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# ALTERATIONS IN NUTRITIONAL VALUE OF THE DOMESTIC PIGEON (*COLUMBA LIVIA DOMESTICA*) MUSCLES BY HELMINTHS INFECTION

By

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**Abstract**

Worldwide, poultry, and its byproducts are frequently among the most important sources of protein for people. This study evaluated the biochemical alterations in infected pigeons' muscles caused by cestode, *Raillietina* sp. and nematode, *Ascaridia columbae* infection. Between January and December 2021, 354 pigeons (*Columba livia domestica*) were randomly purchased from four Buraydah, Unaizah, Ar-Rass, and Al-Bukairiyah Cities (Al-Qassim Province).

The biochemical analysis results showed a marked drop in lipid and protein levels, but a significant rise in glucose levels infected pigeons' muscles compared to healthy ones.

**Key words:** Saudi Arabia, Pigeon, Helminths, Lipids, Carbohydrates, Protein.

## Introduction

Generally speaking, domestic pigeons live among people for food, amusement, and research studies (Mansur *et al,* 2019). They cohabit with other birds, domestic animals and humans exposed to different pathogens (parasites, bacteria and others) and thus are reservoirs for zoonosis (Attia *et al,* 2021). They frequently find resting spots in building tops and windows of homes (Marques *et al,* 2007). Both helminthes and protozoa are one of the major impediments in rearing of pet birds, and must be treated (Zloch *et al*, 2021). Helminthes commonly infect multiple hosts in succession as the birds die before oviposition (Daniel *et al,* 2021). Parasitosis cause marked biochemical changes among birds and regular anti-helminthic use is indicated (Radfar *et al,* 2012).

The current study aimed to evaluate the biochemical alterations by helminthic infection on pigeons' muscles.

## Materials and Methods

Study area: Al Qassim Province is situated about 400 kilometers (250 miles) northwest of Saudi Arabia's Capital, Riyadh, between 25°48′22.68°N and 42°52′23.52°E. The district is typical desert weather in winter, cold & wet, and in summer, scorching & less humidity.

Collections: Between January and December of 2021, 354 pigeons (*Columba livia domestica*) were purchased weekly from markets in cities of Buraydah (Capital), Unaizah, Ar-Rass, and Al-Buk-airiyah (Al-Qassim Province). They were transported alive to lab, macroscopically examined for ecto-parasites. They were divided into: G1:150-350 gm, young less than one year and G2:351-550gm, adult more than one year.

Necropsy of pigeons: Following the ethical approved declared by Helsinki (2013) for dealing with experimental animals, pigeons were dissected out. Body cavity was carefully opened by a longitudinal incision, and internal organ contents were removed individually into a Petri-dish with normal saline solution at 37°C. The recovered helminthes were separated into cestodes and nematodes.

Estimation of lipids: Small muscle portions were removed using Folch, Lees, and Stanley's procedure (1957). 250 mg of pigeon tissue were extracted using a 3:1 ethanol-ether mixture, placed in 15 ml test tubes, and left in a 65°C water bath for two hours. The mixture was chilled and centrifuged after 30 minutes. Then 6ml. of 3:1:1 ethanol-ether mixture was added, left to residue, and heated to 65°C for two hours. After centrifuging the residue, the filtrate that had al-

ready been decanted was filled with the supernatant, which was then transferred to a new tube. The left residue was mixed with 6ml of a 1:1 chloroform-methanol mixture and heated to 65°C for an hour. After centrifuging the residue, the filtrate was filled with the supernatant was transferred to a new tube. A mixture of methanol and chloroform was used, and total volume was fixed at 25ml as a rough estimation of the overall lipid content.

Sulfo-phospho-vanillin reaction (SPV): Technique (Chabrol and Charonnat, 1973) explai-ned the colorimetric detection of total lipids in sera: 180 µL of concentrated sulfuric acid was used to dilute 20 µL of samples, both with and without oleic acid, and the test tubes were then incubated at 100ºC for ten minutes. The tubes were allowed to cool to ambient temperature. Each tube received 0.5 mL of PV reagent for color development, and the mixture was incubated at 37ºC for 15 minutes. Within a dimly lit enclosure, after being moved to 96-well polystyrene microplates, samples were kept for 45 minutes. Finally, using a multilabel plate reader (Perkin Elmer, USA) to measure the absorbance at 530nm, data were expressed as absorbance. A calibration curve was created using pure oleic acid and replicate estimations.

