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IMPACT OF DIETARY ADDITION WITH ROCKET (*Eruca sativa*) SEEDS ON EMBRYONIC DEVELOPMENT, HATCHABILITY AND SOME PHYSIOLOGICAL PARAMETERS FOR SILVER SABAHIA CHICKEN STRAIN

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ABSTRACT: Current study was performed to determine the impacts of supplementing different levels of *Eruca sativa* seeds (*ESs*) in the basal diet of laying hens on embryonic development, hatchability (%), chick quality and some physiological status of Sliver Sabahia chickens strain. A total of 300 laying chickens and 40 cocks aged 30 week were housed individually in single cages and distributed randomly to 4 treatment groups (75 females and 10 males per each). Laying hens were fed different experimental four diets groups. The first diet as control group, while 2nd , 3rd and 4th group had fed the basal diet supplemented with 1.0 , 2.0 and 3.0 % *ES*s/kg diet, respectively. At 32 wks of age, hens were artificially inseminated twice a week with semen from cocks received the control diet. Eggs were daily collected from each group for 7 days at 34, 36 and 38 wks of age. A total of one thousand hatching eggs (250 egg per each treatment) representing the 4 experimental groups were incubated. The acquired results indicated that adding *ESs* at a level of 2.0 % or 3.0 % to the basal diet significantly ($P \leq 0.01$) reduced egg weight loss (%) during 0-18 days of incubation , as well as the relative yolk sac weight, embryonic mortality , and pipped egg (%). Meanwhile, it significantly increased embryonic weight (%), hatchability (%), and quality of the hatched chicks. Additionally, it led to improved most blood parameters values for hatched chicks and lower plasma cholesterol and malondialdehyde, besides higher concentration of total antioxidant. This improvement in all the mentioned parameters was observed to be proportional to the increase in *ESs* levels in the diet, with the 3.0 % level being the most beneficial , followed by 2.0 %.

In conclusion, the data suggested that maternal dietary supplementation with 2.0 % or 3.0 % *ESs* was more effective nutritional tool to improve embryonic development, achieve the best hatchability and obtain chicks with good and health specification.

Key words: Eruca Sativa seeds, maternal diet, embryonic development, hatchability, chick quality

INTRODUCTION

Maternal nutrition directly affects the development and growth of embryos, in addition to influencing certain external characteristics and physiological traits (Yahav and Brake, 2014). Therefore, maternal nutrition is used as a strategy to enhance embryonic growth by providing the essential and important nutrients needed by the embryo with minimizing the harmful effects of other factors (Wang *et al*., 2024).

Bird embryos rely on the nutrients deposited in the egg, as all maternal information is transferred through the egg, which is determined by the nutritional status of the laying hen (Van der Wagt *et al*., 2020). As a result, if there is a deficiency in nutrients deposition in the eggs of breeder hens, this will directly affect embryonic development.

In light of recent studies confirming the importance of maternal nutrition in enhancing fetal progress and growth (Al-Rawe *et al*., 2023), we have incorporated the seeds of an essential natural plant, Rocket (*Eruca sativa*) seeds, as a dietary supplement in maternal diet. These seeds possess significant medicinal and therapeutic properties, which, in turn, promote the overall health of the organism (Nurzynska-Wierdak, 2015).

The *Eruca sativa* (*ESs*) a fast – growing annual plant and classified within the Brassicaceae family (Dolezalova *et al*.,2013). It is considered a promising plant with significant nutritional properties (Al-Rawe *et al*., 2023). *ESs* contain essential nutrients in high proportions, such as proteins (38.78), fats (35%), carbohydrates (38.4), fiber (9.13) and ash contents (5.33%) (Rozan and Boriy, 2022). In addition to primary and secondary metabolites like glucosinolates (glucoerucin and glucoraphanin), flavonoids (quercetin, kaempherol, and isohamnetin) and caroteniods (Bennett *et al*., 2006; Keyata *et al*., 2021). These compounds contribute to improving the health of living organisms due to their antimicrobial, anticarcinogenic and antioxidant properties (Khoobchandani *et al*., 2010; Gulfraz*et al*., 2011; Garg and Sharma, 2014). Moreover, *ESs* are rich in unsaturated fatty acids (85-89.1%), such as oleic, linoleic, and linolenic acids, as well as various vitamins, including E,C,K,B_1,B_2,B_6 and A (Bell and Wagstaff, 2014). These vitamins play a crucial role in reducing the impact of free radicals and protecting against pathogenic microbes, thereby enhancing the health and growth of living organisms (Barillari *et al*., 2005; Jaafar and Jaafar 2019). Additionally, *ESs* contain essential and vital minerals such as calcium, potassium, iron, zinc, sodium, sulfur and copper (Lourenço *et al*., 2019).

In general, several recent studies have proven that natural antioxidants improve embryonic development and reduce embryonic mortality. As a result, both hatchability (%), some internal organ and chick quality at hatch improved (Yang *et a*l., 2021.; Amevor *et al*.,2022.; Araujo and Lara, 2023.; and Du *et al*.,2025)

Elwediny (2024) reported that maternal dietary supplementation with organic zinc, which is present in *ESs*, improved hematological parameters (white blood cell, hemoglobin, packed cell volume) and decreased plasma lipids, cholesterol, and malondialdehyde. Also, laying hens fed diet supplemented with carotenoids-enriched *Spirulina platensis* algae increased values of total antioxidant and high-density lipoprotein by decreased cholesterol and low-densitylipoprotein (Ebtsam *et al*., 2018).

