

Pathological, Bacteriological and Biochemical Studies on the Effect of Formic Acid in Broiler Chickens

Ezzat H. Abo El -Fetouh^{1*}, Nesseran A. Shawky², Ghada M. El Khider², Hassan A. Ali³ and El Sayed Mansour⁴

¹Pathology Department, Animal Health Research Institute, Zagazig Branch

²Chemistry Department, Animal Health Research Institute, Zagazig Branch

³Bacteriology Department, Animal Health Research Institute, Damanhour Branch

⁴Bacteriology Department, Animal Health Research Institute, Zagazig Branch

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Abstract

A total of 100 apparently healthy Hubbard chicks, one day of age were used in this study to investigate the effect of formic acid on body performance. Biochemical and histopathological changes beside its effect on intestinal bacterial growth in broiler were also studied. Cloacal swabs were collected from all chicks for bacteriological examination at day one of age. Eighteen chicks were positive and the distribution of the bacterial agents was 13 for single infection (*E. coli*, *Corynebacterium* species and *Salmonella* species) and 5 in case of mixed infection (*Streptococcus*, *Corynebacterium* species, *Staphylococcus aureus* and *E. coli*). Serological identification of the isolated *E. coli* revealed O78 (4) and O157 (2), while, the obtained *Salmonella* serotypes were *S. Typhimurium* (3) and *S. Enteritidis* (1). Sixty bacterial free chicks were divided into 3 groups (20 birds, each), 1st group served as control. The 2nd and 3rd groups received 1 ml and 2 ml formic acid/ liter drinking water, respectively for 30days. Formic acid in both doses induced a significant increase in body weight gain, total proteins, albumin and globulins coupled with significant decrease in total lipids, cholesterol and triglyceride. Meanwhile, A/G Ratio calcium, phosphorous, magnesium, zinc, sodium and potassium insignificantly increased beside insignificant decrease in liver enzymes (AST, ALT and ALP) uric acid, creatinine and intestinal bacterial content as well as improved the feed consumption and feed conversion rate. Histopathologically, spleen and bursa showed hyperplasia of lymphocytes in white pulp. Hepatic tissue particularly the 3rd group had mild fatty changes and hydropic degeneration. Also renal tubules of undergo mild hydropic degeneration. In conclusion the use of formic acid as feed additive in chicken broiler ration may act as growth promoter and exhibits positive impact on biochemical parameters, intestinal and immune organs histology beside reduction of colonization of bacteria in intestinal wall.

Keywords: Broiler, Formic Acid, Bacteriology, Pathology, Biochemistry

Introduction

Poultry industry is one of the most important sources of protein all over the world [1]. Feed additives induce high growth and efficient feed conversion [2]. Antibiotic growth promoters and antibiotic resistance are clearly connected and increased concern of researchers to use other alternatives like organic acids as feed additives in poultry production [3].

Organic acids have a long history of being utilized as food additives to prevent food

deterioration and extend the shelf life of perishable food ingredients [4]. They are used in poultry diets to elicit a positive response in body growth [5] and as alternative for antibiotic growth promoters [6].

This study aimed to investigate the influence of formic acid on body performance, biochemical parameters beside its pathological effect. Also the changes in populations of bacteria inhabiting the gastrointestinal tract of broiler chickens were investigated.

*Corresponding author e-mail: (ezzataboelfetoh@y.mail.com), Pathology Department, Animal Health Research Institute, Zagazig Branch

Material and Methods

Birds

A total of 100 apparently healthy one day-old Hubbard broiler chicks nearly equal in the live body weight (45.22-48.10 gm) were used in this study. Cloacal swabs were collected from each chick for bacteriological examination.

Experimental design

Post bacteriological examination, 60 chicks free from any bacterial infection were chosen and divided into 3 groups (20 chicks each). The first group served as control group, while, 2nd group received 1 ml formic acid/ liter drinking water and 3rd group received 2 ml formic acid/ liter drinking water for 30 days (from 1st day of age up to 30th day of age)

Body weight

Chicks were individually weighed at the beginning of the experiment and then at 1st day post supplementation for determination of the body weight gain and feed conversion ratio.

