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Effect of Selenium-enriched yeast supplement on the gross morphology and morphometry of the small intestine during post-hatch growth of chicken

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Abstract
The present investigation was conducted to explore the effect of Selenium-enriched yeast supplement on the gross morphology and morphometry of the small intestine during post-hatch growth of chicken. Total eighty numbers of day-old broiler chicks were procured from the authorized breeder and were grouped into control and treatment groups. Each group had four replicates and each replica had ten birds. Selenium-Yeast was mixed with the standard diet of the treatment group at the dose rate of 0.15mg/kg feed. The samples were collected on the 14^{th} and 28^{th} day of the experiment. Results showed that the average body weight gain was higher in the dietary Se-Yeast supplemented group than the control group on both the days of experiments. The average length of the small intestine was increased significantly ($p<0.05$) with the age of both groups. However, when compared between groups, there were significant ($p<0.05$) changes on the 14^{th} day but no difference on the 28^{th} day. The average length of the duodenum and jejunum increased significantly ($p<0.05$) with age. When compared between groups, in case of the duodenal length, no significant ($p<0.05$) difference was found; however, the jejunum showed the opposite result on both the days of the experiment. The ileal average length was more on day 14^{th}. There was no significant ($p<0.05$) difference in length of the ileum between the days and between the groups. The average diameter of jejunal and ileal Peyer's patches had increased with increasing age but no significant differences ($p<0.05$) were noticed between the days and also the groups. In comparison, the diameter of Peyer's patches of the jejunum was higher than the ileum on all days of studies. In conclusion, the Se-yeast had an effect on the small intestine as it increased the length resulting in weight gain and better FCR. The diameter of Peyer's patches of both jejunum and ileum was increased as compared to the control group of birds which ultimately enhanced the better health development and production.

Keywords: Chicken, small intestine, Se-Yeast, gross morphology and morphometry

Introduction
Livestock plays an important role in the economy of developing countries. Domestic fowl or broiler chickens are most widely utilized among all poultry birds worldwide for consumption as well as research purpose. Most commercial broilers grow very fast to gain the body weight quickly and reach to the desired slaughter weight with the help of their low feed conversion ratio, and less physical activity (Eriksson *et al.*, 2008) [6].

The digestive system is one of the most important systems of the body and it is different in different species. It starts at the beak, goes backward in the abdominal cavity, and ends with the cloaca or vent. Relative to body length, the GI tract is much shorter than that of mammalian animals, causing the digesta passes through the entire GI tract faster in poultry than in mammalians (Hughes, 2008) [11].

Selenium-enriched yeast (Se-yeast) is a common organic form of Se which is used as a dietary supplement to intake of this important trace mineral. When yeast is grown in a Se enriched medium it absorbs the Se and converts it into selenomethionine, the organic form. Selenium (Se) is a micro mineral which is an essential element for poultry production and low or absence of Se in the diet leads to poor growth performance, nutritional muscular dystrophy, exudative diathesis, immune deficiencies, and decreased concentrations of the active form of thyroid hormone, reduced antioxidant status in plasma and various organs, and lipid peroxidation (Safdari-Rostamabad *et al*., 2017) [18].

Keeping in view the day to day increasing demand of chickens worldwide and the scanty of literature on the gross changes of the digestive tract with the feed additives, the present study has been undertaken to elucidate the gross morphology and morphometry of the small intestine during post-hatch growth of chicken.

2. Materials and Methods

The present study was carried out in the Department of Veterinary Anatomy and Histology, West Bengal University of Animal and Fishery Sciences, Kolkata, India during 2021-22. The proposed experiment was duly approved by the Institutional animal ethics committee, Faculty of Veterinary and Animal Sciences, WBUAFS, Kolkata. Ref. No. IAEC/61(V).

Experimental design

Eighty numbers of day-old broiler chicks were procured from the authorized breeder and were grouped into control and treatment groups. Each group has four replicates (R-1, R-2, R-3, and R-4). Each replica had ten birds. The starter feed followed by finisher feed and *ad libitum* water supply was provided to both the groups of broiler birds throughout the experimental period. Selenium-Yeast was mixed with the standard diet of the treatment group and the dose rate was 0.15mg/kg feed.