Despite the importance of *ESs* and their various benefits for humans and animals, studied on poultry are limited, especially regarding the effects of these seeds on the development and growth of bird embryos. Therefore, the aim of this current study to evaluate the effectiveness of adding *ESs* to maternal diet on embryonic development, hatching traits and the quality of hatched chicks. Additionally, the study examines

their impact on certain hematological and biochemical blood parameters.

MATERIALS AND METHODS

Ethical approval

The current trial was conducted at El–Sabahia Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt, according to the guidelines of the Departmental Committee of Animal and Poultry Production, and the pronouncement of the Ministry of Agriculture on animal ethics and welfare (Decree No. 27 (1967) that generally enforces the humane treatment of animals.

Experimental Design and Management:

A total of 300 laying chickens and 40 cocks of Silver Sabahia strain at 30 weeks of age were housed individually in single cages and distributed randomly to 4 treatment group (75 females and 10 males per each). Laying hens were fed different experimental diets. The first diet was as control group, while 2nd, 3rd and 4th group had fed the basal diet supplemented with 1.0, 2.0 and 3.0 % *ES*s/kg diet, respectively. The *ES*s (with no preparation) were purchased from the General Company for Agricultural Agencies in Damanhor, Egypt. Table 1 demonstrates the composition of the basal diets according to (NRC, 1994). Feed and fresh water were offered *ad-libitum* thought treatments. Artificial lighting was used to provide birds 16 hrs lighting daily. At 32 wks of age, hens were artificially inseminated twice a week with semen from cocks received the control diet. Eggs were daily collected from each group for 7 days at 34, 36 and 38 wks of age. A total of one thousand hatching eggs (250 egg per each treatment) representing the 4 experimental basal diet supplementations groups were stored in room temperature supplied with fans at 20°C and RH (70%). All eggs were numbered and weighed prior incubation and replicated in three trays for each previously mentioned group. Eggs were set and randomly distributed at different places in the same trolley of the incubator to reduce possible position effect. The set eggs were incubated in Egyptian-made incubator at 99.5 F° temperature and 55% relative humidity (RH) from days 1 to 18 of incubation. On the 18th days of incubation the eggs were transferred singly into pedigree hatching nests and then placed into hatcher for the remainder of period at 98.6°F and 65% RH for 3 days till the hatch.

Collected Data

Egg weight loss

All set eggs were individually weighed again during incubation on the 7th ,14th and 18th days in order to obtain egg weight loss percentages for each incubation time interval.

Relative weights of embryos, yolk sac and eggshell, besides eggshell thickness

At days 7th, 14thand 18th during incubation, eight eggs were randomly chosen from each experimental group (total 96 eggs) weighed and opened then the embryos were separated from the remaining egg contents. Embryos were rinsed in saline and blotted dry on an absorbent paper and the dried embryos were weighed to the nearest 0.001 g .Relative embryo weight, yolk sac weight and eggshell weight were determined as a percentage of set egg weight. Eggshell thickness was measured using the micrometer at 7th, 14th, 18th and 21th days during incubation.

Embryonic mortality, fertility and hatchability percentages

Eggs that failed to hatch and having full opportunity to hatch were broken out and examined macroscopically to estimate the embryonic development and assigned according to their times of death by day as possible. Embryonic mortality percentage expressed as a

percentage of fertile eggs set was recorded every day of incubation and classified into three periods (0-18 and18-21 daysof incubation period). Macroscopic fertility was estimated as a percentage of fertile eggs out of the number of eggs set. Hatchability of total eggs was estimated as a percentage of sound chicks out (Vitality of chicks resulting from the hatching process) of the total set eggs. Also, hatchability of fertile eggs was estimated as a percentage of sound chicks out of the fertile eggs. Also, pipped and addled eggs were estimated

Chick quality measurements

All chicks at day-old were subjected to several quality measuring methods according to observation reported by Tona *et al.* (2003) . Body weight (hatched chicks were weighed to nearest gram on the day of hatch) , chick length (the chick was laid on its ventral side, with the neck and right leg extended to their maximum length, chick length was defined as the length from the tip of the beak to the implantation of the nail on the third toe) , activity (determined by considering whether the chicks laid in the supine position stand quickly), downs and appearance (the dryness and cleanliness conditions of the chick' s body are assessed), retracted yolk (determined with the amount of egg yolk remaining in the navel area), eyes (the brightness of eyes as well as the wideness and elasticity structures of eyelids are assessed) , navel , remaining membrane (the size of the remaining membrane in the navel area is assessed).

Blood hematology and biochemical constituents

At hatch , twenty-four chicks (6 chicks / treatment) were killed for taken blood samples. Blood samples put in heparinized tubes. Half of the blood samples were taken to measure hemoglobin (Hb) content, red blood cells (RBC's), white blood cells (WBC's) counts, heterophil (H) % , lymphocyte (L) %, platelets(10/uL) and packed cell volume (PCV)%. The other blood samples were centrifuged at 3000 rpm for 20 minutes to obtain the plasma. The plasma was stored at -20 ˚C to determine. Plasma total protein (g/dl), cholesterol (mg/dl), low density lipoprotein (LDL) (mg/dl), high density lipoprotein (HDL) (mg/dl), glucose (mg/dl), calcium (mg/dl), total antioxidant capacity (TAC) (mmol/ml) and malondialdehyde (MDA) (nmol/ml) were measured by using available commercial kits.

Yolk sac and internal organs weight of hatched chicks

After blood collection, embryonic yolk sac, liver, intestine, heart, gizzard, spleen, stomach, gallbladder, and fabricius gland were removed and weighed to the nearest 0.1 gm. Relative weights (percentage per chick weight) were determined.

Statistical analysis

This study used an entirely statistical randomization design according to Snedecor and Cochran (1982). All results were subjected to standard statistical one-way analysis of variance (ANOVA) in the Statistical Package for the Social Sciences, version 20 (SPSS, 2011).

