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EFFECTS OF DIETARY SUPPLEMENTATION OF BLACK SEED (*NIGELLA SATIVA*) ON PERFORMANCE, EGG QUALITY AND BLOOD INDICES OF LAYING BIRDS

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Abstract

The aim of this study was to examine the use of *Nigella sativa* on productive performance, egg quality and blood profile of laying birds. Three hundred ISA-Brown point-of-lay pullets were randomly distributed equally into five dietary treatments replicated six times with ten birds per replicate in a Completely Randomized Design (CRD). The diets were supplemented with *Nigella sativa* each at 0, 4, 5, 6 and 7g/kg diet respectively. The feeding trial lasted fourteen weeks. Significant differences ($p<0.05$) were observed in the hen day production, feed conversion ration, egg weight, egg length, egg width, shell thickness, haematological parameters and serum biochemistry indices. In conclusion, 6g/kg black seed supplementation level in the diet of ISA Brown laying birds improved hen day production, egg weight, and egg width. However, 7g/kg black seed supplementation reduced feed conversion ratio, improved the pack cell volume, red blood cell count, reduced and cholesterol level. It is therefore recommended that black seed (*Nigella sativa*) can be added to layers diet at 6g/kg diet as feed additives to enhance production performance and egg width.

Index Terms: Black cumin, Chicken, Haematological parameters, Internal and external egg qualities, Serum biochemistry

1. INTRODUCTION

Synthetic antibiotic growth promoters (SAGPs) have been used for years to improve the profitability of poultry production by helping to control pathogenic bacteria in the gut mucosa, thereby improving weight gain, feed conversion ratio and flock uniformity (Haque, 2020). However, there is a potential development of resistance to repeated use of these drugs by a number of pathogenic bacteria (Ramchandani et al., 2005) thus

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leading to the emergence of antimicrobial drug resistant organisms (Shea, 2003). Phytogenic feed additives (PFAs) have been used as an alternative to SAGPs. The benefit of phytogenic feed additives over synthetic growth promoters is primarily due to natural synergistic influence of all plant agents (Toghyani et al., 2010). Herbal bioactive ingredients had been reported to increase appetite, trigger immune response and have antibacterial, antiviral and antioxidant properties (Toghyani et al., 2010).

In general, phytogenic feed additives consist of variety of plants, such as herbs, spices and essential oils obtained from plant parts. By enhancing appetite and feed consumption, improving endogenous digestive enzyme secretion, activating immune response, antimicrobial and antioxidant properties, the beneficial influence of phytogenic feed additives on poultry health and efficiency could be achieved (Toghyani et al., 2011). The need to reduce cost of production of meat has necessitated the use of plant materials with medicinal properties as feed additives capable of minimizing the influence of pathogenic microbes and improving performance of the animal through better feed utilization (Olayeni et al., 2022). A potential example of PFAs is black seed.

Black seed (*Nigella sativa*) also known as black cumin, black caraway seed, Habbatu Sawda and Habatul Barak “the Blessed Seed”(Hosseinzadeh et al., 2007), is an annual herb of the Ranunculaceae family. The plant is native to the Mediterranean countries and Asia (El-Tahir et al., 1993; Hala et al., 2019) and it is also found growing in some other regions in the world such as in Saudi Arabia, Syria, North Africa and also has been widely cultivated throughout South Europe, Turkey, Pakistan, and India where it is used for culinary and medical purposes (Gali-Muhtasib et al., 2006). This plant has been used for thousands of years in many Asian, Middle Eastern and far Eastern countries as a spice and food preservative as well as a protective and health remedy in traditional folk medicine for the treatment of numerous disorders (Hala et al., 2019). The seeds are commonly eaten alone or in combination with honey and in many food preparations. The oil prepared by compressing the seeds of *Nigella sativa* is used for cooking. *Nigella sativa* does not only promote animal’s health and productive performance, but also plays a significant role as a natural antioxidant and immuno-stimulant (Hammed and Amao, 2022).

