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Effect of *Tenebrio molitor* Supplementation on Performance and Immunity of Broilers under Heat Stress Conditions

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***T. molitor*, Heat stress, Immunity, Production performance, Newcastle disease, Broiler**

ABSTRACT

In broiler production, numerous feed additives have been utilized to promote growth, feed efficiency, immunity, and antioxidant capacity. Similarly, various techniques, including electric fans, cooling pad systems, and water sprinkling through foggers, have been employed to mitigate heat stress in poultry. However, due to cost constraints, these methods may not always be feasible, and alternative nutritional strategies can be followed, including the use of balancing of nutrients. One such nutritional strategy for reducing stress is the incorporation of *Tenebrio molitor* (mealworm) as a feed additive. Therefore, this study was designed to investigate the impact of *T. molitor* as a feed supplement on the performance, immunity, and economics of heat-stressed broilers. A total of 150 (day-old) broilers were randomly allocated to five dietary groups: such as D0 (control), D1, D2, D3, and D4, provided with *T. molitor* at the rate of 0, 0.5, 1.0, 1.5, and 2 g/kg of feed, respectively. The broilers were exposed to temperatures ranging from 32-38˚C during the trial. Results showed the highest significant total weight gain, improved feed conversion ratio, and dressing percentage, and reduced feed intake in the D4 group compared to all other groups. Similarly, the highest antibody titers against the Newcastle disease, and lower mortality rate were calculated in the D4 group than in the remaining groups. Meat quality was not affected. A significant increase in the net profit was observed in the D4 group as compared to the leftover groups. Based on the current findings, it is concluded that *T. molitor* at the rate of 2.0 g/kg in broiler feed improves the production performance, boosts the immune response against the Newcastle disease, and increases the economic return.

INTRODUCTION

The poultry sector in Pakistan plays a crucial role in addressing the protein supply and demand gap. It is a dynamic and well-organized industry that contributes significantly to various aspects of the country's economy. Poultry production in Pakistan contributes approximately 26.9% to the total meat production, 5.76% to the agricultural sector, and 1.29% to the overall GDP (GOP, 2014). Heat stress in the broiler industry has been a great concern for several years because of its deleterious effects on the health and performance of poultry. Heat stress results in increased excretion, reduced feed intake, and reduced bioavailability of essential nutrients, all of which contribute to a compromised immune response and performance (Lara and Rostagno, 2013). High temperature is one of the major reasons that causes stress and adversely affects poultry production (Meremikwu et al., 2013). Generally, the broilers grow well at optimal temperatures of 18-22°C (Sahin et al., 2006), however, prolonged exposure to high temperatures above 30°C adversely affects the physiological functions of broilers (Borges et al., 2004). In broiler production, numerous feed additives have been utilized to promote growth, feed efficiency, immunity, and antioxidant capacity. Several studies (Alzawqari et al., 2016; Khan et al., 2016) have explored the effectiveness of these additives. Additionally, various techniques, such as the use of electric fans, cooling pad systems, and water sprinkling through foggers, have been employed to mitigate heat stress in poultry (Chand et al., 2016). However, due to cost constraints, these methods may not always be feasible. As an alternative, nutritional strategies can be followed, including the use of balancing of nutrient contents and the addition of substances like aspirin, vitamin C, potassium carbonate, and sodium bicarbonate in drinking water. One such nutritional strategy for reducing stress is the incorporation of *T. molitor* as a feed additive (Chand et al., 2017).

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T. molitor can help alleviate heat stress in broilers due to its beneficial effects. They are important sources of protein, fats, essential amino acids, and fatty acids, which can help to improve nutrient intake and utilization in broilers (Hong et al., 2020). *T. molitor* contains chitin, a polysaccharide that has been found to have anti-inflammatory and antioxidant properties, potentially reducing the negative impacts of heat stress on broilers. In addition to chitin, lauric acid and antimicrobial peptides in *T. molitor* meal improve chicken's health by reducing the load of gut microbiota (*Escherichia coli* and *Salmonella* spp.) and increasing the level of *Lactobacillus* spp. and thus minimize the use of antibiotics (Elahi et al., 2020). Research on the effects of *T. molitor* specifically on broilers in heat stress conditions is limited. Therefore, the present study was designed to evaluate the effect of *T. molitor* based diets on the production performance, immunity, meat quality, and economic return of broilers under heat-stress conditions.

MATERIALS AND METHODS

Study area

The present research study was performed at Poultry Research Farm, The University of Agriculture Peshawar, Pakistan.

