THE IMPACT OF INCORPORATING HESPERIDIN (HIS), EITHER INDEPENDENTLY OR IN CONJUNCTION WITH ASCORBIC ACID (ASA), ON SPECIFIC QUALITATIVE EGG CHARACTERISTICS AND PHYSIOLOGICAL BLOOD PARAMETERS OF ISA BROWN LAYING HENS.

Faez M. Turky1 and Ahmed T. Taha2
1Department of Animal Production, College of Agriculture, University of Tikrit, Tikrit, Iraq
Corresponding email: faeizmajed19952006@gmail.com
Dr.att@tu.edu.iq

Abstract

This study examined how adding hesperidin alone or with ascorbic acid affects egg quality and physiological parameters in ISA Brown laying hens. In this experiment, 40 laying hens were randomly assigned to four experimental units of 10 each, with five replicates (2 hens per replicate). Treatments included: CON (control), HIS (250 mg/L hesperidin and ascorbic acid), and HIS+ ASA (125 mg/L hesperidin and 125 mg/L ascorbic acid) in drinking water. The study was conducted over two production periods, each lasting 28 days, with a general average for both periods of egg quality indicators. blood parameters were measured once per month. Most qualitative egg traits (egg weight, relative yolk weight, relative albumen weight, and relative shell weight) were unaffected. Hesperidin treatment resulted in a significant increase in eggshell thickness. All supplemented treatments significantly increased red blood cell count, mean corpuscular volume, and hemoglobin concentration when compared to the control. There were no significant differences in total protein, albumin, globulin, or uric acid levels within the study periods. Treatment with ASA alone or in combination with hesperidin resulted in significantly lower triglyceride and LDL levels. Hesperidin significantly raised HDL levels. Adding HIS significantly reduced Estrogen levels when compared to other options. Adding hesperidin or ascorbic acid did not influence egg quality, the study revealed. Hesperidin thickened eggshells primarily. Hesperidin mainly lowers estrogen. After adding hesperidin, hemoglobin and cell volume decreased and red blood cell numbers increased. In laying chickens, raising HDL and lowering triglycerides improved biochemical blood profiles alone or with hesperidin.

Key words: Hesperidin, ascorbic acid, egg, blood, hens

Introduction

Citrus fruits, naturally contain hesperidin glycoside flavonoid, a cost-effective and abundant byproduct of citrus cultivation [1,2]. Citrus species and plant parts show significant variation in hesperidin content. Liu et al. [3] found that combining hesperidin with certain flavonoids can improve its water solubility despite its low solubility when alone. Musa et al. [4] mention its presence in olive oil and tea, as well as its use in traditional medicine to treat various ailments. Hesperidin was first isolated from the inner part of orange peel in 1828 [5]. Hesperidin can protect ovaries and oocytes from oxidative damage, increase fat deposition and storage in the ovary, and

ISSN 2072-3857

contribute to the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are essential for the maturation and release of eggs, as well as stimulating the ovary to produce and secrete estrogen [6]. [7] showed that hesperidin can improve egg quality, meat quality, intestinal protection efficiency, and both acquired and innate immune responses in poultry. Vitamin E and hesperidin are both antioxidants that protect cells against free radicals [8]. Several studies have shown that flavonoids, like hesperidin, have an important role in growth performance, egg quality, oxidative status, and production performance. Hesperidin has been shown in studies to reduce blood sugar and cholesterol levels. In addition to strengthening the immune system, it has antioxidant properties that are antiviral, anti-inflammatory, anti-allergic, anticancer, and neuroprotective [9,10]. Vitamin C, one of the strongest naturally occurring water-soluble antioxidants, is abundant in a variety of tissues, including the brain [11]. It improves the effects of vitamin E and selenium and acts as an antioxidant by scavenging reactive oxygen species (ROS), such as superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen [12,13]. Vitamin C levels have been demonstrated to decrease while vitamin E levels remain constant in various oxidative stress exposure scenarios [14,15]. However, laying hens suffer from a variety of issues, including immune-related disorders and metabolic diseases caused by excessive liver fat buildup, as well as the increasing negative impacts of reactive oxygen species and free radical activity. These negative repercussions are more obvious in caged chickens than in those raised on the ground, owing to reduced mobility, bone injuries, and increased metabolic activity [16]. The purpose of this study is to investigate the potential benefits of using hesperidin alone or in combination with ascorbic acid to enhance blood indicators, blood cell profiles, lipid and protein characteristics in plasma, and egg quality features. Laying chickens are an ideal subject due to their fatty acid content and lipoprotein composition.