Bacteriological examination

At 1st day post treatment, five chicks from each group were slaughtered and the intestine was exposed ligated at both sides and its contents were taken aseptically. One gram of caecal content was suspended in a tube containing sterile 0.9% normal saline solution (1:1). Then the solutions were mixed on vortex. Serial dilutions of samples were made up to 6th dilution. 0.1 ml of each dilution was poured and spread uniformly on nutrient agar, for total bacterial count and MacConkey's agar for caecal coliform count. All plates were incubated at 37°C for 48 hrs. Colonies were counted by pour plate method [7].

Serological identification

Antisera of for the serotyping of *E. coli* isolates were used for the identification of somatic antigen "O" using slide agglutination test [8].

Serological identification of the isolated strains of *Salmonella* was performed using slide agglutination for identification of somatic

antigen while flagellar antigen was identified by tube agglutination test [8].

Blood samples

At 1st, 7th and 14th day post supplementation, five chicks from each group were slaughtered and blood samples were taken to obtain clear serum for the estimation of the total protein [9] albumin [10] the globulin was calculated as difference between total protein and albumin, total lipid [11], triglyceride [12] cholesterol [13], transaminases (AST and ALT) [14], ALP [15] Uric acid [16] creatinine [17], calcium [18], inorganic phosphorus [19], sodium and potassium [20] and zinc [21].

Pathological examination

Specimens were taken from the internal organs of the sacrificed chicks and fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared and stained with hematoxylin and eosin and examined microscopically [22].

Statistical analysis

The data were analyzed using PASW Statistics (SPSS version 18.0 for Windows [23]). The statistical analysis was performed by analysis of variance (ANOVA) with the fixed effect of Formic acid supplementation and the other investigated parameters as dependent variables. Bacteriological data were transformed to Log₁₀ estimates before further analysis. Duncan's multiple range tests were used for comparing the means.

Results

Bacteriological and biochemical results are summarized in Tables (1-4). The formic acid addition to the 2nd group, intestine showing finger like villi with normal structure that increase in height (Figure1A), but birds of 3rd group had thickening in the columnar epithelium and abundant goblet cells (Figure 1B). Bursa of fabricus in 2nd group showed narrowing of interstitial connective tissue, beside mild hyperplasia in lymphocytes (Figure1C). In 3rd group, the lymphoid tissue of bursa undergone moderate to severe hyperplasia (Figure1D), besides more

narrowing in the interstitial connective tissue. Spleen in 2nd group showed severe hyperplasia in both red and white pulps (Figure1E), liver in 3rd group had mild vacuolar degeneration and sporadic areas of fatty changes (Figure1F), with heterophilic infiltration and hydropic degeneration (Figure1G). In 3rd group, showing

degenerative changes, round cells proliferations, severe fatty change, hemorrhages and coagulative necrosis (Figure1H). Kidneys in 3rd group with more vacuolar and hydropic degeneration of the renal tubules (Figure1I).

Table 1: The proportion of bacterial agents isolated from cloacal swabs of apparently healthy one day-old Hubbard broiler chicks (n=100)

+ ve swabs No.	%	Type	Isolates				Serological identification of isolated <i>E. coli</i> and Salmonellae						
			No	%	Isolated organisms	No	%	<i>E. coli</i> (6)			<i>Salmonella</i> (3)		
								Sero group	No	%	Serogroup	No	%
18	18	Single isolates	13	72.22	<i>E. coli</i>	6	46.15	O78	4	66.67	<i>S. Typhimurium</i>	2	75
					<i>Corynebacterium</i> species	4	30.77	O157	2	33.33	<i>S. Enteritidis.</i>	1	25
					<i>Salmonella</i> species.	3	23.08	Total	6	100		3	100
		Mixed isolates	5	27.78	<i>Streptococcus</i> + <i>Corynebacterium</i> species	2	40						
					<i>Staphylococcus aureus</i> + <i>E. coli</i>	3	60						

Table 2: The effect of formic acid in Microbial balance (log10 CFU/g) in gastrointestinal tract and body performance of Hubbard broiler chickens (n=5)

