Journal of Anatolian Environmental and Animal Sciences
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
DOI: https://doi.org/10.35229/jaes.1210795

JAES
Year: 8, No: 1, 2023 (37-41)
AÇEH
Yıl: 8, Sayı: 1, 2023 (37-41)

ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

The Effects of Ashwagandha (*Withania somnifera*) Root Powder on Performance, Egg Quality and Yolk Lipid Oxidation in Laying Hens

Gözde KILINÇ*
Amasya University, Suluova Vocational Schools, Amasya, Türkiye

Received: *27.12.2022* ***Accepted:*** *23.12.2022* ***Puplished:*** *31.03.2023*

How to cite: **Kılınç, G. (2023**). The Effects of Ashwagandha (*Withania somnifera*) Root Powder on Performance, Egg Quality and Yolk Lipid Oxidation in Laying Hens. *J. Anatolian Env. and Anim. Sciences*, ***8***(1), 40-44. https://doi.org/10.35229/jaes.1210795
Atıf yapmak için: **Kılınç, G. (2023).** Ashwagandha (*Withania somnifera*) Kök Tozunun Yumurtacı Tavuklarda Performans, Yumurta Kalitesi ve Yumurta Sarısı Lipid Peroksidasyonu Üzerine Etkileri. *Anadolu Çev. ve Hay. Dergisi*, ***8***(1), 40-44. https://doi.org/10.35229/jaes.1210795

*: https://orcid.org/0000-0002-8667-3390

***Corresponding author's:**
Gözde KILINÇ
Amasya University, Suluova Vocational Schools, Amasya, Türkiye
✉: gozde.kilinc@amasya.edu.tr

Abstract: This study was conducted to determine the effects of different levels (0, 200, 400, 600 mg kg^{-1}) of ashwagandha (*Withania somnifera*) root powder (ARP) on performance, egg quality and egg lipid peroxidation in laying hens (Lohmann LSL, 36 weeks old) diets. For this purpose, a total of 4 groups were formed, one of which was control (ARP-0) and the other three were experimental groups (ARP-1, ARP-2, ARP-3). A total of 96 hens were distributed to 4-storey cages with 8 replicates in each group and 3 hens in each replicate. The experiment lasted for a total of 2 months. During this period, feed and water were given as *ad-libitum* and 16 hours of light and 8 hours of dark photoperiod was applied daily. The ARP-0 group was fed with basal diet and the experimental groups were fed with diets prepared by adding 200, 400 and 600 mg kg^{-1} levels of ARP to the basal diet, respectively. Except for egg weight, albumen index, Haugh unit, shell weight, yolk L* and b* and TBARs (day 0), other parameters were not affected by ARP in the diet. The highest egg weight ($P<0.05$) was in the ARP-2 group while the highest albumen index ($P<0.05$), Haugh unit ($P<0.05$) and shell weight ($P<0.05$) were in the ARP-1 group. It was determined that egg yolk L* ($P<0.001$) and b* ($P<0.001$) values were highest in ARP-1 group. Moreover, it was observed that all levels of ARP in the diet decreased the yolk TBARs (day 0) value compared to the control group ($P<0.05$). As a result, it can be said that ARP can improve some performance and quality parameters and delay lipid oxidation in laying hens.

Keywords: Antioxidant, ashwagandha, egg quality, laying hens, performance.

Ashwagandha (*Withania somnifera*) Kök Tozunun Yumurtacı Tavuklarda Performans, Yumurta Kalitesi ve Yumurta Sarısı Lipid Peroksidasyonu Üzerine Etkileri

***Sorumlu yazar:**
Gözde KILINÇ
Amasya Üniversitesi, Suluova Meslek Yüksekokulu, Amasya, Türkiye
✉: gozde.kilinc@amasya.edu.tr

