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Effect of Parasitic Infection on Nutritional Parameters of Meat Tissue of *Gallus gallus domesticus*

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Abstract: A crucial part of meeting our nutritional needs is chicken. It has a high protein content and is low in calories, fat, and cholesterol. Additionally, it costs less than other meats like hog, beef, and lamb. India ranks third globally for producing eggs and seventh for producing chicken meat. Each year, more than 50 billion chickens are raised for sustenance, including for their flesh and eggs. One of the biggest risks to the population of chickens in terms of their health is parasite infestation. Parasitic infection may lead to economic loss to the farmers as well as nutritional loss as a diet to humans. Chicken may have poor body condition as a result of the parasitic diseases by limiting feed efficiency, vying for feed, and being affected in terms of health. The present study includes a comparison of nutritional parameters between the control sample (non-infected desi chickens) and parasitic-infected desi chickens from backyard poultry farming collected from various locations in Lucknow, Uttar Pradesh, India. Desi fowls were examined for the presence of ectoparasites and endoparasites using standard methods. Further fat, protein, water, ash, and fatty acid content of muscle tissue of parasitic infected desi fowl and uninfected desi fowl were determined using standard procedures. A decrease in most of the nutritional parameters of the infected chicken were observed as compared to the non-infected control desi chicken.

Keywords: Ectoparasites, Endoparasites, Fatty acids, Biochemical properties, Nutritional parameters, *Gallus gallus domesticus*

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Introduction

The earliest domesticated bird was the chicken, *Gallus gallus domesticus*, which has spent more than 8000 years undergoing the combined forces of man-made and natural selection. (Li *et al.*, 2020). Due to its high nutritional value, high

protein content, low cholesterol, low calorie, and low fat content, chicken flesh is known for its many health benefits. Furthermore, compared to other meats like pig, beef, and lamb, chicken is less expensive (Sujiwo *et al.*, 2018). Because of above

reasons, and the present emphasis on physical fitness, the significance of food labels such as 'Light, Lean, Low-Fat, Reduced-Fat' has increased the demand of poultry meat (John *et al.*, 2016). Among the food animals, poultry is also the finest example of the highest feed conversion rates into high-energy food items (meat and eggs) for human consumption, making it viable to use these animals as a source of revenue.

In India, 498 million chickens are raised at an annual growth rate of 8–10%. Farm women, landless laborers, and marginal farmers are among the rural people that practice the traditional backyard poultry breeding method, which involves a flock size of 5–20 birds and little to no financial input. Backyard poultry producers generate close to 30% of the eggs produced in the nation (Singh *et al.*, 2009). About 75% of the meat and eggs produced in the poultry business are produced by the commercial, or organized sector, while 25% are produced by the unorganized sector.

The production of free-range eggs in Australia is growing quickly; in 2017, it grew by 10.2%, and its projected market value share in supermarkets was 52%. Over the past few decades, there has been a significant growth in the extensive and intensive housing of chickens because of the growing demand for chicken products for human consumption (Permin and Hansen, 1998; Ola-Fadunsin *et al.*, 2019). The domestic economies of the majority of countries benefit considerably and continuously more from poultry production than they do from any other live stock production (Dube *et al.*, 2010, Adang *et al.*, 2014, Ferdushy *et al.*, 2016). Certain intestinal helminth diseases however, can significantly lower the poultry output (Permin *et al.*, 1997; Ruff, 1999).

Free-range birds frequently come into contact with their excrement, which expedites the direct parasite life cycle in them (Wongrakiet *et al.*, 2014). A number of endoparasites such as nematodes, trematodes, cestodes, as well as; ectoparasites such as lice, ticks, and mites can infect chickens. Nematodes are the most

important intestinal worms in the chicken industry due to their effects on human health, the prevalence of pathogenic species, and their economic significance (Ruff, 1999; Macklin, 2013; Bachaya *et al.*, 2015). Infection by parasite causes body weight loss in the chickens that may result in decrease content of nutrients in meat muscles tissue consequently resulting in low nutrients in the diet of humans. Keeping the above in view, this study was designed to determine biochemical changes that may be associated with ectoparasitic and endoparasitic burdens in naturally infected indigenous chickens. This study is aimed at comparing the nutritional parameters of meat tissues of healthy control chickens and parasite-infected chickens reared in backyard rearing system.