Carbohydrates estimation (Kemp and Van Heijningen, 1954): Using an automated homogenizer, 200mg of tissues were homogenized in 5% Trichloroacetic acid, put for 15 minutes in a boiling water bath in order to remove all hexoses and protein, the residue was washed with 5% Trichloroacetic acid, and filtered through Whatman filter paper, and adding centrifugated supernatant. One ml of this solution was added to 3ml of concentrated sulfuric acid, vigorously shakes for 6 minutes in a boiling water bath ($100^{O}C$) and then cooling, a multilabel plate reader measured the absorbance at 560nm (Perkin Elmer, USA).
A calibration curve was done using pure glucose and replicate estimations.

Proteins estimation (Bradford, 1976): A weighed tissue was digested in sodium hydroxide and precipitated with ethanol. After dissolved in 5% Trichloroacetic acid heated at 100°C for 15 minutes and precipitate was centrifuged to extract remaining material. Using bovine serum albumin as a standard, supernatant protein was calculated and absorbance was measured at 625nm using a multi-label plate reader (Perkin Elmer, USA). A calibration curve was done using pure bovine albumin and replicate estimations.

## Results

Pigeons were infected with *Raillietina* sp. (cestode) & *Ascaridia columbae* (nematode)

Total lipids mean concentration of five muscle samples isolated from cestode infected pigeons was (221.24mg/dl), and from nematode infected ones was (121.32mg/dl) and less than control (442.80mg/dl). Both parasites caused significant decrease in lipid muscles' level (P=0.04 & 0.002), respectively.

Total carbohydrates mean concentration of muscles isolated from cestode infected pigeons was (0.943mg/ml), and from nematode infected ones was (0.428 mg/ml), and higher compared to control (0.340mg/ml). Cestodes cause a significant carbohydrates elevation in muscles' level (P=0.025*), but nematodes caused insignificant elevation (P=0.58).

Total proteins mean concentration of muscles isolated from cestodes infected pigeons was (0.186mg/ml), and from infected nematode ones was (0.084mg/ml), and less than control (0.240mg/ml). Cestodes caused insignificant protein decrease in muscles' level (P=0.250), but nematodes showed significant decrease protein (P= 0.0004*).

Details were given in tables (1, 2 & 3) and figures (1, 2, 3, 4, 5 & 6).

Table 1: Mean total concentration of lipids in muscles of infected pigeons compared control

| Helminth's type | Mean total of lipid | Standard deviation | Standard Error | Control | P-value |
|---|---|---|---|---|---|
| Single cestode | 221.24 | 170.6 | 102.66 | 442.80 | 0.04* |
| Single nematode | 121.32 | 76.3 | 45.9 | 442.80 | 0.002* |

Table 2: Mean total concentration of carbohydrates in infected pigeons muscle compared to uninfected pigeon

| Helminth's type | Mean total of carbohydrates | Standard deviation | Standard error | Control | P-value |
|---|---|---|---|---|---|
| Single cestode | 0.934 | 0.377 | 0.168 | 0.340 | 0.025* |
| Single nematode | 0.428 | 0.328 | 0147 | 0.340 | 0.58 |

Table 3: Mean total concentration of protein in infected pigeons muscle compared to uninfected pigeon

| Helminth's type | Mean total of protein | Standard deviation | Standard error | Control | P-value |
|---|---|---|---|---|---|
| Single cestode | 0.186 | 0.0898 | 0.032 | 0.240 | 0.250 |
| Single nematode | 0.084 | 0.040 | 0.014 | 0.240 | 0.0004* |

*Data averages of three experiments, P =significant when P < 0.05.

## Discussion

Generally speaking, pathogens whatever they are effect on the vital activities of all hosts including birds (Anikieva *et al,* 1988).

In the present study, helminthic infection significantly decreased the biochjemical levels among infected pigeons that was claried with nematodes that affected muscle protein and lipid more than cestodes. This agreed with El-Sadawy *et al.* (2009), they reported that nematodes are incapable of building basic amino acids but with limited capacity to build proteins. Also, Shutler *et al.* (2012) found that nematode parasites had multiple negative associations with fat reserves in geese birds. As a result, their primary source of sustenance comes from the host. affects the host. These decreases in lipid and protein contents may be due to the parasites' need and/or due be caused by the host's response immunity to infection, which reduces the nutrients' ability to be absorbed effectively (Al-Hadethi *et al,* 1990; Shani, 2000).

The present levels of protein and lipid agreed with Sonune (2012) who estimated the biochemical content of cestode (*Stilesia* species) on *Ovis bharal* intestines and normal ones reported that parasite's lipid level (12.64mg/gm) was higher than that of infected host's intestinal tissue (11.30mg/gm) and normal intestinal tissue (12.05mg/gm). Also, Pallewad, *et al.* (2015), who calculated the biochemical contents of intestinal tissues of (*Capra hircus L*) infected with trematodes, with the gut tissue had lower quantities of fat and protein. This agreed with Mondal *et al.* (2016), who found that lipid synthesis plays a significant role in helminth parasites. Yuskiv and Yuskiv (2020) found that cestode *B. acheilognathi* in carp intestine damaged feeding processes and altered lipid metabolism.