Öz: Bu çalışma, yumurtacı tavuk (Lohmann LSL, 36 haftalık) karma yemlerine farklı düzeylerde (0, 200, 400, 600 mg kg^{-1}) ashwagandha (*Withania somnifera*) kök tozu (ARP) ilavesinin performans, yumurta kalitesi ve yumurta lipid peroksidasyonu üzerine etkilerini belirlemek üzere yürütüldü. Bu amaçla, biri kontrol (ARP-0) ve diğer üçü deneme grubu (ARP-1, ARP-2, ARP-3) olmak üzere toplam 4 grup oluşturuldu. Her grupta 8 tekerrür ve her tekerrürde ise 3'er adet tavuk olacak şekilde toplam 96 adet tavuk 4 katlı kafeslere dağıtıldı. Deneme toplam 2 ay sürdü. Bu süre içerisinde yem ile su *ad-libitum* olarak verildi ve günlük 16 saat aydınlık 8 saat karanlık fotoperiyot uygulandı. ARP-0 grubu bazal rasyonla, deneme grupları ise bazal yeme sırasıyla ARP'nin 200, 400 ve 600 mg kg^{-1} düzeylerinin ilavesi ile hazırlanmış yemlerle beslendi. Yumurta ağırlığı, ak indeksi, Haugh birimi, kabuk ağırlığı, yumurta sarısı L* ile b* ve TBARs (0. gün) değeri hariç diğer parametreler karma yemdeki ARP'den etkilenmedi. En yüksek yumurta ağırlığı ($P<0.05$) ARP-2; en yüksek ak indeksi ($P<0.05$), Haugh birimi ($P<0.05$) ve kabuk ağırlığı ($P<0.05$) ARP-1 gurubunda oldu. Yumurta sarısı L* ($P<0.001$) ve b* ($P<0.001$) değerlerinin en yüksek ARP-1 grubunda olduğu tespit edildi. Karma yemdeki ARP'nin tüm düzeylerinin kontrol grubuna göre yumurta sarısı TBARs (0. gün) değerini azalttığı ($P<0.05$) tespit edildi. Sonuç olarak, yumurtacı tavuklarda ARP'nin bazı performans ile yumurta kalite parametrelerini iyileştirebileceği ve lipid oksidasyonunu geciktirebileceği söylenebilir.

Anahtar kelimeler: Antioksidan, ashwagandha, performans, yumurta kalitesi, yumurtacı tavuk.

INTRODUCTION

Synthetic antioxidants are frequently used as feed additives (Candan & Bağdatlı, 2017). However, it is known that they can cause health problems in humans due to their residues in animal products (Karasu & Öztürk, 2014). Therefore, natural antioxidants are being investigated as an alternative to synthetic antioxidants (Righi et al., 2021). One of these is plants (Manuelian et al., 2021). It is known that some plants show antioxidant properties with various active substances they contain. Ashwagandha (*Withania somnifera*) is one of them (Gupta & Rana, 2007). Known as Indian ginseng and winter cherry (Gupta & Rana, 2007), this plant is widely used in Ayurvedic medicine (Mishra et al., 2000). This plant is known to have active ingredients such as withanolides, somnitalglucose and withanone (Bhardwaj & Gangwar, 2011; Kushwaha et al., 2012; Vasanthakumar et al., 2015). Furthermore, it has been reported that it contains active ingredients that have antioxidant, antibacterial, hypolipidemic, anti-inflammatory and antitumor effects (Gupta & Rana, 2007). There are different studies investigating the effects of Ashwagandha root powder (ARP) on poultry performance (Akotkar et al., 2007; Bhardwaj & Gangwar, 2011; Biswas et al., 2012; Joshi et al., 2015; Vasanthakumar et al., 2015; Sandeep et al., 2020) and egg quality (Bhardwaj & Gangwar, 2011; Kumar et al., 2020; Kumar 2021). However, very few studies have investigated the effects of poultry products on lipid oxidation. Azimi et al. (2020) reported that ARP decreased thigh MDA (malondialdehit) levels in a study conducted on broilers.

In this study, the effects of different levels (0, 200, 400, 600 mg kg^{-1}) of Ashwagandha root powder supplementation to laying hen diets on performance, egg quality and egg lipid peroxidation were determined.