Groups	Microbial balance (log10 cfu/g) in gastrointestinal tract			Initial body weight	Final body weight	Weight gain	FC	FCR
	Total count	Coliform	Lactobacillus					
	Healthy broiler	8.10±0.92	5.57±0.88					
formic acid 1ml	7.15±0.77	4.59±0.63	2.67±0.18	48.10±1.41	1234.12±3.87**	1186.02±4.07*	1921.74	1.62
acid 2ml	7.04±0.89	4.26±0.58	2.52±0.15	45.32±1.30	1241.95±4.94**	1196.63±6.32**	1928.81	1.61

FC=feed consumption FCR= Feed Conversion rate * Significant at P < 0.05 ** Significant at P < 0.001

Table 3: The effect of formic acid on biochemical parameters of chicken broilers (n=5)

Groups	Liver function							lipid profile				
	T.Protein	Protein profile\ (g/dl)		A/G \ratio	liver enzymes (U/L)			Total lipid (mg/dl)	Cholesterol(mg/dl)	Triglyceride (g/dl)		
Albumin	Globulin	AST	ALT		ALP							
Healthy broiler	5.39±0.31	2.90±0.26	2.49±0.22	1.16±0.18	65.19±3.16	46.04±1.21	27.28±0.84	205.53±2.81	107.17±1.53	112.16±1.13		
formic acid	1ml	1 st day	7.06±0.57*	3.95±0.31*	3.11±0.16*	1.27±0.11	62.54±2.89	44.28±1.48	25.84±0.90	196.05±2.98*	101.26±1.82*	107.20±1.43*
		7 th day	6.78±0.19*	3.70±0.21*	3.08±0.12*	1.20±0.15	63.85±1.65	45.78±1.64	26.04±0.69	197.89±1.18*	102.03±1.12*	108.55±1.04*
		14 th day	5.41±0.22	3.01±0.44	2.40±0.14	1.25±0.16	64.95±1.31	45.97±1.38	27.15±0.89	204.21±2.28	106.05±1.85	111.49±1.83
		1 st day	7.11±0.61*	3.89±0.30*	3.22±0.22*	1.20±0.10	62.94±1.95	45.06±1.56	26.17±0.41	196.13±1.66*	101.32±1.91*	106.95±1.90*
	2ml	7 th day	6.79±0.41*	3.58±0.15*	3.21±0.16*	1.12±0.17	64.10±2.43	45.94±1.82	26.32±0.55	202.69±2.14	103.10±1.16*	108.05±1.06*
		14 th day	5.36±0.28	3.05±0.36	2.31±0.19	1.32±0.12	65.75±2.72	46.16±1.53	27.04±0.68	203.32±2.16	106.24±1.78	113.32±1.21

Table 4: The effect of formic acid in some minerals of chicken broilers (n=5)

Groups	Uric acid(mg/dl)	creatinine(mg/dl)	Ca(mg/dl)	Ph(mg/dl)	Mg(g/dl)	Na(mmol/L)	K(mmol/L)	Zinc(Ug/ml)		
Healthy broiler	5.64±0.44	1.83±0.38	8.78±0.32	5.48±0.62	3.75±0.21	141.60±1.37	4.15±0.49	147.07±7,13		
formic acid	1ml	1 st day	5.25±0.32	1.64±0.21	9.16±0.47	6.19±0.31	3.99±0.44	144.16±1.94	4.97±0.70	154.20±9,50
		7 th day	5.43±0.22	1.70±0.17	8.96±0.34	5.27±0.49	3.89±0.28	142.73±1.84	4.82±0.44	151.42±8,32
		14 th day	5.69±0.24	1.85±0.37	8.80±0.29	5.50±0.22	3.70±0.50	141.63±1.49	4.18±0.28	148.31±9,55
		1 st day	5.16±0.18	1.70±0.21	9.25±0.31	6.21±0.36	4.06±0.37	144.47±1.69	4.74±0.63	152.50±8,96
	2ml	7 th day	5.44±0.21	1.79±0.19	9.11±0.28	6.14±0.42	3.71±0.39	142.09±1.63	4.60±0.63	147.31±5,18
		14 th day	5.55±0.30	1.81±0.15	8.95±0.45	5.43±0.55	3.65±0.47	141.41±1.78	4.20±0.72	146.95±6,92

