

Effects of different acetylsalicylic acid doses on body organs, histopathology, and serum biochemical parameters in broiler birds

Efeito de diferentes doses de ácido acetil salicílico nos órgãos corpóreos, histopatologia e parâmetros bioquímicos sorológicos em frangos de corte

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ABSTRACT

The poultry industry suffers from various diseases or stresses. In poultry, apart from being antipyretic, acetylsalicylic acid (ASA) is widely used to cope with different issues including heat stress, and respiratory and digestive disorders. This study evaluated the beneficial and toxic effects of ASA at different dose levels in broiler birds. To evaluate these toxic and beneficial effects it is necessary to examine the physical and serum biochemical parameters as well as the histopathological changes with tissue sections taken from broiler birds under experimental trial. This study was conducted on 60 one-day-old broiler chicks purchased from a local market in Faisalabad. Chicks were reared for the first 14 days under similar conditions. On the 15th day, birds were randomly divided into 4 groups (1-4) with 15 birds in each group. Group 4 was kept as control, while groups 1, 2, and 3 were treated with ASA at the dose of 300, 600, and 1200 mg/L of drinking water for 21 days. There were 3 samplings performed at 21, 28, and 35 days post-treatment. The live body weight and carcass weight were noted on each sampling. All the visceral organs were recorded for gross pathological changes. The serum samples were collected for biochemical evaluation. Histopathology of all the visceral organs was performed to observe the microscopic changes. A significant ($P < 0.05$) increase in live body weight at a 300mg/L dose was noted after the first 2 samplings. A significant ($P < 0.05$) increase in the relative organ weight was recorded at 1200 mg/L. The groups treated with ASA 600 and 1200 mg/L showed increased ($P < 0.05$) AST, ALT, and creatinine levels from that of the control group. The group treated with 1200 mg/L of ASA showed increased ($P < 0.05$) urea, serum total protein, and albumin level in all the samplings. Histopathological changes revealed swollen hepatocytes, increased sinusoidal spaces in the liver, congestion and abnormal glomerular spaces in the kidney, congestion and alveolar disruption in the lungs, and generation of villi and cellular degeneration in the intestine in a high-dose group. The study concluded that ASA at a low dose can be used for a long time in broilers and has a growth promontory role, while high-level doses cause hepatorenal toxicity.

Keywords: Acetylsalicylic acid. Broiler. Histopathology. Biochemical evaluation, Toxicity.

RESUMO

A indústria avícola é afetada por diversas doenças ou estresses. Particularmente devido às aves serem antipiréticas. O ácido acetil salicílico (AAS) é largamente utilizado com diferentes objetivos que incluem o controle do estresse calórico, bem como a atividade respiratória e digestiva. O propósito deste estudo foi a avaliação dos efeitos benéficos e tóxicos do emprego de diferentes dosagens do AAS em frangos de corte. As variáveis analisadas foram: exame físico, parâmetros bioquímicos, bem como as alterações histopatológicas em seções de tecidos colhidas das aves em um ensaio experimental. O estudo foi conduzido em 60 frangos de corte com um dia de idade adquiridos em um mercado local de Faisalabad que foram criados nos primeiros 14 dias em idênticas condições. Então no 15^o dia as aves foram distribuídas aleatoriamente em quatro grupos identificados pelos números 1 a 4, com 15 aves em cada grupo. O grupo 4 foi mantido como grupo controle e os grupos 1, 2 e 3 foram tratados com AAS, respectivamente, nas doses de 300, 600 e 1200 mg/L de água de bebida, durante 21 dias. Foram realizadas três amostragens nos dias 21, 28 e 35 pós-tratamento. O peso vivo corpóreo e da carcaça foi registrado em cada amostragem. Em todos os órgãos viscerais foi analisada a presença de alterações patológicas. As amostras de soro sanguíneo foram colhidas para a avaliação bioquímica. O exame histopatológico de todos os órgãos viscerais foi realizado para a observação de alterações microscópicas. A partir da segunda amostragem foi observado um aumento significativo ($p < 0,05$) no peso corpóreo na dosagem de 300mg/L. Um significativo aumento