MATERIAL AND METHOD

Ethics committee permission: Ethics committee approval for this study was obtained from Ondokuz Mayıs University Animal Experiments Local Ethics Committee with the date 10.08.2021 and acceptance number 2021/38.

Experiment plan and feeding of animals: This experiment was conducted in the licensed poultry unit of Amasya University Suluova Vocational School. The animal material of the study consisted of 96 Lohmann LSL white laying hens at the age of 36 weeks. A total of 4 groups were formed for the experiment, one as control and the other three as experimental groups. Each group consisted of 8 replicates and 3 hens in each replicate, furthermore, a total of 96 hens were randomly distributed in 4-storey cages. After the groups were formed, the homogeneity of variances was tested for body weight ($P>0.05$). The experiment lasted for a total of 2 months and during this period, feed and water were given to the hens as *ad-libitum*. Lighting was provided for 16 hours daily. The experimental plan is given in Table 1.

Table 1. Experiment plan.

Gruplar	ARP-0	ARP-1	ARP-2	ARP-3
Number of repetitions	8	8	8	8
Number of hens in repetitions	3	3	3	3
Total number of hens	24	24	24	24

ARP-0, ARP-1, ARP-2, ARP-3: Groups fed ashwagandha root powder added to basal diet at 0, 200, 400, and 600 mg kg^{-1} levels, respectively.

The control group (ARP-0) was fed with basal diet and the experimental groups (ARP-1, ARP-2, ARP-3) were fed with the addition of ARP to the basal diet at 200, 400 and 600 mg kg^{-1}, respectively. The basal diet was prepared according to the values recommended by NRC (1994). The nutrient composition of the basal diet was determined by the method reported in AOAC (2000) and metabolic energy value was calculated according to the method developed by Carpenter & Clegg (1956). The nutrient composition of the basal diet is given in Table 2.

Table 2. Chemical composition of basal diet used in the experiment.

Ingredients	kg ton^{-1}	Nutritient content (analyzed)	%
Maize	407.647	Dry matter	88.92
Wheat	200.000	Crude protein	17.70
Sunflower meal (34%)	75.807	Crude fibre	4.14
Soybean meal (46%)	131.217	Ether extract	3.80
Full-fat soybean (34%)	50.000	Crude ash	12.08
Corn gluten (60%)	24.422	Methionine	0.38
Vegetable oil	9.378	Lysine	0.80
Limestone	87.447	Methionine + Cystine	0.70
Dicalcium phosphate	8.414	Arginine	1.12
Salt	3.168	Tryptophan	0.21
Premix*	2.500	Calcium	3.60
Total	1.000	Available phosphorus	0.38
		Sodium	0.15
Calculated ME (kcal kg^{-1})		2737	

*Each 2.5 kg of premix contains 10.000.000 IU Vitamin A, 3.000.000 Vitamin D3, 25.000 mg Vitamin E, 3.000 mg Vitamin K3, 3.000 mg Vitamin B1, 6.000 mg Vitamin B2, 40.000 mg Vitamin B3, 10.000 mg Vitamin B5, 4.000 mg Vitamin B6, 1.000 mg Vitamin B9, 20 mg B12, 50 mg Vitamin H, 300.000 mg Choline, 80.000 mg Mn, 60.000 mg Fe, 60.000 mg Zn, 5.000 mg Cu, 1.500 mg I, 300 mg Co, 150 mg Se, 400.000 mg Phytase, 550.000 mg Xylanese, 650.000 mg Methionine, 2.500 mg canthaxanthin, 1.000 mg apo carotenoic ester.

Ashwagandha root powder was purchased ready-made from a commercial company. To ensure homogeneity, feeds were first made with the additive in small amounts of ARP and then these were added to the whole diets.