Materials and methods :

In this experiment, forty ISA Brown laying hens, aged 40 weeks, were used. The hens were housed in 20 pyramid-shaped metal cages (replicates), with two hens per cage. Each cage measured 50 x 50 x 50 cm and was made of wire mesh. The birds were randomly allocated into four experimental treatments, with two hens per cage, and each treatment had 5 replicates, making each replicate consist of two hens. A nipple-type drinking system was used for the water supply in each cage, and manual trough feeders were used for feeding the birds. The lighting schedule consisted of 15 hours of light and 9 hours of darkness, according to the breeding guidelines. The rearing room was equipped with air exhaust fans and thermometers placed at different locations within the room.

A total of 40 laying hens were randomly assigned to 4 experimental units, with 10 hens per treatment, which included 5 replicates (2 hens per replicate). The experimental treatments were as follows:

.1 Treatment 1 (T1): Control treatment with no addition (CON(

.2 Treatment 2 (T2): Addition of 250 mg of hesperidin (HIS) per liter of water

.3 Treatment 3 (T3): Addition of 250 mg of ascorbic acid (ASA) per liter of water

.4 Treatment 4 (T4): Addition of 125 mg of hesperidin (HIS) per liter of water + 125 mg of ascorbic acid (ASA) per liter of water

ISSN 2072-3857

Bird Feeding:

The birds were fed a diet containing a metabolizable energy of 2750 kcal/kg of feed and a crude protein content of 16.5-17%. Hesperidin and ascorbic acid were added to the drinking water.

Blood Sample Collection:

To study hematological traits, blood samples were collected twice during the study period, after each production phase (every 28 days). Blood was drawn using 5 ml medical syringes from the wing vein of five hens from each treatment. The blood was then transferred into two tubes:

1. The first tube contained an anticoagulant (K-EDTA) for conducting blood count tests, including total red blood cells (TRBC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and total white blood cells (TWBC), which were measured according to the specified method.

2. The second tube also contained an anticoagulant to obtain serum. The samples were placed in a centrifuge at 3600 rpm for 20 minutes. Plasma was then collected and stored at -20°C until laboratory analysis. The tests included measurements of total protein, glucose, albumin, globulin, uric acid, cholesterol, triglycerides, and lipoproteins (HDL, LDL), as well as estrogen hormone levels.

Egg Quality Traits:

Five eggs were randomly selected from each treatment, twice during each productive phase, to Study the following traits:

Egg Weight (g): The average egg weight was measured using a sensitive scale with three decimal places separation.

Relative Yolk Weight: The yolk was separated from the egg white using a large spoon after cutting the chalazae, and the yolk was passed over filter paper to remove any remaining egg white. It was then weighed using a highly accurate electronic scale with three decimal places. The relative yolk weight was calculated as (yolk weight/egg weight) × 100.