The statistical model used was as follows:

$X_{ij}= \mu + T_i + e_{ij}$

Where x_{ij} is the value of the measured variable, μ is the overall mean, T_i is the effect of treatment (i= 4 treatments), and e_{ij} is the random error.

Duncan's multiple range test was implemented to evaluate whether the means of the variables differed significantly or not (Duncan, 1955). Means were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of supplementing the maternal dietary *ESs* on :

1. Egg weight loss (%)

Results in Table 2 represented that there were a significant effect of dietary *ESs* treatment for laying hens on egg weight loss during (0-14) and (0-18) days of incubation. Egg weight loss was significantly (P≤ 0.01) decreased for 3.0 % *ESs* group in compared to other experimental groups.

It is evident from the results of the previous table that the reduction of egg weight loss for group of 3.0 % *ESs* could be due to the increase of shell with membrane thickness for this group compared to all experimental groups in the first part of research on *ESs* (EL-Barbary*et al*., 2023), Besides that, this decline may be attributed to the high quantity of carotinoids content in *ESs* (Abdul-Majeed and Taha, 2019) which can be a good source of Ca to increase egg shell thickness (Englmaierová *et al*., 2013). These results are in accordance with those obtained by Ebtsam *et al*. (2018) who explained the decrease in egg weight loss (%) as a result of supplementing the diet with carotenoids-enriched *Spirulina* algae as a source of antioxidants to their positive role in improving shell thickness. Given that *ESs* contains zinc, copper,iron,selenium, and vitamins such as Vit.E (Zeinab, 2003), one study by Urso *et al*. (2015) demonstrated that supplementing zinc-L-selenomethionine and vitamin E to the diet of broiler breeders decreased egg weight loss during incubation.

2-Relative weights of embryos , yolk sac and eggshell , besides eggshell thickness

Data of embryonic weight at 7^{th},14^{th} and 18^{th} days of incubation in Table 3 revealed that embryo weights increased by increasing *ESs* levels in maternal diet, and this increase is significant for 3.0 % *ESs* group compared with control group. On the contrary, this increase is offset by a decrease in the yolk sac, knowing that this decrease is significant for the previously mentioned group 2.0 % and 3.0 % *ESs* in compared to control group. Also, shell weight decreased (P≤ 0.05) for eggs of 3.0 % rocket seeds group compared with those for control group only at 18^{th} day of incubation. Shell thickness at 18^{th} and 21^{th} days of incubation reduced (P≤ 0.05) for 2.0 and 3.0 % groups compared with control group.

Generally, the observed increase of relative embryonic weight during incubation, as a result of supplementing maternal diet with *ESs*, especially at the higher concentration of *ESs* (3.0 %) could be due to the high nutritional value of *ESs* (El-Barbary *et al*., 2023). These seeds contain various nutrients such as carotenoids, flavonoids, volatile oils, glucosinolates ,minerals,and vitamins (El-Nattat and EL-Kady, 2007; Abdul-Majeed and Taha, 2019). These findings support the results of Urso*et al*. (2015) who indicated that adding vitamin E and zinc-L-selenomethionine to diets of broiler breeder could advance better embryonic development. Also, Ebtsam *et al*. (2018) noted that embryonic weight at day 18 of incubation was significantly largest for a group of eggs produced from hens that were fed a diet containing carotenoids-enriched *Spirulina* algae compared with those for other treatments. Moreover, Pontes *et al*. (2024) observed that the supplementation of quail diets with blend of organic acids, essential oils, curcumin, tannins, vitamin E, and zinc microencapsulated improves embryonic development. As for the decrease in the relative weight of the yolk sac by increasing concentration of *ESs* in the diet ,it may be attributed to the embryo consuming a large amount of the yolk sac to synchronize with the increase in embryonic growth. Dayan *et al*. (2020) demonstrated that assessment of residual yolk sac utilization provides information

on embryonic development and nutritional status, the lower percentage of the residual yolk sac during embryogenesis, indicates better the absorption and utilization of nutrients by the embryo. From days 18^{th}-21^{th} of incubation, the reduction of shell weight (%) and thickness for 2.0 and 3.0 % *ESs* groups could be related to the increase of calcium absorption from egg shell by the embryo to meet the hatching requirements (Ghanem *et al*., 2020). Our results are supported by Alfanso-Torres *et al*. (2009) who accelerated that different percentage of calcium in the egg shell may affect bone development in embryogenesis.

3- **Embryonic mortality , fertility and hatchability percentages**

Impacts of maternal nutrition intervention with *ESs* at different levels on embryonic mortality and hatching traits was illustrated in Table 4. Nutritional addition of *ESs* led to a decrease in percentage of embryonic mortality throughout 0-18^{th} and 18^{th}-21^{th} days of incubation, and this decrease was significant ($P \leq 0.05$) for the 3.0 % *ESs* group when compared to the control group. In addition, the rates of both fertility and hatchability of total and fertile eggs increased significantly ($P \leq 0.05$) as a result of adding 3.0 or 2.0 % *ESs* to the diet, respectively, when compared to control group. While, pipped eggs (%) decreased significantly for the same groups mentioned above. Also, there were no significant effect of maternal dietary *ESs* on addled eggs (%).

