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Research Article
Short Time Preservation of Poultry Viscera Meal and its Potentiality as a Source of Protein in Broiler Diet

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Abstract

Background and Objective: Poultry viscera meal (PVM) is a by-product of poultry slaughterhouses having high protein value at a relatively lower price. However, in developing countries it is a big challenge to maintain the keeping quality of viscera from collection to processing due to small scale availability per day in open market. So, this experiment was designed to find suitable methods for short-term preservation of PVM before processing and recycling it as broiler feed. **Materials and Methods:** Fresh chicken viscera were collected from local market and preserved for 48 h, without preservative at (I) Room temperature (Normal sample), (ii) Chilling temperature (Chilled sample) and with (iii) 0.5% citric acid, (iv) 1.0% citric acid, (v) 0.5% sulfuric acid and (vi) 1.0% sulfuric acid at room temperature which collectively considered as preservative samples. Based on the findings, 1.0% citric acid treated PVM was selected for efficacy study in broiler. Experimental diets contained 0% PVM, 2.5% PVM and 5.0% PVM by replacing similar amount of protein concentrate. **Results:** The lowest free fatty acid and peroxide value was observed at the addition of 1.0% citric acid, whereas pepsin digestibility and microbial load were similar to 1.0% sulfuric acid but lower than others. In the feeding trial, the highest body weight gain was found at 0% PVM but the lowest feed cost was observed at 5.0% PVM. **Conclusion:** Overall, 1.0% citric/sulfuric acid could be added in poultry viscera for short-term preservation and PVM could be included in broiler diet up to 5.0% for cost-effective broiler production.

Key words: Broiler diet, citric acid, free fatty acid, pepsin digestibility, peroxide value

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry viscera meal (PVM) is a powdered product, resulting from the cooking/acid treatment followed by proper drying of the whole gastrointestinal tract in a controlled way. Globally, the consumption of poultry meat is around 123 million tons per year which provide about 10 million tons of empty viscera[1]. Although heads, necks, feet, gizzards, flesh parts, etc. are also rendered for animal feed, still these products are consumed by the human in many countries[2]. However, there is little information available about the use of poultry viscera as human food without proper processing. Higher nutritional value and a relatively lower price of poultry viscera draw animal producers' attention to use it as a replacement of protein concentrate in the feed of dairy cattle[3], beef cattle[4] and poultry[5].

However, poultry is slaughtered mostly in open markets rather than slaughterhouses in developing countries where viscera are generally disposed of in open fields, streams and municipal sewage[6]. Moreover, some other factors trigger its utility; high-fat content is one of them, which reduces its storage time by developing rancidity[7]. As small quantities of viscera are available in open air slaughtering, it needs to be stored for several days to collect enough before industry scale processing. In that case, maintaining the storage quality of raw viscera would be a problem to obtain a quality product due to microbial and biochemical changes[8]. For improving the storage time of poultry viscera meal, a lot of preservation techniques are used viz. direct acidification[2], freezing[9], fermentation[10], fat extraction[11], etc. Both mineral acids (sulfuric acid, phosphoric acids) and organic acids (formic acid) effectively acidify poultry viscera meal[8]. Previous research illustrated that putrefaction and rancidity up to 72 h can be prevented by the use of 1.0-5.0% of sulfuric acid[12]. There is a dearth of information available pertaining to the efficacy of citric acid for acidification of poultry viscera although in poultry ration, its effect as an acidifier has been demonstrated[13].

Though its nutritional composition varies based on the raw materials used during processing, its protein composition and digestibility are comparable to fish meal[14]. Although there are many controversial findings, a previous research demonstrated that broiler diet containing 3.0-12.0% poultry viscera did not show any adverse effect on their production performance[5], even as a replacement of fish meal up to 100%[15]. Based on this information, it can be concluded that sulfuric acid and citric acid might have a positive impact on reducing fat oxidation in poultry viscera and improve its storage life. Therefore, the present study was designed to determine the usefulness of citric acid and sulfuric acid for short time preservation of poultry viscera and its potentiality as a source of protein in broiler diet.

