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Effects of dietary acidifiers on body weight gain and bacterial load in major organs of Swiss Albino mice

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Abstract

Acidifiers are increasingly used in livestock nutrition as cost-effective feed additives to improve animal performance and maintain intestinal health. Their beneficial effects are mediated through several mechanisms involving feed preservation, gastrointestinal pH regulation, microbial modulation, and nutrient metabolism. The present study investigates the effects of dietary acidifiers on body weight gain, bacterial load and major organ health of Swiss Albino Mice. A total of twenty-five 21-day-old Swiss Albino mice were randomly allocated into five groups, with five mice in each group, designated as Groups A, B, C, D, and E. Group E served as the untreated control. Groups A, B, C, and D received Activate liquid® at 1 mL/4 L of drinking water (DW), Antamix ME® at 300 mg/100 g of feed, Avian pH® at 1 mL/L of DW, and Acidifier Vet® at 1 mL/3 L of DW, respectively. The respective acidifiers were administered orally once daily for 45 consecutive days. The results showed that body weight gain was higher in mice treated with the acidifier in Group C than in the control group. The bacterial load was highest in the control group compared with the acidifier-treated groups. In the intestine, the lowest bacterial counts were observed in Groups A and C. Similarly, the highest microbial load in the kidney was detected in the control group, whereas the lowest counts were recorded in Groups A and D. In the liver, the microbial load was also highest in the control group (7× CFU /mL) and lowest in Group D (3×CFU /mL). Overall, the findings suggest that dietary acidifiers may promote body weight gain and reduce microbial loads in selected organs and the gastrointestinal tract. Therefore, acidifiers may serve as potential alternatives to antibiotic growth promoters by creating a more favorable intestinal environment for beneficial microbes.

Keywords: Acidifier, beneficial effect, microbial load, mice.

Introduction

Acidifiers are naturally occurring components in a variety of feeds, are often engaged in feed acidification, and are produced during animal metabolism. These have been used as an in-feed preventive approach to fight against diseases in the feed industry since they have antibacterial, antifungal, and antibacterial properties (Frank 2020). Organic acid therapies containing single acids and mixtures of multiple acids have been exposed to reveal antibacterial properties comparable to antibiotics (Wang *et al.* 2009).

The use of antibiotics in livestock and poultry farming has been debated, as side effects may occur with long-term usage, such as residues in meat and the expansion of bacterial resistance (Muaz *et al.* 2018). Therefore, in response to the emergence of antibiotic resistance and the unreasonable use of antibiotics, several European countries have restricted or banned the use of antibiotics as growth promoters (Frank 2020). Moreover, several alternatives to the use of antibiotics in poultry are under investigation (Inatomi and Otomaru 2018, Salah *et al.* 2019). The withdrawal of antibiotic growth promoters (AGP) from feed induces poor growth performance, but that the supplementation of AGP has adverse effects on the meat quality of broiler chickens (Hamid *et al.* 2019). Among these alternatives, the organic acids (or simply acidifiers) play an important role in the gut health in animals, and also improve nutrient digestibility. Performance and health-promoting impacts have been elucidated for a number of organic acids, such as fumaric acid, formic acid, lactic acid (LA), citric acid, and their salts (Yang *et al.* 2018).

Acidifiers are often used as alternatives to antibiotic growth promoters because of their ability to create a favorable intestinal environment for beneficial microbes which may result in increased nutrient digestibility, increased growth performance, and reduced diarrhea. Dietary acidifiers may be organic or inorganic acids or salts acids (Partanen and Mroz 1999, Papatsiros and Billinis 2012). The heightened public concern over the emergence of antibiotic-resistant strains prompted the exploitation of alternate growth promoters to antibiotics (Dhama *et al.* 2014, Yadav *et al.* 2016). Among such alternatives, one such striking preference includes acidifiers, especially in livestock and poultry sectors. The potential of acidifiers in livestock feed industry is known for decades for their preservative and nutritional qualities (Partanen and Morz 1999, Spratt 1985). The present research was conducted to know the effects of acidifier on body weight gain and bacterial load in different organs, and to observe the gross and histopathological lesions of Swiss albino mice after administration of different acidifier.