T. molitor *drying and rations formulation*

Mature *T. molitor* larvae were obtained from the stock culture maintained on wheat bran at the Entomology Research Laboratory, The University of Agriculture Peshawar. The collected larvae were starved for 24 h to reduce the gut contents, then killed and dried in an oven at 60 ºC for 24-36 h. The larvae were finally ground into fine powder through the electric grinder. The powdered larvae were mixed (g/kg) into the broiler feeds in mesh form.

Broilers husbandry and dietary groups

A total of 150 broiler chicks (day old) were obtained from the commercial hatchery for this experiment and were distributed randomly into five groups, each replicated three times (10 birds replicate^{-1}) followed by a Completely Randomized Design (CRD). The five dietary groups were D0 (control), D1, D2, D3, and D4 provided with dried *T. molitor* at the rate of 0, 0.5, 1.0, 1.5, 2.0 g kg^{-1} feed respectively as per the procedure of NRC (1994) to fulfill the broilers needs. The broilers were vaccinated against the Newcastle disease. This experiment lasted for six weeks including one week of adaptation period. During the experiment, the broilers had ad libitum access to the feed and water. The broilers were kept under natural heat-stressed conditions (32-38 ºC, 60-80% R.H). Maximum and minimum teleprompter and humidity within the sheds were recorded daily at six hours intervals (Chand et al., 2017).

Production performance parameters

The body weight gain was measured as the difference between the initial weight and the final weight e.g., Weight gain= Final weight – Initial weight. Feed intake was computed as a difference between the total provided feed and feed left over. The feed conversion ratio (FCR) was measured by the formula e.g., FCR = Feed intake ÷ Weight gain (Shuaib et al., 2020). The mortality rate was computed at the end of the experiment with the help of the given formula e.g., Mortality rate (%) = Total dead broilers ÷ total broilers×100

Antibody titers against Newcastle disease

At the end of the experiment, blood samples were collected from three randomly selected broilers from each treatment. Antibody titer against ND virus in all the tested groups was estimated according to the standard procedures of OIE (2012).

Dressing percentage and organoleptic study

At the end of the experiment, three broilers were randomly selected, weighed, and slaughtered. After the removal of non-edible parts (neck, head, feet, and internal organs) the dressing percentage was calculated as; Dressing (%) = Dressed weight ÷ Live broiler weight x 100. For the organoleptic study, broiler meat (breast, legs, and thighs) fed with *T. molitor* was cooked up to 82°C. The cooked meat was presented to a group of staff members and students for assessment as per the procedures of Khan et al. (2016). The samples were evaluated based on color, taste, flavor, juiciness, and tenderness.

Economics

The economic feasibility of the present study was assessed by computing the expenditure and economic return. The feed cost was estimated by considering the feed cost including the cost of *T. molitor*. The net return was computed as a difference between the overall production cost (labor cost, feed cost, chicken cost, and miscellaneous costs) and gross return. The gross return was estimated according to the market price per kilogram of broiler meat on a live-weight basis. All these calculations were carried out in Pakistan rupee (PKR).

Statistical analysis

The data was analyzed with the help of the statistical package statistic 8.1. The means were compared by using the LSD test at a 5% significance level (Steel and Torrie, 1980).

RESULTS

Table I shows the effect of *T. molitor* supplementation on the feed intake (FI), weight gain (WG) and feed conversion ratio (FCR), dressing %, antibody titers against NDV, mortality and organoleptic properties of meat of broilers under heat stress conditions. The highest significant total weight gain, improved feed conversion ratio, and reduced feed intake were calculated in the D4 group compared to all other groups. The highest dressing %, and antibody titers against the Newcastle disease was recorded in the D4 group while the lowest dressing % and antibody titers against the Newcastle disease was recorded in the D0 group. The addition of *T. molitor* in poultry feeds improved the defense mechanism of broilers against the Newcastle disease. The highest ($p<0.05$), and lower mortality rate was calculated in the D4 group than in the remaining groups. The result of the organoleptic study of the heat-stressed broilers fed with mealworms is shown in Table I. At the end of the experiment, the color, flavor, tenderness, taste, and juiciness of broiler meat were assessed by a group of staff members and students. All the parameters were non-significant in all groups indicating that *T. molitor* inclusion in poultry feed does not affect the quality of broiler meat when compared with the control group (D0). Table II shows the economics of broiler production in response to the supplementation of *T. molitor* in commercial poultry feeds. The highest ($p<0.05$) total cost of production, gross return, and profit margin was observed in the D4 group as compared to the remaining groups.

Table I. Effect of *T. molitor* supplementation on production performance, dressing (%), antibody titer against ND virus, mortality and organoleptic qualities of heat stressed broilers (Mean±SD).