MATERIALS AND METHODS

This study was conducted at Shahjalal Animal Nutrition Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh 2202, Bangladesh from January to April 2020. The experimental procedures, animal handling and the collection of samples were reviewed and approved by the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University, Mymensingh 2202 (AWEEC/BAU/2020/31).

Experiment 1

Experimental groups: Fresh poultry viscera were collected from the local market and a part was kept in room temperature (25-30°C) and another part at chilling temperature (4°C). After that, a certain volume of poultry viscera minced through a 5.0 mm sieve using a meat mincer (TORREY 32, Mexico). Immediately after mincing, different concentrations of citric acid and sulfuric acid (0.5 and 1.0%) were added and stored at room temperature. Experimental groups consisted of the (i) Normal sample (1000 g fresh poultry viscera without preservative at room temperature), (ii) Chilled sample (1000 g fresh poultry viscera without preservative at chilling temperature); (iii) 0.5% Citric acid (5 g citric acid +1000 mL water +1000 g fresh poultry viscera), (iv) 1.0% Citric acid (10gm citric acid + 1000 mL water + 1000 g fresh poultry viscera), (v) 0.5% Sulfuric acid (5 mL H_2SO_4 +1000 mL water +1000 g fresh poultry viscera) and (vi) 1.0% Sulfuric acid (10 mL H_2SO_4+1000 mL water+1000 g fresh poultry viscera). Viscera treated with both citric and sulfuric acids (0.5 and 1.0%) were collectively considered as preservative samples. All the samples were kept in glass jars up to 48 h.

Determination of storage quality: The pH, peroxide value (POV) and free fatty acids (FFA) of viscera were monitored at 0, 24, 48 h of storage. In contrast, pepsin digestibility and microbial quality were determined at 24 and 48 h of storage. An amount of 5 g viscera was mixed with 45 mL of distilled water and homogenised for 1 min by using a grinder (MG-300HM, Taishan AISON Electronics Co, China). After that, it was centrifuged at 2000×g for 15 min and the pH was measured using a pH meter (HI-2211, Hanna Instruments, USA). To determine peroxide value (POV), 3 g viscera were heated at 60°C for 30 min and 30 mL acetic acid-chloroform

solution (3:2, v/v) was used for dissolving fat. The POV was calculated according to procedure described by Rahman *et al.*[16] and expressed as milliequivalent peroxide per kilogram of the sample (meq kg^{-1}). Free fatty acid (FFA) was determined by mixing 5 g of the homogenised viscera with 30 mL of chloroform, centrifuging in a vortex machine for 1 min. Then, 1% phenolphthalein indicator was added in the filtrate and titrated it against 0.1N alcoholic KOH according to the method described by Rahman *et al.*[16]. The pepsin digestibility was determined using 0.2% pepsin (0.2 g of 1:10,000 activity pepsin; Sigma-Aldrich Co., USA) according to the method described by AOAC[17]. Total viable count (TVC) and total coliform count (TCC) were determined according to procedure described by Rima *et al.*[18] and expressed as log of colony-forming units per gram (Log CFU g^{-1}).

Experiment 2

Preparation of poultry viscera meal: Fresh viscera were collected from the local market and mixed with 1.0% citric acid immediately as described in the previous experiment. After that, viscera were kept in a hot air oven at 50°C for 24 h, followed by 70°C for 72 h. Then viscera were ground using locally made grinder fitted with US #20 sieves (850-µm openings). Poultry visceral meal contained 93.1% dry matter and 57.3% crude protein, 37.4% ether extract, 4.7% Ash, 0.85% calcium, 0.86% available phosphorus and 0.04% sulfur on dry matter basis was ready to be fed.

Experimental birds and management: A total of 150 straight run day-old broiler chicks (Cobb 500; initial body weight 46±0.5 g) were distributed in a completely randomized design consisting of three experimental groups with five replications having thirty birds per replicate. The experiment was continued for 28 days. The experimental diets were (I) 5% protein concentrate +0% PVM, (ii) 2.5% protein concentrate +2.50% PVM and (iii) 0% protein concentrate +5.0% PVM. Diets were formulated considering iso-nitrogenous and iso-caloric (Table 1).