Collection of specimens and preservation

For the present study, on the 14th post-hatch day, 4 numbers of birds (one bird from each replica) from each group were euthanized by injecting an overdose of Sodium pentobarbital IP at the dose rate of 120 mg/kg (Gourdan, 2016) [9], and samples were harvested carefully immediately after being sacrificed. Then on the 28th day, again 4 birds from each group were sacrificed followed the same procedure, and samples were collected.

Gross morphometry

The live body weight was measured in the morning on the day of sacrifice by the digital weighing machine. The feed conversion ratio (FCR) was calculated on the 14th and 28th days. Grossly the length of the small intestine was measured after separation from the body and carefully uncoiled. Measurements were made with the help of a measuring scale after harvesting. The diameter of Peyer's patches was measured by the digital slide calipers. All the recorded data were analyzed statistically as per the standard method given by Snedecor and Cochran (1994) [21], with the help of SPSS 17.0 software.

3. Results and Discussion

The live body weight of individual poultry birds from the control group as well as from the treatment group was measured on the 14th and 28th day and the average body weight (Mean±SE) was 458.092±11.604gm and 1284.804±18.267gm in the control groups and 466.758±5.220gm and 1312.648±5.452gm in the treatment groups on day 14th and 28th respectively (Table 1). It was shown that the average body weight was higher in the dietary Se-Yeast supplemented (0.15mg/kg feed) group than the control group on both days of experiments. This was in accordance with the results of Upton *et al.* (2008) [23] where they offered 0.2 ppm Se-yeast in the experimental diet. However, El-Sheikh *et al.* (2010) [5] observed a similar result after supplementation of organic selenium at 0.2 and 0.3 mg/kg diet which significantly (P≤0.05) increased the live body weight of Bandarah chicks during the experimental period. El-Sebai (2000) [4] also noticed that the inorganic selenium supplementation at 0.5ppm level to the basal diet increased body weight significantly as compared with the control group of broiler chickens. However, Wang and Xu (2008) [25], Swain *et al*. (2000) [22], and Niu *et al*. (2009) [14] noticed that dietary Se levels did not influence body weight. The average body weight gain was more in the Se-Yeast supplemented groups as compared to control groups on both the days of sacrifice and the data presented in table 1. Similar results were documented by adding organic selenium in the basal diet by Pardechi *et al.* (2020) [16], Rutz *et al*. (2003) [17], Anciuti *et al*. (2004) [3] and Shabani *et al*. (2019) [20]. Santin *et al*. (2001) [19] and He *et al*. (2021) [10] observed the improvement of daily weight gain with the addition of yeast cell wall and live yeast in the diet respectively. The FCR was calculated on the 14th and 28th days and was presented in table 1. The feed conversion was better in the Se-Yeast supplemented group as compared to the control group on both days but with no significant difference. Wang and Xu (2008) [25], Wang (2009) [24], Niu *et al*. (2009) [14], and Pardechi *et al.* (2020) [16] found an improvement in the FCR of dietary Se supplement (0.2 mg Se kg−1) as compared with the control group and had significant difference $P<0.05$). Ghosh *et al*. (2012) [8] noted that the feed conversion ratio was better (p = 0.039) in the yeast cell wall supplemented group compared with the other groups. Moreover, it was found that the feed conversion efficiency was decreased with the advancement of the age of both groups.

Grossly, the small intestine of poultry birds was a tube-like structure that began at the gizzard (ventriculus) and extended up to the ileo-caecal-colic junction. It was long and consisted of a coiled mass formed by a series of loops and suspended within the body cavity (thoraco-abdominal) from the dorsal abdominal wall by a thin transparent membrane called the mesentery. This membrane carried the blood vessels associated with the intestine. The small intestine of both groups consisted of three main parts, the duodenum, jejunum, and ileum (Fig.1 & 2). Eyhab *et al.* (2017) [17] and Kadhim *et al.* (2018) [12] observed similar findings in mallard and broiler birds respectively. The length of the whole small intestine of both groups was recorded in every sacrifice on the days viz: 14th and 28th and the average length (Mean±SE) was presented in table 2. The average length of the small intestine of control and experimental groups was increased significantly ($p<0.05$) from day 14th to day 28th of the experiment (Fig. 8 & 9). Ahmadi *et al*. (2018) [1] showed that supplementation of nano selenium in chicken feeds resulted in simultaneously increasing the length of the small intestine. Nasrin *et al.* (2012) [13] studied that the average lengths of the small intestine were increased from day 1 to day 28. However, when compared between the groups, there were significant ($p<0.05$) changes on the 14th day but on the 28th day no significant differences were found. Yang *et al.* (2007) [26] revealed that depending on the dosage level and the age of birds, Mannan-Oligosaccharides (MOS) seemed to reduce the relative length of the small intestine.