Materials and methods

Ethical clearance: All experimental mice described in this manuscript were conducted in accordance with the principles of care and handling of experimental animals approved by the ethical committee of the Faculty of Veterinary Medicine and Animal Science, Gazipur Agricultural University (GAU/ AREC/ 2023/27).

Statement of the experiment: The experiment was conducted in the laboratory of the Department of Pathobiology, Gazipur Agricultural University, Gazipur-1706, during November 2021 to June, 2022.

Experimental animals: The experiment was carried out on Swiss Albino mice (*Mus musculus*). Twenty-five (25) mice of 7 days old were bought from the Animal Resource Center, ICDDR, B. The collected mice had neither any developmental disorders, detectable genital diseases nor other diseases that may cause any problem in the experiment or affect the result of the experiment.

Rearing and care: The mice were adapted at Parasitology Laboratory, FVMAS, GAU for the period of 14 days before being used for the experiment. The mice were housed in compartmentalized rectangular cages wrapped with wire mesh. The cages were kept in well-ventilated room at 28±2 ºC and a relative humidity of 70-80% with natural day and light. The mice were cared in proper hygienic conditions, with experimental and normal feeding *ad libitum*. Standard mouse-pellets were used as normal feed collected from ICDDR, B. During the experimental period, uniformity of the management practices was maintained as much as possible.

Experimental design: Twenty-five 21-day-old mice were randomly selected and allocated into five groups (n = 5 per group), designated as Groups A, B, C, D, and E. Group E served as the untreated control. Groups A, B, C, and D received Activate liquid® (Navana Pharmaceuticals PLC, Bangladesh) at 1 mL/4 L of drinking water (DW), Antamix ME® at 300 mg/100 g of feed, Avian pH® at 1 mL/1 L of DW, and Acidifier® Vet at 1 mL/3 L of DW, respectively (Table 01). The acidifier preparations were administered orally according to the respective treatment doses. Mice in Group E were provided with a standard diet and water *ad libitum* throughout the experimental period.

Experimental procedure: Group A, B, C and D were given orally Activate liquid 1ml/4L water, Antamix ME -300 mg/100g feed, Avian pH-1ml/1L water and Acidifier vet-1ml/3L doses of the acidifiers were administered orally once daily for 45 days to sample groups, respectively (Table 01). Group-E (control group) was supplied with normal feeds and *ad libitum* water.

Sample collection: After administration of acidifier drugs for 45 days, these mice were sacrificed through anesthesia by administering Jasocaine (Lidnocaine HCL,1%) and after those different important organs (Intestine, kidney and liver) were collected from the mice of both control and sample groups to investigate the effects of acidifier.

Sample preservation: Immediately after euthanized and killing, commercially available cotton tipped surface swabs were used after sterilization by autoclaving for the collection of swabs of different organs for bacteriological study. The vital organs were collected as soon as possible with the help of scalpel and scissors avoiding any destruction of the organs. The specimens then were collected and fixed in the 10% formalin solution.

Bacteriological colony count: After collection of bacteriological samples of the test tubes containing swabs

Table 1. Acidifiers (Organic acid) used in the study

Commercial name of acidifier	Organic acid	Manufacturers
Activate liquid®	2-hydroxy 4-methylthiobutyric acid and organic acid	Navana Pharmaceuticals PLC
Antamix ME®	Formic acid, lactic acid	DR. ECKEL
Avian pH®	Formic acid, lactic acid, propionic acid	Sk+F
Acidifier vet®	Lactic acid, ciric acid, formic acid, acetic acid, phosphoric acid	ACME

were hold in a test tube rack and placed in the incubator. The temperature was set to 37°C and the samples were incubated for 24 hours. From the incubated Nutrient Broth, cultures were made in Nutrient agar and incubated for overnight and counted the bacteria colony.

Gross and Histopathological study: The gross lesions of different organs were recorded carefully during necropsy. Tissue samples of intestine, kidney and liver were fixed in 10% neutral buffered formalin and further processed for histopathological staining with hematoxylin and eosin stains followed by standard producer (Alam *et al.* 2020).

Photomicrography: Photomicrography was taken using photomicrographic camera facilities facilitated by Department of Gynecology, Obstetrics and Reproductive Health of GAU.