As it appears from data of Table 4, the reduction of embryonic mortality and pipped egg percentages, a long with the improvement in hatchability rate in the dietary supplementation groups of *ESs*, especially for the 3.0 % *ESs* group compared to control group could be due to the positive effect of *ESs* as a good source of calcium in improving shell thickness, as reported by El-Barbary *et al*. (2023). Moreover, *ESs* are rich in bioactive glucosinolates compounds (Bell *et al*., 2015), carotenoids and vitamins (E,C, A, and B) that works as antioxidants (Al-haj *et al*., 2019). These compounds are transferred from the maternal diet to the eggs, then to the embryos (Surai *et al*., 2016), and subsequently work to protect embryonic cells from oxidative stress through three mechanisms: First, by reducing harmful enzymes; Second, by eliminating free radicals in the cell and Third by increasing cellular antioxidant defenses (Barillari *et al*., 2005). Also, Kumar *et al*. (2023) confirmed the importance of the aforementioned antioxidants in protecting embryos during embryonic development . Furthermore, Mariey *et al*. (2012) and Ebtsam *et al*. (2018) have demonstrated the role of these compounds in increasing both fertility and hatchability percentages. Results of Basuony *et al*. (2020) also confirmed our data, they concluded that *in ovo* injection with omega 3, which is present in *ESs* with a large amount, increased the percentage of hatching by increasing the energy production during the late phase of embryogenesis.

4- **Chick quality measurements**

Effects of maternal dietary supplementation of 1.0 ,2.0 and 3.0 % *ESs* on quality of Silver Sabahia hatched chicks according to the record scores given by Tona *et al*.(2003) are shown in Table 5. Adding *ESs* to the diet at different levels significantly ($P \leq 0.01$) increased quality characteristics of hatched chicks compared to the control group.

Maternal nutrition is considered one of the factors affecting day-old-chick quality (Bruggeman *et al*., 2007). So, the observed results could potentially be attributed to the high nutritional value of *ESs* (carotenoids, vitamins, minerals, and fatty acids) .These include carotenoids (Ebtsam *et al*.,2018), particularly canthaxanthin, as well as

vitamins and zinc, all of which have a positive impact on weight and quality of hatched chicks (Pontes *et al*., 2024). Also, vitamin supplementation via the maternal diet increased bone size, ash and bone mechanical properties of embryo and chicks at hatch (Torres and Korver, 2018). Furthermore, maternal dietary vitamins and carotenoids have been reported to be effectively deposited in the egg yolk (Leeson and Caston, 2003), and influence the antioxidant status of embryos and newly-hatched chicks (Lin *et al*., 2005; Rosa *et al*., 2012). Koppenol *et al*.(2014) reported that maternal transition of omega-3 fatty acids, which is rich in *ESs*, improved chick quality. Also, Basuony *et al*. (2020) recorded that *in ovo* injection with omega 3-fatty acids increased body weight of hatched chicks. During embryogenesis, the presence of omega 3-PUFA , may enhance the proliferation of myoblasts which in turn increase the muscular growth of chicks (Tako *et al*.(2004) and Lopes *et al*. (2006)).

5- **Blood hematology**

Table 6 displays that plasma Hb and PCV (%) were significantly increased ($P \leq 0.01$) for chicks of 2.0 and 3.0 % *ESs* groups compared to those for 1.0 % *ESs* and control groups at hatch. Also, plasma WBC's was significantly ($P \leq 0.01$) highest in chicks for 3.0 % *ESs* group compared to those for other experimental groups except for 2.0 % *ESs* group. While, chicks of 2.0 and 3.0 % *ESs* groups represented significant decrease for plasma heterophil (%) and H/L ratio compared with those for control group. Moreover, there were no significant differences between all chicks for experimental groups with respect to plasma RBC's, lymphocyte (%), and platelets .

The enhancement of most hematological parameters for newly hatched chicks for supplemented groups with *ESs* could be interpreted with the maternal influence for dietary supplementation. Backed by this idea, Khawaja *et al*. (2012) demonstrated that nutrition is one of the factors affecting hematological measurements in birds. Also, in the first part of the study by El-Barbari *et al*. (2023) , attributed the increase in blood plasma Hb to the presence of iron and essential vitamins in *ESs* , which play a role in the synthesis of hemoglobin in the body (Khalil *et al*.,2015), and an increase in Hb and PCV (%) enhance the oxygen-carrring capacity of the blood, thus improving the health of the chicks (Farahat *et al*.,2009). Also, Badway (1998) reported that iron is essential for metabolic enzymes biosynthesis as cytochromes superoxide. As for the increase in value of plasma WBC's count with a decrease in heterophil (%) and H/L ratio, it may be due to that *ESs* have immune-stimulatory effects and work to improve immune functions (Karadas *et al*.,2005). These effects could be due to the presence of phtochemicals such as flavonoids, phenolics, glucosinolate, vitamins, carotenoids and others in *ESs* (Al-Qudah *et al*., 2018), which enhance digestion and the absorption of nutrients (Al-Shammari and Batkowska, 2021), thereby improving immunity and overall health. Supporting to the previous results, Hassan *et al*.(2023) observed that adding 0.2 g/Kg diet of *ESs* significantly increased Hb, PCV (%) and WBC's count compared to control group, with no significant differences between treatments regarding RBC's . Also, they proved that addition of *ESs* to broiler diet had a positive effect on hematological and immunological parameters. Moreover, Ebtsam *et al*. (2024) indicated that *in ovo* injection with *Spirulina* as a source of carotenoids and vitamins (C,E,A,D) at 14^{th} day of incubation increased values of Hb and WBC's counts , while, decreased the values of H/L ratio of the day-old chicks.

