

Effects of Sodium Acetate and Sodium Propionate Supplemented Diets on Growth Performance and Gut Histomorphology of Broiler Starters

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Target Audience: *Animal scientists and researchers, Feed millers, Broiler farmers*

Abstract

In a 21-day feeding trial, 240 one-day old broiler chicks were distributed into five treatments replicated six times with eight birds each to evaluate the effect of sodium acetate, sodium propionate and their combination on growth performance and gut histomorphological parameters of broiler starter. Diet 1 was a basal diet, diets 2, 3, 4 and 5 contained 0.1g/kg oxytetracycline, 4g/kg sodium acetate, 4g/kg sodium propionate and combination of 4g/kg sodium acetate + 4g/kg sodium propionate respectively. Performance indices, morphological measurements and histological examinations were assayed for. Birds fed sodium acetate diet had significantly ($P < 0.05$) higher final weight compared to those fed other diets. However, birds fed sodium propionate diets and the combination were identical with birds fed other treatments. Improved villus height was recorded for birds fed sodium acetate, which did not differ significantly from those fed the combination. Sample sectioning of liver of birds fed antibiotics revealed dissociation of hepatic cords while sodium propionate showed hepatic necrosis. However sodium acetate and the combination showed no observable lesion. Bursa of fabricius of birds fed antibiotics revealed evidence of fibrosis while those fed sodium propionate showed scanty populated follicles. It was concluded that organic salt is a viable feed supplement in broiler diet.

Keywords: Broiler starter, Growth performance, Gut histomorphology and Organic salts

Introduction

Poultry are vulnerable to potentially pathogenic microorganisms such as *Escherichia coli*, *Salmonella ssp.*, and *Clostridium*, which invade the small intestine to compete with the host for nutrients. Antibiotics have been given in sub-therapeutic dosage to prevent some of this specific intestinal pathology and to stabilise the intestinal microflora. Over the years, the use of antibiotics as supplements in broiler diet around the world became detrimental to the health of man because of the development of resistance by pathogenic bacteria and the result of residual problem in the tissues of birds and animals (1). The search for alternative growth

promoters to phase out the use of antibacterial growth promoters has made researchers to reconsider the complexity of the gut ecosystem and the need to clarify the continuous interaction among the feed ingredients, the host and the intestinal microbiota (2; 3).

Organic acids and their salts are generally considered as safe and have been approved by most member states of European Union to be used as the feed additives in animal production. Amongst the organic acids, short chain fatty acids (SCFA) are considered as potential alternative to antibiotic growth promoter (4). Gut microflora appears to be the target for the growth-promoting effects of this alternative. It has been shown that gut

microflora has significant effects on host nutrition, health and growth performance by interacting with nutrient utilisation and the development of gut system of the host (5). The small intestine is a critical digestive organ involved in nutrient absorption, the development of this organ is essential to poultry health and performance (6). Therefore modifications of the gastrointestinal microflora to reduce pathogen attachment may have a profound effect on the structure of the intestinal wall (4).

The beneficial effect of organic acids particularly the short chain fatty acids (acetic acid, propionic acid and butyric acid) on the productive traits of broiler chicken has been demonstrated in many studies (7). Organic acid supplementation markedly enhances the intestinal absorption area by improving the villi growth in height and width at 21 and 42 days (8). Moreover, these histological alterations appear early (before 21 days of age) and transiently in duodenum and jejunum but display stability afterward in the ileal portion, hence providing significantly higher magnification rates for ileal villi in exposed birds. Organic acids have been adjudged to positively affect the gastrointestinal tract, mineral utilization and as a result, the performance (9).

The application of organic acids supplementation in broiler chicken diet has been studied extensively as compared to organic acid salts. It was therefore the aim of this study to determine the effect of feeding sodium acetate and sodium propionate as a substitute for antibiotic growth promoter on the growth performance and gut histomorphology of broiler starter.

Materials and Methods

Experimental site

The experiment was carried out at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Oyo State in the South

West geopolitical zone of Nigeria, within the tropical rain forest region.