Statistical analysis: Statistical analysis was performed using SPSS (IBM@ Version 21.0, USA). All results are represented as the Means ± S.D. For the comparison, one-way analysis of variance (ANOVA) was carried. Differences were considered to be statistically significant when the *p* value was less the 0.05.

Results and discussion

After administration of acidifiers drugs mixed with feed and water, body weight gain observed at 10 days interval up to 6 weeks. Acidifiers were increased the body weight of mice progressively (Table 2). The following table represented the beneficial effect of different acidifiers on body weight gain. In case of control group (group E), the body weight gaining rate was lower than the other treated groups. In this table Group C showed the highest body weight gaining rate. Analysis of variance of the results revealed that the differences in the changes of body weight gaining rate among the different groups (both control and treated) was significant ($p<0.05$*).

The bacterial culture on nutrient media were presented in the Table.03. The highest number of microorganisms were found in Control group. The lowest number of organisms were found in Group A (activate liquid) and one sample of intestine kidney and liver were associated with microorganisms.

Table 2. Body weight gain of mice of different group at different age ($p<0.05$*)

Group	10 days (gm) Mean±SD	20 days (gm) Mean±SD	30 days (gm) Mean±SD	40 days (gm) Mean±SD	45 days (gm) Mean±SD
A	20.55 ± 2.47	25.39±4.94	30.2±6.29	26.45±6.03	30.16±6.94
B	16.2±3.25	20.16±4.72	27.11±7.59	26.3±7.26	30.6 ±8.81
C*	20.6±2.94	23.3±2.78	34.5±4.69	32.81±4.68	38.59±6.27
D	17.11±1.08	20.9±5.42	26.08±7.58	24.31±7.37	28.3±8.7
E	22.03±1.75	24.88±1.93	30.5±2.02	29.06±1.01	29.65±0.92

Table 3. Cultural prevalence of microorganisms in different organs of mice

Sl. No.	Name of Organs	Total swabs (Each group contain 5 samples)	Microorganisms found in different group (Cultural Prevalence %)				
			Group A	Group B	Group C	Group D	Group E
01.	Intestine	25	1(20%)	2(40%)	2(40%)	3(60%)	4 (80%)
02.	Kidneys	25	1(20%)	1(20%)	2(40%)	2(40%)	4(80%)
03.	Liver	25	2(40%)	1(20%)	1(20%)	3(60%)	5 (100%)

The effects of acidifiers drugs on microbiological load of intestine of mice are presented in the Table 04 and Figure 01. Organisms were formed round; white dew drops like colonies on nutrient agar. The lowest number of organisms were found in Group A and Group C which were 6.4× CFU/ ml and 119× CFU/ml, respectively but the highest number of microorganisms were found in the Control group that was, 496×CFU/ ml.

The effects of acidifiers drugs on bacterial load of kidneys of mice are presented in the Table 04 and Figure 01. Organisms form round, white dew drops like colonies on nutrient agar. The highest number of microorganisms were showing in the Control group that was 5×CFU/ml in comparison to others treated groups and the lowest microorganisms found in Group A and D which was 10×CFU/ml and 652×CFU/ml.

The effects of acidifiers drugs on bacterial load of liver of mice were presented in the table 04 Figure 01. Organisms were showed round, white dew drop like colonies on nutrient agar. The highest number of microorganisms were found in the Control group which was 7×CFU/ml in comparison to others treated groups (Figure 01) and the lowest microorganisms found in Group D which was 3× CFU/ml.

The gross anatomical effects of acidifiers drugs on intestine of mice were presented. Intestine of all treated groups were showed no morphological changes (Figure 02) but slight hemorrhage with thin wall of intestine was observed in Control group. The gross anatomical effects of acidifier drugs on kidneys of mice were presented. Kidneys of Group A, Group B and Group C groups were showed no morphological changes (Figure 02) but slight hemorrhage with congestion of kidneys in Group D was observed. Control group of kidneys were showed congestion and fragile. The gross anatomical effects of acidifier drugs on liver of mice are presented in the Figure. Liver of all treated groups (Group A, Group B, Group C and Group D) were showed no morphological changes (Figure 02) but swelling and congestion were found on the liver of control group (Figure 02).