Duodenum

The duodenum was the first and bulging part of the small intestine which was an elongated tube in shape, and creamy pale pinkish in appearance and consisted of three parts viz; descending part, middle part, and ascending part. The pancreas was lied between these two limbs of the loop and attached to each limb which generally held the two limbs together. Similar findings were observed by Zaher *et al.* (2012) [27] in quail, Nasrin *et al.* (2012) [13] and Kadhim *et al.* (2018) [12] in broilers, Okpe *et al.* (2016) [15] in African pied crow and Eyhab *et al.* (2017) [17] in mallard. On the serosal surface, many fine networks of blood capillaries were clearly visible of both the groups of birds (Fig.1). With the advancement of the age, the capillaries became more prominent in the mesentery of both groups. The left side of the duodenum was related to the right side of the ventriculus, the right side of the duodenum was contacted with the right lobe of the liver and right lateral body wall, and dorsally it was related with jejunum, ileum, and caecum.

The average length (Mean±SE) of the duodenum at different ages of individual groups was measured and presented in table 2. The average length of the duodenum was increased significantly (p<0.05) from the 14th to the 28th day of the control and experimental groups (Fig. 8 & 9). A similar result was documented by Ahmadi *et al.* (2018) [1] in chicken with nano selenium supplement. Ahmed *et al.* (2016) studied on goats and observed that the selenium yeast did not alter (P > 0.05) the lengths of the duodenum. However, when compared between the groups, there was no significant (p<0.05) difference on both the days of the sacrifice.

Jejunum

The jejunum was the longest part of the small intestine, pinkish-grey in colour, which was found in cone shaped coils form, known as jejunal loops. The cone had centripetal coils, sigmoid flexure, and centrifugal coils. A small projection was on the outer surface of approximately the middle of the jejunum, known as Meckel's Diverticulum, which was the remnant of the yolk sac (Fig.1). Zaher *et al.* (2012) [27], Okpe *et al.* (2016) [15] and Eyhab *et al.* (2017) [17] noticed similar observations in quail, African pied crow and in mallard respectively. The blood capillaries on the serosal surface were clearly visible and they were continuous with the mesenteric blood vessels (Fig.2). The loops were suspended by mesentery with the dorsal wall of the body cavity.

The average length (Mean±SE) of the jejunum at different ages of both groups was measured and presented in table 2 and it was concluded that on the day 28th the average length of the jejunum was increased significantly (p<0.05) from the day 14th of both the groups. Similar result was reported by Ahmadi *et al.* (2018) [1] in chicken with nano selenium supplement. However, Ahmed *et al.* (2016) studied on goats and observed that the selenium yeast did not alter (P > 0.05) the lengths of the jejunum. Moreover, when compared between groups, there was a significant (p<0.05) difference of both the experimental days, and that was presented graphically (Fig. 8 & 9).

Grossly, the Peyer's patches (PP) were studied by two separate chemical treatments viz. 10% Glacial Acetic Acid and crystal violet (Fig.5 and Fig.6) and their average diameter (Mean±SE) were recorded on the 14th and 28th day of rearing and presented in table 3. The average diameter gradually increased with increasing age (Fig.7). No significant differences (p<0.05) were noticed between the days and groups. The distributions of PP were more just before and after Meckel's Diverticulum as per visual estimation. No supportive literature was identified in this regard.

Ileum

The ileum was the terminal and smallest part of the small intestine. Ileum was not clearly demarcated from the jejunum and continued from the caudal part of the jejunum to the caeco-colic junction. It had a tube-like structure and creamy white in colour. Okpe *et al.* (2016) [15] and Eyhab *et al.* (2017) [17] noticed similar observations in African pied crow and in mallard respectively. The diameter of the ileum was less than the other two parts of the small intestine as observed under visual observation in both the groups. It was located ventral to the cloaca on the right side of the body cavity and both the right and left side of more than half of the length of ileum attached with the caecum by the adipose tissues (Fig.1). The average length (Mean±SE) of the ileum at different ages of different groups was measured and presented in table 2. It was concluded from the table that the average length of the ileum was more on day 14th compared with day 28th. There was no significant (p<0.05) difference in length of the ileum between the days and between the groups (Fig. 8 & 9). This was in accordance with the result of Ahmed *et al*. (2016), in goats. But it was contrary to the result of Ahmadi *et al*. (2018) [1] in chicken with nano selenium supplementation.