6- **Biochemical blood constituents**

Data presented in Table 7 showed that supplementation hen's diet with *ESs* had no significant effect on plasma total protein of hatched chicks. Plasma cholesterol concentration of baby chicks decreased significantly ($P \leq 0.01$) with increasing the level of *ESs* in the maternals diet as compared to of control group. Values of MDA were significantly ($P \leq 0.01$) reduced for 2.0 and 3.0 % *ESs* groups in compared to those for control group. In addition, the lowest significant ($P \leq 0.01$) values for LDL plasma concentration were recorded for the same groups mentioned above compared to those for 1.0 % *ESs* and control groups. Chicks of 3.0 % *ESs* group had highest ($P \leq 0.05$) values of plasma glucose compared with those for control group. While , there were no significant differences among chicks groups of 2.0 and 3.0 % *ESs*. Plasma calcium were elevated for 3.0 % *ESs* group ($P \leq 0.05$) compared with those for control group, besides, chicks of 1.0 , 2.0 and 3.0 *% ESs* groups did not represent statistical difference in the same trait. Values of plasma HDL and TAC increased ($P \leq 0.05$) for hatched chicks of 2.0 and 3.0 % *ESs* groups compared to control group.

From the previous table it is clear that some blood parameters of hatched chicks are similar to those found in the blood of their mothers, as stated in the first part of the research (El-Barbary *et al*.,2023). This similarity in the measurements confirms the maternal influence (Surai *et al*., 2016). The decrease in plasma total cholesterol and LDL and the increase in HDL is due to *ESs*containing ß-carotene (Bassyouni *et al*., 2022), which in turn works to reduce cholesterol in the blood by replacing it in the transport of lipoproteins and releasing them into blood circulation ((Yeum and Russell, 2002 ; Salma *et al*., 2007). Also, the presence of glucosinolate compounds, flavonoids (mainly appin and luteolin) and unsaturated fatty acids (linoleic and linolenic acids) in *ESs* (Gugliandolo*et al*., 2018 ; Abdul-Majeed and Taha, 2019) contributed to inhibiting lipid oxidation by suppressing the secretion of Co enzyme A (El-Fadaly *et al*., 2017), which plays a role in cholesterol formation. Consequently, this led to an increase in level of HDL and a decrease in LDL (Abou El-Maaty *et al*., 2021).

The results reported herein regarding to an increase plasma glucose for chicks of 3.0 % *ESs* group is because containing *ESs* protein with high concentration (Bell and Wagstaff, 2014) and most types of vitamins B groups (B_{12} , B_6 , B_2 and B_1) (Gulfraz*et al*., 2011), since vitamin B_1 plays an important role in the synthesis of glucose from protein and stimulate the glycolysis of stored glycogen (Elwyn and Bursztein, 1993). Furthermore, vitamin B_2 helps break down dietary carbohydrates by acting as a cofactor for function of several enzymes (Kitakoshi *et al*., 2007). Moreover, the observed increase of plasma calcium for hatched chicks of the same aforementioned group compared with the others could be due to the increase of the amount of calcium in *ESs* (1223.5 mg/100g) (Rozan and Boriy, 2022).

As for the increase in total antioxidants and the decrease in malondialdehyde in chicks' plasma as a result of maternal diets with *ESs* ,it could due to the richness of *ESs* in carotenes and glucosinolates, which work as antioxidants (Kim *et al*., 2004). Also, Erucin is considered the main glucosinolate in *ESs* , which works to protect cells from oxidative stress by stimulating P enzymes, removing free radicals accumulated in cells, and acting as a precursor to sulforaphene, which is considered a catalyst for removing toxins from cells and increasing cellular antioxidants (Barillari *et al*., 2005). Also, there are many important vitamins

in *ESs*, such as vitamins E,A, and C act as antioxidant (El-Nattat and El-Kady, 2007 ; El-Sahn *et al*., 2024), which transfer from the feed to the egg, then to the embryo's tissues and blood (Surai, 2012) and can be regulated by dietary means.

Due to the lack of research on the effect of adding *ESs* to maternal diet on blood parameters of hatched chicks, we attempted to explore additives with similar components to those found in *ESs*, such as *Spirulina* algae and annatto seeds, this was confirmed by Ebtsam *et al*.(2024) who mentioned that injecting eggs with *Spirulina* as a potent and effective antioxidant led to an increasethe levels of glucose and HDL , as well as TAC. Conversely, it reduced the levels of LDL and MDA in plasma of day-old chicks. Also, Al-Saeedi *et al*.(2025) observed that *in ovo* injection with annatto seed extract decreased values of plasma cholesterol and MDA , while , increased values of plasma glucose for hatched chicks and enhanced the antioxidant levels , this may due to presence tocopherols, tocorinols, carotenoids and many vitamins (such as E, A), which considered natural antioxidants (Appiah- Nkansah, 2011).

7- **Yolk sac and internal organs weight of hatched chicks**

As is clear from Table 8, the data of relative weight of yolk sac represented significant ($P \leq 0.01$) decrease for hatched chicks of 2.0 and 3.0 % *ESs* groups compared to control group. While, relative weight of liver was significantly ($P \leq 0.01$) increased for chicks hatched from eggs produced from hens fed different levels of *ESs* in compared to control group. Also, chicks of 3.0 % *ESs* group represented significant ($P \leq 0.01$) increase of relative weight of intestine compared with those of other experimental groups. Moreover, all experimental groups did not represent any statistical differences with respect to relative weight of heart, gizzard, spleen , stomach, gallbladder, and fabricious gland.