Black seed oil is scientifically proven to contain many naturally occurring ingredients, such as carbohydrates, proteins, glucose, rhamnose, xylose, arabinose, and vitamins, particularly thiamine, niacin, riboflavin, pyridoxine, and folic acid. In addition, *Nigella sativa* seeds are also reported to be a source of crude fiber, minerals (such as calcium, iron, and potassium), fatty acids (such as oleic, linoleic, and palmitic acids), aliphatic alcohols, terpenoids, unsaturated hydroxy ketones, and alkaloids (such as nigellidine, nigellimine, and nigellicine). The oil of *Nigella sativa* seeds contains thymoquinone, dithymoquinone, thymohydroquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine, and alpha-hederin (El-Naggar et al., 2010).

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The use of herbal medicinal products and supplements has increased tremendously over the past three decades (Martins, 2014) because of its natural properties, which are less toxic. Herbal medicines derived from plant extracts are also increasingly utilized to treat a wide variety of clinical diseases (Gupta 2004; Ahirwar et al., 2009). Black seed can be of great benefit and value as natural feed additives in poultry feeding, particularly for layers chicken that suffers lots of losses in both performance and survival as a result of adverse effect of pathogenic microorganism causing disease. This study consider the effects of dietary supplementation of black seed (Nigella sativa) on performance and egg quality of laying birds.

2. MATERIALS AND METHODS

2.1 Experimental location and feed preparation

The experiment was conducted at the Poultry Unit of Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, in derived savanna zone of Nigeria. It is located between latitude 8007'N and 8012'N and longitude 4004'E and 4015'E. The altitude ranges from 300m and 600m above sea level while mean temperature and annual rainfall are 270C and 1247mm (Ojediran et al., 2022).

2.2 Procurement of Test Ingredient

The test ingredient, Black Seeds (*Nigella sativa*) were purchased from a reputable local store in Ogbomoso and were used in the feed directly with no further processing.

Table 1: Composition of experimental feeds and their calculated nutrient analysis

	T1	**T2**	**T3**	**T4**	**T5**
Ingredient (%)	**(0g/kg diet)**	**(4g/kg diet)**	**(5g/kg diet)**	**(6g/kg diet)**	**(7g/kg diet)**
Maize	40	40	40	40	40
*Concentrate (Layers)	40	40	40	40	40
Wheat offal	20	20	20	20	20
Black seed (g/kg)	-	+	+	+	+
Total	**100**	**100**	**100**	**100**	**100**
Nutrients					
Metablizable Energy (kcal/kg)	2788	2788	2788	2788	2788
Crude Protein	17.4	17.4	17.4	17.4	17.4
Crude Fibre	4.55	4.55	4.55	4.55	4.55
Fat	4.7	4.7	4.7	4.7	4.7
Calcium	4.8	4.8	4.8	4.8	4.8

*Concentrate that was used contain: fat min: 6%; Calcium Min: 12%; Protein Min: 25.0%; Metabolizable Energy: 2600Kcal/kg

2.3 Experimental Birds and Management

A total of three hundred (300) ISA Brown point of lay (18weeks old), 1.7kg of body weight were used for the experiment. The birds were purchased from a reputable commercial farm). The birds were weighed individually and housed in a battery cage pen and

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acclimatized for two (2) weeks before the beginning of the feeding experiment. The birds were randomly divided to five experimental dietary treatments. Each treatment was replicated three (6) times with ten (10) birds per replicate in a Completely Randomized Design (CRD) experiment. The birds were given access to dietary treatments and fresh clean water *ad libitum* for a period of fourteen weeks.

2.4 Data Collection

The following parameter was determined:

2.4.1 Performance indices

Data were collected on the performance indices of the birds, such as feed intake, weight gain and feed conversion ratio. Feed intake is the differential between feed offered and left over. It was measured as the amount of feed consumed by a layer per day. This was monitored daily throughout the period of the experiment. The weight gain was the measure of the difference between the final and initial weight. This was done on weekly basis. Feed conversion ratio (FCR) was estimated as the relationship between the feed an animal consumes and the weight that the animal gains by consuming the feed. It also determined the productivity and efficiency of an animal.

And they are expressed in the mathematical form.