no peso relativo dos órgãos foi registrado na dosagem de 1200 mg/L. Os grupos tratados com dosagens de 600 e 1200 mg/L de AAS, apresentaram aumento significativo dos níveis de AST, ALT e de creatinina quando comparados ao grupo controle. O grupo tratado com 1200 mg/L de AAS apresentou um aumento significativo ($P,0,05$) de uréia, proteína sérica total e de albumina em todas as amostragens. No grupo de alta dosagem foram observadas alterações histopatológicas constituídas por aumento dos hepatócitos, aumento dos espaços sinusoidais no fígado, congestão e anormalidades nos espaços glomerulares nos rins, congestão e ruptura alveolar nos pulmões, degeneração das vilosidades e celular nos intestinos. A conclusão obtida foi que em frangos de corte uma baixa dosagem do AAS pode ser utilizada por um período de longa duração, apresentando um efeito promotor do crescimento, contudo as doses elevadas determinam toxicidade hepática e renal.

Palavras-chave: Ácido acetil salicílico. Frangos de corte. Histopatologia. Avaliação bioquímica e toxicidade

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Introduction

The poultry industry has played a vibrant role in the economy of Pakistan to compensate for the protein demand. Since 1960, commercial poultry farming has flourished in Pakistan and grew rapidly over the years (Hussain et al., 2015). About 1.5 million people in Pakistan are currently engaged in this sector. The overall contribution of poultry farming in the livestock sector is 12.7%, whereas it contributes 7.5% to agriculture (Pakistan, 2018).

Acetylsalicylic acid (ASA) was discovered about 2 centuries earlier from plants and is considered the drug of choice for the treatment of different conditions (Thomas et al., 1966). ASA is commonly used as an analgesic for the treatment of different abnormal conditions in poultry as well as in livestock (De-Caterina & Renda, 2012). ASA as an anti-cancerous chattel has also been reported (Rothwell et al., 2012). Non-steroidal anti-inflammatory drugs (NSAIDs) also have characteristics to overcome the effects of myocardial infarction. (Solomon et al., 2002). Heat shock proteins (Hsps) also correlate to non-steroidal anti-inflammatory

drugs. In most cases, heat stress factors cause cell injury. In this situation, ASA has a role in avoiding myocardial injury by controlling the heat shock protein expression (Ghavami et al., 2002).

The most common use of ASA is to mask fever and general illness conditions. The liver converted the deacetylated form into the active form of salicylic acid (Amann & Peskar, 2002). The synthesis of prostaglandin is reduced through cyclooxygenase inhibition by salicylic acid (Ohdo et al., 1995). In the case of broiler birds, a 0.05% dose rate of aspirin in feed is sufficient to decrease the body temperature of broiler birds up to 3°C when the birds are reared from 4 to 8 weeks (Adams & Rogler, 1968). An amount of 1000 mg/kg of ASA helps to reduce the heat stress in broiler birds for 3 weeks peroral route, but this amount is not sufficient to improve the body weight (Freeman et al., 1983).

Considering these facts, it is necessary to evaluate the beneficial and toxic effects of ASA at different dose levels in broiler birds. To evaluate these toxic and beneficial effects, though, it is necessary to examine the physical and serum biochemical parameters as well as the histopathological changes with tissue sections taken from broiler birds under experimental trial.

Materials and Methods

A total of 60 one-day-old broiler chicks (cobb strain) were purchased from a commercially available hatchery and reared at the faculty's experimental poultry shed at the University of Agriculture Faisalabad. Before the arrival of chicks, the shed was disinfected and fumigated. Hatchery-vaccinated day-old chicks were then transferred to the shed in an optimal environment. This trial was conducted in the winter season but the birds were in a controlled environment where the temperature was adjusted to their body requirement according to their age. The drinking water intake was almost double the intake of feed and the birds were given feed ad libitum. The locally recommended vaccination schedule was followed to prevent infectious diseases. Birds were divided into 4 different groups with

15 birds in each group on the 15th day, i.e., group 1 (300 mg/L acetylsalicylic acids), group 2 (600 mg/L acetylsalicylic acids), group 3 (1200 mg/L acetylsalicylic acid) were treatment groups and group 4 was control group. These doses were made with salicylic acid and administered in drinking water. Water and ASA consumption are presented in Table 1.