Moreover, Coles (1967) and Twij (2012) reported that the quick loss of protein associated with diarrhea may be the cause of the drop in protein levels, which might be attributed to a state of blood protein insufficiency brought on by an increase in intestinal velocity. Deka and Borah (2008) reported decrease in total serum protein and albumin in *Ascaridia galli* infected quails and hens. Nabavi *et al.* (2013) reported that the overall protein decrease in all blood samples from nematode *(Hadjelia truncate*) pigeons, was an indicator of clinical and hematological changes mal-absorption and bad digestion by infection. Anah and Anah (2015) found a significant drop in the percentage of protein content in the blood of quail birds (*C. coturnix*) infected with nematodes (*A. galli*). Besides, Abd Alhadi and Al-Awady (2020) observed a drop in the protein concentration in the blood samples of nematode infected pigeons. The reduction of digestive secretions and tissue protein leakage in the bowel were due to this decline.

The present results of protein content agreed with Hassan *et al.* (2015), who found a marked reduction in muscle and liver protein of helminthic infected Koshar fish. Nabi *et al.* (2017) found that fish infected with *Pomphorhynchus kashmirensis* parasite had less protein. Again, Nabi *et al.* (2021) reported a significant decrease in protein content in fish's muscle, intestine, and liver due to infection that minimized intestinal food absorption. But, this disagreed with Abd Alhadi and Al-Awady (2020), who reported that nematode-infected birds (*Fulica atra*) had higher blood protein levels than control. The migra-

tory birds' unique physiological makeup explained the protein levels increase; they stored large amounts in their nutrient stores for emergency while migration over long distances (Lindstrom and Piersma, 1993).

In the present study, carbohydrates caused a considerable rise in muscle with cestode but a less increase with nematodes. This agreed with Sukhdeo and Mettrick (1984), who found that nematode, affected the glucose amount in rat's colon with marked reduced in its absorption. Glucose consumption increase in infected host's muscle activities reserve lipids' conversion to glycogen by gluconeogenesis in muscles **(**Hantoush *et al,* 2001). Also, Tyutin and Izvekova (2013) reported that the accumulation of glycogen in infected host skeletal muscles was due to its elevated insulin hormone activity. This agreed with Mondal *et al.* (2016), who reported increased activity and intensity of cestode parasites in pigeons' intestines. Besides, Abd-Alhadi and Al-Awady (2020) found an increase in glucose concentration in blood samples of helminth-infected domestic pigeons, which gave birds' ability to store large amounts of blood glucose to combat infection by changing physiological aspects.

Previously, Reddy and Benarjee (2011) reported that helminthiasis caused increased levels of glycogen as carbs in infected fish muscles opposed breaking down process and absorbing food, leading to metabolism disruptions. Nisar *et al.* (2012) found that the cestode in infected pigeons affects glucose levels in the pancreas, liver, and spleen fell, they increased in the gut and stomach. The cestodes feed on glucose and extra glucose deposited in its body as glycogen and thus the most affected organ is the crop. Pallewad *et al.* (2015) reported that the glycogen levels in healthy and trematoda infected domestic goat, *Capra hircus* L, was significantly less in infected ones due parasites' requirements and inability to have energy from food.

## Conclusion

Helminthes have an impact on nutritional value and overall health, increasing deaths. and consequently economic loss.

There was decrease in lipid and protein levels, but marked increase in carbohydrates.

*Authors' contributions*: Material preparation, data collection and analysis were performed by Al Damigh and Alahmadi, The manuscript draft was prepared by Al Damigh and Hassan. All authors wrote, revised and approved the manuscript for publication.


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## Explanation of figures

Fig. 1: A- Saudi Arabia map showing Al-Qassim region highlighted in yellow color, B-Map of locations in Al-Qassim region.
Fig. 2: Standard curve of oleic acid.
Fig. 3: Standard curve of glucose.
Fig. 4: Standard curve of albumin**.**
Fig. 5: Mean total concentration of lipids in infected pigeons muscles (cestodes & nematodes) compared to uninfected pigeon.
Fig. 6: Mean total concentration of carbohydrates in infected pigeons muscles (cestodes & nematodes) compared to uninfected pigeon.
Fig. 7: Mean total concentration of protein in infected pigeons muscles (cestodes and nematodes) compared to uninfected pigeon.