Relative Egg White and Shell Weight: The relative weights of the egg white and shell, as well as the yolk index and albumen index, were calculated according to the method of Parmar et al. [17 .[

Statistical Analysis:

After data collection, statistical analysis was conducted using a Completely Randomized Design (CRD) to assess the effect of the treatments on the studied traits in this experiment. Duncan's multiple range test [18] was used to determine the significance of differences between treatment means for the studied traits. Statistical analysis was performed using [19.[

Results and Discussion:

Treatment with hesperidin, ascorbic acid, or their combination had no significant influence on egg weight, relative yolk weight, egg white weight, or relative shell weight, according to the statistical analysis in Table 1. In the second and fourth treatments—hesperidin either alone or in combination with ascorbic acid—average shell thickness showed notable increases compared to the control treatment , over the second research period, which ran eight weeks. For most measures of egg quality, chickens given either hesperidin, ascorbic acid, or both showed no appreciable variations. Either alone or in combination with ascorbic acid, hesperidin significantly raised average shell thickness during the second productive phase as compared to the control treatment. Since average shell thickness has an extremely substantial negative correlation with egg production rate, the great increase can be attributed to reduced egg production rates

[20,21]. Moreover, hesperidin possesses estrogenic properties that allows it to interact with estrogen receptors in the body and increase estrogenic activity, so supporting physiological processes that depend on this hormone , like calcium metabolism and bone calcium content [22]. Increased calcium absorption in the small intestine and build-up in bones and eggshells have been associated with estrogen [23]. Genetic expression of calcium transport proteins includes TRPV6 and calbindin D9K, which encourage calcium entry into intestinal cells and the circulation and determine calcium transport and absorption from the small intestine to the bloodstream. The main source of calcium for the eggshell, estrogen has been demonstrated to boost the synthesis of these proteins, maybe affecting osteoblast cells and boosting bone development [24.[

Table (1) The impact of incorporating hesperidin (HIS) either independently or in conjunction with ascorbic acid (ASA) on specific qualitative egg characteristics of ISA Brown laying hens

Treatments	Egg weight (gm)		
	P1(40-43 w)	P2(44-47 w)	TP (40-47 w)
CON (T1)	59.14± 2.24 a	63.22± 3.07 a	61.18± 2.58 a
HIS (T2)	57.71± 3.47 a	60.72± 0.89 a	59.22± 1.93 a
ASA (T3)	60.67± 1.12 a	59.68± 1.89 a	60.17± 0.95 a
HIS +ASA (T4)	57.38± 1.21 a	59.04± 0.70 a	58.21± 0.84 a
P- value	N. S	N. S	N. S
Treatments	Yolk%		
	P1(40-43 w)	P2(44-47 w)	TP (40-47 w)
CON (T1)	25.17± 1.26 a	25.83± 0.70 a	25.50± 0.93 a
HIS (T2)	26.80± 0.61 a	25.79± 0.93 a	26.29± 0.40 a
ASA (T3)	27.31± 1.26 a	25.93± 1.43 a	26.62± 1.22 a
HIS +ASA (T4)	25.52± 0.98 a	26.80± 1.22 a	26.16± 0.99 a
P- value	N. S	N. S	N. S
Treatments	Albumin%		
	P1(40-43 w)	P2(44-47 w)	TP (40-47 w)
	61.04± 1.76 a	63.21± 1.01 a	62.13± 1.32 a
HIS (T2)	62.51± 1.57 a	64.12± 0.59 a	63.32± 0.64 a
ASA (T3)	62.29± 1.25 a	63.75± 1.23 a	63.02± 1.16 a
HIS +ASA (T4)	63.59± 1.04 a	63.53± 1.65 a	63.46± 1.26 a
P- value	N. S	N. S	N. S
Treatments	Shell thickness(mm)		
	P1(40-43 w)	P2(44-47 w)	TP (40-47 w)
CON (T1)	0.48± 0.00 a	0.38± 0.01 b	0.43± 0.01 a
HIS (T2)	0.45± 0.00 a	0.45± 0.01 a	0.45± 0.00 a
ASA (T3)	0.47± 0.01 a	0.42± 0.01 ab	0.45± 0.01 a
HIS +ASA(T4)	0.48± 0.01 a	0.43± 0.01 a	0.46± 0.00 a