Experimental diets and management of birds

Two hundred and forty one-day old Abor Acre broiler chicken was purchased from a reputable commercial hatchery. The birds were randomly distributed into one of the five dietary treatments with five replicate of eight birds each in a completely randomised design.

Treatment 1 was a basal diet with no supplement (negative control) while treatment 2 comprised of a basal diet +0.01% oxytetracycline (positive control). Treatments 3 and 4 comprised the basal diets with 0.4% sodium acetate and 0.4% sodium propionate respectively while treatment 5 comprised of the basal diet, 0.4% sodium acetate and 0.4% sodium propionate.

Parameters measured and calculated

The growth performance parameters that were measured included feed intake, body weight gain and feed efficiency.

Gut morphology

At week 3, two birds per replicate were sacrificed and eviscerated. The ileal tissues were cut and kept in pre-labeled polyvinyl bottle filled formalin for preservation and measurements were taken with the aid of the graticle in micrometers (*%m*) according to the methods of (10).

Gut histology

The appropriately labeled samples (liver and bursa of fabricus) were dissected, appropriately labeled and fixed in 10% neutral buffered formalin for histological examination as described by (11).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using (12) at significance

level ($P < 0.05$) and means were separated using Tukey's HSD test.

Results

Growth performance indices of broiler chicks fed antibiotics and organic salt supplemented diets

The results of the effect of dietary supplementation of antibiotic growth promoter and organic salts on broiler growth performance are shown in Table 2.

Birds fed sodium acetate supplemented diet had significantly ($P < 0.05$) higher final weight (368.18g/b) compared to those fed the control and antibiotic supplemented diets. However, birds fed sodium propionate diets and the combination of sodium acetate and sodium propionate were identical with other treatment groups. A similar trend was recorded in the weight gain of birds on dietary treatments. Diets had no significant influence on the feed intake and gain: feed of birds on the experimental group.

Gut morphology of broiler chicks fed antibiotics and organic salt supplemented diets

The results of the gut morphology of the ileum of broiler chicken fed dietary antibiotics and organic acid salts are shown in Table 3.

Improved villus height was recorded for birds in sodium acetate supplemented diet which did not differ significantly from those on the blend. Meanwhile low villus height was observed in birds in the control diet, antibiotic and sodium propionate supplemented diets. Crypt depth of birds on the control and antibiotic diets were similar and significantly ($P < 0.05$) lower than what was recorded for birds on the blend. Similar trend was recorded for muscular cell width except that the control did not differ significantly ($P > 0.05$) from the blend. There were no significant differences ($P > 0.05$) observed in the villus: crypt ratio of broiler chicken on the dietary treatments.

Similar result was also observed for villus width of birds on dietary treatments.

Discussion

Performance of broiler chicks fed antibiotics and organic salt supplemented diets

The results of this present study showed that dietary organic salt supplemented diets did not have a significant effect on the performance of broilers in the starter phase. Several organic acids and their salts have been reported to improve growth performance when supplemented in diets (13; 14). (13), reported that body weight was increased, and feed intake and feed conversion ratio were improved when butyric acid was used. The improvement in body weight gain and feed efficiency by potassium di-formate (KDF) supplementation was discussed by (14), who concluded that double salts of organic acids, such as potassium di-formate (KDF) and sodium di-formate (NaDF), which reach the small intestine, have been shown to have a significant impact on nutrient utilisation. Better ileal digestibility of nutrients as also been reported by. (15) and (16) to be the reason for improved growth performance of broiler chicken. The same was recorded by (17) who reported that dietary supplementation of KDF at 6% significantly increased BWG and FI from 16 to 35 days post-hatch. Similarly, (18) reported that the addition of di-formate at (0.1%, 0.3% and 0.5%) was found to enhance individual live weight and FCR with increasing dosage, the best results in respect of these parameters were obtained for a dosage of 0.3% di-formate. (19) recorded that dietary KDF supplementation at 1% significantly increased the body weight of broiler chickens. (20) studied the results of 17 trials with NaDF inclusion, which ranged from 0.1% to 0.6% and concluded that the dietary NaDF could improve broiler production worldwide. However these results did not comply with (21) who found that adding KDF