Parameters	Control (D0)	Supplementation of *T. molitor* at (g/kg^{-1})				P. value
		0.5 (D1)	1.0 (D2)	1.5 (D3)	2.0 (D4)	
Production performance						
Feed intake (g)	3106.7±7.47^{a}	3095.9±5.18^{b}	3087.9±9.74^{b}	3073.7±4.32^{c}	3066.8±5.66^{c}	0.001
Weight gain (g)	1789.2±8.33^{e}	1817.3±8.01^{d}	1870.7±6.91^{c}	1903.3±5.67^{b}	1925.8±9.01^{a}	0.007
Feed conversion ratio	1.74±0.21^{a}	1.70±0.30^{ab}	1.65±0.24^{b}	1.61±0.33^{bc}	1.59±0.35^{c}	0.001
Dressing (%)	64.18±1.06^{d}	65.52±2.28^{cd}	67.46± 0.85^{bc}	68.32±1.04^{b}	71.12±0.76^{a}	0.002
Antibody titer against ND virus	4.24±0.06^{c}	5.15±0.05^{b}	5.21±0.02^{b}	6.24±0.10^{a}	6.33±0.20^{a}	0.001
Mortality	5.51±0.16^{a}	4.21±0.10^{b}	4.16±0.04^{b}	3.34±0.07^{c}	3.31±0.05^{c}	0.003
Organoleptic qualities of meat						
Taste	2.34±0.16	2.33±0.13	2.37±0.05	2.34±0.03	2.36±0.12	0.021
Tenderness	2.39±0.25	2.36±0.21	2.38±0.14	2.41±0.08	2.38±0.04	0.035
Juiciness	2.28±0.05	2.31±0.09	2.32±0.08	2.35±0.12	2.33±0.10	0.067
Color	2.43±0.12	2.41±1.04	2.42±0.21	2.43±0.24	2.42±0.19	0.0721
Flavor	2.52±0.06	2.44±0.03	2.51±0.22	2.55±0.04	2.47±0.07	0.012

Means with different letters within the row differs at P < 0.05.

Table II. Economics (PKR) of *T. molitor* supplementation in broilers diets (Mean±SD).

Parameters	Control (D0)	Supplementation of *T. molitor* at (g/kg^{-1})				P. value
		0.5 (D1)	1.0 (D2)	1.5 (D3)	2.0 (D4)	
MW cost	0.00±0.00^{d}	2.34±0.81^{c}	4.65±0.67^{c}	6.95±0.79^{b}	9.48±0.05^{a}	0.002
Feed cost/kg	110.2±2.87^{d}	112.7±1.92^{cd}	114.1±1.99^{bc}	117.7±0.93^{b}	120.6±0.71^{a}	0.001
Total feed cost/bird	345.0±2.69^{e}	351.5±4.46^{d}	355.8±6.23^{c}	359.7±3.26^{b}	366.3±3.37^{a}	0.0041
Operational charges	65.00	65.00	65.00	65.00	65.00	----
Total cost of production	410.0±4.56^{d}	416.5±3.31^{c}	420.8±1.78^{c}	424.7±4.77^{b}	431.3±3.51^{a}	0.002
Gross return	447.3±3.69^{e}	455.5±6.31^{d}	467.6±3.19^{c}	475.8±6.05^{b}	485.7±7.48^{a}	0.001
Net profit	37.22±7.54^{d}	39.07±2.38^{d}	46.84±5.17^{c}	51.11±3.25^{b}	54.35±4.18^{a}	0.004

Means with different letters within the column differs at P < 0.05. For group description see Table I. Average price of broiler Rs. 250/kg. Average price of dried *T. molitor* Rs 150/100 gram. PKR= Pakistan rupees. MW= Meal worms.