Determination of performance parameters: Hens were weighed at the beginning and end of the experiment, hence, the change in body weight (g) was calculated. The feeds were weighed daily at the same time and eggs were counted daily. At the end of the experiment, the remaining feeds and eggs were weighed. Then egg production (%), feed intake (g), egg mass (g) and feed conversion ratio (g feed/g egg) were calculated. Egg production was calculated by dividing the total number of eggs in each group by the number of animals. Egg mass was determined by multiplying the egg weight by egg production and then dividing it by 100. Besides, the feed

conversion ratio was calculated by dividing the feed intake by the egg mass.

Determination of egg quality parameters: To determine egg internal and external quality parameters, 20 eggs were randomly selected on the last two days of the experiment. Eggs and shells were weighed with a 0.01 g precision balance and weights were recorded as g. Egg width (mm) and length (mm), yolk diameter (mm), albumen width (mm) and length were measured with a digital caliper. Egg yolk height (mm) and albumen height (mm) were measured with a tripod micrometer. Shape index (%) (Sarıca & Erensayın, 2009), yolk index (%) (Yalçın et al., 2008), albumen index (%) (Yalçın et al., 2010), and Haugh unit (Ryu et al., 2011) were calculated from these values. To determine the shell thickness (µm), samples were taken from three different parts (sharp, medium, and blunt) of the shells and measured with a digital micrometer and the average was given.

Shape index = [egg width/egg length] × 100

Yolk index = [yolk height/yolk width] × 100

Albumen index = [egg albumen height] / [(albumen length + albumen width) / 2] × 100

Haugh unit = 100 log (H + 7.57-1.7$W^{0.37}$), (H = egg height; W = egg weight)

Colorimetry was used to determine egg yolk L*, a*, b* values. L* value indicates the range of dark (0-50) and light (51-100) values in terms of light transmittance; a positive value indicates red and a negative value indicates green for a*, a positive value indicates yellow and a negative value indicates blue for b* (Peşman et al., 2022).

Determination of egg yolk TBARs value: An important criterion affecting the shelf life of eggs is the rancidity of the lipids it contains. As an indicator of this rancidity, egg yolk TBARs (thiobarbituric acid reactive substances) value was determined at 2 different periods, day 0 and day 28. At the end of the experiment, a total of 48 eggs, 12 eggs from each group, were randomly taken and analyzed for malondialdehyde (MDA) for day 0 value on the same day, and for day 28 after storing at +4°C for 28 days. Moreover, 12 ml of TCA (trichloroacetic acid) solution (7.5% TCA, 0.1% EDTA, 0.1% propyl gallate) was added to egg yolk samples (2 g) and homogenized at ultra-turrax for 20-25 s and filtered through Whatmann 1 filter paper. In addition, 3 ml of the filtrate was taken into glass tubes and 3 ml of 0.02 M TBA (thiobarbituric acid) solution was added. These tubes containing the solution were kept in a water-bath at 100°C for 40 minutes and then cooled under tap water. The cooled tubes were centrifuged at 2000 rpm for 5 minutes and the absorbance values were read at 530 nm wavelength in a spectrophotometer (Kılıç & Richards, 2003). TBARs values were calculated from the formula below and given as µmol MDA/kg egg.

TBARS = [(absorbance/k(0.06) × 2/1000) × 6.8] × 1000/ sample weight

k= Value obtained from the standard curve

Statistical analysis: Analysis of variance (One-Way Analysis of Variance) and comparisons between groups (Duncan's test) were performed using SPSS 22.0 package program. Furthermore, linear and quadratic effects of increasing doses of ashwagandha root powder (200, 400, 600 mg kg^{-1}) were determined by polynomial analysis. The effects (significance) of the groups were evaluated at P<0.05 level (IBM., Corp., 2011). In addition, the number of animals used in the study was determined by G*Power analysis.

RESULTS

Results of performance parameters

The results of the study on performance parameters (body weight, body weight change, egg weight, egg production, feed conversion ratio, and daily feed intake) are given in Table 3.

Table 3. Effect of Ashwagandha root powder on performance parameters.