Materials and Methods

Study Area:

The study area comprises the urban area of Lucknow (26°51'N 80°57'E) which is the capital of Uttar Pradesh in India. The study areas for collection of samples are shown in Figure 1. Mohanlalgunj, Talibagh, Lucknow cantt, Janakipuram, Indira nagar, Ghalia, Gomtinagar, Alambagh, Ahamamu, Ghushwal, Kalan, Aliganj, and Bara Imambara are the locations from where the birds samples were collected. The main vocations of the rural populations who reside in urban spaces, are farmland and subsidiary farming viz. fruit, fishing, livestock rearing especially poultry, production.

Ethical Consideration:

The owner, who owns local chicken coops, gave his verbal consent for conducting the studies and willingly sold the chickens for further lab procedures. The chicken's age and weight were also sought from the owner and recorded. The price of each chicken was predetermined.

Sampling:

630 dsesi chickens (*Gallus gallus domesticus*), reared in backyards, were randomly selected in different seasons in a two-year time period from

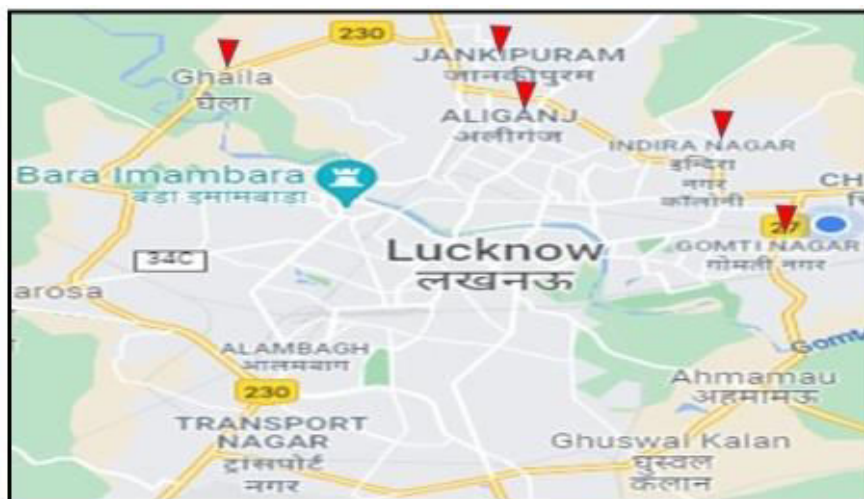


Fig. 1: Selected study areas of Lucknow for sample collection.

2021 to 2023, from different sites in Lucknow, Uttar Pradesh, India. The domestic chickens were purchased from the local people practicing backyard poultry rearing from the different selected study sites. The chickens that were chosen were not gender-restricted. During the study period, adults aged 32 weeks were generally collected. Age was recorded as determined by the owner.

Processing of desi chicken for the detection of ecto- and endoparasites:

Chickens were inspected for the presence of ecto and endoparasites as per standard protocols. On the basis of the number of parasites present in a single bird, these chickens were classified into four categories, as non-infected samples (control), low parasitic infected samples, medium parasitic infected samples and highly infected samples as described later.

Chemical analysis:

The muscle tissue samples were extirpated from the breast and thigh muscle tissue of slaughtered chicken and were ground in a meat grinder. Minced samples were covered with an aluminium sheet to prevent exposure to light and frozen at -20 °C in PE plastic bags. Samples were defrosted at 20 °C in a thermostatic bath before proceeding for further biochemical analysis. Following

methods were used to measure the nutritional content of both infected and non-infected (control) samples:

Moisture content:

The moisture content of meat samples was measured using the Association of Official Analytical Collaboration (AOAC) 950.46(B)-1950 technique. 2g of homogenized meat sample was uniformly dispersed in a tiny aluminum dish (about 50 mm in diameter and 40 mm in depth). The dish containing meat sample was then dried in an air oven for 2 to 4 h at 125 °C with the lids off. Then, dish was covered with the appropriate lids and allowed to cool in desiccators till its weight become constant. The moisture content was calculated using the following formula:

$$\text{Moisture} = \frac{\text{Wet Meat Wt.} - \text{Dried Meat Wt.}}{\text{Wet Meat Wt.}} \times 100$$

Fatty acid:

Fatty acid content was determined by converting fatty acid into fatty acid methyl esters, which were then separated and quantified by gas chromatography. 10 g of homogenized meat sample was mixed with 1 g of anhydrous Na₂SO₄, then dried in an oven at 45° to 55 °C (Zzaman *et al.*, 2017). The lipids were extracted using the Folch *et al.* (1957) technique. The extraction solvent used for extracting lipids was removed

using a rotary evaporator, and the resulting crude fat was then frozen (-180 °C) in a dark bottle with a cap for future analysis (Wagner *et al.*, 2008; Asgary *et al.*, 2009). FAME (Fatty Acid Methyl Ester) is created using boron trifluoride and methyl esters (adapted from AOAC Method 969.33). For this, 500 mg of the of the extracted fat sample was added to 100 ml of the boiling flask. The boiling chip and 8 ml of methanolic NaOH solution were added and then refluxed for 5–10 min to get rid of any remaining fat globules. A condenser was used to add 9 ml of the BF₃ solution, and boiling was continued for 2 min. A condenser was used to add 5 ml of hexane, which was then heated for another minute. 15 ml of saturated NaCl solution was added when the boiling flask was removed and the fluid was still warm. The flask was tightly covered and violently shaken for 15 sec. Hexane solution was floated into the neck of the flask by adding more saturated NaCl solution. The floated hexane solution was separated, and 1 ml of this was taken in a syringe. Then, injected 1 ml of hexane solution into the gas chromatograph after adding anhydrous Na₂SO₄ to eliminate the water (Nielsen, 2017b).

Ash content:

Ash content determines the inorganic matter remained after burning off organic matter. It is performed in two steps-- removal of water by charring and burning off organic matter by ashing in muffle furnace (Perez *et al.*, 1981; Ismail, 2017).

Around 5 g meat sample was weighed into the crucible. Before that, crucible and lid were initially placed in the furnace at 550°C for an entire night to make sure that any impurities on the surface of crucible and lids got burned off, and then cooled in desiccators for 30 min and weighed. Then, the sample was heated in crucible with half covered with lid, at low flame on Bunsen burner. When fumes production stopped, the crucible was placed in muffle furnace at 550 °C for overnight. After that crucible with ash was cooled down in desiccators and weighed.

Ash Content (%) = $\text{Wt of ash} \times 100 / \text{Wt of the sample}$.

Protein content:

The Bradford (1976) protein assay was used to determine the protein content, which is based on the concept that binding the dye molecule, Coomassie brilliant blue (CBB) G-250 to proteins results in a shift in dye absorption spectra that results in a visible color change.

Firstly, Bradford reagent was prepared by adding around 100 mg of CBB dye (G250) in 50 ml of ethanol (95%) and 100 ml of orthophosphoric acid (85%) in a beaker that was wrapped by aluminum foil to exclude light from entering and was diluted to the 1 liter solution. Dye solution was filtered through Whatman no.1 before it was used.

Secondly, BSA standard protein solutions were prepared by adding 1 mg per ml of stock BSA solution to 4 ml of distilled water. Then, a series of working solutions was prepared by adding the BSA standard solution prepared above by pipetting into 11 test tubes with increasing concentrations (0.00 to 0.1 µg), and distilled water was added to these test tubes to make a volume up to 5 ml. After that, 2.5 ml of Bradford reagent was added to each of the test tubes. Then these test tubes were kept in the dark for 10 min. An optical density(OD) reading was taken by the spectrophotometer at 595 nm against blank. A graph was plotted between the absorbance of standards and their concentration. The amount of protein in the unknown meat sample was found by using this curve.

0.02 g of homogenized meat sample was weighed in a tiny centrifuge tube, and distilled water was added to fill it to make one milliliter volume, then centrifuged at 12000 rpm at 4 °C. Supernatant was transferred to another centrifuge tube and used for quantification of protein using above mentioned a procedure The above mentioned process was repeated by adding reagents, measuring OD by a spectrophotometer, and calculating the protein on curve.

Fat Content:

Fat content was determined by Soxhlet method (AOAC 991.36). It is based on concept of extracting the fat from meat sample using organic solvent, then weight of extracted fat is determined.

The meat sample was dried for 5 h in an oven at 102 °C and then cooled in desiccators. The 5 g of dried meat sample was inserted in the thimble flask. Thimble was placed into a Soxhlet extractor. A clean, dry 150-ml round bottom flask was weighed and filled with around 90 ml petroleum ether. The extraction equipment was then inserted into the electrical heating mantle. The solvent was heated in the flask until it boiled (the heat source was adjusted such that the solvent drips from the condenser into the sample chamber at a rate of approximately 6 drops per second). The extraction process was continued for 6 h. After that, the heat source was disconnected from extraction unit and then the extractor was dismantled from condenser. The flask was placed in an oven at 60 to 80 °C to evaporate the solvent and content was dried until a consistent weight was achieved (around in 1 to 2 h). The flask was cooled in a desiccator before the content was weighed.

$$\% \text{ crude fat} = (W2 - W1) \times 100/S.$$

Weight of empty flask (g) = W1; Weight of flask with extracted fat (g) = W2; Weight of sample = S.