The basal diet was offered along with different doses of the ASA in drinking water for 21 days, continuously. The total duration of the trial was 35 days.

There were 3 slaughters planned. Five birds were slaughtered on the 7th, 14th, and 21 days post-treatment. The live body weight and carcass weight were recorded on every sampling. Relative weights of all visceral organs were also noted. After slaughtering, the gross lesions were noted and the organ samples (liver, kidney, spleen, lungs, intestine, heart, bursa, and thymus) were collected for histopathology in 10% neutral buffered formalin. The standard protocol of fixation, dehydration, cleaning, embedding, cutting, and staining was adopted for the processing of tissues (Bancroft & Gamble, 2007). Blood samples were collected and serum was separated to perform the serum biochemical parameters. Serum biochemical parameters (ALT, AST, urea, creatinine, total proteins, albumin, and globulin) were examined through a commercially available kit of Merk Company*.

Results

Live and carcass weight

In the group treated with ASA, the dose of 300 mg/L showed an increase ($P<0.05$) in live body weight after the first 2 samplings, while all other treatment groups showed a non-significant difference. Carcass weight of birds showed a non-significant difference in all treatment groups than that of control groups after all samplings (Table 2).

Relative organ weight

The relative weight of the liver was increased ($P<0.05$) in birds treated with 1200 mg/L than that of the control group

in sampling 1, while in sampling 2, the group treated with ASA the dose of 600 and 900 mg/L showed an increased ($P<0.05$) relative liver weight from that of the control group (Table 2).

In sampling 1, the group treated with ASA with the dose of 600 and 1200 mg/L showed increased ($P<0.05$) relative kidney weight from that of the control group, while in sampling 2, the group treated with ASA 1200 mg/L showed an increased ($P<0.05$) relative kidney weight.

The group treated with 1200 mg/L of ASA showed increased ($P<0.05$) relative heart weight at 2nd sampling.

The relative weight of the intestine and bursa showed a non-significant difference from that of the control group in all the 3 samplings.

In sampling 1, the relative weight of the lungs was increased ($P<0.05$) from that of the control group in all the treatment groups, while in sampling 2, only the group treated with 1200 mg/L of ASA showed an increase ($P<0.05$) relative weight of lungs (Table 2).

In sampling, I, the group treated with 1200 mg/L of ASA showed increased ($P<0.05$) relative spleen and thymus weight from that of the control group.

Serum biochemistry

In sampling 1, the groups treated with ASA 600 and 1200 mg/L showed increased ($P<0.05$) AST levels from that of the control group. While in sampling 2 and 3, the group treated with ASA the dose of 1200 mg/L showed an increased ($P<0.05$) AST level from that of the control group.

The ALT and creatinine levels were increased ($P<0.05$) in the groups treated with ASA at the dose of 600 and 1200 mg/L from that of the control group in all the samplings (Table 3).

The group treated with 1200 mg/L of ASA showed increased ($P<0.05$) urea, serum total protein, and albumin level in all the samplings (Table 3).

In sampling 1, all the treated groups showed increased ($P<0.05$) globulins levels from that of the control group.

Table 1 – Daily consumption of acetylsalicylic acid (ASA) administered in drinking water by broilers^a for 3 consecutive weeks

Groups	Consumption of ASA per day by birds in drinking water		
	1 st week	2 nd week	3 rd week
300mg/L ASA	20mg	40mg	60mg
600 mg/L ASA	40mg	80mg	120mg
1200 mg/L ASA	80mg	160mg	240mg
0 mg/L ASA	—	—	—

^a: The number of birds in each group was 15. ASA =acetylsalicylic acid.