to diet at 0, 0.3%, 0.6%, 0.9% and 1.2% linearly reduced feed intake and weight gain. Meanwhile, in most cases growth performance of chicks was unaffected by the addition of an organic acid to diet (22). In this study, growth performance was not statistically affected by dietary treatments. In agreement with the present study, (23) and (24) reported that at the end of the study period, there was no significant improvement in performance of broilers when organic acid and antibiotic were used. (25) using various levels of butyric acid in the diet showed that at the end of the experiment (35days) feed intake and feed conversion ratio were not affected. Thus from the above result it may be revealed that sole organic acid salt supplementation did not improve body weight, body weight gain and feed intake, rather organic acid salt reduced feed intake specially sodium propionate. This result also corroborates the observation of (26) where they studied several organic acid including formic and propionic acids and showed that only propionic acid had a significant action in reducing feed intake. (27) indicated that propionic acid plays a role in satiation regulatory system since intraperitoneal injection of organic acid in broiler suspended intake for 0.5 to 1.5 hours. Previous researchers (28; 29; 30) also reported no effect on body weight of broiler chicken with the use of formate and propionic acid in broiler diet. (31) studied the effects of organic acid as an alternative feed additive to an antibiotic growth promoter (bacitracin) on the performance of broiler chicks and found that weight gain and feed intake were not affected by dietary treatments. In the current study, antibiotics did not differ significantly from the control and the organic acid salts (sodium propionate and its blend). This finding contradicts the finding of (32) who concluded that organic acid are more efficient than antibiotic growth promoters in improving broiler performance. (33) also reported that

antibiotics showed better performance than that of the control group. (34) reported that addition of antibiotic to broiler diet caused the weight to significantly decrease compared with that of control group. (35) and (36) found depressed body weight gain in birds fed diets supplemented with acetic acid. (37) found no significant effects of dietary acetic acid on body weight of broiler chickens. (38), in agreement with the results of (39), did not find positive effects of organic acid (formic acid) on performance. It can be concluded, in agreement with (40), that the inconsistencies of reports on the effects of these feed additives on performance may either be due to variation in the level of acids used, variation in specific acid, variation in feed ingredient, birds characteristics such as species or environmental conditions. It has been suggested that in cases of well-nourished healthy chicks, housing at a moderate stocking density and dietary inclusion of organic acid was ineffective on bird's growth performance. On the other hand beneficial effects counter stress conditions and ailments (41).

Gut morphology of broiler chicks fed organic salt supplemented diets

Different morphological features in the broilers fed the experimental diets were observed during the period of study. The villus height of broiler chicken on sodium acetate was improved than those on the control, antibiotics, sodium propionate and the blend. This indicates that sodium acetate positively affected the villus height compared to other treatments. Regarding the relationship between performance and gut morphology, (42) reported that significant correlations were not observed between performance and villus height or crypt depth. (43) and. (44) reported that antibiotic supplementation caused low villi height. The decrease in villi height could be attributed to the suppressing effect of the antibiotic for the beneficial bacteria, these

bacteria play a major role in growth and maturation of the villi and usually equated with excellent gut health, high absorptive efficiency and healthier intestinal tract of chickens. (1) reported higher villus height in the ileum with the diet based on organic acid salts compared with diet fed without mannan oligosaccharide plus organic acid salt. The increase of villus height of different segment of small intestine may be attributed to the role of the intestinal epithelium as a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen. Also, (45) and (1) explained that increased villus height with most of the organic acids was attributed to the fact that organic acids reduce the growth of many pathogenic or non-pathogenic intestinal bacteria, decreasing the intestinal colonization and infectious processes, ultimately decreasing the inflammatory reactions at the intestinal mucosa, which increases the villus height and functions of secretion, digestion and absorption of nutrients by the mucosa. Stressors, pathogen and chemical substances, among others, cause disturbances in the normal micro flora or in the intestinal epithelium that may alter the permeability of this natural barrier, facilitating the invasion of pathogens and prejudicial substances, modifying the metabolism, the ability to digest and absorb nutrients, leading to chronic inflammatory processes at the intestinal mucosa (46). The more the villus and the larger the villus heights, the more the surface area available for the absorption of nutrients from the intestine, which will in turn result in a better growth and development of the host as more nutrients are quickly utilized, while the decrease of depth of the crypts can indicate a decrease of enterocyte replacement and a decrease in cellular turnover (47; 48).