ISSN 2072-3857

P- value	N. S	*	N. S
Treatments	Shell weight%		
	P1(40-43 w)	P2(44-47 w)	TP (40-47 w)
CON (T1)	10.44± 0.87 a	10.94± 0.44 a	10.69± 0.65 a
HIS (T2)	10.68± 1.21 a	10.07± 0.37 a	10.38± 0.77 a
ASA (T3)	10.40± 0.20 a	10.31± 0.35 a	10.36± 0.09 a
HIS +ASA(T4)	11.07± 0.34 a	9.66± 0.44 a	10.37± 0.37 a
P- value	N. S	N. S	N. S

N.S) refers to absence of significant differences between the means at a probability level of ($p \leq 0.05$.(
(*)indicates the presence of significant differences between the means at a probability level of ($p \leq 0.05$.(
CON = Control treatment without addition, HIS= Addition of hesperidin at a rate of (250 mg/L of water), ASA= Addition of Ascorbic acid at a rate of (250 mg/L of water), HIS +ASA : Addition of hesperidin with Ascorbic acid at a rate of (125 + 125 mg/L of water.(

Effect of Adding Hesperidin Alone or with Ascorbic acid on Some Physiological Blood Parameters of ISA Brown Laying Hens

Based on statistical research, Table 2 shows that hesperidin, alone or in combination with vitamin C, affects Isa Brown laying hens' blood profiles. Blood hemoglobin and packed cell volume showed no change. Hesperidin alone or in combination with ascorbic acid (HIS + ASA) significantly reduced MCV and MCH levels. Hesperidin alone or with ascorbic acid did not affect glucose, protein, albumin, globulin, or cholesterol. The third and fourth treatments considerably lowered LDL and triglycerides compared to the first and second. The hesperidin and ascorbic acid treatments did not substantially change HDL levels compared to the control, even though the latter performed better. Hesperidin therapy decreased medication-induced estrogen effects more than other therapies. Hesperidin's estrogen-reducing effect may explain therapy's red blood cell growth, alone or with vitamin C. Oxygen, estrogen, and testosterone affect avian red blood cell production, according to [25]. Estrogen directly inhibits kidney erythropoietin synthesis, lowering bone marrow red blood cell formation. Thus, lower estrogen levels may enhance red blood cell count. Hesperidin's antioxidant qualities may have caused red blood cells to diminish in the bone marrow or beyond maturity. Reduced metabolic activity may explain the drop in egg production by reducing reactive oxygen species and free radicals [26]. The control therapy had a numerical edge in MCV and MCH values, while PCV and Hb levels were similar. Increased cell size and hemoglobin content may explain why PCV and Hb are similar [27]. Total red blood cell count correlated negatively with PCV, Hb, MCV, and MCH. Studies show that oxidants connected to LDL may cause lipoprotein oxidation, therefore increasing the risk of atherosclerosis, or plaque development in blood vessels. A hydrophilic antioxidant is vitamin C. By lowering LDL oxidation and neutralizing free radicals, ascorbic acid helps arteries to avoid cholesterol accumulation [28]. It could affect lipid metabolism by changing the metabolic routes taken in the liver. Reducing the low-density lipoproteins (LDL) formation by HMG-CoA reductase, which controls cholesterol synthesis, might help increase its enzymatic activity. Through

better liver cholesterol metabolism, ascorbic acid may lower LDL levels [29]. The increased egg production connected with ascorbic acid intake may lead to the removal of low-density lipoproteins during yolk formation. Hesperidin might affect the liver, the main organ in charge of controlling HDL and LDL among other lipoprotein levels in the blood. By turning on enzymatic pathways that enable efficient lipid and cholesterol metabolism, hesperidin might enhance liver performance. Through improved cholesterol metabolism, hesperidin can raise blood levels of HDL, a protein that helps excess cholesterol move to the liver for elimination. It is expected that Hesperidin's stimulation of hepatic enzymes linked to cholesterol synthesis and distribution—including HMG-CoA reductase and ACAT (Acyl-CoA: cholesterol acyltransferase)—will help to balance low- and high-density lipoproteins (HDL/LDL). The drop in estrogen levels might be related to the lowering of FSH and LH, which have little effect on the granulosa cells in the ovary that produce estrogen [30]. Hesperidin's estrogenic effect interacts with estrogen receptors in the basal cells of the anterior pituitary gland, thereby reducing the production of FSH and LH levels