Table 2 – Live, carcass, and relative organ weight (mean±S.D) of birds treated with three different doses of acetylsalicylic acid (300, 600, and 1200 mg/L DW)

Groups		Group 1 (Control)	Group 2 (300mg/L)	Group 3 (600mg/L)	Group 4 (1200mg/L)	
Live body weight	S1	919.50 ± 31.96	1076.67±25.17*	1015.00±157.955	861.25±40.73	
	S2	1050.00±10.00	1322.67±80.75*	1133.50±121.612	997.50±140.13	
	S3	1346.00±22.71	1461.00±58.02	1255.67±80.311	1170.00±170.00	
Carcass weight	S1	720.66±46.71	758.00±84.071	699.33 ± 172.86	618.75±37.87	
	S2	835.66±36.83	882.00±32.924	819.50 ± 119.14	739.00±88.67	
	S3	930.50±37.17	1033.67±30.97	876.33 ± 127.64	818.00±127.42	
Body organ weight						
	Liver	S1	2.69 ± 0.139	2.85 ± 0.37	2.51 ± 0.47	3.80 ± 0.11*
		S2	3.05 ± 0.146	3.16 ± 0.22	3.82 ± 0.326*	3.94 ± 0.56*
S3		3.00 ± 0.081	3.30 ± 0.17	3.33 ± 0.546	3.28 ± 0.44	
Kidney	S1	0.66 ± 0.23	0.62 ± 0.08	0.41 ± 0.05*	0.87 ± 0.10*	
	S2	0.68 ± 0.01	0.57 ± 0.03	0.65 ± 0.09	0.98 ± 0.15*	
	S3	0.61 ± 0.02	0.68 ± 0.04	0.66 ± 0.05	0.65 ± 0.08	
Heart	S1	0.58 ± 0.08	0.56 ± 0.04	0.50 ± 0.06	0.54 ± 0.06	
	S2	0.55 ± 0.05	0.65 ± 0.02	0.64 ± 0.04	0.79 ± 0.12*	
	S3	0.64 ± 0.09	0.49 ± 0.09	0.54 ± 0.06	0.55 ± 0.08	
Intestine	S1	6.34 ± 0.56	4.11 ± 0.10	4.71 ± 0.54	5.49 ± 0.43	
	S2	6.27 ± 0.09	4.71 ± 0.22	5.84 ± 0.72	6.60 ± 0.86	
	S3	7.04 ± 0.55	6.47 ± 0.73	6.71 ± 0.32	5.98 ± 0.89	
Lungs	S1	0.25 ± 0.05	0.50 ± 0.02*	0.62 ± 0.08*	0.73 ± 0.01*	
	S2	0.49 ± 0.13	0.50 ± 0.02	0.51 ± 0.53	0.68 ± 0.13*	
	S3	0.50 ± 0.04	0.47 ± 0.03	0.52 ± 0.02	0.50 ± 0.063	
Spleen	S1	0.04 ± 0.01	0.08 ± 0.01	0.08 ± 0.03	0.12 ± 0.05*	
	S2	0.14 ± 0.04	0.13 ± 0.04	0.15 ± 0.03	0.16 ± 0.04	
	S3	0.12 ± 0.04	0.10 ± 0.02	0.10 ± 0.01	0.15 ± 0.012	
Thymus	S1	0.25 ± 0.01	0.26 ± 0.01	0.31 ± 0.03	0.40 ± 0.06*	
	S2	0.24 ± 0.03	0.30 ± 0.03	0.22 ± 0.04	0.19 ± 0.02	
	S3	0.19 ± 0.01	0.18 ± 0.42	0.27 ± 0.01	0.26 ± 0.09	
Bursa	S1	0.19 ± 0.04	0.15 ± 0.02	0.16 ± 0.03	0.19 ± 0.02	
	S2	0.12 ± 0.01	0.13 ± 0.01	0.10 ± 0.17	0.12 ± 0.01	
	S3	0.14 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.14 ± 0.01	

The values with asterisk showed significant differences from the control group. These asterisks are added with the comparison of control group as it has shown significant changes to the control group.