Parameters	Groups				SEM	P values		
	ARP-0	ARP-1	ARP-2	ARP-3		C	L	Q
IBW	1458.00	1454.38	1449.25	1533.50	18.42	0.328	0.186	0.239
FBW	1389.88	1421.00	1421.54	1455.33	11.28	0.244	0.056	0.952
BWG	-68.13	-33.38	-27.71	-35.04	14.02	0.753	0.424	0.473
EW	61.50^{c}	63.90^{ab}	64.36^{a}	62.01^{bc}	0.412	**0.025**	0.547	**0.003**
EP	88.90	90.69	91.15	91.91	1.11	0.819	0.365	0.824
FI	112.22	112.16	112.19	115.52	0.722	0.268	0.128	0.242
FCR	2.07	1.94	1.92	2.02	0.028	0.215	0.532	**0.046**

a, b, c: The averages with different superscripts in the same row differ significantly (P<0.05).
SEM: Standard Error of Mean; C: Combined; L: Linear; Q: Quadratic; ARP-0, ARP-1, ARP-2, ARP-3: Groups fed ashwagandha root powder added to basal diet at 0, 200, 400, and 600 mg kg^{-1} levels, respectively. IBW: Initial Body Weight, g; FBW: Final Body Weight, g; BWG: Body Weight Gain, g; EW: Egg Weight, g; EP; Egg Production, %; FI: Feed Intake, g/day/hen; FCR: Feed Conversion Ratio, g feed/g egg.

It was observed that ARP in laying hen diets had no significant effect on performance parameters except egg weight. In the polynomial analysis, it was determined that ARP had a quadratic effect on egg weight and feed conversion ratio. Moreover, egg weight was the highest (64.36 g) in the ARP-2 group (P<0.05). Feed conversion ratio decreased numerically in all groups compared to the control group, thus, ARP improved feed conversion (P>0.05). This decrease was especially remarkable in the ARP-2 group. Kumar (2021) reported that ARP did not affect body weight change in laying hens and our study was in agreement with that. In contrast to the present study, Sandeep et al. (2020) reported that ashwagandha root powder (0.25, 0.5, 0.75, 1%) affected feed intake, egg mass and egg production. In the same study, Sandeep et al. (2020) reported that ARP did not affect the feed conversion ratio. This finding coincides with our study result. In another study, Biswas et al. (2012) reported that ARP increased feed intake in laying hens. Furthermore, Akotkar et al. (2007) reported that ARP affected body weight gain, feed intake and feed conversion ratio in broilers. Joshi et

al. (2015) reported that ARP did not affect feed intake and feed conversion ratio in broilers. In the present study, the numerical decrease in feed conversion ratio (P>0.05) and increase in egg weight (p<0.05) may be due to the bioactive components in ARP.

Results of egg quality parameters

The findings of the study on egg internal and external quality parameters (egg weight, shape index, yolk index, albumen index, Haugh unit, shell weight, shell thickness and yolk L*, a*, b* values) are given in Table 4.

Table 4. Effects of Ashwagandha root powder on egg quality

	Groups					P values		
Parameters	**ARP-0**	**ARP-1**	**ARP-2**	**ARP-3**	**SEM**	**C**	**L**	**Q**
EW (g)	61.35^{c}	65.92^{a}	64.08^{ab}	63.12^{bc}	0.450	**0.002**	0.346	**0.001**
SI	75.08	74.74	75.00	74.00	0.286	0.534	0.249	0.561
YI	39.48	40.52	40.83	40.04	0.254	0.260	0.382	0.073
AI	7.87^{b}	9.24^{a}	8.67^{ab}	8.06^{b}	0.196	**0.054**	0.992	**0.011**
HU	79.50^{b}	84.68^{a}	83.04^{ab}	80.10^{b}	0.759	**0.045**	0.981	**0.007**
SW (g)	6.72^{c}	7.47^{a}	7.14^{ab}	6.99^{bc}	0.068	**0.001**	0390	**0.000**
ST (µm)	0.353	0.353	0.357	0.361	0.002	0.608	0.214	0.627
L*	49.88^{b}	53.44^{a}	49.74^{b}	53.07^{a}	0.371	**0.000**	**0.037**	0.854
a*	14.19	14.26	14.05	13.29	0.145	0064	**0.023**	0.146
b*	34.89^{b}	38.97^{a}	36.07^{b}	38.55^{a}	0.358	**0.000**	**0.003**	0.179