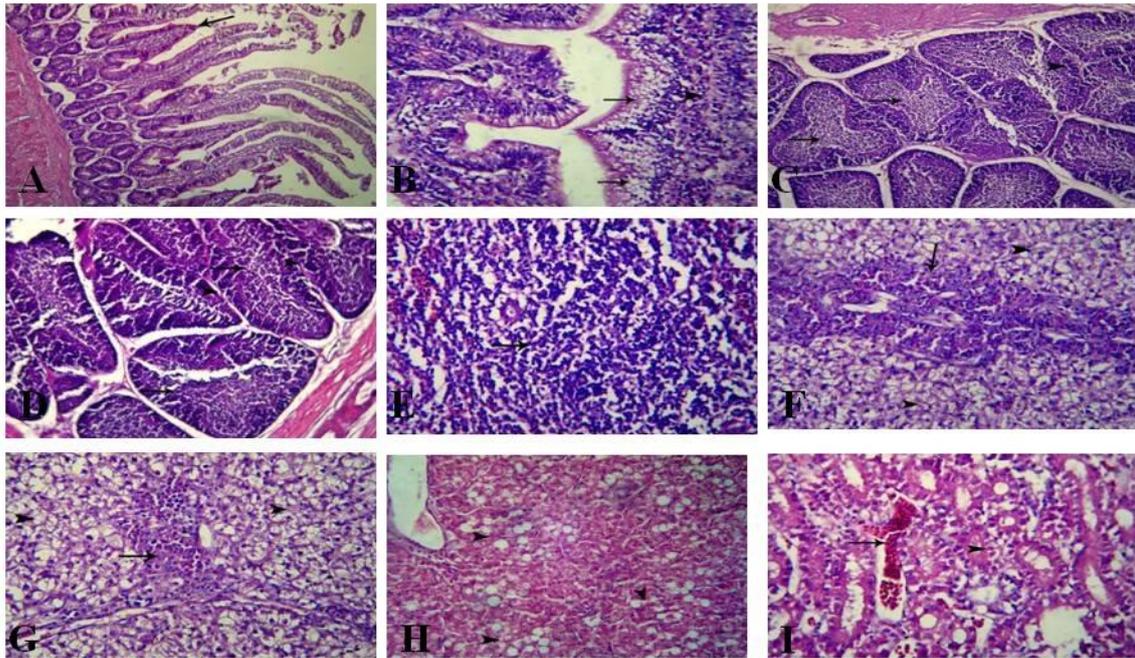


Figure 1: The pathological findings in the tissues of broiler chickens received formic acid in their ration: A) Small intestine of chickens, 2nd group show finger like villi with elongation (H&EX 200); **B)** thickening in the lining epithelium and abundant goblet of small intestine of chickens, 3rd group (H&EX 400); **C)** Bursa of fabricus of chickens 2nd group show mild hyperplasia (H&EX 200); **D)** Bursa of fabricus of chickens 3rd group, had hyperplasia (H&EX 200); **E)** Spleen of chickens, 3rd group, with severe hyperplasia (H&E X 400); **F)** liver of chickens, 2nd group, show heterophilic infiltration, vacuolar degeneration and mild fatty changes (H&EX 200) group, showed heterophilic infiltration, vacuolar degeneration and mild fatty changes (H&EX 200); **G)**, 2nd group, hydropic degeneration, fatty change and heterophilic infiltration in liver of chickens (H&E X 400); **H)** liver of chickens, 3rd group, fatty changes (H&E X 400) and **I)** Kidneys of chickens, 2nd group, with vacuolar degeneration & congestion (H&EX 400).

Discussion

Broiler chickens supplemented with formic acid in the drinking water with both doses showed a significant increase in body weight gain and improved feed conversion rate. Our results were in conformity with those previously reported in broilers received formic acid [24-25]. Acidified increased body weight gain [26]. Growth promoting effect of formic acid may be due to its positive effect on digestion by inducing a slower passage of feed in the intestinal tract, a more efficient absorption of the necessary nutrients [27]. Also the improved body weight gain was explained previously by the decrease in the number of pathogenic bacteria in small intestine [28] and the beneficial effect of acidifiers on gut flora [29].

Our findings revealed that, broilers

supplemented with formic acid in both doses showed significant increase in total proteins, albumin and globulin beside insignificant decrease in A/G Ratio. Similar increase in serum protein was recorded Adil *et al.* [30] in chickens fed organic acid. Increase globulin in broilers supplying with organic acids [31]. Increase in total protein in broiler chickens in our study may be due to organic acids increased gastric proteolysis and improved the digestibility and absorption of protein and amino acids as reported earlier by Samanta *et al.* [32].

