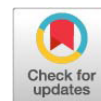


Research Article



The Use of Black Cumin (*Nigella sativa*) as Feed Supplement for Broiler Chickens Raised in Open Cages in Humid Tropical Areas

MOHAMMAD HASIL TAMZIL*, SULAIMAN NGONGU DEPAMEDE, I GEDE NANO SEPTIAN

Faculty of Animal Science, University of Mataram, Jalan Majapahit No. 62 Mataram, Lombok, West Nusa Tenggara, Indonesia.

Abstract | Black cumin is an herb rich in bioactive compounds that can be used as a substitute for chemical-based drugs including antibiotics. The study aims to determine the effect of black cumin supplementation in feed on the growth performance of broiler chickens raised in open cages in humid tropical areas. The study was designed using a Completely Randomized Design with a one-way pattern using 200 DOC CP 707 unsex broiler chickens. The chickens were divided into 4 treatments, each with 5 replications, so that each replication consisted of 10 chicks. The control treatment group of chickens were chickens not given black cumin supplementation in their feed, while the 2nd, 3rd and 4th treatment groups were each given black cumin supplementation of 600 mg, 1200 mg and 1800 mg/kg feed, respectively. Maintenance was carried out in an open cage for 4 weeks with feed and drinking water provided ad libitum. The results of the study found that black cumin supplementation in broiler chicken feed did not affect rectal temperature, HSP 70 density, feed consumption, blood weight, head and neck weight, leg weight, abdominal fat weight, and percentage of back weight and percentage of chest weight ($p > 0.05$), but it can increase body weight gain, reduce feed conversion, increase slaughter weight, feather weight and internal content weight ($p < 0.01$), carcass weight ($p < 0.05$), percentage of wing weight and percentage of thigh weight ($p < 0.01$). In the practice of broiler chicken cultivation in open cages in humid tropical areas, black cumin supplementation of 1200 mg/kg feed is recommended.

Keywords | Black cumin, Body weight, Broiler, HSP 70, Rectal temperature

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***Correspondence** | Mohammad Hasil Tamzil, Faculty of Animal Science, University of Mataram, Jalan Majapahit No. 62 Mataram, Lombok, West Nusa Tenggara, Indonesia; **Email:** m.h.tamzil@unram.ac.id

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INTRODUCTION

It is evident that the poultry industry continues to rely on the utilisation of synthetic compounds as pharmaceuticals and feed supplements. The utilisation of antibiotics and chemical compounds as feed additives has been demonstrated to result in the presence of residues in meat, thereby exerting a detrimental effect on human health (Arsene *et al.*, 2022). In contrast, black cumin has been utilised as a medicinal agent to treat various diseases in humans for millennia (Salehi *et al.*, 2021). Black cumin is characterised by a high content of volatile

fatty acids (0.5-1.6%), crude protein (20-26.7%), and a number of amino acids (Kooti *et al.*, 2016; El-Naggar *et al.*, 2017). Moreover, black cumin has been found to contain significant quantities of crude fat, crude fibre, and a variety of essential minerals, including Fe, Na, Cu, Zn, P, Ca, and vitamin C, as well as vitamins B complex and B9, and various digestive enzymes. From a pharmacological perspective, black cumin is characterised by the presence of active compounds, namely volatile oils, including thymoquinone, dithymoquinone, and thymol (Noviadi and Irwani, 2017; Salehi *et al.*, 2021). Furthermore, black cumin has been found to contain linoleic acid, oleic acid,

palmitic acid, and trans-anethole, in addition to other minor constituents, including nigellimine, nigellidine, nigellimine, and nigellimine N-oxide (Soleimani *et al.*, 2008). Thymoquinone has been shown to act as an antioxidant, thus helping to protect cells from oxidative damage by acting as a free radical scavenger (Al-Naqeep *et al.*, 2009; Khader and Eckl, 2014; Alzahrani *et al.*, 2024), and can be used to optimize the functioning of the digestive organs in poultry (Singh *et al.*, 2018). Thymoquinone and thymohydroquinone have been shown to inhibit non-enzymatic lipid peroxidation *in vitro* in iron-ascorbate-induced hippocampal homogenates (Mizuno and Fukuhara, 2022). Black cumin oil and extract have been shown to possess antioxidant properties, which have the capacity to modulate oxidative stress. Oxidative stress has been identified as a contributing factor in the development of diseases and disorders that are associated with this process. In this instance, research has demonstrated that both black cumin seed oil and solvent extract have antioxidant activity *in vitro* (Toma *et al.*, 2015; Mohammed *et al.*, 2016; Abedi *et al.*, 2017; Singhal *et al.*, 2017). The utilisation of black cumin as a medicinal agent has been documented on numerous occasions (Yimer *et al.*, 2019), however, there is a paucity of literature concerning the utilisation of the substance as a pharmaceutical agent or a feed supplement in poultry nutrition. Poultry is classified as homeothermic (warm-blooded) livestock, and almost all parts of its body are covered in feathers. Poultry farming in humid tropical regions, such as Lombok Island, is vulnerable to heat stress attacks. The maximum environmental temperature that can be recorded on Lombok Island is 31.8 °C, and the relative humidity can reach 93% (BMKG NTB, 2023). Far above the comfortable temperature for poultry life which ranges from 18-22°C (Pawar *et al.*, 2016). The utilisation of black cumin as a supplementary feed in the context of broiler maintenance is widely regarded as a multifaceted nutritional strategy. It is believed to function as an antioxidant, thereby reducing oxidative stress, and as a growth stimulant, promoting optimal development. How effective is it?