Experimental birds were kept in a floor pen (4 cm sawdust bedding) having floor space of 0.91 m^2 (120 cm×76 cm) for ten birds. The brooding temperature was maintained at 32°C in 1st week and after that, it gradually decreased by 3°C each week until it reached to 21°C. Feed intake (FI) was calculated based on the difference between the offered and residual feed in the feeder at the end of the week. The body weight (BW) of an individual bird was measured weekly and total body weight gain (BWG) was calculated based on the difference between the initial and final body weights. The feed conversion ratio (FCR-kg FI kg^{-1} LWG) was determined cumulatively through the collected data. Performance efficiency index (PEI) was calculated according to the equation described by Martins *et al.*[19]:

$$\text{PEI}(\%)=\frac{\text{Body weight (kg)}\times\text{livability (\%)}}{\text{Age (days)}\times\text{feed conversion ratio}}\times 100$$

Table 1: Formulation of diet (%) in different dietary treatment groups for broiler

Ingredients	0% PVM	2.5% PVM	5.0% PVM
Maize	52.50	52.00	50.00
Rice polish	2.00	2.00	1.50
Protein concentrate	5.00	2.50	-
Poultry viscera meal	-	2.50	5.00
Soybean meal	31.50	32.00	33.00
Mustard oil cake	3.80	3.80	3.80
Soybean oil	3.00	3.00	3.80
DCP	1.40	1.70	1.70
Limestone	-	-	0.40
Methionine	0.05	0.05	0.05
Vit-mineral premix[1]	0.25	0.25	0.25
Common salt	0.50	0.50	0.50
Nutrient composition (%)			
ME (kcal kg^{-1})[2]	2997.00	2988.00	2993.00
Crude protein	22.89	22.87	22.89
Crude fibre	4.88	4.81	4.67
Ether extract	6.70	6.03	7.04
Ash	7.50	7.56	7.52
Calcium[2]	1.09	1.04	1.05
Avail. phosporus[2]	0.45	0.46	0.47

[1]Square Broiler Premix (Square Pharmaceutical Ltd, Bangladesh) contained per kilogram: 5000000 IU vitamin A, 1000000 IU vitamin D_3, 8000 mg vitamin E, 1600 mg vitamin K, 1000 mg vitamin B_1, 2000 mg vitamin B_2, 1600 mg vitamin B_6, 16000 mcg nicotinic acid, 5000 mg pantothenic acid, 4800 mcg vitamin B_{12}, 320 mg folic acid, 40 mg biotin, 160 mg cobalt, 4000 mg cupper, 16000 mg iron, 160 mg iron, 24000 mg manganese, 20000 mg zinc, 60 mg selenium and limestone carrier. [2]Calculated value

Sample collection and analysis: The proximate components of feeds and viscera meal were analyzed in triplicate, according to AOAC[20]. At the end of the feeding trial, five birds from each group were sacrificed to collect blood and carcass samples. Around 5 mL of the blood sample was collected in a sodium-heparinised tube and kept it in an icebox until centrifugation. Samples were centrifuged at 6000×g for 15 min for plasma separation and the plasma was stored at -20°C. Plasma glucose, urea nitrogen (BUN) and total protein were analyzed using different enzymatic kits in a bio-analyser (Urit-810, URIT Medical Electronic Group Co, Ltd, China).

Statistical analysis: Data were analyzed using the MIXED model (Experiment 1) and one-way ANOVA (Experiment 2) using SPSS 2011 Statistical Software Program (Version 20.0; IBM Corp, NY, USA). Experimental results were presented as Mean±standard deviation. Variation among the treatments in

different storage periods was determined using Duncan Multiple Range Test (DMRT) with a significance level of 0.05. Correlation coefficients (r) among the treatment, period and storage quality parameters were tested by Pearson correlation analysis using 2-tailed test of significance.

RESULTS

Changes of pH during storage: The pH values changed ($p<0.05$) with the increase of storage time (0, 24, 48 h) and due to the addition of different preservatives (Table 2). Initially, similar pH values were found in both normal and chilled samples but reduced in preservative samples due to addition of acid ($p<0.05$), pH values were similar at 0.5% level of citric and sulfuric acids but different at 1.0% level ($p<0.05$). The pH value decreased ($p<0.05$) with the increase in the duration of preservation, in every case except normal sample. At the end of 48 h of observation, the lowest pH value was found in 1.0% sulfuric acid group.