In this study, the organic acid salts showed better improvement in the crypt than the control and the antibiotics. There were more crypts of the treated group than the

control and antibiotics. (48) reported that decreased crypts depth may lead to poor nutrient absorption, increased secretion in the gastrointestinal tract and lower performance. Increased crypt depth indicates that mucosal secretion (49) and cell turnover (50) are high. Improved mucous will improve the protection of the epithelium of the bird as well as prevent the influx of pathogens through the epithelial lining, while high cell turnover will result in the improvement of villus height. This corroborates the report of (51) who observed that villus height depends upon cellular multiplication and differentiation from the crypts and on the migration of mature cells along the villus axis, accompanied by the cellular shedding from the apex. Villus crypt is regarded as the villus factory, and deeper crypt indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins and high demand for tissue (50).

Histology of liver of birds on experimental diets

The liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism and its portal location within the circulatory system (52).

The histopathological investigation of antibiotic growth promoter in the liver revealed dissociation of hepatic cords, widening of sinusoidal spaces, and severe congestion with mild presence of neutrophils and kupffer cell hyperplasia. According to (53) increased sinusoidal congestion in some liver samples was observed in the deceased birds with aging but mildly present in healthy birds when they were reared under different management conditions. Liver of birds on sodium propionate showed sinusoidal congestion, kupffer cell hyperplasia and widened focal area of hepatic necrosis. Among the histopathological changes of liver, sodium propionate showed the presence of hepatic

necrosis, this has also been reported by (53) as multifocal necrosis in liver tissues which were obtained from healthy birds at slaughter describes that the birds reared under high temperature indirectly affects the liver tissue. Usually hepatic necroses are more evident in older age groups of birds subjected to either sudden death or slaughter. Birds on antibiotic growth promoter and sodium propionate showed Kuffper cell hyperplasia. Kupffer cells, also known as stellate macrophages are specialized macrophages located in the liver, lining the walls of the sinusoids that form part of the mononuclear phagocyte system. The hyperplasia could be as a result of degeneration or loss of enterocytes from this cell lining nutrient digestion by enzymes as well as absorption of electrolytes and nutrients is interrupted resulting in maldigestion, malabsorption and osmotic retention of water (54).. No histological visible lesions were observed in sodium acetate and the blend.

Histology of bursa of fabricius of birds on experimental diets

Histological studies revealed some structural changes in the bursa of fabricius examined from the broiler chicks fed antibiotics and organic acid salts. The plates show the effect of dietary treatments on histology of chick bursa of fabricius. Bursa of fabricius is a primary lymphoid organ in birds and it is composed of about 15 plicae (folds), each contains numerous bursa follicles. The cortex and medulla form the two distinct areas in these follicles. It can be seen from the study that the bursa follicles of the control group elongated with few large lymphocytes and had abundant medullary area. This area is composed of apparently undifferentiated epithelial cells (pale-staining) and small lymphocytes or lymphoblasts. This feature was also described by (55). In this study, it can be seen that antibiotics enhanced the activity of bursa follicles as compared to birds fed organic

salts. According to the work of (55), this hyperactivity is accompanied by the presence of many large lymphocytes in the cortex area concomitant with many lumens between the follicles. These lumens are abundant in this treatment and to less extent in organic acid treatment. In general, the lumens are responsible for the phagocytic processes and for maintaining the B-cell production. It is also same age- related degenerative areas that could be seen with high frequency respectively. The result is presumably because the bursa of fabricius plays an important role in developing immunity against Gumboro in chickens. With an attenuated strain of Gumboro virus colonising the bursa, it atrophied by three to six times between 8 and 10 days post infection. The recovery phase could last up to 35 days, depending on the virulence of the virus strains. The size of the bursa of fabricius becomes smaller as body weight increases, resulting in a lower bursa weight to bodyweight ratio, linked mostly with improved absorption of nutrients by the addition of organic acid.