Table 3 – Serum biochemistry (mean±S.D) of birds treated with three different doses of (300, acetylsalicylic acid (600 and 1200 mg/L DW)

Groups		Group 1 (Control)	Group 2 (300mg/L)	Group 3 (600mg/L)	Group 4 (1200mg/L)
AST	S1	140.50 ± 8.34	133.33 ± 6.50	169.50 ± 8.09*	189.75 ± 6.50*
	S2	142.66 ± 3.05	143.66 ± 7.75	145.50 ± 6.19	195.01 ± 7.88*
	S3	141.01±15.84	139.50±6.28	142.75 ± 5.60	191.50 ± 6.86*
ALT	S1	2.17 ± 0.12	2.40 ± 0.36	7.30 ± 0.46*	12.35 ± 0.23*
	S2	2.26 ± 0.15	2.01 ± 0.10	6.35 ± 0.23*	12.90 ± 0.35*
	S3	3.10 ± 0.20	2.92 ± 0.46	8.77 ± 1.49*	11.05 ± 0.46*
Urea	S1	25.50 ± 3.67	24.3 ± 6.66	25.75 ± 4.73	40.25 ± 3.57*
	S2	26.33 ± 3.05	24.33 ± 4.93	24.75 ± 3.66	47.75 ± 7.17*
	S3	27.25 ± 5.78	31.25 ± 9.87	28.25 ± 7.84	44.01 ± 2.01*
Creatinine	S1	0.21 ± 0.04	0.26 ± 0.06	1.23 ± 0.09*	1.78 ± 0.08*
	S2	0.21 ± 0.02	0.21 ± 0.03	1.20 ± 0.02*	1.92 ± 0.01*
	S3	0.22 ± 0.03	0.25 ± 0.06	1.23 ± 0.02*	1.84 ± 0.04*
Total Proteins	S1	3.32 ± 0.09	3.51 ± 0.22	3.87 ± 0.47	5.28 ± 0.26*
	S2	4.11 ± 0.25	3.54 ± 0.19	4.08 ± 0.49	5.09 ± 0.13*
	S3	3.60 ± 0.73	3.49 ± 0.31	3.38 ± 0.61	5.64 ± 0.43*
Albumin	S1	2.91 ± 0.29	2.42 ± 0.22	2.60 ± 0.59	3.87 ± 0.17*
	S2	3.06 ± 0.20	2.46 ± 0.17	2.89 ± 0.49	3.93 ± 0.15*
	S3	2.39 ± 0.59	2.27 ± 0.30	2.25 ± 0.71	3.76 ± 0.25*
Globulin	S1	0.40 ± 0.21	1.09 ± 0.04*	1.27 ± 0.16*	1.41 ± 0.421*
	S2	1.04 ± 0.08	1.08 ± 0.03	1.18 ± 0.05	1.16 ± 0.09
	S3	1.21 ± 0.29	1.22 ± 0.22	1.12 ± 0.14	1.88 ± 0.67

The values with asterisk showed a significant difference from the control group. These asterisks are added with the comparison of control group as it has shown significant changes to the control group.

Histopathological results

Liver

At 300 mg/L dosage, no typical lesions were recorded (Figure 1) in contrast to 600 mg/L dosages, where hepatocytes exhibited a mild type of degeneration. In the case of 1200 mg/L dosage, hepatocytes were swollen with dense nuclei, and sinusoidal spaces were found in a few places (Figure 2).

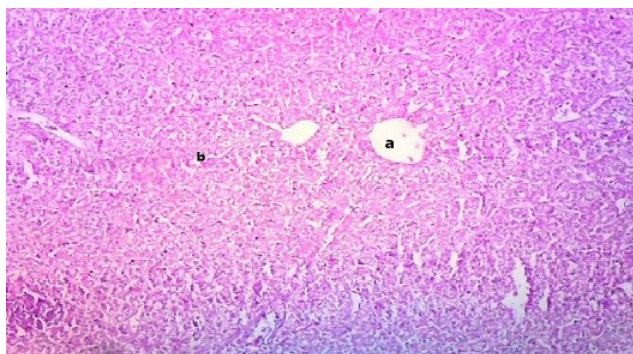


Figure 1 – (a) Histopathological pictures of the liver showed normal central vein and (b) normal sinusoidal spaces, of birds treated with acetylsalicylic acid (300 mg/L).