The average diameter (Mean±SE) of Peyer's patches was recorded in table 3. It was concluded that from the 14th to 28th day the diameters of both the groups had been increased but no significant differences were found between the days and groups. In comparison, grossly the diameter of Peyer's patches of the jejunum was higher (p<0.05) than the ileum on all days of studies. No supportive research article was noticed in this regard.

Table 1: Measurement of average body weight (Mean±SE) and the FCR of both the control and treatment group of poultry birds on the day 14th and 28th.

Days	Groups	Body weight (gms)	Body weight gain	FCR
14th	Control	458.092^a±11.604	265.443^m±11.895	1.461±0.101
	Treatment	466.758^a±5.220	267.54^m±4.268	1.236±0.065
28th	Control	1284.804^b±18.267	449.673^n±7.237	1.833±0.038
	Treatment	1312.648^b±5.452	452.374^n±21.155	1.551±0.160

N.B. Column wise similar superscripts did not differ significantly (p<0.05).

Table 2: Measurement (Mean±SE) of the length (cm) of the small intestine and the different segments of the control and treatment group of poultry birds on the day 14th and 28th.

Age (Days)	Groups	Length (cm)			
		Small intestine	Intestinal segments		
			Duodenum	Jejunum	Ileum
14th	Control	103.733^a±2.341	21.300^m±0.923	68.133^p±2.195	14.300^x±0.437
	Treatment	108.733^b±3.109	22.16^m ±0.701	72.167^q±2.820	14.333^x±0.607
28th	Control	144.167^c±2.339	28.233^n±0.919	102.667^r±2.201	13.267^x±0.440
	Treatment	146.467^c±3.110	27.967^n±0.711	105.500^s±2.820	13.000^x±0.609

N.B. Column wise similar superscripts did not differ significantly (p<0.05).

Table 3: Measurement of the average diameter (Mean±SE) of Peyer's patches (in mm) of control and treatment group of poultry birds on the day 14th and 28th.

Serosal view of organs	Day 14th		Day 28th	
	Control	Treatment	Control	Treatment
Jejunum	$8.674^{a}\pm0.309$	$8.998^{a}\pm0.122$	$8.926^{a}\pm0.146$	$8.948^{a}\pm0.295$
Ileum	$6.112^{m}\pm0.106$	$6.938^{m}\pm0.195$	$7.806^{m}\pm0.340$	$8.242^{m}\pm0.302$

N.B. Row wise similar superscripts did not differ significantly ($p<0.05$).

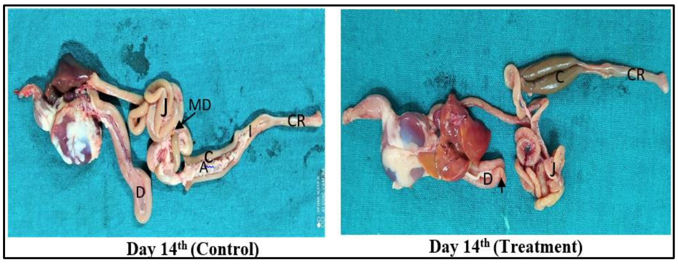

Fig 1: Photograph showing the different segments of intestine of both the control and treatment group of chicken of day 14th. D- duodenum, arrow-blood capillary, J- jejunum, MD-Meckel's diverticulum, I- ileum, A- adipose tissue, C- caecum, CR- colorectum.

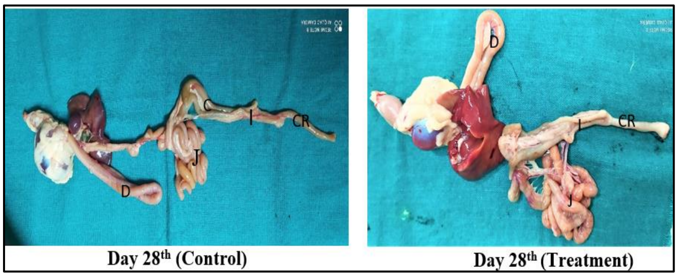

Fig 2: Photograph showing the different segments of intestine of both the control and treatment group of chicken of day 28th. D- duodenum, J- jejunum, I- ileum, C- caecum, CR- colorectum.