MATERIALS AND METHODS

RESEARCH LOCATION

The process of chick rearing was centred at the Teaching Farm of the Faculty of Animal Science, University of Mataram, which is located in Lingsar Village, Lingsar District, West Lombok (approximately 12 km from the University of Mataram campus). Concurrently, HSP 70 density measurements were conducted at the Immunobiology Laboratory of the University of Mataram.

RESEARCH PROCEDURE

The study was designed using a completely randomized

design with a one-way pattern, and 200 DOC broiler chickens of the CP 707 strain were used. The chickens were divided into four treatments, each with five replications, so that each replication consisted of ten chicks. The first treatment group of chickens was not given black cumin supplementation in their feed (control), while the second, third and fourth treatment groups were given black cumin supplementation of 600 mg, 1200 mg and 1800 mg/kg of feed, respectively. Each replicate group of chickens was confined to a one-square-metre enclosure that contained a food and water receptacle, as well as a heating lamp. The box is placed in an open cage building with a litter base. The cage walls are composed of materials that facilitate adequate ventilation at the front and back, thereby ensuring unobstructed air circulation both inside and outside the cage. Maintenance is carried out for a period of four weeks, during which time the subjects are provided with feed and drinking water *ad libitum*.

PREPARATION OF EXPERIMENTAL FEED

The type of feed utilised in this study is a commercial feed produced by PT. Japfa Comfeed Indonesia, while the black cumin used is ground black cumin seeds purchased at a herbal shop in Mataram City. The incorporation of black cumin into the feed of each group of treatment chickens is conducted on a periodic basis. The mixing process entails the incorporation of black cumin into the feed, in accordance with the stipulated dosage. In the preliminary phase, a modest quantity of culture should be prepared, which is subsequently to be amalgamated with a more substantial volume of feed.

BLOOD SAMPLING

Rectal temperature measurements and blood sampling for the purpose of measuring the HSP 70 density variable are carried out during the day, around 13:00, when the chickens are 21 days old. Rectal temperature was measured by inserting a digital thermometer through the rectum, while blood was taken through the wing vein (brachial vein) using a 1 cc insulin syringe. The blood was subsequently stored at a temperature of 3°C for a period of 16 hours.

SAMPLING FOR CARCASS AND NON-CARCASS WEIGHT MEASUREMENT AND PRIMAL CARCASS CUT WEIGHT

The measurement of cut weight, carcass and non-carcass weight, and primal carcass cut weight, was carried out by means of sampling when the chickens were 4 weeks old. In each treatment group, two samples were obtained from each replication. The objective of this procedure was to ensure that the body weights of the chickens were close to the mean body weight of the chickens in each replication group.

OBSERVED VARIABLES

The variables measured in the study were:

1. Air temperature in the cage: The cage was subject to

- daily measurement of its air temperature, with the minimum and maximum recorded using a digital thermometer.
2. The rectal temperature is measured: Rectal temperature data was obtained by measuring the body temperature of each sample chicken by inserting a body heat thermometer into the rectum. Concurrently, rectal temperature measurements were obtained in conjunction with blood sampling for the purpose of determining the density of heat-shock protein 70.
3. The following data set concerns the consumption of feed: The feed consumption of each group of chickens within each replication of the treatment was determined by measuring the total weight of feed consumed on a weekly basis. The quantity of feed consumed was determined by calculating the difference between the total amount of feed provided at the commencement of the week and the residual feed remaining at the conclusion of the week.
4. Body weight: The body weight of each chicken in the treatment and replication groups was calculated on a weekly basis at the conclusion of the study.
5. The subject has exhibited an increase in body mass: The calculation of body weight gain was performed by deducting the subject's body weight from their body weight in the previous week.
6. Feed conversion: Feed conversion was calculated by dividing the amount of feed consumption by the body weight gain in the same period.
7. Slaughter weight: Slaughter weight was calculated by weighing each chicken before slaughter.
8. Blood weight: Blood weight was calculated by subtracting the slaughter weight from the weight after slaughter.
9. Feather weight. Feather weight is calculated by subtracting the weight after slaughter from the weight of the chicken after the feathers have been removed.
10. Internal content weight: Internal content weight is calculated by weighing all the internal contents of each sample chicken.
11. Head and neck weight: Head and neck weight is calculated by weighing the head and neck parts cut at the last neck bone area (before the clavicle bone).
12. Leg weight: Leg weight is calculated by weighing the leg parts cut at the joint area between the tibia and metatarsus bones.
13. Abdominal fat weight: Abdominal fat weight was calculated by weighing all the abdominal fat in the abdomen of each sample chicken.
14. Carcass weight: Carcass weight is obtained by weighing the carcass of each sample chicken.
15. Carcass primal cuts: Carcass primal cuts are obtained by weighing all carcass primal cuts, namely back weight, wing weight, chest weight, upper thigh weight, and lower thigh weight.
16. HSP70 density. HSP 70 density measurement is carried out by immediately centrifuging the collected blood for 5 minutes at 3000 rpm to obtain serum. Furthermore, the serum is analyzed using an ELISA (Enzyme Linked Immunosorbent Assay) kit produced by Depamede *et al.* (2022) (<https://pdki-indonesia.dgip.go.id/detail/02c105fdee50e91655>). The analysis method is as follows, the ELISA plate is dicoted (dropped) with HSP70 polyclonal antibody (Ab-HSP70) with a concentration of 10µg/ml in a pH 9.0 carbonate-bicarbonate buffer solution of 100 µl per ELISA plate well. After incubation for 12-14 hours at 4°C, the Ab-HSP70 solution was discarded, then the plate was washed once using phosphate buffered saline solution containing 0.05% tween-20 (TBST). Furthermore, the plate wells were blocked using 300 µl of 2% skimmed milk for 60 minutes at 37°C, then the plate was washed using TBST solution three times. After washing, each well of the plate was filled with 50 µl of chicken serum sample that had been diluted using PBST solution containing 1% BSA, then incubated for 60 minutes at 37°C. After incubation, the plate was washed using PBST three times, then each well was filled with 50 µl of secondary antibody solution (goat-anti chicken IgY-HRP; Invitrogen-USA) with a dilution of 10,000 times, then the plate was incubated for 60 minutes at 37°C. Furthermore, all samples were discarded and the plate was washed using PBST three times, then each well was filled with 50 µl of TMB substrate solution (3,3',5,5'-Tetramethylbenzidine), continued with incubation in a dark room for 15 minutes and ended with the addition of 1 M H₂SO₄ solution to stop the reaction. The reaction results were read using an ELISA photoreader at OD450.

STATISTICAL ANALYSIS

The data obtained were tabulated and analyzed using Analysis of Variance and the Least Significant Difference further test, using the assistance of the SPSS program version IBM SPSS Statistics 19.

RESULTS AND DISCUSSION

As demonstrated in Table 1, the data indicates that the supplementation of black cumin in feed does not exert an effect on body temperature (rectal temperature), and Heat Shock Protein (HSP) 70 density, and feed consumption ($p > 0.05$). However, it has been observed to increase body weight and body weight gain, and to suppress feed conversion ($p < 0.05$) of broiler chickens kept in open cages. The absence of effect of black cumin supplementation in feed on rectal temperature and HSP 70 density indicates that the experimental chickens

did not suffer from heat stress, even though they were kept in open cages. The mean rectal temperature of the experimental broiler chickens was between 41.12 and 41.62°C, thus remaining within the normal temperature range (40.5-41.5°C) (Tamzil *et al.*, 2019) or between 41-42oC (Ruzic *et al.*, 2023). It is widely accepted that broiler chickens are classified as homeothermic animals. In order to thrive, these chickens require a comfortable temperature range of 18–24°C (Aluwong *et al.*, 2017), In cases where the ambient temperature exceeds the optimal range for poultry, specifically broiler chickens, the animals are susceptible to a condition referred to as heat stress. A hallmark of this condition is an elevation in the bird’s rectal temperature (Aluwong *et al.*, 2017; Biutifasari, 2022). In addition to measuring rectal temperature, another method of determining the level of heat stress in poultry is to assess the density of Heat Shock Protein (HSP) 70 (Tamzil *et al.*, 2019). The density level of HSP 70 in the sample chickens from the control group is equivalent to the density level of HSP 70 in the treatment group. Consequently, it can be concluded that not all experimental chickens are experiencing heat stress. This condition arises as a consequence of the maintenance being conducted within an open cage that is equipped with effective cage ventilation. This ensures unobstructed air circulation both inside and