Data Analysis:

Data of chemical test performed on muscle tissues of *Gallus gallus domesticus* was collected for each category of parasitic infection and Microsoft excel and SPSS20 software was used for statistical analysis of data. Normality test data was done for all the categories. Then one way ANNOVA test was applied.

Results and Discussion

The production of chickens is severely hampered by parasites, which causes significant economic losses to the farmers and nutrition losses. To find the impact of parasites on nutrition or biochemical components of meat muscle tissue of *Gallus gallus domesticus*, 630 desi chickens (*Gallus gallus*

domesticus) that were reared in backyards, were randomly selected in different seasons in a two-year time period from 2021 to 2023, from different sites in Lucknow, UP. These chickens were inspected for ecto- and endoparasites as per standard protocols. On the basis of the number of parasites present in a single bird, chickens were classified into four categories as shown in Table 1. Non-infected samples (control), low infected samples were those which have number of parasites less than 33, medium-infected samples were those where the number of parasites present varies from 33 to 66, and highly infected samples were those where the level of infection was above 66.

It was found that 91 desi chickens were not infected by any ecto- and endo -parasites and were considered as control samples. While 310 chickens had a low infection, 184 chickens had a medium level of parasitic infection, and 45 chickens were highly infected (Table 2).

After inspection for ectoparasites, chickens were slaughtered at a butcher shop under standard protocol, the bodies of the host birds were dissected, and various organs, including the alimentary canal, were checked for endoparasites. Meat samples that were dissected from slaughtered chickens were ground. Minced samples were covered with an aluminum sheet to prevent exposure to light and frozen at -20 °C in PE plastic bags. Samples were defrosted at 20 °C in a thermostatic bath before proceeding for further biochemical analysis. The sample size for each category was kept at 31 (n = 31). Different biochemical parameters, viz., fat, protein, ash, moisture, fiber, and fatty acids, were performed on thawed meat samples using the standard methods. Chemical tests were performed on the muscle tissue of 31 samples from each category of level of parasitic infection in desi chickens, viz., non-infected, low-infected, medium-infected, and highly infected samples.

It was found that fat content decreases as the severity of infection increases, as shown in Table 3 and Figure 2. The control samples exhibited the

Table 1: Level of infections in chicken on the basis of number of parasites present in single bird

S. No.	Level of infection	Number of parasites present in one bird
1.	Non infected(control)	0
2.	Low	<33
3.	Medium	34 to 66
4.	High	>66

Table 2: Number of birds infected with different level of parasitic infection

S. No.	Category	Number of birds infected	Percentage of birds infected
1	Not infected	91	14.4
2	Low infection	310	49.2
3	Medium Infection	184	29.2
4	High infection	45	7.1

Table 3: Fat % of meat muscle tissue of *Gallus gallus domesticus* infected with different level of infection (n=31)

Parameter (%age)	Control	Low parasitic infected samples	Medium parasitic infected samples	High parasitic infected samples
Fat	7.10	5.79	3.25	2.01

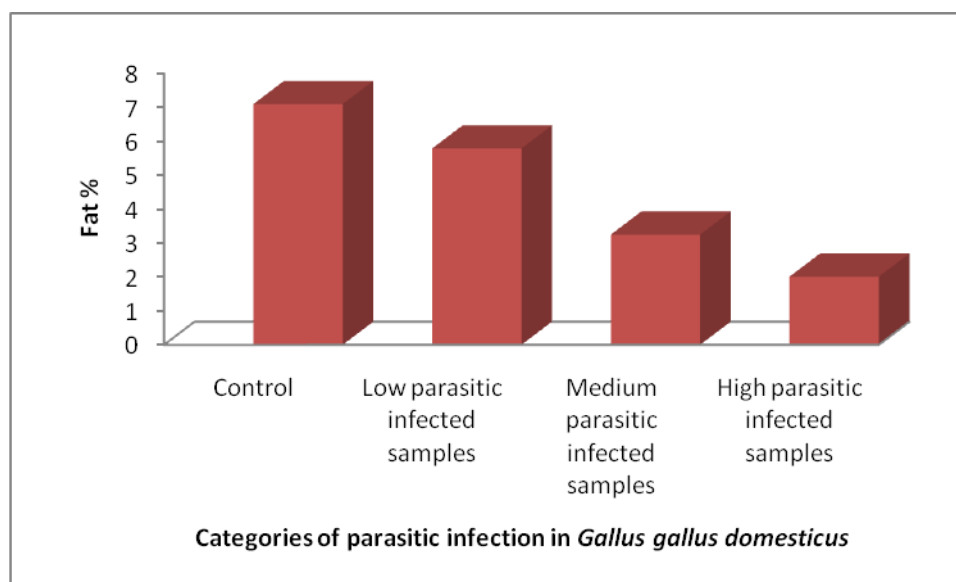


Fig. 2: Fat % of meat muscle tissue of *Gallus gallus domesticus* infected with different level of infection.