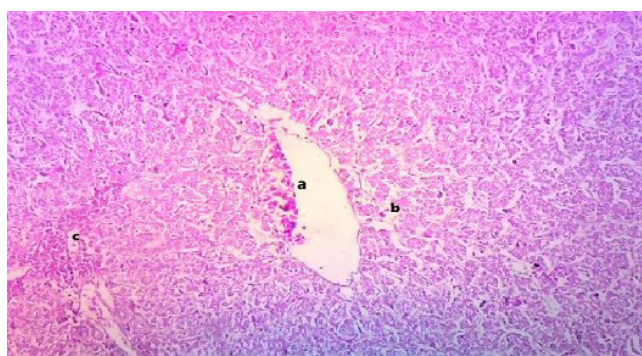


Figure 2 – (a) Histopathological pictures of the liver showed mild congestion (b) along with hepatocytes degeneration, in birds treated with acetylsalicylic acid 1200 mg/L.

Kidney

No typical lesions were seen in the case of 300 mg/L and 600 mg/L of ASA in the kidney (Figure 3). Meanwhile, in the case of 1200 mg/L dose level, the epithelium had mild congestion, and abnormal glomerular spaces were noted (Figure 4).

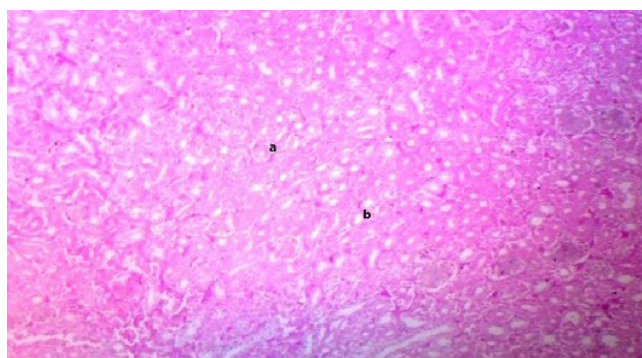


Figure 3 – (a) Histopathological picture of the kidney showed normal glomerulus in bowman capsule and (b) normal glomerular spaces, of birds treated with acetylsalicylic acid 300 and 600 mg/L.

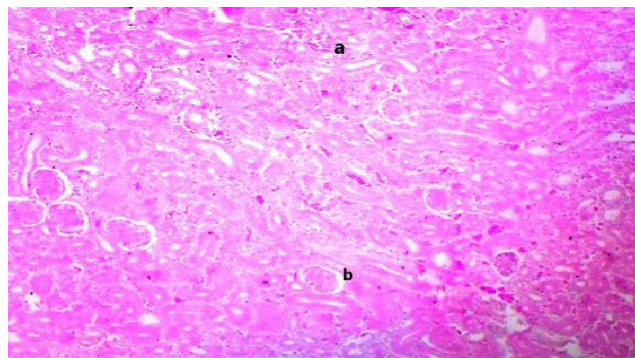


Figure 4 – (a) Histopathological picture of the kidney showed congestion and degeneration of glomerulus (b) and abnormal glomerular spaces in bowman capsule of birds treated with acetylsalicylic acid 1200 mg/L.

Lungs

The structure of the lungs was quite normal in the case of 300 mg/L and 600 mg/L doses of ASA (Figure 5). In the case of 1200 mg/L level, the lung's parenchyma indicated the lesions of congestion at most places with disrupted alveolar spaces (Figure 6).

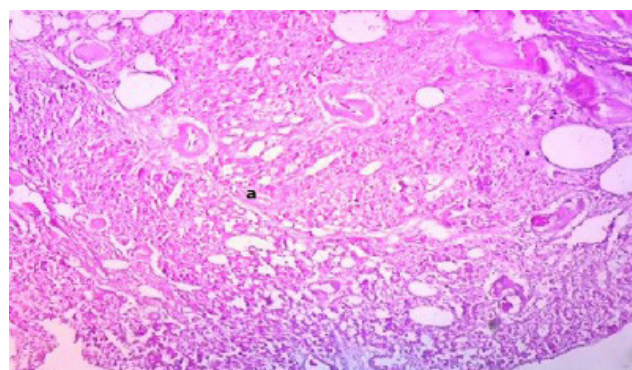


Figure 5 – (a) Histopathological picture of lungs showed normal alveolar spaces of birds treated with acetylsalicylic acid 300 and 600 mg/L.