- Weight gain per bird = Final weight (g) - Initial weight (g)
- Feed intake (g) = Feed offered (g) - Left Over (g)
- Average feed intake (g/birds) = feed intake/Number of birds
- Hen-day percentage = $\frac{\text{Number of eggs produced}}{\text{Number of birds}} \times \frac{100}{\text{No of days}}$
- Mortality = $\frac{\text{Number of dead birds}}{\text{Total number of birds incurred}} \times 100$
- Feed conversion ratio = $\frac{\text{Total amount of feed consume by the flock}}{\text{Amount of weight gain or amount of egg produce}}$

2.4.2 Egg Quality Analysis

Data were collected on the external and internal parameters. Egg were collected twice daily at 10.00am and 4.00pm. Eggs collected from each treatment were weighed at the end of each day using a sensitive scale. At the end of each 2 days period, two eggs were selected at random from each replicate. Egg length and width were measured with vernier sliding calipers and the result was used to compute egg shape index.

$$\text{Egg shape index} = \frac{\text{Egg width}}{\text{Egg length}} \times 100$$

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Each egg were broken out at the equatorial region to assess the internal egg quality parameters (Yolk index, yolk color, yolk weight, Albumen height etc). Egg shells were cleaned out with tissue paper and allowed to dry for 20minutes, cooled in the open air of the room. Shell weight was taken using an electronic scale. Each egg shell thickness was measured at three different places (tip, mid and broad end) with the aid of a micrometer screw guage.

The internal egg qualities that were measured include egg albumen and yolk heights using spherometer. The yolk diameter was taken as the maximum cross sectional diameter of the yolk using spherometer, read in millimeters. Yolk was carefully separated from albumen with yolk cups. The yolk was carefully rolled on a damp paper to remove any adhering albumen prior to weighing. Yolk index was calculated as the ratio of yolk height to the length of the egg broken onto a flat surface. Albumen height was measured as the height of the outer circumference of the white with a spherometer. Yolk weight was the weight of separated yolk measured using the sensitive scale in grams. Yolk percentage was calculated as yolk weight to total egg weight x 100. Albumen weight was the weight of the albumen measured using the sensitive scale in grams. Albumen percentage was calculated as albumen weight to total egg weight x 100. Haugh unit is the measured of internal quality of an egg. This was determined using the equation (Cotta, 1997).

$HU = 100 \log (H + 7.57 - 1.7W0.37)$ Where HU=Haugh unit, H= Albumen height (mm), W= Egg weight (g)

Yolk colour was measured using the DSM yolk colour fan on the yolk separated from the albumen.

2.4.3 Blood Analysis

At the end of the experiment, two birds from each replicate were randomly selected and bled through the wing blood veins. Blood sample for hematological analysis was collected into bottle containing ethylene diamene tetra-acetic acid (EDTA) as anti-coagulants while blood sample meant for serum biochemical analysis was collected into plain bottles free of EDTA. The haematological parameters measured include: hemoglobin (Hb) (Coles, 1986), red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets. Serum biochemical parameters include: albumin globulin, total protein (Adeyemi and Soetan 2018), triglyceride, total cholesterol (Rouschlau et al. 1974), alkaline phosphatase, alanine amino transferase and aspartate amino transferase.

2.5 Chemical analysis

The proximate analysis of the experimental diets were carried out according to the methods of AOAC (2005).

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2.6 Statistical Analysis

All data collected were subjected to One-way analysis of variance (ANOVA) using SAS (2000) software package. The variations in means were separated using the Duncan Multiple Range Test of the same statistical package and considered significant at p<0.05.

3. RESULTS

3.1 Performance indices of layers

The effect of black seed supplementation on the performance indices of layers is shown in Table 2. There were significant (p<0.05) differences in the value obtained for hen day production, and feed conversion ratio while initial body weight, final body weight, weight changes, and feed intake does not differ significantly (p>0.05). The bird fed with 6g/kg black seed supplementation had the highest (p<0.05) value for hen day production, those fed 5g/kg black seed supplementation had the least value while those offered the other diets were comparable (p >0.05). The 6g/kg and 7g/kg group also had lower values (p<0.05) for feed conversion ratio but those fed 0g/kg, 4g/kg and 5g/kg supplementation level had higher values.