outside the cage, thereby creating a relatively comfortable atmosphere for the chickens. The maximum environmental temperature recorded in the cage during the study was 30.1°C, which falls within the temperature range that is tolerable for chickens. However, it has been established that temperatures in excess of 34°C can lead to an increase in rectal temperature and HSP70 density (Khan *et al.*, 2021), and subsequently affecting the body’s homeostasis balance (Biswas *et al.*, 2024). It has been demonstrated that increased HSP density functions as a molecular chaperone, thereby assisting organisms in coping with both internal and external stresses (Akbarian *et al.*, 2016; Balakrishnan *et al.*, 2023, Goel *et al.*, 2025).

The data presented in Table 1 also demonstrates that the supplementation of black cumin in the ration resulted in increased body weight and weight gain, as well as suppressed feed conversion (P<0.01). It can thus be concluded that the supplementation of feed with black cumin has a positive effect on the performance of broiler chickens. The supplementation of black cumin at levels of up to 600 mg/kg of feed has been demonstrated to result in enhancements in growth parameters and a reduction in feed conversion efficiency. A highly significant effect on growth and feed conversion was observed in black cumin

Table 1: Effect of black cumin supplementation on body temperature, HSP 70 density, and growth performance of broiler chickens raised in open cages.

Variables	Level of black cumin supplementation in feed (mg/kg feed)				p-value
	0	600	1200	1800	
Body temperature (°C)	41.12	41.36	41.28	41.62	0.296
HSP 70 density (µg/mL)	0.442	0.461	0.432	0.451	0.084
Feed consumption (g/head)	2641.25 ^{ab}	2529.72 ^a	2500.4 ^a	2817.02 ^b	0.795
Body weight (g/head)	1453.10 ^a	1554.40 ^b	1588.20 ^b	1711.66 ^c	0.000
Body weight gain (g/head)	1376.50 ^a	1481.20 ^b	1544.00 ^b	1635.50 ^c	0.000
Feed conversion	1.92 ^a	1.71 ^b	1.65 ^b	1.72 ^b	0.001
Slaughter weight (g/head)	1496.00 ^a	1493.00 ^a	1503.00 ^a	1744.00 ^b	0.003
Blood weight (%)	2.02	1.95	1.91	1.91	0.565
Feather weight (%)	7.67 ^a	7.83 ^a	7.23 ^a	11.04 ^b	0.01
Head-neck weight (%)	7.10	7,34	6.90	7.66	0.659
Leg weight (%)	4.83	5.01	5.01	5.07	0.547
Giblet weight (%)	9.022 ^a	9.02 ^a	11.19 ^{ab}	15.52 ^b	0.000
Adominal fat weight (%)	2.93	2.93	2.83	2.93	0.701
Carcass weight (g/head)	1052 ^a	1048 ^a	1053 ^a	1272 ^b	0.045
Carcass weight (%)	66.35 ^a	65.82 ^a	64.86 ^a	55.73 ^b	0.000
Percentage weight of carcass primal cuts					
Back weight (%)	25.43	24,19	24.60	25.43	0.437
Wing weight (%)	16.45 ^a	16.79 ^a	18.16 ^b	15.96 ^a	0.002
Chest weight (%)	30.26	31.51	30.57	33.25	0.076
Thigh weight (%)	27.08 ^a	27.50 ^a	28.23 ^b	25.30 ^a	0.014