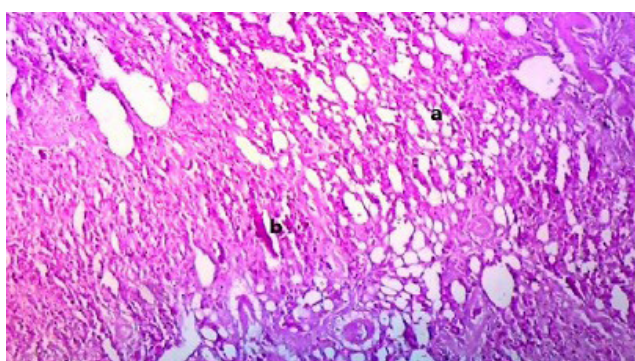


Figure 6 – (a) Histopathological picture of lungs showed disrupted alveolar spaces (b) along with mild congestion areas of birds treated with acetylsalicylic acid 1200 mg/L.

Intestine

The intestine was affected with ASA at all dose levels (300, 600, and 1200 mg/L). At the 300 mg/L dose level, intestinal cells and villi were disrupted in a few places (Figure 7). Degeneration

of villi was also found, while the mucosa, submucosa, and muscularis were normal. At 600 mg/L dose, a mild type of cellular degeneration was found, yet the mucosa, submucosa, and muscularis were normal. The intestine was severely affected in the case of the 1200 mg/L dose level (Figure 8). The intestinal epithelium was disrupted in a few places. The villi were also damaged and had not reached their full length in a few places.

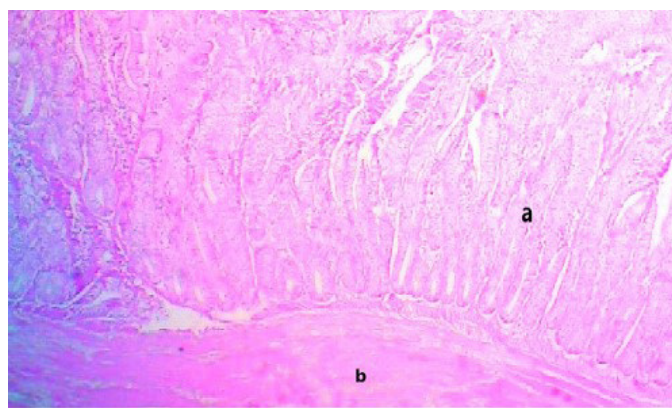


Figure 7 – (a) Histopathological picture of intestine showed normal villi length and (b) villus epithelium of birds treated with acetylsalicylic acid 300 mg/L.

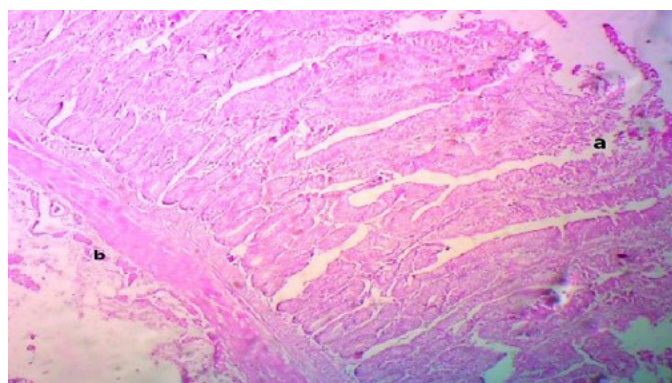


Figure 8 – (a) Histopathological picture of intestine showed disrupted and degenerated villi of the intestine (b) along with distorted epithelium of birds treated with acetylsalicylic acid 1200 mg/L.

Discussion

ASA is used in poultry for the treatment of respiratory and digestive problems in humans and animals (Balog & Hester, 1991). No side effects of ASA treatment have been reported in birds (Pożniak et al., 2010). The present study was conducted to evaluate the effect of 3 different levels of ASA in broiler birds. The treatment was carried out for 3 weeks. We then observed gross pathological, histopathological, and serum biochemical changes in the birds at 7th, 14th, and 21-days post-treatment and compared results with the control group.

