbal plant, can be used to improve the performance of older laying hens, extend their productive lives, and so raise the flock's economic yield. Furthermore, the antioxidative state, hormonal profile, steroidogenesis, and egg quality of aged laying hens improved.

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