Table 4: Protein % of meat muscle tissue of *Gallus gallus domesticus* infected with different level of infection (n=31)

Parameter (%age)	Control	Low parasitic infected samples	Medium parasitic infected samples	High parasitic infected samples
Protein	21.76	16.05	15.14	12.96

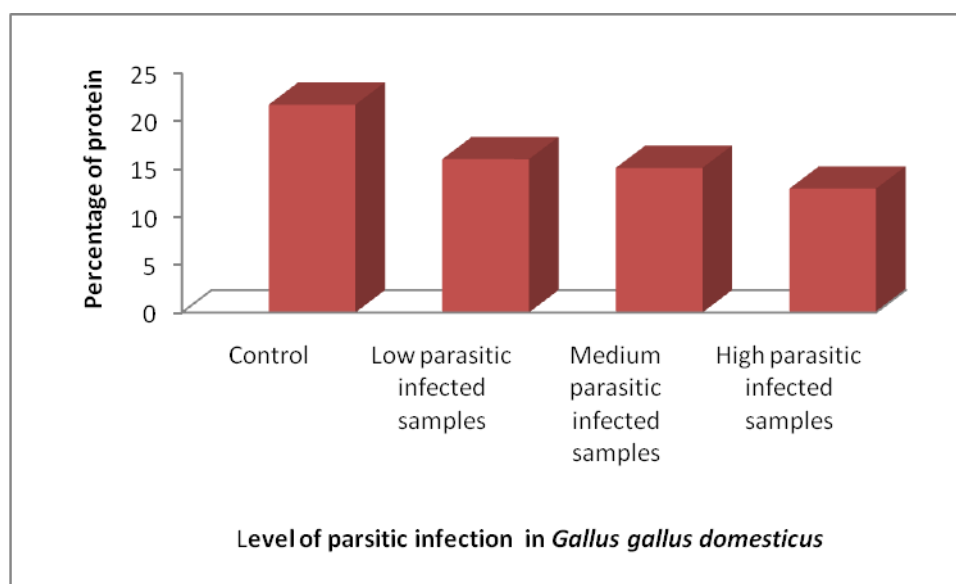


Fig. 3: Protein % of meat muscle tissue of *Gallus gallus domesticus* infected with different level of infection (n=31).

highest fat percentage (7.10%), low parasitic infected samples have 5.79%, the medium-infected samples have 3.25%, and the highly infected samples showed the lowest fat content as 2.01%. The content of fat is significantly decreased with the increase in parasitic infection ($P < 0.05$). Similar results were also observed in previous studies as described hereafter. It is observed that lipid content was lower in hens with a high level of infection as compared to the non-infected birds (Sharma *et al.*, 2018). A similar result was earlier reported by Trbhuvan *et al.* (2022) that highly infected hens have a lower liver lipid content of 2.72 % as compared to uninfected hens that have 4.46% ($p < 0.01$). This decline in fat content may be attributed to metabolic changes and alterations in nutrient absorption associated with parasitic infections (Houdijk *et al.*, 2012).

Similarly, protein content decreases with increase of infection severity as shown in Table 4 and Figure 3. The control samples exhibit the highest protein percentage (21.76%), while low infected samples have 16.05% protein content, the medium-infected samples have 15.14%, and the highly infected samples show the lowest protein content (12.96%). It was found that there is a significant decrease in protein content with the increase in level of infection ($p < 0.05$). Similar results were also found by Aade (2022) who reported that protein content of intestine muscle tissue of non-infected *Gallus gallus domesticus* was 15.55 mg/g, while that of parasitic-infected intestinal tissue of *Gallus gallus domesticus* was 10.33%. Similar results were also reported by Skallerup *et al.* (2005) who reported that parasitic-infected desi chicken had 19.34%

Table 5: Ash % of meat muscle tissue of *Gallus gallus domesticus* infected with different level of infection (n=31)

Parameter (%age)	Control	Low parasitic infected samples	Medium parasitic infected samples	High parasitic infected samples
Ash	1.40	1.75	1.81	1.91