Conclusion and Applications

In conclusion, the present study showed that

1. Supplementation of sodium acetate, sodium propionate and its blend in broiler starter diets fed to birds for 21days had no effect on the growth performance and relative organ weight of the birds.
2. Organic salts significantly increased the villus height, crypt depth of the ileum of the birds. Although the supplementation of sodium propionate in the diet stimulated some histological changes in the liver and bursa of fabricius, it is not conclusive in determining the effects of these organic salts in related organs.
3. The use of sodium acetate and sodium propionate at an inclusion level of

4g/kg is recommended for broiler chicks for improved growth performance and gut health.

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Table 1: Gross composition (g/kg) of starter diets fed to broiler chicks.

Ingredients g/kg	Negative control (Basal diet)	Positive control (Antibiotics)	NC sodium acetate	+ NC sodium propionate	+ NC acetate + sodium propionate
Corn	555.00	555.00	555.00	555.00	555.00
Soyabean meal	372.00	372.00	372.00	372.00	372.00
Fish meal	25.00	25.00	25.00	25.00	25.00
Wheat offal	15.00	14.895	11.00	11.00	7.00
Dicalcium phosphate	15.00	15.00	15.00	15.00	15.00
Premixes	2.50	2.50	2.50	2.50	2.50
Limestone	8.00	8.00	8.00	8.00	8.00
DL-methionine	2.50	2.50	2.50	2.50	2.50
L-lysine	2.50	2.50	2.50	2.50	2.50
Salt	2.50	2.50	2.50	2.50	2.50
Antibiotics	0.00	0.105	0.00	0.00	0.00
Sodium acetate	0.00	0.00	4.00	0.00	4.00
Sodium propionate	0.00	0.00	0.00	4.00	4.00
TOTAL	1000	1000	1000	1000	1000
Calculated nutrient (g/kg)					
Crude protein	232.29	232.27	231.61	231.78	230.93
Energy ME(kcal/kg)	3009.82	3009.62	3002.34	3002.34	2994.86
Crude fat	37.01	37.00	36.83	36.83	36.83
Crude fiber	39.70	39.69	39.36	39.36	39.36
Calcium	8.75	8.75	8.74	8.74	8.74
Total phosphorus	7.37	7.37	7.32	7.32	7.32
Non-phytate P	3.93	3.93	3.93	3.93	3.93
Ca:NPP	2.23	2.23	2.23	2.23	2.23

*Supplied the following per kg diet: vitamin A, 5484 IU; vitamin D3, 2643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06mg; Cu, 4.44mg; Fe 44.1mg; Zn, 44.1mg; Se, 300µg.

Table 2: Performance indices of broiler chicks fed antibiotics and organic salt supplemented diets (starter phase)

Parameters	Treatments					P Value
	Control	Antibiotics	Sodium Acetate	Sodium Propionate	Sodium Acetate + Sodium Propionate	
Initial weight (g/bird)	37.25 ± 1.92	38.63 ± 2.05	37.88 ± 2.50	38.69 ± 2.50	38.54 ± 1.90	0.7487
Final weight (g/bird)	336.26 ± 12.88 ^b	336.51 ± 12.05 ^b	368.18 ± 10.22 ^a	341.65 ± 27.44 ^{ab}	364.27 ± 17.85 ^{ab}	0.0046
Weight gain (g/b/day)	14.24 ± 0.53 ^b	14.19 ± 0.52 ^b	15.73 ± 0.52 ^a	14.43 ± 1.36 ^{ab}	15.51 ± 0.86 ^{ab}	0.0050
Feed intake (g/b/day)	23.38 ± 4.77	23.72 ± 3.43	24.99 ± 6.74	21.94 ± 4.99	28.21 ± 1.26	0.2102
Gain: Feed	0.63 ± 0.13	0.61 ± 0.09	0.66 ± 0.13	0.70 ± 0.24	0.55 ± 0.03	0.4529

^{a,b,c} Means in the same row with different superscripts are significantly (P<0.05) different.