DISCUSSION

Results of the current experiment demonstrated that the growth, performance, and immunity of broilers were significantly affected by higher ambient temperature while in inclusion of *T. molitor* in poultry feed improved these parameters. In high temperatures, the broiler's ability to dissipate heat is drastically affected and their appetite tends to decrease as a part of their physiological response to avoid the metabolic heat production to cope with the heat (Chand et al., 2017). This reduction in feed intake can negatively affect the overall growth, productivity, and health of broilers (Imtiaz et al., 2014). Our findings can be compared with the earlier work of Adam et al. (1962) who reported reduced feed intake and weight gain in broilers exposed to high temperatures. Bartlett and Smith (2003) also agreed with the current findings. According to Lara and Rostagno (2013), broilers maintained in hot environments reduce their feed consumption which ultimately results in lower weight. The high protein, vitamin, and mineral contents found in *T. molitor* make them a great addition to a balanced diet which can induce satiety, resulting in decreased feed intake in broilers to minimize body heat production. In the present study, the reason for lower intake might be the presence of chitin in feeds which may alter its overall palatability, leading to reduced intake as reported by Khan et al. (2023) and Bovera et al. (2015). Current findings revealed that *T. molitor* can be added to poultry feed without affecting the body weight of broilers. The broiler's weight was significantly higher in *T. molitor* augmented feed compared to the control. These results follow Khan et al. (2023) and Biasato et al. (2017) who stated that *T. molitor* has an important role in growth increment, nutrient utilization, and feed conversion efficiency (FCE). The superior body weight gain in supplemented diets is probably due to the beneficial effect of mealworm chitin which improves the broiler's health. The high protein content in mealworms may promote muscle development and weight gain, even in challenging environmental conditions, as documented by Khan et al. (2023) and Imtiaz et al. (2014). These findings highlight the potential benefits of incorporating mealworms into broiler diets for improved growth and performance. Our results showed that *T. molitor* supplementation in feeds significantly improved the FCR of broilers at the rate of 1.5 to 2 g/kg feed compared to control. The results align with findings reported by Hussain et al. (2017) and Khan et al. (2023) who found improved FCR and higher body weight for broilers fed on *T. molitor*-based feeds than basal diet which may lack some essential nutrients or contain compounds that are harder to digest for broilers and hence affect the efficiency of broilers. The results are also supported by Moreki et al. (2012).

Present results indicated that the inclusion of *T. molitor* in broiler feeds up to 2% had no adverse effects on the quality of meat. The flavor, color, juiciness, and taste of broiler meat were non-significant among all the experimental groups. The results are consistent with those of Aniebo et al. (2011) and Khan et al. (2016) who observed no effects on flavor, taste, color, juiciness, and tenderness of broiler meat fed on *T. molitor*-supplemented diets. Similarly, Elahi et al. (2020) and Zadeh et al. (2019) did not find any adverse effect of replacing soybean with mealworms on the carcass quality and broiler's organs weight. The dressing percentage and antibody titer against Newcastle disease of broilers were significantly affected by mealworm. Hussain et al. (2017) found better dressing percentage and antibody titer against ND in birds fed at a higher level of mealworms. This improvement may be attributed to the high protein contents and beneficial amino acids profile of mealworms can help improve the overall carcass composition and enhance dressing percentage in broilers as reported by Khan et al. (2023. Moreover, the chitin, lauric acid, and antimicrobial peptides (AMP) of mealworms may also improve the broiler's health (Gasco et al., 2018). Our results are also in agreement with those of Farooqi et al. (2005) who found similar results in the case of betaine supplementation on the broiler's immunity. The presence of insect chitin in supplemented feeds improves broilers health by reducing the load of gut microbiota (*Escherichia coli* and *Salmonella* spp.) and increasing *Lactobacillus* spp. *T. molitor* contains antioxidants including carotenoids and phenolic compounds which have potential health benefits and may help protect against oxidative stress. Results of our investigation showed positive effects of *T. molitor* on the final output of broilers compared to control. These results are comparable with Hussain et al. (2017) who reported higher net profit in broilers fed on higher levels of *T. molitor*. The feed cost was slightly higher in all the supplemented groups, but the potential improvements in broiler performance associated with mealworm inclusion offset the initial increased cost with the highest gross return and net profit. These findings are also in line with the results of some earlier research conducted by Khatun et al. (2005) and Choudhury et al. (1998). However, Dutta et al. (2012) observed the lowest feed cost when the silkworm meals replaced 100% fish meal proteins indicating that incorporating a high quantity silk worms in poultry feed reduces feed costs.

CONCLUSION

It was concluded that the use of *T. molitor* in poultry feeds improved the overall performance of broilers through their positive effects on nutrient digestibility, reduced the gut microbiota, and boosted immune response against the prevalent infectious diseases under heat stress conditions. Regular use of *T. molitor* at the rate of 2.0 g/kg in broiler feed is recommended for improved performance and high economic return. Further research is required to explore the effects of partial or complete replacement of soybean meal with *T. molitor* on the production performance of heat-stressed broilers.

DECLARATIONS

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IBR approval

The experimental work was approved by the Board of Studies meeting (Ent. No.624//UAP) dated 16-01-2022, Department of Entomology, Faculty of Crop Protection Sciences, The University of Agriculture Peshawar, KP, Pakistan.

Ethical statement

The study was approved by the Ethical Committee of the Department of Entomology, Faculty of Crop Protection Sciences, The University of Agriculture, Peshawar, before the practical execution of this experiment (Ent.600/UAP dated; 06-10-2021).

Statement of conflict of interest

The authors have declared no conflict of interest.

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