Table 2: The effect of black seed supplementation on performance indices of layers

Parameters	Black seed supplementation levels (g/kg)					SEM
	0g/kg	4g/kg	5g/kg	6g/kg	7g/kg	
Initial Body Weight (g/b)	1717.01	1726.16	1718.16	1723.75	1727.46	32.71
Final Body Weight (g/b)	2015.23	1994.42	1976.20	1976.54	1978.33	35.77
Total Weight Changes (g/b)	298.22	268.26	258.04	252.79	250.87	33.85
Feed intake (g/b)	143.07	132.44	137.21	131.62	136.61	1.32
Hen Day Production (%)	90.17^{ab}	93.75^{ab}	81.55^{b}	95.83^{a}	87.79^{ab}	1.94
Feed Conversion Ratio	2.15^{a}	2.12^{a}	2.12^{a}	2.01^{b}	2.00^{c}	0.04
Mortality (%)	0.00	0.00	0.00	0.00	0.00	0.00

^{abc}-means the same row bearing superscript differ significantly (P<0.05)

3.2 Egg external qualities of laying birds fed diet supplemented with black seeds (*Nigella Sativa*)

Table 3 shows egg qualities of laying birds fed dietary black seed supplementation. It was observed that the Egg weight, egg length, egg width and shell thickness were all significantly affected (p<0.05) by the dietary treatments. The birds fed 6g/kg diet had higher value (p>0.05), those fed 4 and 5g/kg were lower while those fed the control and 7g/kg diet were comparable for egg weight. For egg length and width, those fed the control diet had the highest egg length (p<0.05) while those given 5g/kg had the least. Birds given 5g/kg have the highest (p<0.05) shell thickness while 4g/kg has the lowest (p<0.05) value. Meanwhile, the control group possesses egg shell thickness similar to all other treatment groups.

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Table 3: Egg external qualities of laying birds fed diet supplemented with black seeds (*Nigella Sativa*)

Parameters	Black seed supplementation levels (g/kg)					SEM
	0g/kg	4g/kg	5g/kg	6g/kg	7g/kg	
Egg weight (g)	57.67^{ab}	57.33^{b}	57.33^{b}	60.67^{a}	55.67^{ab}	0.70
Egg Length(cm)	5.45^{a}	5.33^{ab}	5.30^{b}	5.41^{ab}	5.37^{ab}	0.20
Egg width(cm)	3.61^{a}	3.60^{a}	5.30^{b}	3.60^{a}	3.56^{ab}	0.20
Shell weight(g)	7.33	7.33	7.67	7.33	7.67	0.10
Shell thickness (mm)	0.35^{abc}	0.33^{c}	0.38^{a}	0.37^{b}	0.37^{b}	0.10

^{abc}-means the same row bearing superscript differ significantly (P<0.05). SEM- Standard error of the mean

3.3 Internal egg qualities of laying birds fed diet supplemented with black seeds (*Nigella Sativa*)

Table 4 shows internal egg qualities of laying birds fed dietary black seed supplementation. Yolk and albumen weight, albumen weight, yolk weight, albumen height, yolk length, and haugh unit (HU), were not significantly affected (p>0.05) by black seed supplementation.

Table 4: Egg internal qualities of laying birds fed diet supplemented with black seeds (*Nigella Sativa*)

Parameters	Black seed supplementation levels (g/kg)					SEM
	0g/kg	4g/kg	5g/kg	6g/kg	7g/kg	
Yolk + Albumen weight (g)	50.42	49.21	48.44	49.42	47.75	0.59
Albumen weight (g)	36.23	35.73	34.9	36.19	34	0.49
Yolk weight (g)	14.19	13.42	13.54	13.9	13.75	0.21
Albumen height (mm)	16.39	16.59	16.17	16.32	16.71	0.08
Yolk Height (cm)	1.38	1.39	1.35	1.31	1.35	0.01
Yolk length (cm)	3.65	3.5	3.61	3.86	3.61	0.06
Haugh unit	91.31	92.02	90.89	91.26	92.45	0.25