In this study, there were no significant change found in all the treated groups but the Group A, Group B and Group D showed mucus in the lumen in the intestine (Figure 03) and congestion and mucus in the lamina of intestine found in Group C. In control group hemorrhage were found in the glandular layer of intestine and also showed necrosis and infiltration of heterophils in the intestine. Histopathologically, kidneys of mice of Group A, Group B and Group C were showed mild fatty change and mild haemorrhages (Figures 03). Lymphocyte infiltration was

Table 4. Bacterial load of different organs of mice after using of dietary acidifiers

Group (Name of Acidifier)	Colony forming unit (CFU)/ml		
	Intestine	Kidney	Liver
Group A (Activate liquid®)	6.4×10^{3}	10×10^{4}	94×10^{6}
Group B (Antamix ME®)	23×10^{4}	763×10^{3}	632×10^{6}
Group C (Avian pH®)	119×10^{3}	856×10^{3}	256×10^{6}
Group D (Acidifier vet®)	130×10^{4}	652×10^{3}	3×10^{6}
Group E (Control)	496×10^{4}	5×10^{8}	7×10^{8}

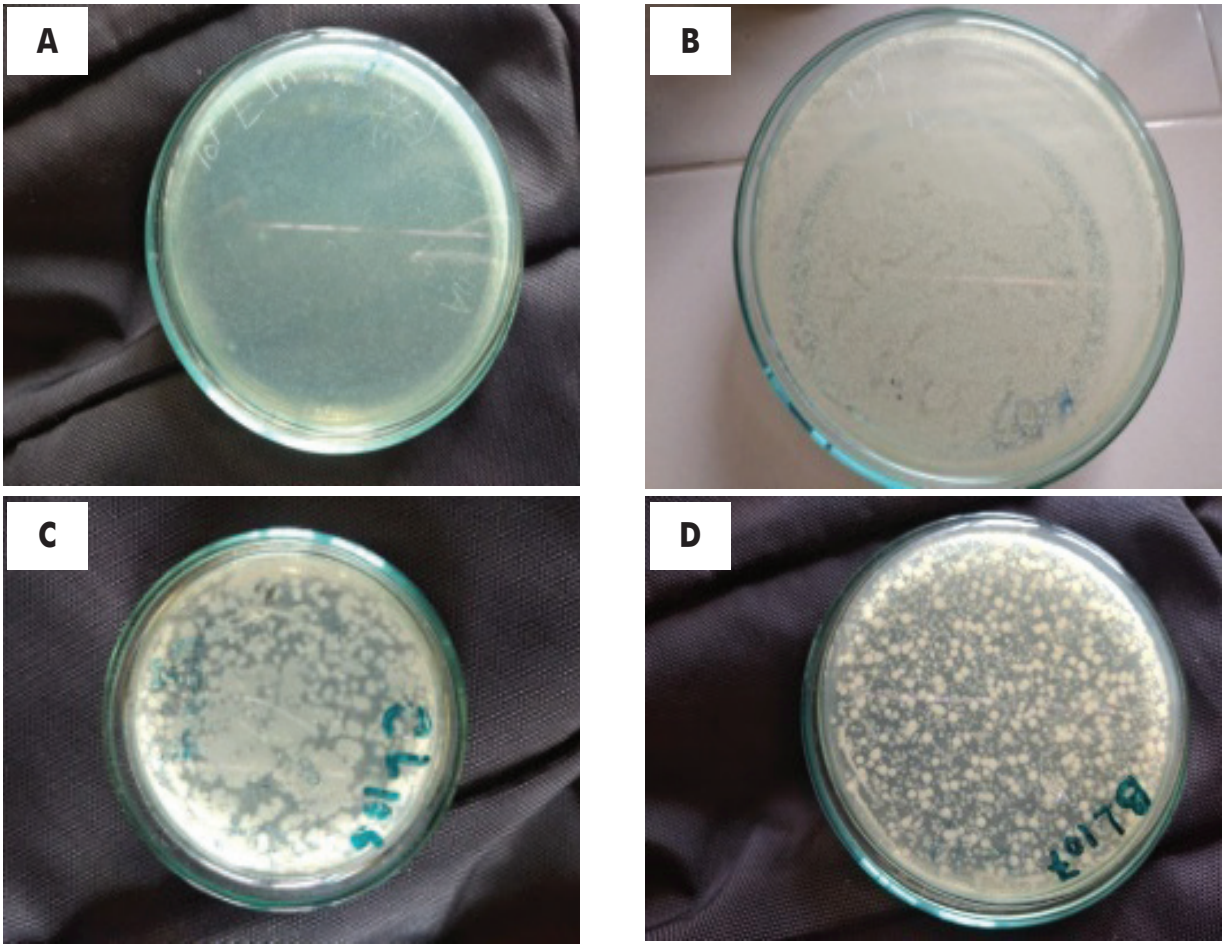
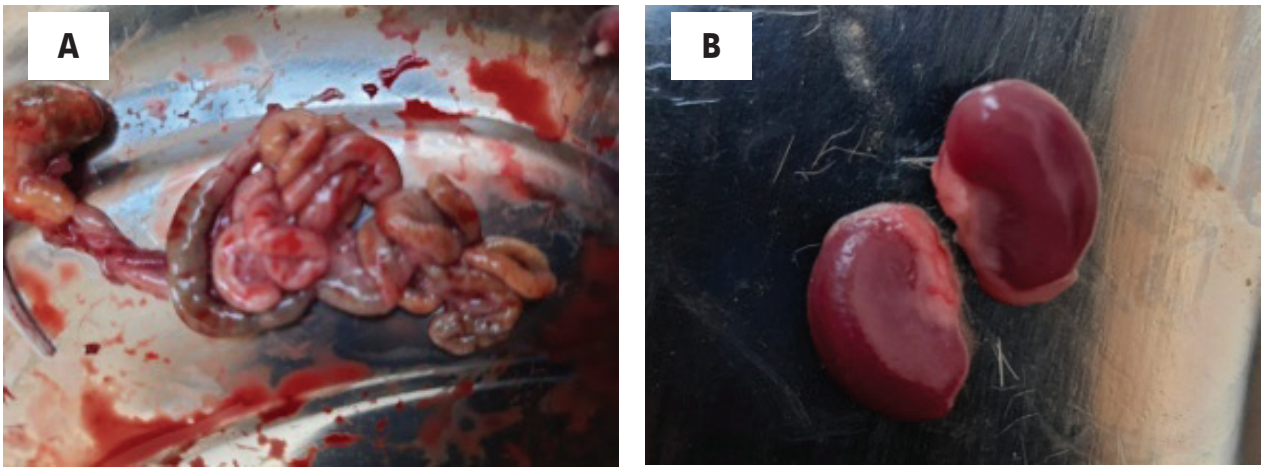