Changes of free fatty acid value during storage: Initially, free fatty acid (FFA) value was similar ($p>0.05$) in all groups but increased periodically (Table 3). During preservation, the FFA value of the normal and chilled samples increased more rapidly compared to preservative samples which indicate accelerated fat oxidation in these samples. There was a negative correlation between the concentration of acids and the FFA values in preservative samples. Moreover, citric acid reduced ($p<0.05$) FFA value more efficiently compared to sulfuric acid at both concentrations.

Changes of peroxide value during storage: Initially, peroxide values (POV) were similar ($p>0.05$) among the treatment groups but found different ($p<0.05$) at 24 and 48 h of preservation (Table 4). Like FFA value, POV was also higher ($p<0.05$) in fresh and chilled samples during preservation and showed a negative correlation with the concentration of acids in preservative samples. The lowest POV was observed in 1.0% citric acid group up to 48 h of preservation.

Pepsin digestibility: An increasing trend of digestibility was observed due to acidification (citric and sulfuric acid respectively) at different level. At 24 h of observation, 1.0% citric acid group exhibited 0.5-6% higher pepsin digestibility

Table 2: Effect of different preservatives on pH value of poultry viscera meal during storage up to 48 h of observation

	Time (h)			p-value		
Treatments	0 h	24 h	48 h	T	P	T × P
Normal sample	5.98±0.01[a]	6.00±0.02[a]	6.04±0.01[a]			
Chilled sample	5.99±0.03[a]	5.96±0.02[b]	5.89±0.01[b]			
Preservative samples						
0.5% citric acid	5.41±0.02[b]	5.14±0.04[d]	5.04±0.03[d]	<0.001	<0.001	<0.001
1.0% citric acid	4.89±0.01[d]	4.71±0.02[e]	4.65±0.02[e]			
0.5% sulfuric acid	5.46±0.03[b]	5.25±0.04[c]	5.11±0.02[c]			
1.0% sulfuric acid	4.98±0.07[c]	4.07±0.04[f]	3.89±0.02[f]			

[a-f]Means with dissimilar superscripts are significantly different ($p<0.05$) in same column, T: Treatment, P: Period, T×P: Treatment×period. Normal sample: fresh poultry viscera without preservative at room temperature; Chilled sample: fresh poultry viscera without preservative at 4°C; 0.5% Citric acid: 5gm citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 1.0% Citric acid: 10 g citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 0.5% Sulfuric acid: 5 mL H_2SO_4 acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 1.0% Sulfuric acid: 10 mL H_2SO_4 acid +1000 mL water +1000 g fresh poultry viscera at room temperature

Table 3: Effect of different preservatives on free fatty acid (%) of poultry viscera meal during storage up to 48 h of observation

	Time (h)			p-value		
Treatments	0 h	24 h	48 h	T	P	T × P
Normal sample	0.070±0.000	1.413±0.025[a]	3.979±0.002[a]			
Chilled sample	0.067±0.006	1.064±0.003[b]	1.691±0.003[b]			
Preservative samples						
0.5% citric acid	0.070±0.000	0.913±0.004[d]	1.178±0.004[d]	<0.001	<0.001	<0.001
1.0% citric acid	0.065±0.006	0.563±0.004[f]	0.842±0.004[f]			
0.5% sulfuric acid	0.067±0.005	0.974±0.004[c]	1.198±0.004[c]			
1.0% sulfuric acid	0.064±0.006	0.673±0.003[e]	0.891±0.005[e]			

[a-f]Means with dissimilar superscripts are significantly different ($p<0.05$) in same column, T: treatment, P: period, T×P: treatment×period. Normal sample: Fresh poultry viscera without preservative at room temperature, Chilled sample: Fresh poultry viscera without preservative at 4°C, 0.5% Citric acid: 5 g citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature, 1.0% Citric acid: 10 g citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 0.5% Sulfuric acid: 5 mL H_2SO_4 acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 1.0% Sulfuric acid: 10 mL H_2SO_4 acid +1000 mL water +1000 g fresh poultry viscera at room temperature