^{abc}-means the same row bearing superscript differ significantly (P<0.05). SEM- Standard error of the mean

3.4 Haematological parameters of laying birds fed diet supplemented with black seeds (*Nigella Sativa*)

The haematological indices of layers fed diet supplemented with varying levels of black seed is presented in Table 5. Result showed significant (p<0.05) differences in all haematological parameters measured. The highest and lowest values (p<0.05) of packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and platelets is observed in the control diet and 6g/kg of black seed respectively. Birds fed diet supplemented with 5g/kg of black seed has a higher (p<0.05) mean corpuscular volume (MCV) compared to other dietary treatments which have similar values (p>0.05). For mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration,

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birds fed 4g/kg diet had higher values ($p<0.05$). Diets supplemented with black seed had lower values for platelet compared with those fed the diet without black seed (control).

Table 5: Haematological parameters of laying bird fed diet supplemented with black seed (*Nigella Sativa*)

Parameters	Black seed supplementation levels (g/kg)					SEM
	0g/kg	4g/kg	5g/kg	6g/kg	7g/kg	
Packed cell volume (%)	32.60^{a}	26.75^{b}	14.05^{c}	12.58^{c}	30.65^{a}	1.95
Haemoglobin (g/dl)	10.25^{a}	10.36^{a}	8.41^{c}	7.98^{c}	9.35^{b}	0.66
Red blood cells (x10^{6}/mm^{3})	2.59^{a}	2.11^{b}	1.10^{c}	1.03^{c}	2.45^{a}	0.15
White blood cell (x10^{3}/mm^{3})	28.455^{a}	27.175^{b}	17.030^{c}	16.701^{c}	27.910^{ab}	12.4
Mean corpuscular volume (fl)	125.79^{b}	125.36^{b}	129.00^{a}	125.70^{b}	125.40^{b}	0.33
Mean corpuscular haemoglobin (pg)	36.10^{d}	40.35^{a}	40.30^{b}	39.98^{c}	38.25^{c}	0.93
Mean corpuscular haemoglobin concentration (%)	31.15^{b}	33.60^{a}	31.10^{b}	30.89^{c}	30.50^{c}	0.88
Platelets (x10^{3}/mm^{3})	31.61^{a}	20.01^{b}	15.98^{c}	14.99^{c}	16.50^{b}	2.45

^{abcd} means a row with different superscript differed ($p<0.05$) significantly.

3.5 Serum Biochemical indices of laying bird fed diet supplemented with black seed (*Nigella Sativa*)

Table 6 shows the serum biochemical indices of fed diet supplemented with black seeds (*Nigella Sativa*). Significant differences ($p<0.05$) were observed in all the serum biochemical parameters. The layers fed diet with 0g/kg black seed had high ($p<0.05$) albumen and triglyceride values than all other dietary treatments. The globulin and total protein was highest ($p<0.05$) in 6g/kg diet and lowest in the control group. The cholesterol content ranged from 63.40mg/dl (4g/kg) to 118.33mg/dl (5g/kg) ($p<0.05$). The triglyceride values range ($p<0.05$) from 52.01mg/dl (4g/kg) to 194.95mg/dl (control). Birds fed 4g/kg of black seed recorded the highest ($p<0.05$) values of alanine transaminase and aspartate transaminase compared to other dietary treatments. Birds fed diets 6g/kg and 7g/mg had higher alkaline phosphatase value ($p<0.05$), those fed diet with 0g/kg and 5g/kg were lower ($p>0.05$) while those fed 4g/kg were comparable.