a,b: The averages with different superscripts in the same row differ significantly (P<0.05).
SEM: Standard Error of Mean; C: Combined; L: Linear; Q: Quadratic; ARP-0, ARP-1, ARP-2, ARP-3: Groups fed ashwagandha root powder added to basal diet at 0, 200, 400, and 600 mg kg^{-1}levels, respectively. EW: Egg Weight; SI: Shape Index; YI: Yolk Index; AI: Albumen Index; HU: Haugh Unit; SW: Shell Weight; ST: Shell Thickness; L*: Lightness; a*: Redness; b*: Yellowness.

When egg quality parameters were evaluated, except egg weight, albumen index, Haugh unit, shell weight, and yolk L* and b* value, other quality parameters were not affected by ARP in the diet. The highest egg weight (65.92 g), albumen index (9.24), Haugh unit (84.68) and shell weight (7.47 g) were in the ARP-1 group (P<0.05). There was also a significant difference in yolk L* and b* values between the groups (P<0.001). The highest egg yolk L* and b* values were in ARP-1 group. In the polynomial analysis, ARP had a quadratic effect on egg weight, albumen index, Haugh unit and shell weight, and there was a linear effect on yolk L*, a* and b* values. In the present study, the increase in Haugh unit and albumen index indicates that ARP has a positive effect on egg shelf life. It has been reported that albumen quality depends on the amount of β-ovomucin secreted in the magnum and antioxidant substances may protect β-ovomucin, resulting in an increase in albumen height (Özgan, 2009). Therefore, ARP is thought to increase albumen index and Haugh unit. The increase in eggshell weight may have resulted from the increase in egg weight. In addition, bioactive components of ARP may have increased Ca absorption. In contrast to the present study, Kumar et al. (2020) reported that different levels of ARP (0.25, 0.5, 0.75, 1%) did not affect the egg albumen index and Haugh unit. In another study, Kumar (2021) reported that ARP in laying hen diets did not affect shape index and shell thickness.

Results of egg yolk TBARs value: The results of the egg yolk TBARs value of the study are given in Table 5.

Table 5. Effect of Ashwagandha root powder on egg yolk TBARs.

	Groups				SEM	P values		
Parameters	**ARP-0**	**ARP-1**	**JLE-2**	**ARP-3**		**C**	**L**	**Q**
TBARs (day 0)	4.187^{a}	2.744^{b}	2.924^{b}	2.939^{b}	0.211	**0.048**	**0.047**	0.067
TBARs (day 28)	3.519	3.131	2.984	2.548	0.167	0.230	**0.045**	0.941

a,b: The averages with different superscripts in the same row differ significantly (P<0.05).
SEM: Standard Error of Mean; C: Combined; L: Linear; Q: Quadratic; ARP-0, ARP-1, ARP-2, ARP-3: Groups fed ashwagandha root powder added to basal diet at 0, 200, 400, and 600 mg kg^{-1} levels, respectively. TBARs: Thiobarbituric Acid Reactive substances.

There was a significant difference in yolk TBARs levels between the groups only on day 0. When the TBARs values of the eggs analyzed immediately at the end of the experiment (day 0) were examined, it was revealed that all levels of ashwagandha decreased the egg yolk TBARs values (P<0.05) and slowed down the lipid oxidation compared to the control group. In the polynomial analysis, it was determined that ashwagandha root powder had a linear effect on egg yolk TBARs value at day 0 and 28. There are a limited number of studies investigating the effects of ashwagandha on lipid oxidation in poultry. Azimi et al. (2020) reported that ARP decreased MDA levels in thigh in a study conducted on broilers.