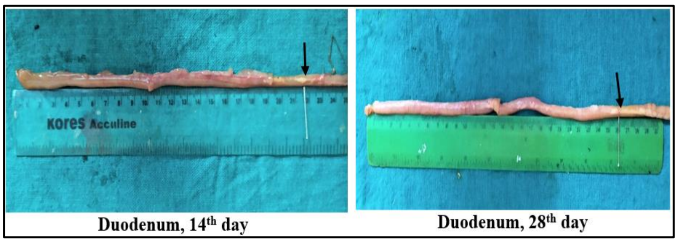

Fig 3: Photograph showing the measurement of the duodenum by the measuring scale of both the 14 and 28 days old chicken.

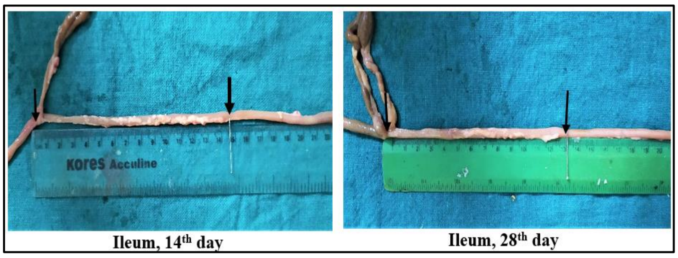

Fig 4: Photograph showing the measurement of the ileum by the measuring scale of both the 14 and 28 days old chicken.

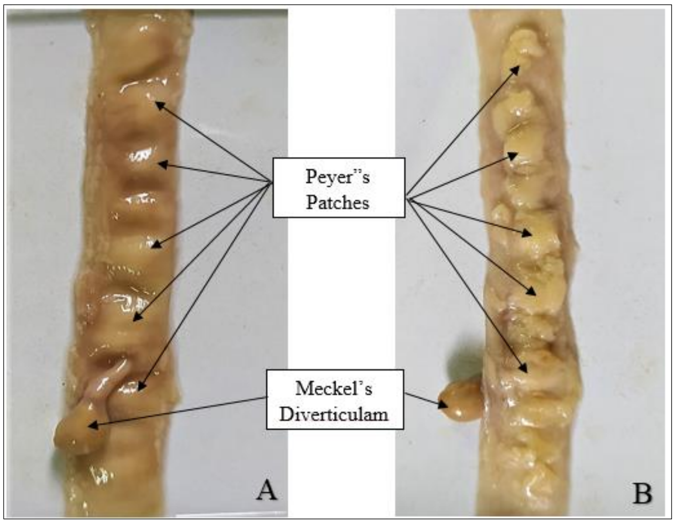

Fig 5: Photograph showing gross serosal (A) and mucosal (B) view of Peyer's patches of jejunum after 24 hours treatment with 10% glacial acetic acid.

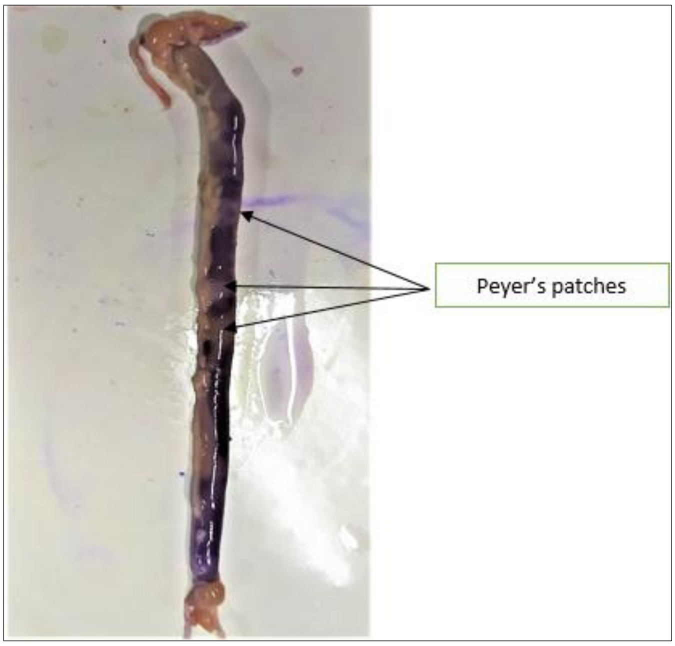

Fig 6: Photograph showing gross serosal view of Peyer's patches of jejunum after treatment with Crystal violet (Cationic dye) for 2 to 3 minutes.