Table 6: Serum Biochemical indices of laying bird fed diet supplemented with black seed (*Nigella Sativa*)

Parameters	Black seed supplementation levels (g/kg)					SEM
	0g/kg	4g/kg	5g/kg	6g/kg	7g/kg	
Albumin (g/dl)	2.39^{a}	2.19^{c}	2.31^{b}	2.30^{b}	2.10^{d}	0.04
Globulin (g/dl)	0.23^{c}	1.15^{b}	0.35^{c}	1.71^{a}	0.43^{c}	0.14
Total protein (g/dl)	5.62^{c}	5.80^{b}	5.67^{c}	6.01^{a}	5.36^{d}	0.15
Cholesterol (mg/dl)	101.21^{b}	63.40d^{c}	118.33^{a}	97.73^{c}	64.31^{d}	4.98
Triglycerides (mg/dl)	194.95^{a}	52.01^{d}	129.80^{b}	72.47^{c}	133.67^{b}	11.80
Alanine transaminase (IU/L)	23.07^{c}	36.26^{a}	18.73^{d}	30.83^{b}	12.81^{e}	1.94
Aspartate transaminase (IU/L)	147.33^{b}	167.89^{a}	138.12^{c}	94.16^{d}	95.80^{d}	8.80
Alkaline phosphatise (IU/L)	25.92^{b}	27.54^{ab}	25.98^{b}	27.95^{a}	26.69^{a}	0.29

^{abcde} -means within a row different superscript differ ($p<0.05$).

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4. DISCUSSION

Results of the study on hen day production, and feed conversion ratio were in agreement with the work of Aydın et al., (2006) who supplemented layer diet with black seed. This could be as a results of low supplementation level of black seed. In contrast, El Bagir et al., (2006) reported that inclusion of black seeds in the diet caused a significant increase effect in egg weight and body weight in laying hens. However, Akhtar et al., (2003) showed that black seed supplementation (15 g kg^{-1}) in layer diets significantly increased egg production.

Khodary et al., (1996) showed that addition of black seed to laying hen diets significantly increase egg weight. As shown in this present study, black seed supplementation significantly affect the egg weight and it is in consistent with Akhtar et al., (2003) and Aydin et al.,(2006) who observed that black seed supplementation at the level of 5, 10, 15, and 30g/kg significantly increased egg weight in laying hens. Moreover, Aydin et al., (2008), Yalçin et al., (2009) and Khan et al., (2013) reported that dietary inclusion of black seed at the levels of 10 to 15, 30 and 30 to 50 g/kg respectively, increased egg weight. Although, Boka et al., (2014) showed that supplementation of black seed up to 30 g/kg of laying hen diets had no effect on egg weight. But, Gharaghani et al., (2015) and Vakili and Majidzadeh (2016) showed that dietary inclusion of black seed in laying hen diets increased egg weight either under heat stress or normal ambient temperature. In contrary to this present study, El-Kaiaty et al., (2003) reported that inclusion of 2g/kg of black seed in laying hen diets insignificantly improved both egg weight and egg mass. It could be observed from the experiment that no mortality was recorded throughout the experimental period which was similar to the report of Akhtar et al., (2003) who reported that mortality rate decreased from 16.67 to 4.17% by dietary supplementation of Nigella sativa seeds.

It was also observed in this present study that addition of black seed had no significant effect on the shell weight, this is in line with Aydin et al., (2006) who observed that 10 and 20g/kg supplementation did not affect the percentage of egg yolk weight, albumen and shell weight.

Black seeds supplementation at vary levels of 4,5,6, and 7g/kg in the layers diet had no effect on all the internal egg quality parameters such as yolk + albumen weight, albumen weight, yolk weight, albumen height, yolk height, yolk length and Haugh unit. This present study agreed with the report of Bolukbasi et al., (2009) who reported that dietary supplementation of Nigella sativa seed had no significant effect on ratio of yolk, albumen weight and height. Although, highest numerical value was observed in control group in term of yolk + albumen weight, albumen weight, and yolk weight. This implied that black seed effect were not significant. Furthermore, Denli et al., (2004) also observed non-significant differences in the yolk weight when layer feed was supplemented at 0.5 to 5g/kg. The significant different might be due to low supplementation level of black seed used in this study. Although, the results indicated that the use of black seed in the diet was not able to improve internal egg quality of the laying hens.