Analysis of lipids profile of the broiler received formic acid showed significant decrease total lipids, cholesterol and triglyceride in broilers. Alike the findings of Kamal and Ragaa [33] who supplemented broiler with organic acid. Serum total lipids and triglyceride significantly decreased by

dietary acidifiers [34-35]. Organic acid induced the decrease in total lipid in hens [36]. Organic acids decreased total lipid cholesterol and triglyceride in quail [37].

The obtained results showed that formic acid resulted in insignificant increase in calcium, phosphorous, magnesium, zinc, sodium and potassium. The obtained results are in agreement with the results in a study conducted in broilers received formic acid [6]. Acidic anion has been shown to complex with calcium, inorganic phosphorous, zinc, magnesium, sodium and potassium which results in an improved digestibility of these minerals [38]. Also, it was reported that organic acid improved digestibility of calcium, phosphorous, magnesium, zinc, sodium and potassium in broiler chickens [39]. Insignificant increase in these minerals may be due to organic acids induce lowering of gastrointestinal tract pH, which lead to increased absorption of these minerals from the gut into the blood stream [40].

Formic acid induced the insignificant decrease in liver enzymes (AST, ALT and ALP), uric acid and creatinine. These results are in full agreement with Adil *et al.* [41] in broiler received organic acid and with Abdel-Azeem *et al.* [42] in growing rabbits received citric acid. Organic acids up to 3% had no effects on liver and kidney function in broiler [43]. Reduced liver enzymes could be resulted from improvement of the physiological condition of liver and the increase in hepatic metabolic reserve [44].

The present investigation revealed that broiler chickens supplied with formic acid in both doses show insignificant reduction in total bacterial count, Coliform (*E. coli* and other coliform) and Lactobacillus in intestinal tract. Same observation was previously recorded where other organic acid (Galli acid) induced the reduction in the total bacterial count in intestine [45]. Also, organic acids reduced colonization of pathogens on the intestinal wall [46]. Moreover, Gauthier [47] stated that organic acids cause a reduction of the bacteria in the colon. Organic acids can penetrate the bacterial cell wall and disrupt the

normal physiology of certain types of bacteria [48]. In addition, organic acids supplementation has pH reducing properties in various gastrointestinal segments of broiler chicken lead to reduction of pathogenic bacteria [34]. The way of action of organic acids seems to be related to a reduction of pH in the upper intestinal tract, interfering with the growth of undesirable bacteria and modifying the intestinal flora [49].

The histomorphological changes in villi could be considered as an indicator for a responding in the functioning activity of the absorptive organs (villi) and healthy elongated villi in chickens lead to high absorption efficiency as in [50]. Tappenden and McBurney [51] stated that increased villi heights with the most organic acids was attributed to the reduction of many intestinal pathogens or non pathogens growth and decreasing the inflammatory reactions at the intestinal mucosa. In the immune organs (bursa and spleen) hyperplasia of lymphocytes in different cases was observed. Similar observations were previously recorded [52].

Conclusion

It could be concluded that, formic acid supplementation of great value on modern poultry production as it act as growth promoter and exhibits some benefits effect on the biochemical parameters ,intestine and the immune organs histology beside inhibition of colonization of pathogenic bacteria in intestinal wall of chickens.

Conflict of interest

The authors declare no conflict of interest.

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الملخص العربي
دراسات باثولوجيه بكتريولوجيه وبيوكيميائيه على
تأثير حمض الفورميك في كتاكيت التسمين

عزت ابو الفتوح حموده^١, نسرين احمد شوقى^٢, وغاده محمد الخضر^٢, حسن عبد السلام على^٣, السيد منصور^٤