Figure 1. Microbial load 6.4× 10³cfu/ml on intestine of mice in group A. Bacterial load 763 ×10³ cfu/ml on kidneys of mice in group B. Bacterial load 256×106 cfu/ml on liver of mice in group C. Bacterial load 7×108 cfu/ml on liver of mice in control group D.



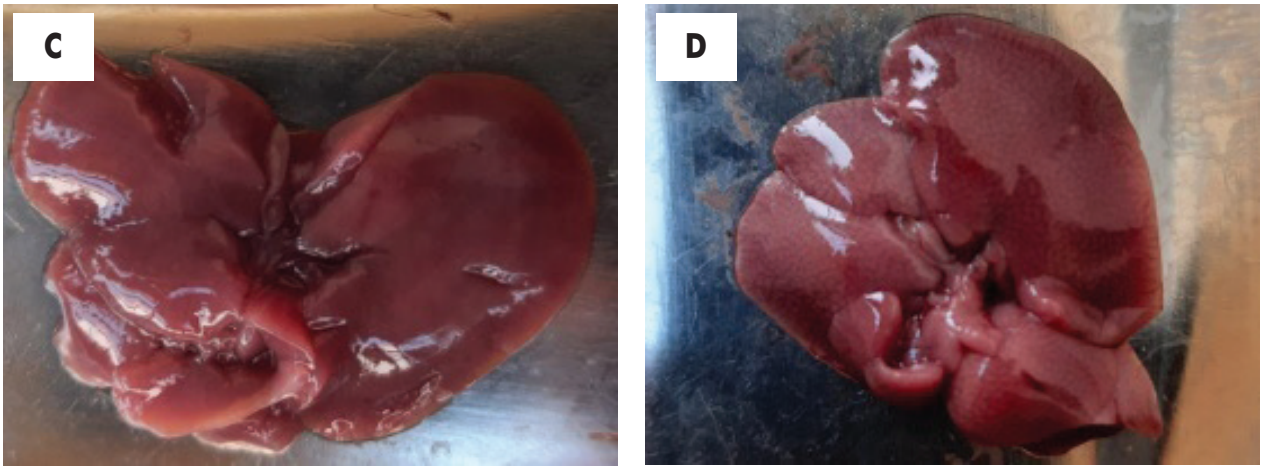


Figure 2. No morphological changes in intestine group A. No morphological changes in kidney group B. No morphological changes in liver group C. Liver of mice showing swelling and congestion in Group D.

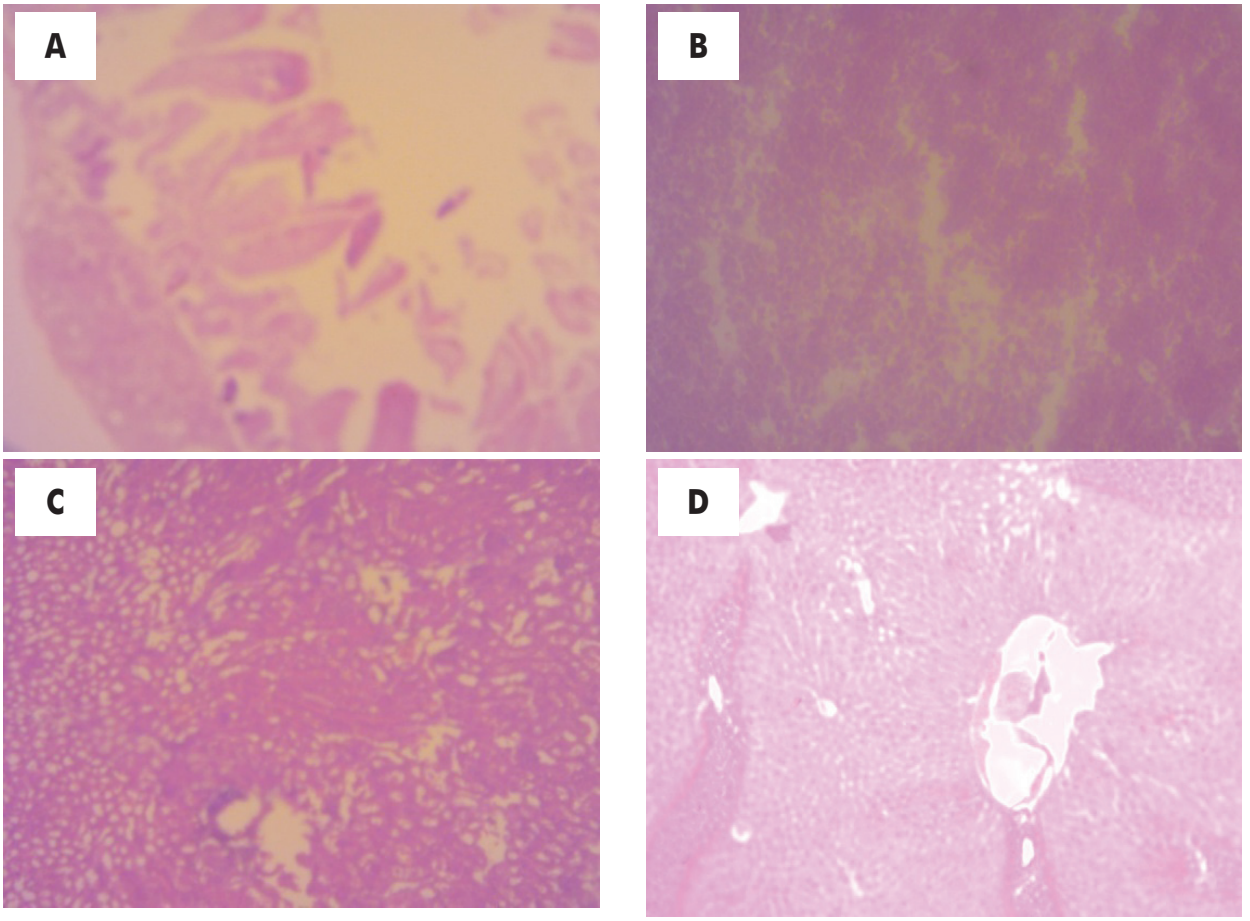


Figure 3. Figure A showed the accumulation of mucus in the intestine in group A (10X). Figure showed necrosis and infiltration of heterophils in the intestine of control Group (40X) Figure C showed the fatty changes and mild haemorrhages in kidney of Group B (40X) Figure showed the fatty change and lymphocytic infiltration in Group A (10X) in stain H & E.