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Supplementation of layers diet with 7g/kg of black seed increased the levels of white blood cell. The results agreed with those obtained by Khodary et al., (1996) that laying hens fed diets supplemented with black seed at levels ranging from 1 up to 3g/kg and the values of white blood cell counts increased as the level of black seed increased. Black seed increased the level of red blood cell counts of layers fed diets supplemented with 7g/kg of black seed, similarly Shewita and Taha (2011) observed a marked elevation in red blood cell counts in chick dietary supplemented with 4, 6 and 10g black seed/kg for 42days. Layers fed 4g/kg diet of black seed had increased level of haemoglobin content. This result was in harmony with Mehdi et al., (2010) who reported that black seed increased haemoglobin content at 2g/kg black seed supplementation level.

Supplementation of layers diets with black seed increased the value of packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration(MCHC) containing 7g/kg, 6g/kg, 5g/kg and 4g/kg of black seed respectively. Also, the similar findings were recorded by khan et al., (2012) who recorded a significant increase in the PCV, MCV, MCH and MCHC when broiler chicks were vaccinated against Newcastle disease and supplemented with 1.25, 2.5 or 5.0% black seed for 42days.

The albumin content in birds with black seed supplementation was lower but higher serum albumin level were recorded in group that received 0g/kg black seed diet and same results were recorded by Hassan and Abotaleb, (2007) who found increased serum albumin level. Supplementation of 6g/kg of diet increased the level of total protein. Hassan and ragab, (2007) reported that adding 1% black seed to layer chickens diet increased total protein. El-ghamry et al., (2002) found no significant effects on total protein when adding 0.2, 0.4% black seeds as well as 2% black seed (Al-Homidan et al., 2002) in broiler diets.Supplementation of 6g/kg of black seeds increased globulin content which is in contrast with the finding of Hassan and Ragab, (2007) who reported that adding 0.4% black seeds had no significant effect on globulin.

Black seed increased the level of alkaline phosphatase of the laying hens fed diets with 6g/kg. On the contrary, Khan et al., (2012) study showed that black seed cumin seed is more effective in reducing the levels of ALP enzymes. Layers fed 4g/kg of black seeds had increase levels of ALT and AST while birds fed with 7g/kg of black seed has lowest value of ALT and AST. Similar hen fed diets containing 100g/kg of black seeds had increased levels of ALT, (Al-homidan et al., 2002) and AST activities but Toghyani et al., (2010), reported that AST and ALT were not statistically influenced by supplementation of black seeds in broiler diets. This suggests that the liver may not be adversely affected at the level of supplementation (Ojediran et al., 2015).

The cholesterol values in the present study ranged from 97 to 118.33mg.dl-1, being in accordance with average values reported in the literature to usual cholesterol levels in chickens can range from 100 to 250mg.dl-1 (Swenson and O'Reece, 1996). In some situations, elevation of serum cholesterol may not be related to pathological changes (Alonso-Alvarez, 2005). Supplementation of layers diet with 4g/kg black seeds reduced

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the cholesterol content when compared to control diet. Results agreed with those obtained by Tolbaand and Hassan (2003). Hassan and Abotaleb, (2007) and Al-batawi et al., (2009) also recorded that black seeds decreased the serum levels of total cholesterol and triglycerides. Black seeds reduced the triglycerides content of laying hens while Togyyani et al., (2010) found that serum triglycerides were not significantly affected by black seeds supplementation. This is unlike the hypercholesterolaemic effect of the Polyalthia longifolia extract on broiler chickens as reported by Shittu et al., (2022) which was not the case when Ojediran et al., (2017) supplemented cockerel diet with *Moringa oleifera*.

5. CONCLUSION

It could be concluded that black seed (*Nigella sativa*) supplemention favours feed conversion ration and egg weight. With varying levels of black seed up to 7g/kg, no adverse effects were recorded on external egg qualities, internal egg qualities, haematological and serum biochemical indices. Also black seed reduces the concentration of cholesterol and tryglycerides while total protein content was increased. It can be recommended from the results that black seed can be incorporated in the diets of laying birds up to 7g/kg supplementation level. Further researches should be conducted to establish gut microbial activities of black seed at different levels of inclusion.

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