As can be seen from data of Tables (3,5 and 8) that the decrease of embryonic yolk sac (%) for 2.0 and 3.0 % *ESs* groups compared with control group could be due to the absorption of great amount of nutrients from the yolk sac. These increase of absorbed nutrients in previous mentioned groups could be the reason for increase of metabolic pathway and consequently increase of chicks weight. As for the increase in liver weight (%) due to the addition of *ESs* in the maternal diet , it was attributed to the fact that *ESs* contains glucosinolates, which have various biological activities including antimicrobial, antioxidant and anti-inflammatory properties (Kim *et al*., 2004). These bioactive components can improve liver function (Abou- El Khair*et al*., 2014). The intestinal weight of hatched chick from the eggs produced from hens supplemented with 3.0 %*ESs* was higher than other groups . This suggested that the intestinal development of the chicks benefited from the antioxidant constituents of *ESs*. The use of antioxidants embryonic development has been studied to confer oxidative protection on the intestines and other groups from radicals that could impair development before hatching (Surai,1999) . Also, this may be due to the increase in beneficial microbes in the intestines and the enhancement of digestion and absorption processes in the embryos (Abdelli *et al*., 2021). Supporting to results and interpretation herein, Pontes *et al*. (2024) found that supplementing the maternal dietary quails with a blend of organic acids, essential oils, curcumin, tannins, vitamin E, and zinc microencapsulated increased relative liver and intestinal weights for embryos compared with control diet. These phytogenics stimulate the gastrointestinal system, leading to increased production of digestive enzymes (Saleh *et al*., 2021). Regarding

this effect, in recent studies, zinc and vitamin E were used, alone and combined , in poultry and laying hens diets, affording satisfactory effects on animal growth and gastrointestinal (Zhao *et al*., 2021 ; Xiao *et al*., 2022).

CONCLUSIONS

Maternal dietary addition of 2.0 or 3.0% *ESs* improved embryonic development and increased hatchability by decreasing embryonic mortality, which was achieved by increasing the antioxidant status of the egg yolk and tissues of the embryos and improving some hematological and biochemical blood parameters of one-day-chicks. Therefore, *ESs* could be used as a dietary supplement to enhance hatchability and chick quality in poultry production. The suitable *ESs* level for laying breeder diet was 3.0 %. We need to conduct extensive research to better understand the impact of adding different levels of *ESs* to maternal diet on embryonic development, as there no studies available in this field.

Table (1): Ingredient and chemical composition (g/kg) of the experimental diet for laying hens through 30-38 weeks of age

Ingredients	**(%)**
Corn	66.33
Soybean meal (48% CP)	24.2
Limestone	7.5
Dicalcium phosphate	1.32
Vit + Min Premix*	0.25
NaCl	0.25
DL-methionine	0.15
Total	100
Chemical composition calculated**	
Metabolizable energy (kcal/kg)	2700
Dry matter (%)	90.73
Crude protein (%)	16.97
Crude fat (%)	2.45
Crude fiber (%)	3.96
Ash (%)	6.37
Nitorgen free extract (%)	60.98

*Vit+Min mixture provides per kilogram of diet: Vitamin A, 12000 IU;Vitamin E, 10 IU; Menadione, 3 mg; Vit. D3, 2200 ICU; riboflavin, 10mg; Ca Pantothenate, 10 mg; Nicotinic acid, 20 mg; Choline chloride, 500mg; Vitamin B12, 10 µg; Vitamin B6, 1.5 mg; Vitamin B1, 2.2 mg; Folicacid, 1 mg; Biotin, 50 µg.

*Trace mineral (milligrams per kilogram ofdiet): Mn, 55; Zn, 50; Fe, 30; Cu, 10; Se, 0.10;Antioxidant, 3 mg. CP:Crude protein.

**These values were calculated according to NRC (1994)

Table (2): The impact of maternal dietary addition of *Eruca sativa* seeds on egg weight loss during incubation

Traits	*Eruca* seeds levels group					
	Control	**1%**	**2%**	**3%**	**SEM**	**P value**
Intial set egg weight (g)	49.82	49.50	49.70	49.10	0.45	0.706
Egg weight, (g) at						
7 d	47.82	47.50	47.70	47.10	0.45	0.706
14 d	44.82	44.50	44.70	44.63	0.46	0.970
18 d	43.67	43.49	44.05	44.13	0.41	0.653
Egg weight loss (%)						
0 -7 days	4.02	4.06	4.04	4.08	0.04	0.677
0 -14 days	10.05 ^{a}	10.15 ^{a}	10.09^{a}	9.12 ^{b}	0.13	0.000
0 -18 days	12.34 ^{a}	12.13 ^{a}	11.36 ^{b}	10.13^{c}	0.16	0.000

^{abc} Means in the same row having different superscripts are significantly different ($P \leq 0.05$). SEM: Standard error of the means. P value : Probability level.

Table (3): The impact of maternal dietary addition of *Eruca sativa* seeds on embryonic development during incubation

Traits	*Eruca* seeds levels group					
	Control	**1%**	**2%**	**3%**	**SEM**	**P value**
Intial set egg weight (g)	49.82	49.50	49.70	49.10	0.45	0.706
At day 7 of incubation						
Egg weight (g)	47.84	47.57	47.70	47.11	0.27	0.333
Embryonic weight (%)	2.55 ^{c}	3.13 ^{b}	3.24^{b}	4.12^{a}	0.16	0.000
Yolk sac (%)	64.65 ^{a}	60.10^{ab}	58.08^{b}	57.06 ^{b}	1.60	0.021
Shell weight (%)	10.99	10.98	10.60	10.06	0.26	0.143
Shell thickness (mm)	0.37	0.35	0.35	0.35	0.01	0.330
At day 14 of incubation						
Egg weight (g)	44.82	44.59	44.70	44.60	0.51	0.991
Embryonic weight (%)	32.11^{b}	33.24^{b}	34.34^{b}	37.46^{a}	0.59	0.002
Yolk sac (%)	30.59^{a}	24.56^{b}	24.81^{b}	22.91^{b}	1.39	0.049
Shell weight (%)	10.67	10.96	10.58	9.83	0.71	0.795
Shell thickness (mm)	0.36	0.35	0.35	0.32	0.01	0.327
At day 18 of incubation						
Egg weight (g)	43.68	43.50	44.05	44.17	0.51	0.805
Embryonic weight (%)	52.96 ^{b}	57.20^{ab}	57.74 ^{ab}	58.96^{a}	1.37	0.034
Yolk sac (%)	21.52^{a}	18.44^{b}	17.34^{b}	17.19^{b}	0.66	0.001
Shell weight (%)	10.54^{a}	10.01^{ab}	9.65^{ab}	9.04^{b}	0.40	0.047
Shell thickness (mm)	0.36^{a}	0.35^{a}	0.32^{b}	0.31^{b}	0.01	0.015
At day 21of incubation						
Shell thickness (mm)	0.33^{a}	0.32^{ab}	0.31 b^{c}	0.29^{c}	0.01	0.020