CONCLUSION

The results showed that ARP increased egg weight and improved FCR. Furthermore, ARP had positive effects on the Haugh unit and albumen index, which are important indicators of egg freshness. It was also observed that ARP could contribute to the improvement of egg shelf life by slowing down yolk lipid oxidation (on day 0). In addition, it increased yolk L* and b* values, which are one of the egg quality criteria. It was also determined that ARP increased shell weight, which is one of the egg's external quality factors. As a result, it was concluded that ARP in laying hen diets can positively affect some performance and egg quality parameters.

REFERENCES

Akotkar, N.S., Sarag, A. N., Rekhate, D.H. & Dhok, A.P. (2007). Effect of supplementation of Ashwagandha (Withania somnifera) on performance of broilers. *Indian Journal of Poultry Science*, ***42***(1), 92-94.

AOAC. (2000). Official methods of analysis (17th ed.). Assosication of Official Analytical Chemists, AOAC *International*, Maryland, USA.

Azimi, V., Mirakzahi., M.T. & Saleh, H. (2020). Hydroalcoholic extract of Withania somnifera leaf and α-tocopherol acetate in diets containing oxidised oil: effects on growth performance, immune response, and oxidative status in broiler chickens, *Italian Journal of Animal Science*, ***19***(1), 917-928. DOI: 10.1080/1828051X.2020.1808537

Bhardwaj, R.K. & Gangwar, S.K. (2011). Effect of dietary supplementation of Withania somnifera on egg production and egg quality parameters in Japanese Quails *International Journal of Advanced Biological Research*, ***1***, 32-34.

Biswas, P., Sharma, R.K. & Biswas, A. (2012). Effect of dietary supplementation of Ashwagandha (Withania somnifera) and ascorbic acid on production performance of broiler birds. *Indian Journal of Poultry Science*, ***47***(3), 403-405.

IBM Corp. (2011). IBM SPSS Statistics for Windows, Version 20.0. IBM Corporation, Armonk, NY.

Candan, T. & Bağdatlı, A. (2017). Use of natural antioxidants in poultry meat. *Celal Bayar University Journal of Science*, ***13***(2), 279-291. DOI: 10.18466/cbayarfbe.319752

Carpenter, K.J. & Clegg, K.M. (1956). The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. *Journal of the Science of Food and Agriculture*, ***7***(1), 45-51. DOI: 10.1002/jsfa.2740070109

Gupta, G.L. & Rana, A.C. (2007). PHCOG MAG.: Plant review Withania somnifera (Ashwagandha): A review. *Pharmacognosy Reviews*, ***1***(1), 129-136.

Joshi, S.S., Ingle, P.B., Bhagwat, S.R., Pawar, M.M., Prajapati, K.B. & Kulkarni, R.C. (2015). Effect of dietary addition of ashwagandha (Withania somnifera) and guduchi (Tinospora cordifolia) powder on broiler performance. *Indian Journal of Animal Sciences*, ***85***(12), 1358-1361.

Karasu, K. & Öztürk, E. (2014). Tıbbi ve aromatik bitkilerin kanatlılarda antioksidan ve antimikrobiyal etkileri. *Türk Tarım ve Doğa Bilimleri Dergisi*, ***1***(Özel Sayı-2), 1766-1772.

Kılıç, B. & Richards, M.P., (2003). Lipid oxidation in poultry doner kebap: prooxidative and antioxidative factors. *Journal of Food Science*, ***68***(2), 686-689. DOI: 10.1111/j.1365-2621.2003.tb05732.x

Kumar, S., Berwal, R.S. & Sihag, S. (2020). Effect of dietary supplementation of ashwagandha root powder on production performance, egg quality and blood biochemical constituents of laying hens. *Indian Journal of Animal Nutrition*, ***37***(4), 352-357. DOI: 10.5958/2231-6744.2020.00057.2

Kumar, S. (2021). Effect of dietary supplementation of Ashwagandha (Withania somnifera) root powder on external egg parameters and body weight changes in laying hens. *The Pharma Innovation Journal*, **10**(9), 475-478.