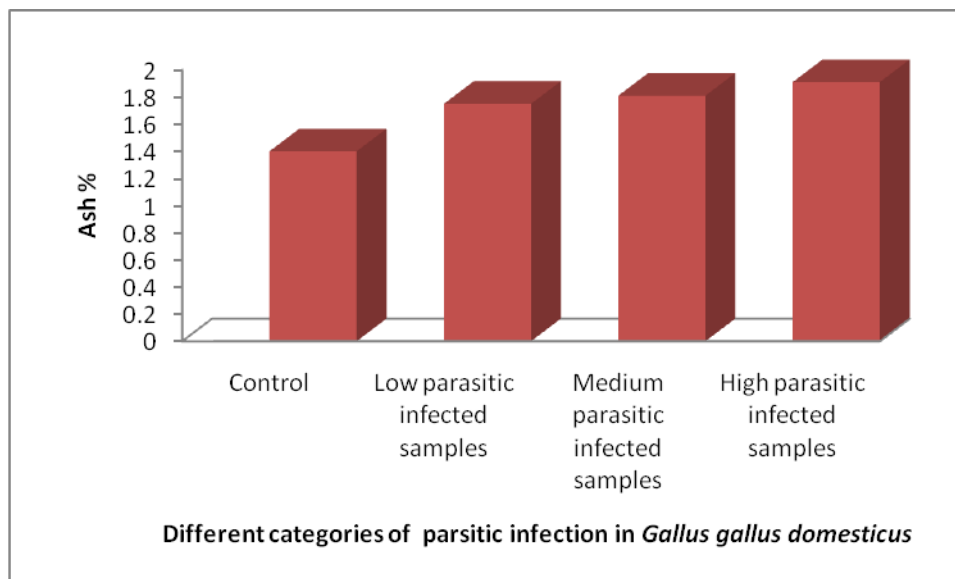


Fig. 4: Ash % of meat muscle tissue of *Gallus gallus domesticus* infected with different level of infection (n=31).

Table 6: Moisture % of meat muscle tissue of *Gallus gallus domesticus* infected with different level of infection (n=31)

Parameter (%age)	Control	Low parasitic infected samples	Medium parasitic infected samples	High parasitic infected samples
Moisture	70.70	72.73	74.1	77.49

protein content, while non-infected chickens had 21.55 mg/g protein content. It was reported in earlier studies that parasitic infections can lead to decreased protein synthesis, increased protein breakdown, and impaired nutrient utilization, contributing to reduced protein levels in infected tissues (McDowell *et al.*, 2019). It has also been reported that *Ascaridia galli* infection reduces

dietary absorption of protein in chickens which might be due to reduced digestibility by presence of parasites (Walker *et al.*, 1976).

The ash content also shows fluctuations across different parasitic infection levels of *Gallus gallus domesticus*, as shown in Table 5 and Figure 4, with the highest percentage of ash observed in the highly infected samples (1.91%); and medium-

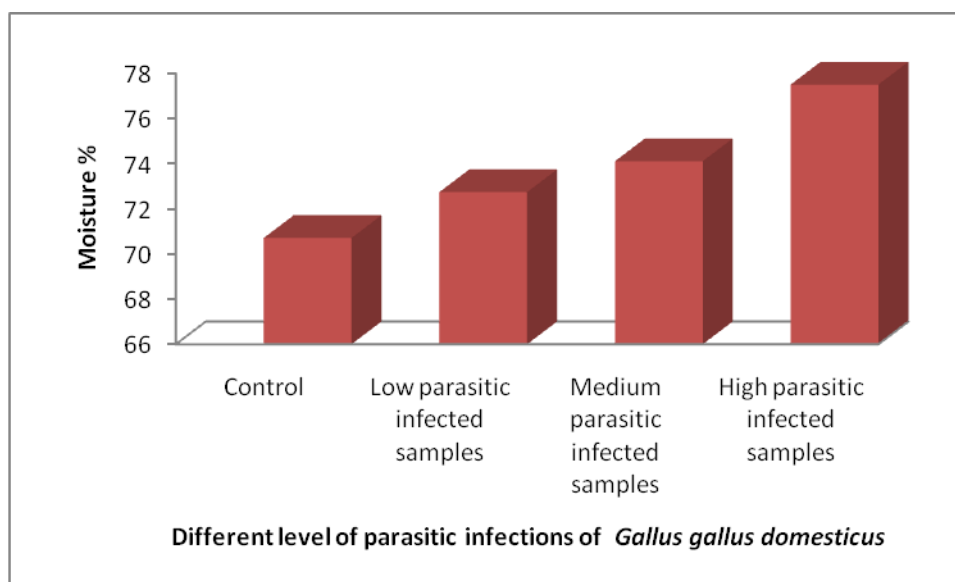


Fig. 5: Moisture % of meat muscle tissue of *Gallus gallus domesticus* infected with different level of infection (n=31).