Table 4: Effect of different preservatives on peroxide value (meq kg^{-1}) of poultry viscera meal during storage up to 48 h

	Time (h)			p-value		
Treatments	0 h	24 h	48 h	T	P	T × P
Normal sample	3.65±0.01	14.05±1.44[a]	24.33±2.61[a]			
Chilled sample	3.66±0.01	7.11±0.19[b]	14.48±0.84[b]			
Preservative samples						
0.5% citric acid	3.64±0.01	5.26±0.19[d]	9.51±0.39[d]	<0.001	<0.001	<0.001
1.0% citric acid	3.65±0.01	4.34±0.31[f]	7.14±0.13[f]			
0.5% sulfuric acid	3.65±0.01	5.90±0.18[c]	9.81±0.25[c]			
1.0% sulfuric acid	3.66±0.01	4.71±0.19[e]	7.57±0.28[e]			

[a-f]Means with dissimilar superscripts are significantly different (p<0.05) in same column, T: treatment, P: period, T×P: Treatment×period. Normal sample: fresh poultry viscera without preservative at room temperature; Chilled sample: fresh poultry viscera without preservative at 4°C, 0.5% Citric acid: 5gm citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 1.0% Citric acid: 10 g citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature, 0.5% Sulfuric acid: 5 mL H_2SO_4 acid +1000 mL water +1000 g fresh poultry viscera at room temperature, 1.0% Sulfuric acid: 10 mL H_2SO_4 acid +1000 mL water +1000 g fresh poultry viscera at room temperature

Table 5: Effect of different preservatives on total viable count (TVC) and total coliform count (TCC) of poultry viscera meal during storage up to 48 h

	TVC (log CFU g^{-1})		TCC (log CFU g^{-1})	
Treatment	24 h	48 h	24 h	48 h
Normal sample	9.41±0.02[a]	11.44±0.21[a]	9.51±0.14[a]	11.36±0.18[a]
Chilled sample	8.42±0.14[b]	10.59±0.12[b]	9.52±0.01[a]	10.34±0.16[b]
Preservative samples				
0.5% citric acid	7.85±0.19[c]	8.59±0.15[c]	7.56±0.04[b]	8.54±0.11[c]
1.0% citric acid	5.91±0.06[d]	7.16±0.05[d]	5.98±0.08[d]	7.21±0.09[d]
0.5% sulfuric acid	7.79±0.14[c]	8.75±0.04[c]	7.22±0.12[c]	8.61±0.03[c]
1.0% sulfuric acid	5.85±0.08[d]	6.89±0.09[d]	4.58±0.14[e]	7.14±013[d]
SEM	0.384	0.469	0.538	0.466
p-value	<0.001	<0.001	<0.001	<0.001

[a-e]Means with dissimilar superscripts are significantly different (p<0.05) in same column, TVC: Total viable count, TCC: Total coliform count. Normal sample: Fresh poultry viscera without preservative at room temperature; Chilled sample: fresh poultry viscera without preservative at 4°C; 0.5% Citric acid: 5 g citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 1.0% Citric acid: 10 g citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 0.5% Sulfuric acid: 5 mL H_2SO_4 acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 1.0% Sulfuric acid: 10 mL H_2SO_4 acid +1000 mL water +1000 g fresh poultry viscera at room temperature

compared to other preservative samples and 6-8% higher than chilled and normal samples (Fig. 1). However, 1.0% sulfuric acid provided better digestibility at 48 h which was 2-9% higher than those of the other preservative samples and ~14% higher than chilled and normal samples at respective duration.

Microbial quality: Both total viable count (TVC) and total coliform count (TCC) were reduced (p<0.05) in preservative samples at 24 and 48 h of observation compared to respective normal and chilled samples (Table 5). However, the similar concentration of citric and sulfuric acids exhibited similar (p>0.05) results and the lowest TVC and TCC counts were recorded at 1.0% concentration in both observations. Compared to normal sample, 1.0% citric/sulfuric acid sample reduced 39% TVC and 44% TCC up to 48 h of observations.