Table (2) The impact of incorporating hesperidin (his) either independently or in conjunction with ascorbic acid (ASA) on some physiological blood parameters of ISA brown laying hens

Treatment group / traits	CON	HIS	ASA	HIS +ASA	*P-value*
RBC ($X10^6$ /µL)	1.16± 0.08b	1.62± 0.62a	1.62±0.04a	1.68± 0.11a	*
PCV (%)	25.20± 0.33a	25.50± 0.50a	25.00± 0.70a	25.50± 0.61a	N. S
Hb(g/100mL)	8.63± 0.10a	8.72± 0.17a	8.57± 0.21a	8.72± 0.18a	N. S
MCV ($\mu m^{3)}$	223.94± 14.65a	171.26± 11.65b	160.63± 9.54b	156.23± 11.17b	*
MCH (pg)	76.75± 4.99a	58.59± 3.86b	55.11± 3.13b	53.47± 3.75b	*
Glucose (mg/100ml)	171.59± 19.31a	196.92± 7.95a	190.64± 14.32a	180.00± 21.91a	N. S
T- protein(g/100ml)	5.04± 0.32a	5.32± 0.18a	5.38± 0.31a	4.9± 0.22a	N. S
Albumin (g/100ml)	2.40± 0.76a	2.74± 0.07a	2.71± 0.27a	2.66± 0.08a	N. S
Globulin (g/100ml)	2.64± 0.16a	2.58± 0.18a	2.67± 0.19a	2.24± 0.23a	N. S
Cholesterol (mg/100ml)	183.00± 6.42a	198.60± 10.95a	191.40± 11.11a	174.50± 14.24a	N. S
Tri glyceride (mg/100ml)	718.45± 32.37a	769.36± 38.09a	565.53± 36.59b	571.71± 32.41b	*
LDL (mg/100ml)	143.69± 6.47a	153.87± 7.61a	113.10± 7.31b	114.34± 6.48b	*

ISSN 2072-3857

HDL (mg/100ml)	53.62± 1.80ab	57.80± 2.84a	44.47± 3.27b	49.73± 4.44ab	*
Estrogen (pg./ml)	407.26± 34.95a	222.83± 1.85b	403.33± 3.00a	343.16± 13.90a	*

) N.S) refers to the absence of significant differences between the means at a probability level of ($p \leq 0.05$.(

(*)indicates the presence of significant differences between the means at a probability level of ($p \leq 0.05$.(

CON = Control treatment without addition, HIS= Addition of hesperidin at a rate of (250 mg/L of water), ASA= Addition of Ascorbic acid at a rate of (250 mg/L of water), HIS +ASA : Addition of hesperidin with Ascorbic acid at a rate of (125 + 125 mg/L of water.(

Conclusion :

The investigation's results indicated that the addition of either hesperidin or ascorbic acid did not influence the qualitative qualities of the eggs. Nonetheless, Hesperidin's effects were mostly focused on the thickening of eggshells. The primary impact of hesperidin was a decrease in estrogen levels. The overall blood count distinctly changed upon the introduction of hesperidin; hemoglobin levels and cell volume decreased, whilst the red blood cell counts significantly rose. Individually or in conjunction with hesperidin, elevating high-density lipoprotein levels and diminishing triglyceride levels improved the biochemical blood profiles of laying chickens.

Acknowledgments:

The researchers want to sincerely thank you to the Deanship of the College of Agriculture at Tikrit University for providing necessary tools for field and laboratory environments inside the Animal Production Department, therefore enabling their great scientific study.

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ISSN 2072-3857