A significant ($P < 0.05$) increase in live body weight at 300 mg/L doses of ASA was recorded in the control group on the 7th and 14th days post-treatment. The significant increase in live weight in the ASA dose of 300 mg/L treated group after the first 2 samplings indicated a growth promotory effect, while the difference during the last week became non-significant. Al-Obaidi & Al-Shadeedi (2010) reported that 0.2% ASA in broiler diets improved live body weight. Similarly, Al-Mashhadani et al. (1988) also observed a significant increase in the live body weight of broilers and the dose of 100 mg/kg of aspirin in feed. However, the present study results indicated a non-significant difference in all the treatment groups in carcass weight from that of the control group in all samplings.

Relative liver and kidney weight of ASA at the dose of 600 and 1200 mg/L was significantly increased at the 7th and 14th days post-treatment from that of the control group. Pożniak et al. (2010) have also reported that the liver and kidney were affected when they administered ASA at the dose of 200 mg/kg for 20 days. Pandey et al. (2015) also reported the hypertrophy of the kidney by the use of ASA at the dose of 4000 ppm in the feed of broiler birds for 21 consecutive days. The relative weight of the heart was significantly increased ($P < 0.05$) in the 1200 mg/L dose group at 14 days post-treatment. On the 7th day post-treatment, the relative weight of lungs was also significantly increased in 300 and 1200 mg/L treated groups, while on the 14th-day post-treatment, only the 1200 mg/L treated group showed significantly increased relative lung weight. The relative organ weight of the spleen and thymus showed significantly increased weight on the 7th-day post-treatment. The relative weight of most of the visceral organs (liver, kidney, heart, spleen, and bursa) was increased when given the ASA dose of 600 and 1200 mg/L during all the samplings. These results indicated that at the 300 mg/L dose, the organ's weight increased because of the increased metabolic activity, while the decreased relative weight in the 1200 mg/L treated group may have been due to the pathological changes. Studies related to toxic effects on relative organ weight in broilers were rarely published.

The level of AST was higher ($P < 0.05$) in 600 and 1200 mg/L treated groups on the 7th-day post-treatment, while on the 14th and 21-days post-treatment, only the 1200 mg/L group showed an increased ($P < 0.05$) AST level. The ALT level was also high in both 600 and 1200 mg/L in all samplings. The higher level of ALT and AST were attributed to the histopathological changes present in the liver that include congestion, cell swelling, necrosis, and other degenerative changes.

The level of urea was also increased ($P < 0.05$) in a 1200 mg/L treated group, while the creatinine level was higher ($P < 0.05$) in both 600 and 1200 mg/L treated groups in all samplings. The histopathological findings at 300 mg/L showed a normal appearance of the liver and kidney as their ALT, AST, urea, and creatinine levels also support their non-toxic effect. The increase in levels of urea and creatinine was attributed to kidney damage. The histopathological changes in the kidney support each other as they showed congestion along with abnormal glomerular space and degenerative changes. The serum total proteins and albumin levels were increased ($P < 0.05$) in ASA at the dose of 600 and 1200 mg/L as compared to the control group after 21 days post-treatment. The level of globulin was significantly increased in all the treatment groups after the 7th-day post-treatment. Histopathological change in immune organs showed lymphocytic depletion along with bursal and thymic atrophy. In the group treated with ASA, the dose of 1200 mg/L showed severe congestion in the spleen and thymus of the birds. There was no significant change recorded in the group treated with ASA at the dose of 300 mg/L.

The group treated with ASA with the dose of 300 mg/L showed mild congestion, while the group treated with 600 mg/L

showed moderate to severely congested areas along with villus atrophy. The group treated with 1200 mg/L showed severe histopathological changes that include congestion, degenerative changes in the intestine, and atrophied villus structure. The lungs section showed a normal appearance in 300 and 600 mg/L treated groups under the microscope, while the 1200 mg/L treated group showed disruption of alveolar structure.

Conclusion

The results of the present study suggested that ASA in the daily dose of 300 mg/L can be used continuously for 21 days (for a short period) as it can give a beneficial effect on body weight as well as carcass weight, while the higher levels may be used continuously but can be harmful to growth and organs.

Conflict of Interest

There is no conflict of interest

Ethics Statement

This research is approved by ethical committee of University of Agriculture, Faisalabad. This research is not against the animal rights

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