All the storage parameters in this experiment were positively correlated (p<0.01) with pH change except digestibility. Due to acidification FFA, POV, TVC and TCC were reduced and the digestibility was increased (Table 6). Besides, these parameters were strongly influenced by preservatives rather than the duration of preservation.

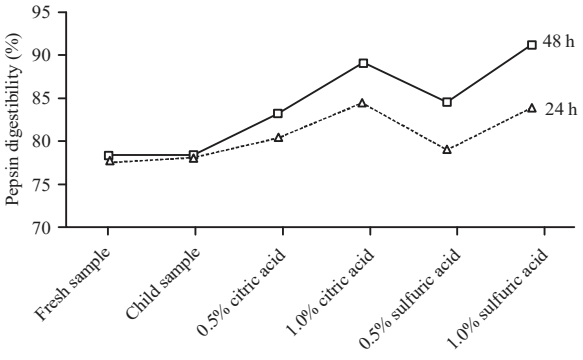

Fig. 1: Effect of different preservatives on pepsin digestibility (%) of poultry viscera meal during storage up to 48 h

Normal sample: fresh poultry viscera without preservative at room temperature; Chilled sample: fresh poultry viscera without preservative at 4°C, 0.5% Citric acid: 5 g citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 1.0% Citric acid: 10 g citric acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 0.5% Sulfuric acid: 5 mL H_2SO_4 acid +1000 mL water +1000 g fresh poultry viscera at room temperature; 1.0% Sulfuric acid: 10 mL H_2SO_4 acid +1000 mL water + 1000 g fresh poultry viscera at room temperature

Table 6: Correlation coefficients (r) for the relationship of treatment, period and storage quality parameters

	Treatment	Period	pH	FFA	POV	Digestibility[1]	TVC	TCC
Treatments	1	0.000	-0.831**	-0.369**	-0.465**	0.717**	-0.763**	-0.739**
Period	0.000	1	-0.221	0.707**	0.654**	0.415	0.453*	0.404
pH	-0.831**	-0.221	1	0.247**	0.361**	-0.862**	0.813**	0.791**

[1]Pepsin digestibility, FFA: free fatty acid, POV: peroxide value, TVC: total viable count, TCC: total coliform count. **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed)

Table 7: Effect of poultry viscera meal on growth performance of broiler at 28 days

	Treatments				
Age	0% PVM	2.5% PVM	5.0% PVM	SEM	p-value
Growth performance					
BWG (g)	1045.90±12.3[a]	988.00±22.9[b]	967.90±14.91[b]	10.90	0.000
FI (g)	1705.70±79.3	1622.10±14.6	1603.10±59.4	20.60	0.082
FCR	1.63±0.08	1.64±0.04	1.66±0.07	0.020	0.862
PEI (%)	231.90±9.3	225.40±9.1	212.10±11.9	3.630	0.059
FC kg^{-1} BW[a]	0.67±0.02[a]	0.63±0.01[b]	0.60±0.01[c]	0.010	0.004
Carcass characteristics (%)					
Dressing percentage	62.96±0.09[a]	62.91±0.37[a]	61.84±0.25[b]	0.190	0.003
Breast	18.54±0.07[a]	18.48±0.09[a]	17.88±0.26[b]	0.110	0.005
Thigh	8.20±0.08[a]	8.19±0.07[a]	8.01±0.05[b]	0.040	0.025
Drumstick	4.31±0.08	4.29±0.12	4.32±0.12	0.030	0.943
Liver	3.36±0.07	3.39±0.15	3.51±0.11	0.040	0.317
Kidney	0.22±0.01	0.22±0.02	0.22±0.04	0.010	1.000
Bursa	0.04±0.003	0.04±0.006	0.04±0.004	0.001	0.930
Spleen	0.09±0.004	0.09±0.004	0.09±0.003	0.001	0.806
Thymus	0.11±0.002	0.12±0.002	0.11±0.004	0.001	0.971
Plasma metabolites (mg dL^{-1})					
Glucose	237.06±12.81	231.59±16.49	237.84±3.91	3.674	0.800
Total protein	2535.30±168.1	2575.70±232.3	2573.20±152.1	54.51	0.958
BUN	6.70±0.24	6.78±0.37	6.69±0.31	0.102	0.951