Table 3: Morphological characteristics of broiler chicks fed antibiotics and organic salt supplemented diets

Parameters	Treatments					P Value
	Control	Antibiotics	Sodium Acetate	Sodium Propionate	Sodium Acetate + Sodium Propionate	
Villus height (µm)	673.70 ± 176.67 ^{bc}	445.8 ± 187.95 ^c	1169.6 ± 272.32 ^a	555.9 ± 309.77 ^c	975.5 ± 234.63 ^{ab}	<0.0001
Crypt depth (µm)	66.17 ± 28.83 ^b	66.05 ± 26.28 ^b	124.25 ± 40.70 ^{ab}	77.19 ± 44.49 ^{ab}	129.26 ± 30.78 ^a	0.0047
Villus: Crypt ratio	10.89 ± 2.73	6.68 ± 0.75	9.99 ± 3.08	8.56 ± 4.54	7.96 ± 2.73	0.1576
Muscular Cell Width	229.97 ± 67.16 ^{ab}	138.16 ± 16.65 ^b	280.44 ± 173.21 ^{ab}	244.21 ± 151.99 ^{ab}	382.81 ± 106.60 ^a	0.0237
Villus Width	108.06 ± 16.01	149.28 ± 58.16	115.87 ± 21.11	96.18 ± 37.35	93.00 ± 28.92	0.0758

^{a,b,c} Means in the same row with different superscripts are significantly (P<0.05) different

Histopathological readings of photomicrographs of liver and Bursa of fabricius

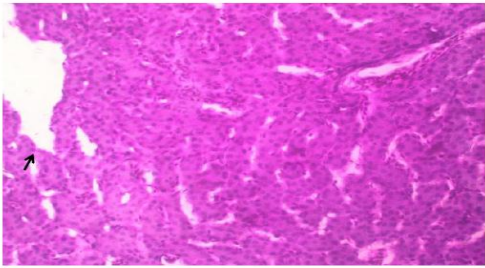


Plate 1 showed the sample sectioning of liver of birds fed the control diet
Liver: Moderate congestion with mild hepatocellular swelling (black arrow).

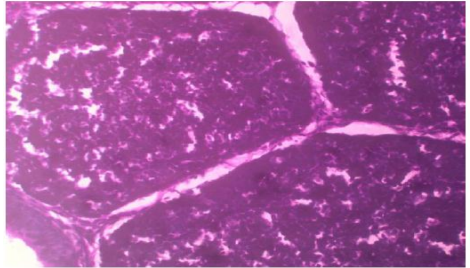


Plate 2 showed the sample sectioning of bursa of fabricius of birds fed the control diet
Bursa: No visible lesion.

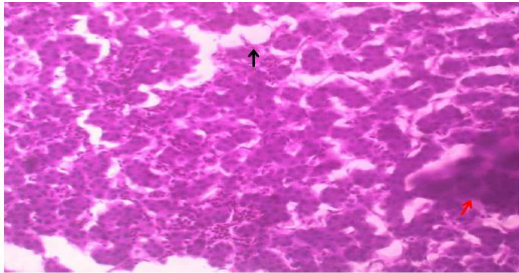


Plate 3 showed the sample sectioning of liver of birds fed antibiotics diet
Liver: Dissociation of hepatic cords (**black arrow**), widening of sinusoidal spaces, and severe congestion with mild presence of neutrophils (**red arrow**) and kuffer cell hyperplasia. i.e. hepatitis

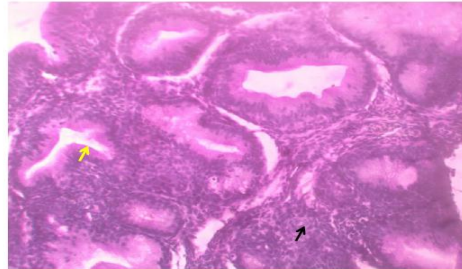


Plate 4 showed the sample sectioning of bursa of fabricius of birds fed antibiotics diet
Bursa: Markedly scanty populated germinal centres (yellow arrow), prominent inter-follicular area with evidence of fibrosis (black arrow).