supplementation of 1800 mg/kg of feed. This biological phenomenon is attributable to the accumulation of the benefits of black cumin, which has antioxidant properties (Khader and Eckl, 2014; Alzahrani *et al.*, 2024), an immune system booster (Younus, 2018), an anti-inflammatory (Alzahrani *et al.*, 2024), increasing feed digestibility (Asghar *et al.*, 2022; Saputri *et al.*, 2024), and a stress controller (Hafeez *et al.*, 2024). A number of compounds that function in synergy are present in black cumin, including thymoquinone (TQ), thymohydroquinone (THQ), dithymoquinone, thymol, carvacrol, α - and β -pinene, d-limonene, d-citronellol, and p-cymene. Of all these compounds, the one that exerts the greatest influence on the pharmacological effects is the quinine component, which is the most abundant in TQ. It has been established that TQ exerts a role in a number of bioactivities, including anticonvulsant, antioxidant, anti-inflammatory, anticancer, antibacterial and antifungal properties (Forouzanfar *et al.*, 2014). It has been established that TQ, in conjunction with THQ, exerts a suppressive effect on non-enzymatic lipid peroxidation in *in vitro* settings, specifically within the context of hippocampal homogenates, when induced by iron-ascorbate (Hosseinzadeh *et al.* 2007). Thymoquinone compounds present in black cumin have been demonstrated to exert a positive effect on the augmentation of levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), total antioxidant (TAC) enzymes, and the reduction of cortisol concentrations. The present study investigates the levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and malondialdehyde (MDA) in chickens suffering from stress, and compares these levels with those recorded in non-stressed chickens (Khader and Eckl, 2014; Jomova *et al.*, 2024; Alzahrani *et al.*, 2024). The results of this study also found that supplementation of black cumin in feed of 1800 mg/kg feed increased the percentage of feather weight, internal content weight, and reduced carcass weight ($P < 0.01$). This indicates that although black cumin supplementation up to a threshold of 1800 mg/kg feed produced the highest body weight and body weight gain ($P < 0.01$), the resulting body weight was partly in the form of increased feather weight and internal weight. Therefore, black cumin supplementation of 1800 mg/kg feed is not recommended in broiler chicken farming practices. This increase in feather weight is hypothesised to be attributable to the influence of omega-3 and omega-6 contained in black cumin, which stimulate feather growth and health. The group of chickens that received black cumin supplementation at a dose of 1800 mg/kg feed produced the highest percentage of feather weight (Alagawany *et al.*, 2019). The development of these feathers is further facilitated by the presence of vitamin E, which plays a pivotal role as an antioxidant, and potassium, magnesium and phosphorus, which play a pivotal role in strengthening

the feather structure (van Emous and van Krimpen, 2019). Meanwhile, the phenomenon of increased internal weight resulting from the supplementation of black cumin at a dose of 1800 mg per kg of feed has been observed. This phenomenon can be attributed to the stimulatory effect of the black cumin content on enzymes and hormones that facilitate growth, thereby promoting enhanced blood flow and the subsequent multiplication and enlargement of internal organ tissue (Hannan *et al.*, 2021). The data presented in Table 1 further demonstrate that the supplementation of black cumin up to a threshold of 1800 mg per kg of feed results in a reduction of the percentage of wing weight and thigh weight. However, supplementation at the level of 1200 mg per kg of feed leads to an increase in the percentage of wing weight and thigh weight (upper thigh and lower thigh). The findings of this study suggest that the supplementation of black cumin in broiler feed, at a range of 1200 to 1800 mg per kg of feed, is effective. The current study found that the threshold was still below the recommendations of previous researchers. These researchers had suggested the addition of black cumin to the feed of broiler chickens, at a rate of 3 grams per kilogram, with a view to increasing their growth (Shewita and Taha, 2019; Hafeez *et al.*, 2024). The disparity in the recommendations provided by these studies indicates that further research is required to ascertain the optimal threshold for the incorporation of black cumin in broiler feed.

CONCLUSIONS AND RECOMMENDATIONS

The study concluded that black cumin supplementation in feed can increase body weight gain, slaughter weight, feather weight, internal content weight, carcass weight, wing weight percentage and thigh weight percentage, and reduce feed conversion. However, no effects were observed on rectal temperature, HSP 70 density, feed consumption, blood weight, head and neck weight, leg weight, and abdominal fat weight. In the context of broiler chicken husbandry in open cages in humid tropical regions, the recommendation is for supplementation with black cumin at a dosage of 1200 mg/kg of feed.

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NOVELTY STATEMENT

The use of synthetic compounds as antibiotics and feed supplements remains common practice in broiler chicken farming systems to this day. However, the inclusion of chemical additives in animal feed is known to leave residues

in the meat, which may pose health risks to humans. As a safer alternative, black cumin (*Nigella sativa*) has been identified as a source of bioactive compounds with potential benefits for poultry health. Supplementation with black cumin has been shown to improve the growth performance of broiler chickens raised in open-house systems under humid tropical climates. Therefore, the findings of this study present a novel contribution and hold promising potential for broader application in broiler farming systems in humid tropical environments.

AUTHOR'S CONTRIBUTION

Mohammad Hasil Tamzil served as the originator of the research topic idea. He also played an active role in designing and conducting the research from start to finish, as well as preparing the report and developing it into a scientific article. Sulaiman Ngongu Depamede contributed by completing laboratory tasks, particularly in the analysis of HSP70 concentration. I Gede Nano Septian was actively involved in daily data collection in the poultry house.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

This article was written without the use of AI.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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