^١قسم الباثولوجيا-معهد بحوث صحة الحيوان-الزقازيق

^٢قسم الكيمياء -معهد بحوث صحة الحيوان-الزقازيق

^٣قسم البكتريولوجيا-معهد بحوث صحة الحيوان-دمنهور

^٤قسم البكتريولوجيا -معهد بحوث صحة الحيوان-الزقازيق

كان الهدف من هذه الدراسة هو معرفة تأثير حمض الفورمك على وزن الجسم على بعض الوظائف البيوكيميائية والتغيرات الباثولوجيه بجانب تأثير حمص الفورمك على نمو البكتيريا. لذلك تم تجميع ١٠٠ مسحه من فتحة المجمع من كتاكيت هيرد عمر يوم واحد تتمتع بصحة جيدة وذلك لفحصها بكتريولوجيا. وبالفحص البكتريولوجي للمسحات الماخوذة من الكتاكيت السليمه وجد عدد ١٨ عينة تم عزل بكتيريا منها موزعه كالاتى عدوى مفردة ١٣ (٧٢.٢٢%) وعدوى مختلطة ٥ (٢٧.٧٨). وباجراء الاختبارات السيرولوجية تم التعرف على الميكروب القولوني O78 فى عدد ٤ مسحة وO157 فى عدد ٢ مسحة ووجد السالمونيلا تيفينيوريم فى عدد ٣ مسحة والسالمونيلا انتيديريس فى عدد ١ مسحة.

تم استخدام عدد ٦٠ كتكوت خاليه من اى نوع من البكتيريا فى تلك التجربه وتم تقسيمها إلى ٣ مجموعات متساوية تحتوى كلا منها على ٢٠ كتكوت. المجموعة الاولى استخدمت كمجموعة ضابطة, المجموعة الثانية كتاكيت تم اعطائهم حمض الفورمك بجرعه ١ملى/لتر من مياه الشرب والمجموعة الثالثه كتاكيت تم اعطائهم حمض الفورمك بجرعه ٢ ملى/لتر من مياه الشرب. يتم اعطاء حمض الفورمك لمدة ثلاثون يوما من اليوم الاول من العمر حتى اليوم الثلاثون من العمر. يتم دراسة كفاءة حمض الفورمك على وزن الجسم ومعدل التحويل الغذائى. يتم جمع عينات دم عند اليوم الاول, السابع والرابع عشر من نهايه استخدام حمض الفورمك. لقياس بعض الوظائف البيوكيميائيه. يتم ذبح ٥ كتاكيت من كل مجموعه بعد نهايه استخدام حمض الفورمك ويتم ربط الامعاء من الجانبين واخذها لفحص مكوناتها بكتريولوجيا لدراسه تأثير حمض الفورمك على البكتيريا الموجوده بالامعاء ويتم تجميع اجزاء من الامعاء, الكبد والكلى والطحال والبرسا عند اليوم الاول, السابع والرابع عشر من نهايه استخدام حمض الفورمك لدراسه تأثير حمض الفورمك على تلك الاعضاء باثولوجيا.

وقد اظهرت النتائج أن استخدام حمض الفورمك بالجرعتين ادى الى حدوث زياده معنويه فى وزن الجسم المكتسب , البروتين الكلى, الزلال والجلوبولين بجانب نقص معنوى فى الدهون الكليه, الكليستيرول, الدهون الثلاثيه وزياده غير معنويه فى مستوى الكالسيوم, الفسفور, الصوديوم, الماغنسيوم والپوتاسيوم والزنك ونقص غير معنوى فى نشاط انزيمات الكبد (AST, ALT, ALP) حمض اليوريك والكرياتينين وتقليل اعداد البكتيريا الموجوده بالامعاء وتحسن فى معدل التحويل الغذائى. باثولوجيا حمض الفورمك أدى الى زياده فى طول وسك الخملات كما أدى الى نشاط قوى فى معظم الأجهزة المناعيه

يتبين لنا من تلك الدراسة أن حمض الفورمك له دور فى صناعه الدواجن الحديثه حيث انه يعمل كمحفز للنمو حيث انه ادى الى تحسن ملحوظ فى وزن الجسم المكتسب ومعامل التحويل الغذائى وادى الى تحسن وظائف الكبد والكلى وتقليل الدهون والكليستيرول والدهون الثلاثيه فى مصل الدم وتقليل عدد البكتيريا الموجوده بالامعاء وتحسن فى جدار الامعاء والتي تم تأكيدها باثولوجيا. لذلك نوصى باستخدام حمض الفورمك نظرا لقله اثاره الضاره على الجسم وتأثيره المهم على وزن الجسم ومعامل التحويل الغذائى