Table 7: Fatty acid composition in the muscle tissue of control and infected chickens, revealing notable alterations in fatty acid profiles across different infection levels

Common name	Fatty acids %	Control	Low infection	Medium infection	High infection
Myristic acid	C14:0	5.26	3.40	1.66	0
Stearic Acid	C18:0	45.48	42.61	40.87	32.00
Oleic acid	C18:1	14.51	12.89	9.91	5.99
Linoleic acid	C18:2	42.47	39.99	37	22.49
Linolenic acid	C18:3	23.91	22.00	0	0
Arachidic acid	C20:0	13.93	12.04	0	0
Eicoenoic acid	C20:1	0.92	0.87	0	0
Docosanoic acid	C22:0	1.10	0.94	0	0
Lignocerc acid	C24:0	3.84	3.02	0	0

infected has 1.81%, low infected samples have 1.40% and lowest (1.40%) in non-infected meat muscle samples. Ash content significantly increases with the level of infection in chickens ($p < 0.05$). It was observed that the ash content represents the mineral content of the meat and may vary due to changes in tissue integrity and mineral metabolism associated with infection (Nasir *et al.*, 2018).

The moisture content of meat muscle tissue varied with the level of parasitic infection in birds. It was observed in the present study that moisture content increased significantly with the level of infection, as shown in Table 6 and Figure 5.

Similar results were also observed in previous studies, where moisture content increases with the infection of parasites on the host, as observed by Yampolskiy (1981). Similar result were also reported by Valieva *et al.* (2014) who reported substantial increase in moisture and ash content in meat muscle tissue of host with parasitic infections.

In this study the alterations in nutrient composition of infected meat samples were tested for the potential impact of parasitic infections on the nutritional quality of poultry meat. Effective parasite control measures and proper management practices are crucial to minimize such

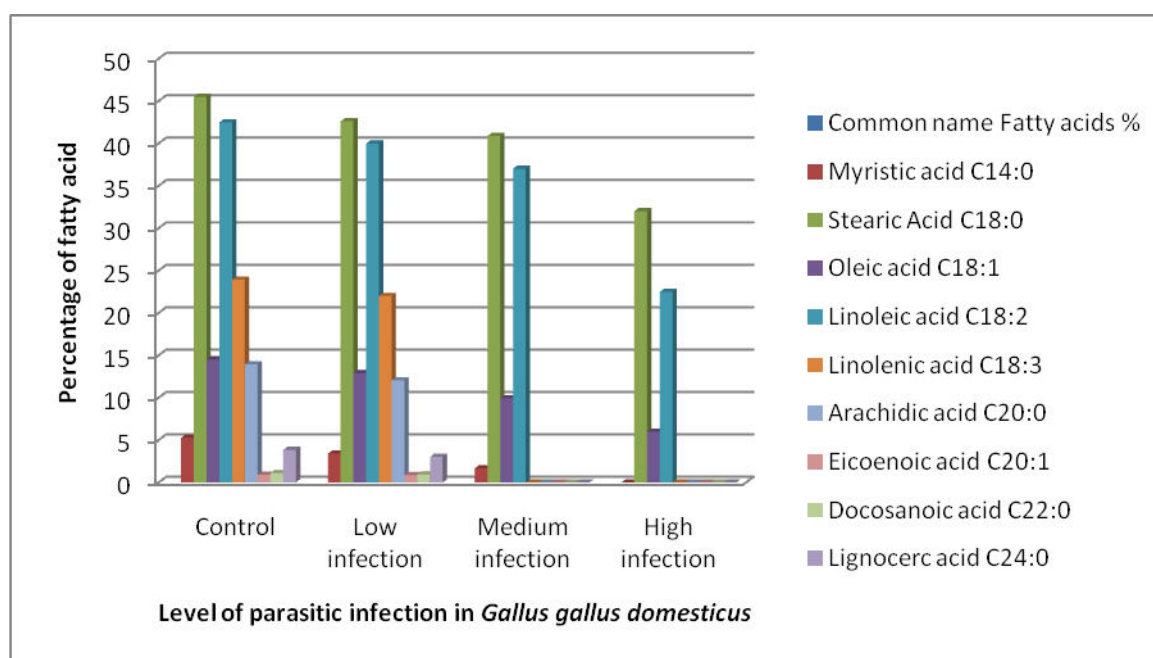


Fig. 6: Fatty acid composition in the muscle tissue of control and infected chickens, revealing notable alterations in fatty acid profiles across different infection levels.

nutritional losses and to ensure the production of safe and nutritious poultry products.