[a-b]Means with dissimilar superscripts are significantly different (p<0.05), BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio, PEI: Performance efficiency index, FC: Feed cost, BUN: Blood urea nitrogen; 0% PVM: Diet having 0% poultry viscera meal and 5.0% protein concentrate, 2.5% PVM: Diet having 2.5% poultry viscera meal and 2.5% protein concentrate, 5.0% PVM: Diet having 5.0% poultry viscera meal and 0% protein concentrate. [a]feed cost was calculated in US dollar (1 US dollar = 84.98 Bangladeshi taka)

***In vivo* feeding trial in broiler:** No difference (p>0.05) was found in FI, FCR and PEI among the dietary groups except BWG and feed cost per kg of BW (Table 7). The BWG was the highest in 0% PVM group, intermediate in 2.5% PVM and the lowest in 5.0% PVM groups. However, the lowest feed cost per kg of BW was observed in 5.0% PVM group followed by 2.5% and 0% PVM groups, respectively. Besides, 0 and 2.5% PVM group exhibited similar (p>0.05) dressing percentage, breast meat and thigh meat percentage which was higher (p<0.05) than 5.0% PVM group. The relative weight of immune organs and plasma metabolites were not different (p<0.05) among the treatment groups.

DISCUSSION

Changes of pH during storage: The stability of pH tends to reduce the microbial activity which preserves the keeping quality of protein meal[21]. In this experiment, the pH values were reduced in all treatment samples during storage except normal sample which exhibited the opposite trend. However, the increased pH in normal sample during preservation might be due to the decomposition of protein by proteolytic bacteria[22]. During decomposition, the protein was converted to amino acids which were further degraded into ammonia. Finally, the accumulation of this ammonia in the normal sample during preservation was responsible for increasing pH. The lower temperature in the chilled sample and addition of acids in preservative samples might limit the growth and proliferation of proteolytic bacteria which resulting lower pH[22]. Furthermore, the reduction rate of pH in chilled sample was lower than the preservative samples (2% vs 10%) which indicated that the growth of spoilage bacteria was declined rather stopped at 4°C[23]. In preservative samples, the pH reduced rapidly during the first 24 h of acidification compared to 48 h (5% vs 3%). Cai *et al.*[8] also reported rapid pH reduction of formic and phosphoric acid-treated offal meal during the first 24 h after acidification at room temperature. Some potential factors might be responsible for rapid pH reduction

in preservative samples during the first 24 h of acidification, including rapid penetration of acid into offal tissue and lower buffering capacity of offal[24]. However, the reason for pH reduction in preservative samples due to the increase in storage time is important to understand. Immediate after acidification, the preservative samples had pH ranged from 4.89-5.41 which might promote the growth of *Lactobacillus* bacteria (most prominent microbes in chicken ileum) at room temperature and reduced pH during the storage period[25].

Changes of free fatty acid value during storage: Hydrolysis of fat by enzymatic and microbial degradation produces FFA which provides an idea about lipid stability during storage[16]. The FFA value of meat sample below 1.2% is considered acceptable. In this experiment, all the preservative samples had FFA value lower than 1.2 up to 48 h of observation which was in consistence with the findings of Ibrahim *et al.*[26] and Mir *et al.*[27] who reported that citrus food extracts and organic acids/salts reduced FFA value of meat products during storage. These findings could be justified in a way that antioxidative properties of citric acid[13] might be responsible for reducing fat oxidation in the citric acid group. Sulfuric acid increased H^+ ion concentration which might trigger the activity of lipolytic microbes[28]. Consequently, it reduced the lipolytic enzymes availability and lower autoxidation of lipids which reduced FFA values in sulfuric acid-treated samples.