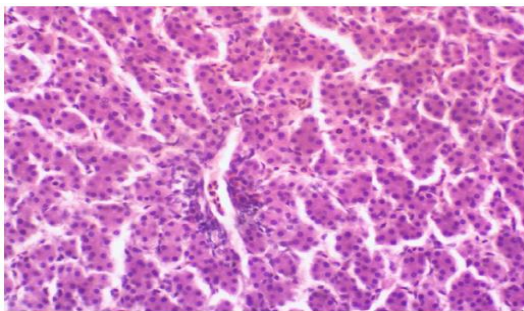


Plate 5 showed the sample sectioning of liver of birds fed sodium acetate diet
Liver: No visible lesion

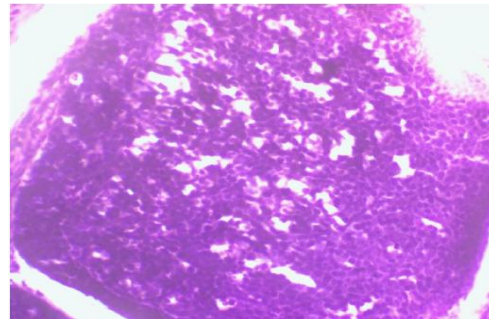


Plate 6 showed the sample sectioning of bursa of fabricius of birds fed sodium acetate diet
Bursa: No visible lesion.

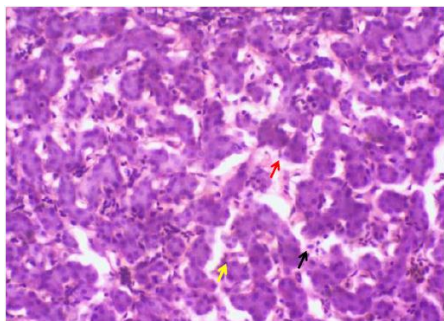


Plate 7 showed the sample sectioning of liver of birds fed sodium propionate diet

Liver: Sinusoidal congestion (black arrow), kupffer cell hyperplasia (yellow arrow), widened and focal area of hepatic necrosis (red arrow)

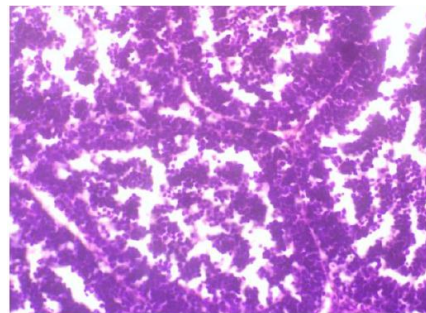


Plate 8 showed the sample sectioning of bursa of fabricus of birds fed sodium propionate diet

Bursa: Scanty populated follicles (plasma cells)

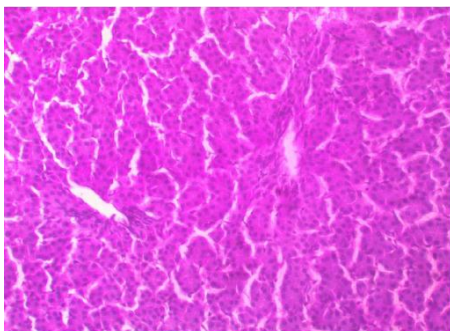


Plate 9 showed the sample sectioning of liver of birds fed sodium acetate and sodium propionate diet

Liver: No visible lesion

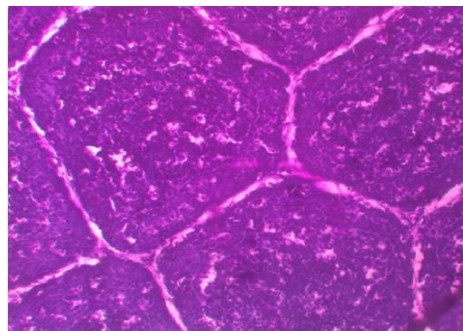


Plate 10 showed the sample sectioning of bursa of fabricus of birds fed sodium acetate and sodium propionate diet

Bursa: No visible lesion