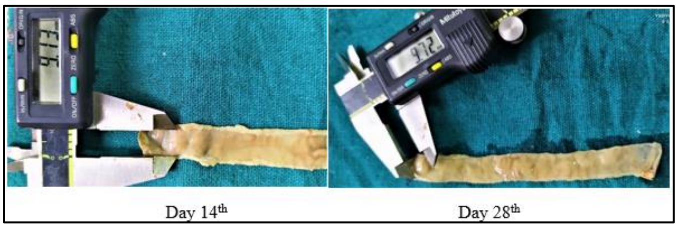

Fig 7: Photograph showing the gross serosal view of Peyer's patches of jejunum where the diameter was measured by slide calipers of each day of the experiment (day 14th and 28th). The diameters were slightly increased with the advancement of age.

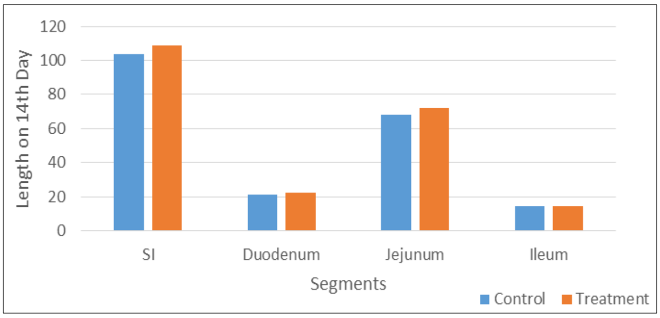

Fig 8: Graphical presentation of the average length of the small intestine, duodenum, jejunum and ileum on day 14th of both the control and treatment group of the poultry birds. SI-small intestine.

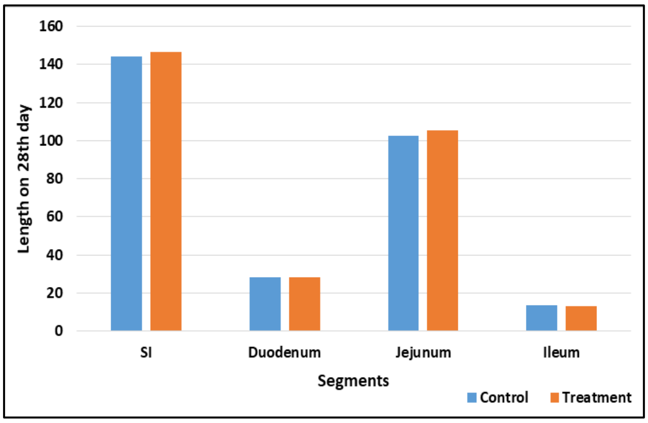

Fig 9: Graphical presentation of the average length of the small intestine, duodenum, jejunum and ileum on day 28th of both the control and treatment group of the poultry birds. SI-small intestine.

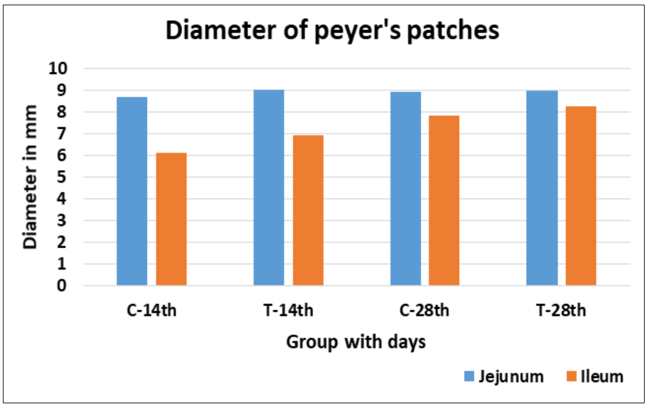

Fig 10: Graphical presentation of average diameter of the peyer's patches of the jejunum and ileum on the day 14th and 28th of the poultry birds. C-control, T-Treatment.

4. Conclusion

The Se-yeast, at the dose rate of 0.15mg/kg feed, had an effect on the small intestine as it increased the length resulting in weight gain and better FCR. The diameter of Peyer's patches of both jejunum and ileum was increased as compared to the control group of birds which ultimately enhanced the better health development and production.

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