In the present studies, it was observed that the content of different saturated and unsaturated fatty acids decreases with the increase in the level of infection by parasites in *Gallus gallus domesticus* (Table 7; Fig. 6).

Myristic acid (C14:0) content demonstrated a significant decrease with increasing infection severity. The control samples exhibited the highest myristic acid percentage (5.26%), whereas the highly infected samples showed the absence of myristic acid content (0%). The myristic acid levels could be attributed to metabolic changes and alterations in fatty acid metabolism associated with parasitic infections (Caboni *et al.*, 2018).

Stearic acid (C18:0) content also exhibited a gradual decrease with increasing infection severity. The control samples had the highest stearic acid concentration (45.48%), while the highly infected samples showed the lowest stearic acid content (32.0%). Parasitic infections might lead to alterations in lipid metabolism, resulting in

reduced stearic acid levels in infected tissues (Pantzaris *et al.*, 2019).

Linoleic acid (C18:2) content also showed a gradual decline with increasing infection severity. The control samples exhibited the highest linoleic acid percentage (42.47%), while the highly infected samples showed the lowest linoleic acid content (22.49%). Parasitic infections might interfered with the metabolism of essential fatty acids, contributing to reduced linoleic acid levels in infected tissue, as reported by Caboni *et al.* (2018).

Linolenic acid (C18:3), arachidic acid (C20:0), eicoenoic acid (C20:1), docosanoic acid (C22:0), and lignoceric acid (C24:0) were not detected in the highly infected samples, indicating a complete absence of these fatty acids in severely infected muscle tissues. This absence may reflect significant disruptions in fatty acid metabolism and synthesis pathways.

Similar results were also reported in earlier studies, where fatty acid content decreased with the infection of parasites in chickens as reported

by Chao *et al.*, (2020) that myristoleic acid, eicoenoic acid, and arachidonic fatty acid content were decreased in infected bird as compared to the non-infected bird by parasitic infection in *Gallus gallus domesticus*. It was observed in earlier studies that cestodes lost their capacity for *de novo* synthesis of lipids and became entirely dependent on the host. It is also reported that cestodes are able to absorb both short- and long-chain fatty acids through a mixture of diffusion and mediated transport (Mondal *et al.*, 2016). A similar result was also observed by Valieva (2014) that decrease in monounsaturated and polyunsaturated fatty acids in the muscle tissue of the host animal. In the same way, it has also been experimentally observed by Yampolskiy (1981) that helminthiasis cause a reduction in the content of general protein in liver, muscle, blood, and other tissues, causing damage to carbohydrate and fatty acid exchanges.

Overall, the findings of this study suggest that parasitic infections have a profound impact on the fatty acid composition of poultry meat. Proper management practices and effective parasite control measures are essential to minimize such alterations and ensure the production of safe and nutritious poultry products. The alterations in nutritional parameters and fatty acid composition observed in infected meat samples highlight the potential impact of parasitic infections on the nutritional quality of poultry meat. Increased moisture content and decreased fat, protein, and specific fatty acid levels in infected samples suggest tissue damage and metabolic alterations associated with parasitic infections. These findings emphasize the importance of effective parasite control strategies in poultry management to ensure the production of safe and nutritious poultry products (Yampolskiy *et al.*, 1981; Houdijk *et al.*, 2012; Mondal *et al.*, 2016; Caboni *et al.*, 2018; McDowell *et al.*, 2019).

Conclusion

The findings of this study showed that there was a significant impact of parasites on meat quality of desi chickens (*Gallus gallus domesticus*) in

Lucknow. In conclusion, this study underscores the necessity of implementing comprehensive parasite control measures, including regular monitoring, proper sanitation practices, and the judicious use of acaricides and dewormers, to mitigate the detrimental effects of parasites on chicken health, productivity, and meat quality. Further research is warranted to explore additional biochemical indicators influenced by parasite infections and to develop integrated control strategies aimed at enhancing the welfare and productivity of poultry. Present study revealed that native chickens sold in various Lucknow markets, despite appearing healthy, harbor a range of ecto- and endoparasites, many of which are subclinically ill. Parasite infections have the potential to impact biochemical indicators that could serve as diagnostic tools in naturally infected hens. Implementing integrated control strategies is imperative to enhance the welfare and productivity of native chicken breeds. To prevent the spread of infection and disease to existing flocks on farms, it is crucial to ensure that chickens purchased for rearing or restocking at markets undergo proper treatment and isolation protocols. Further research is warranted to deepen our understanding of how parasites influence biochemical variables.

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