Kushwaha, S., Betsy, A. & Chawla, P. (2012). Effect of Ashwagandha (Withania somnifera) root powder supplementation in treatment of hypertension. *Studies on Ethno-Medicine*, ***6***(2), 111-115. DOI: 10.1080/09735070.2012.11886427

Manuelian, C.L., Pitino, R., Simoni, M., Mavrommatis, A., De Marchi, M., Righi, F. & Tsiplakou, E. (2021). Plant feed additives as natural alternatives to the use of synthetic antioxidant vitamins on livestock mammals' performances, health, and oxidative status: A Review of the Literature in the Last 20 Years. *Antioxidants*, ***10***(9), 1461. DOI: 10.3390/antiox10091461

Mishra, L.C., Singh, B.B. & Dagenais, S. (2000). Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review. *Alternative Medicine Review*, ***5***(4), 334-346.

NRC. (1994). Nutrient requirements of poultry. 9th rev. Ed., National Academy Press. Washington, D.C.

Özgan, A. (2008). *Fonksiyonel Yumurta Eldesinde Üzüm Çekirdeği Yağının Kullanım Olanakları*. Yüksek Lisans Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü. Adana, Türkiye, 69s.

Peşman, E., Yıldız, H.E., Boran Torun, S. & Dönmez Çavdar, A. (2022). Atık gazete kağıdı liflerinin biyokompozit üretiminde kullanılabilirliği. *Artvin Çoruh Üniversitesi, Orman Fakültesi Dergisi*, ***23***(1), 45-50. DOI: 10.17474/artvinofd.1027482

Righi, F., Pitino, R., Manuelian, C.L., Simoni, M., Quarantelli, A., De Marchi, M. & Tsiplakou, E. (2021). Plant feed additives as natural alternatives to the use of synthetic antioxidant vitamins on poultry performances, health, and oxidative status: A review of the literature in the last 20 years. *Antioxidants*, ***10***(5), 659. DOI: 10.3390/antiox10050659

Ryu, K.N., No, H.K. & Prinyawiwatkul, W. (2011). Internal quality and shelf life of eggs coated with oils from different sources. *Journal of Food Science*, ***76***(5), 325-329. DOI: 10.1111/j.1750-3841.2011.02177.x

Sandeep, K., Berwal, R.S. & Ravi, K. (2020). Effect of dietary supplementation of Ashwagandha root powder on production performance of laying hens. *Haryana Veterinarian*, ***59***(2), 201-205.

Sarıca, M. & Erensayın, C., (2009). Tavukçuluk ürünleri: tavukçuluk bilimi, yetiştirme, besleme ve hastalıklar. Ed: Türkoğlu, M. ve Sarıca M., ***89***-139. Bey Ofset Matbaacılık, Ankara.

Vasanthakumar, P., Pangayarselvi, B., Sasikumar, P., Chandrasekaran, D., Doraisamy, K.A. & Purushothaman, M.R. (2015). Performance of broilers fed ashwagandha (Withania somnifera) incorporated diets during summer season for alleviating heat stress. *Indian Journal of Animal Research*, ***2646***, 1-3. DOI: 10.5958/0976-0555.2015.00082.5

Yalçın, S., Özsoy, B., Erol, H. & Yalçın, S. (2008). Yeast culture supplementation to laying hen diets containing soybean meal or sunflower seed meal its effect on performance, egg quality traits, and blood chemistry. *Journal of Applied Poultry Research*, ***17***(2), 229-236. DOI: 10.3382/Japr.2007-00064

Yalçın, S., Erol, H., Özsoy, B., Onbaşılar, İ., Yalçın S. & Üner, A. (2010). Effects of glycerol on performance, egg traits, some blood parametersa and antibody production to SRBC of laying hens. *Livestock Science*, ***129***, 129-134. DOI: 10.1016/j.livsci.2010.01.014