^{abc} Means in the same row having different superscripts are significantly different ($P \leq 0.05$). SEM: Standard error of the means. P value : Probability level.

Table (4): The impact of maternal dietary addition of *Eruca sativa* seeds on embryonic mortality and hatching traits

Traits	*Eruca* seeds levels group					
	Control	**1%**	**2%**	**3%**	**SEM**	**P value**
Embryonic mortality (0- 18) %	12.33 ^{a}	9.31^{ab}	5.24 ^{ab}	2.67 ^{b}	2.27	0.043
Embryonic mortality (18- 21) %	7.00 ^{a}	5.33^{ab}	4.33 ^{ab}	2.33 ^{b}	0.63	0.048
Macroscopic fertility (%)	76.57^{b}	82.37^{ab}	85.25^{a}	88.22^{a}	1.78	0.021
Hatchability of total egg (%)	61.46^{c}	70.00^{bc}	76.63^{ab}	82.96^{a}	2.34	0.003
Hatchability of fertile egg (%)	80.67^{c}	85.36^{bc}	90.43^{ab}	95.00^{a}	1.43	0.001
Pipped eggs (%)	4.00^{a}	3.00^{ab}	2.00 ^{bc}	1.00 ^{c}	0.43	0.014
Addled eggs (%)	3.00	2.33	2.33	1.33	0.48	0.210

^{abc} Means in the same row having different superscripts are significantly different ($P \leq 0.05$). SEM: Standard error of the means. P value : Probability level.

Table (5): The impact of maternal dietary addition of *Eruca sativa* seeds on hatched chick quality traits

Traits	*Eruca* seeds levels group					
	Control	**1%**	**2%**	**3%**	**SEM**	**P value**
Chick weight (gm)	33.39 ^{d}	35.05 ^{c}	36.46 ^{b}	37.97 ^{a}	0.29	0.000
Chick length (cm)	13.73 ^{c}	14.68 ^{b}	14.92 ^{b}	16.00 ^{a}	0.11	0.000
Activity (good)	91.60 ^{b}	98.70 ^{a}	99.10 ^{a}	99.60 ^{a}	0.50	0.000
Downs and appearance (Clean and dry)	93.70 ^{b}	99.40 ^{a}	99.70 ^{a}	99.80 ^{a}	0.41	0.000
Retracted yolk (Body with normal swallowed yolk)	97.70 ^{b}	100.00^{a}	100.00^{a}	100.00^{a}	0.11	0.000
Eyes (Opened and bright)	88.20 ^{b}	97.40 ^{a}	97.90^{a}	99.00 ^{a}	0.72	0.000
Navel (Completely closed and Clean)	92.50 ^{c}	97.50 ^{b}	97.90^{ab}	99.07 ^{a}	0.41	0.000
Remaining membrane (No membrane)	91.40 ^{c}	97.20 ^{b}	97.60^{b}	99.47 ^{a}	0.59	0.000

^{abcd}Means in the same row having different superscripts are significantly different ($P \leq 0.05$). SEM: Standard error of the means. P value : Probability level.

Table (6): The impact of maternal dietary addition of *Eruca sativa* seeds on hematological parameters (within normal range) of hatched chick

Traits	*Eruca* seeds levels group					
	Control	**1%**	**2%**	**3%**	**SEM**	**P value**
Hb (g/dL)	10.88^{b}	11.13^{b}	12.85^{a}	13.50^{a}	0.45	0.004
PCV (%)	35.89 ^{b}	36.71 ^{b}	42.41^{a}	44.55 ^{a}	1.49	0.004
WBC's (10^3/mm^3)	6.03 ^{b}	6.34 ^{b}	7.06 ^{ab}	8.02 ^{a}	0.30	0.005
RBC's (10^6/mm^3)	2.08	2.34	2.46	2.30	0.30	0.701
Heterophils (H, %)	25.65 ^{a}	21.30^{ab}	17.73 ^{b}	14.65 ^{b}	1.84	0.027
Lymphocytes (L, %)	65.50	65.75	67.75	71.75	4.34	0.783
H/L ratio	0.39 ^{a}	0.32 ^{ab}	0.27 ^{bc}	0.21 ^{c}	0.03	0.013
Platelets (10/uL)	4.50	4.75	5.25	6.00	1.40	0.896

^{abc}Means in the same row having different superscripts are significantly different ($p \leq 0.05$). SEM: Standard error of the mean, P value : Probability level, Hb: Hemoglobin concentration, RBC's: Red blood cell, WBC's: White blood cell, PCV: Packed cell volume.