observed in mice of Group D and Control group. In my study, Group A, Group B, Group C, Group D were showed fatty change and lymphocytic infiltration (Figure 03). The liver of control group showed the congestion in the central vein of the liver and heterophilic infiltration (Figure 03).

Acidifiers are often used as alternatives of antibiotic growth promoters because of their ability to create a positive intestinal environment for beneficial microbes which may result in improved nutrient digestibility, increased growth performance, and reduced diarrhea. (Partanen and Mroz 1999, Papatsiros and Billinis 2012). In this present study, the effects of acidifiers on the body weight gain, bacterial load in 3 different organs (liver, kidney, intestine), gross and histopathological changes of these organs of Swiss albino mice were observed.

In term of bacterial load, loads of microbes were higher in Control Group than that of treated acidifier group. In case of intestine, the lowest number of organisms were found in group A and group C which were 6.4×CFU/ ml and 119×CFU/ml but the highest number of microorganisms were found in the Control group that was, 496× CFU/ ml. In kidney, the highest number of microorganisms were recorded in the control group E that was 5×CFU/ ml in comparison to others treated groups and the lowest microorganisms were found in group A and D which was 10× CFU/ml and 652×CFU/ml. In case of liver, the highest number of microorganisms were found in the control group E which was 7×CFU/ ml in comparison to others treated groups and the lowest microorganisms found in group D which was 3× CFU/ml. This result was supported by others authors (Brzóska *et al.* 2013, Yakhkeshi *et al.* 2014).

In case of Control Group, the body weight gaining rate was lower than the acidifier drugs treated groups. I found gradual increase of the body weight after giving the dose of acidifier drugs of treated groups were observed. Mice of Group C which were provided Avian pH drug showed the highest body weight gaining rate. The similar results were described in previous reports (Fascina *et al.* 2012; Brzóska *et al.* 2013, Yang *et al.* 2019).

In this study no significant gross lesion except some haemorrages and congestion in Control Group. In case of histopathology of liver Group A, Group B, Group C, Group D were showed fatty change, congestion and lymphocytic infiltration. The histology of liver of Control group showed the congestion of vein of the liver and heterophilic infiltration. These findings were supported by others authors (Suiryanrayna and Ramana 2015).

Histopathologically, kidneys of mice of Group A, Group B and Group C were showed mild fatty change and mild haemorrhages. Lymphocyte infiltration was observed in mice of Group D and Control group. This result was supported by other authors (Kum *et al.* 2010). In case of histopathology of intestine, Group A, Group B and Group D were showed mucus in the lumen and muscularies of intestine, congestion and mucus in the lamina of intestine found in Group C. In case of Control group were showed the hemorrhages in the glandular layer of intestine (10X) and also showing necrosis and infiltration of heterophils in the intestine (40X). These findings were supported by others authors (Hernandez *et al.* 2006, Li *et al.* 2019).

Conclusions

In conclusion, acidifier supplementation showed beneficial effects on microbial control, as evidenced by the reduced microbial load in the liver compared with the control group, with the lowest microbial count observed in Group D. These findings indicate that dietary acidifiers have potential as alternatives to antibiotic growth promoters and may contribute to improv gut health, nutrient utilization, immune function, and overall animal performance. Nevertheless, further well-controlled studies involving larger sample sizes are needed to validate these findings and establish the most effective acidifier types and supplementation levels.

Conflict of interests

The authors declare no conflict of interests.

Author contribution

MM: conceptualization, data curation, investigation, methodology, visualization and writing original draft. MTI: data curation, formal analysis, investigation, methodology and writing original draft. ANMAR: research, resources, writing, review and editing. MGH: investigation, methodology, supervision, writing and review.

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