Table (7): The impact of maternal dietary addition of *Eruca sativa* seeds for laying hens on blood parameters (within normal range) of hatched chick

Traits	*Eruca* seeds levels group					
	Control	**1%**	**2%**	**3%**	**SEM**	**P value**
Total Protein (g/dl)	6.47	6.55	6.57	6.64	0.10	0.648
Cholesterol (mg/dl)	149.97 ^{a}	139.97 ^{b}	135.50^{bc}	132.00^{c}	1.19	0. 000
LDL (mg/dl)	48.75 ^{a}	44.25 ^{a}	30.00 ^{b}	30.00 ^{b}	2.70	0.001
HDL (mg/dl)	37.75 ^{b}	41.00 ^{b}	55.75 ^{a}	60.75 ^{a}	3.82	0.005
Glucose (mg/dl)	180.00^{b}	195.75 ^{b}	202.25^{ab}	221.75^{a}	6.31	0.010
Calcium (mg/dl)	10.66 ^{b}	10.88^{ab}	11.21^{ab}	11.70 ^{a}	0.24	0.035
TAC (mmol/ml)	1.70 ^{b}	1.20 ^{ab}	2.45 ^{a}	2.73 ^{a}	0.18	0.028
MDA (nmol/ml)	3.29 ^{a}	2.83 $^{a\,b}$	2.44 ^{b}	2.08 ^{b}	0.23	0.022

^{abc} Means in the same row having different superscripts are significantly different (P≤ 0.05). SEM: Standard error of the mean, P value : Probability level, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TAC:Total antioxidant capacity, MDA: Malondialdehyde.

Table (8): The impact of maternal dietary addition of *Eruca sativa* seeds on relative weight of yolk sac and internal organs for hatched chicks

Traits	*Eruca* seeds levels group					
	Control	**1%**	**2%**	**3%**	**SEM**	**P value**
Yolk sac (%)	14.36 ^{a}	12.21^{ab}	11.83 ^{b}	9.80 ^{b}	0.78	0.006
Liver (%)	2.08 ^{b}	2.83 ^{a}	2.93 ^{a}	2.96 ^{a}	0.09	0.000
Intestine (%)	4.04 ^{b}	4.02 ^{b}	4.43 ^{b}	5.39 ^{a}	0.26	0.004
Heart (%)	0.78	0.79	0.81	0.87	0.05	0.571
Gizzard (%)	4.71	4.99	5.12	5.13	0.24	0.622
Spleen (%)	0.07	0.06	0.06	0.06	0.01	0.222
Stomach (%)	0.80	0.80	0.83	0.82	0.06	0.153
Gallbladder (%)	0.17	0.16	0.15	0.14	0.02	0.765
Fabricius gland (%)	0.16	0.17	0.17	0.15	0.03	0.838

^{ab} Means in the same row having different superscripts are significantly different (P ≤ 0.05). SEM: Standard error of the means. P value : Probability level.

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الملخص العربى

تأثير الأضافة الغذائية ببذور الجرجير(*Eruca sativa*) على تطور الأجنة و نسبة الفقس و بعض المعايير الفسيولوجية لسلالة دجاج الصبحية فضى

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تم اجراء هذه الدراسة لتحديد تأثير اضافة مستويات مختلفة من بذور الجرجير (*ESs*) الى العليقة الأساسية للدجاج البياض على تطور الأجنة , و نسبة الفقس (%), و جودة الكتاكيت , و بعض الخصائص الفسيولوجية لسلالة دجاج الصبحية فضى . تم استخدام 300 دجاجة بياضة و 40 ديك بعمر 30 اسبوع . وتم توزيعها عشوائيا الى 4 مجموعات تجريبية (75 انثى و 10 ذكور لكل مجموعة) . تم تغذية الدجاج البياض بأربعة أنظمة غذائية تجريبية مختلفة : المجموعة الأولى كانت مجموعة الكنترول , بينما تم تغذية المجموعات الثانية و الثالثة و الرابعة على العليقة الأساسية مضافا اليها 1,0 % و2,0 % و 3,0 % من بذور الجرجير على التوالى . عند عمر 32 اسبوع تم تلقيح الدجاجات صناعيا مرتين اسبوعيا باستخدام السائل المنوى من الديوك التى تغذت على العليقة الكنترول . تم جمع البيض يوميا من كل مجموعة لمدة 7 أيام عند أعمار 34و36و38 أسبوعا . تم تحضين 1000 بيضة (250 بيضة لكل معاملة) لتمثل المجموعات التجريبية الأربعة . أظهرت النتائج أن اضافة بذور الجرجير (*ESs*) بمستوى 2,0 % أو 3,0 % الى العليقة الأساسية قللت معنويا ($P\leq 0.01$) من الفقد فى وزن البيض (%) خلال الأيام 0-18 من التحضين , وكذلك من الوزن النسبى لكيس الصفار , معدل نفوق الأجنة (%) , و نسبة البيض الناقر . فى المقابل , أدت هذه الأضافة الى زيادة وزن الأجنة معنويا (%) و نسبة الفقس (%) وجودة الكتاكيت الفاقسة . علاوة على ذلك , حسنت معظم قيم معاييرالدم للكتاكيت الفاقسة و خفضت نسبة الكوليسترول فى البلازما , و المانولندهيد ,الى جانب زيادة تركيز مضادات الأكسدة الكلية . لوحظ أن هذا التحسن فى جميع المعايير المذكورة كان متناسبا مع زيادة مستويات بذور الجرجير فى العلف , حيث كانت نسبة 3,0% هى الأفضل يليها 2,0% .
الخلاصة, تشير البيانات الى أن اضافة 2,0% أو3,0 % من بذور الجرجير الى العلف تعتبر أداة غذائية فعالة لتحسين تطور الأجنة ,و تحقيق أفضل نسبة فقس , و انتاج كتاكيت ذات جودة صحية عالية و مواصفات جيدة .