Changes of peroxide value during storage: Extremely rancid products had higher POV due to the oxidation of peroxides which produced initially from lipid oxidation[16]. The POV of meat sample below 10 meq kg^{-1} is considered as acceptable[29] and the results of this study indicated that all the preservative samples were well controlled up to 48 h of observation. Cagdas and Kumcuoglu[29] and Racanicci *et al.*[30] reported that the application of natural and commercial antioxidants reduced POV of meat products during storage. Like FFA values of viscera meal, the POV in preservative samples were reduced due to antioxidative action of citric acid[13] and anti-lipolytic activity of sulfuric acid[28].

Pepsin digestibility: Pepsin digestibility is used in practice as an estimator of the amino acid bioavailability in protein meal. Nourmohammadi and Afzali[31] reported that acidification (pH 3.5-4.0) of protein meal had higher digestibility in the broiler. The application of organic acid in the poultry diet for improving protein digestibility has been examined by researchers[13]. Ao[32] reported that the addition of citric acid in soybean meal boosted protein digestibility in the *in vitro* trial. In this experiment, we found better pepsin digestibility in 1.0% citric/sulfuric acid at both observations. Higher pepsin digestibility in 1.0% citric/sulfuric acid group could be justified by the finding of Omogbenigun *et al.*[33] who reported that lower pH accelerated the conversion of pepsinogen to pepsin in broiler and improved protein digestibility in broiler.

Microbial quality: Microbial growth and survival were influenced by pH and the proliferation of most pH-sensitive bacteria (*E. coli*, *Salmonella* and *Clostridium perfringens*) is minimised[34] below pH 5. In this experiment, lower TVC and TCC were found in 1.0% citric/sulfuric acid group which was in accordance with the findings of Ahmad *et al.*[35]. However, the pH value of 1.0% citric acid group was higher ($p<0.05$) than 1.0% sulfuric acid group at 48 h of observation. Previous study illustrated that, pH reduction obviously not the sole factor for the antibacterial activity of citric acid in meat samples[36]. The disassociation value of sulfuric acid is much lower than citric acid[37]. Nevertheless, it is assumed that undissociated/partially disassociated citrate entered the microbial body rapidly and then completely disassociated inside the microbes[36] resulting in improved bacteriostatic activity in 1.0% citric acid group. Moreover, the FFA and POV are the good indices for understating microbial activity in protein meal[16] and under the present experimental condition preservative samples had lower FFA and POV which reflected that acidic condition might trigger the microbial activities and resulting lower lipid oxidation in these samples.

***In vivo* feeding trial in broiler:** Previous findings illustrated that the inclusion of poultry offal meal in broiler diet improved growth performance[4,35]. Moreover, poultry by-products meal as a replacement of fish meal in broiler diet did not affect growth performance[15]. In the current study, the poultry viscera meal was replaced by protein concentrate and observed comparatively lower growth rate in treated groups. Commercial protein concentrate has higher digestible crude protein, gross energy, phosphorous and several amino acids[38]. In contrast, a previous study reported some potential anti-nutritional factors and lack of nutrients uniformity[14]. Poultry viscera meal contained around 85% pepsin digestibility (Fig. 1) which was comparatively lower than protein concentrate (>95%). Furthermore, Xavier *et al.*[39] reported that poultry by-product meal significantly reduced nutrient

digestibility in chicks from four to seven days of age due to the presence of higher lipids. However, the lower growth performance of broiler due to PVM was compensated by the reduced feed cost. It was reduced up to 6-9% and the lowest value was found in the 5.0% PVM group, which was consistent with the result of Da Silva *et al.*[5]. Breast meat and thigh meat percentage were similar up to 50% replacement and reduced at 100% replacement which might be due to the higher nitrogen digestibility of protein concentrate compared to viscera meal. Moreover, in this study, the results about liver and pancreas functions obtained by the inclusion/replacement of poultry by-product meal in broiler diet do not agree with previous studies[15,39]. In this experiment, all treated groups exhibited similar weight for liver, kidney and immune organs, indicating that no physiological abnormalities happened due to replacement.

CONCLUSION

Both 1.0% citric/sulfuric acid prevented lipid oxidation in PVM and maintained storage quality. The pH value, FFA and POV did not exceed the acceptable level in both groups up to 48 hours of observation, so storage time could be extended though further research is needed. Finally, it is concluded that 1.0% of citric/sulfuric acid could be added in poultry viscera to improve the storage quality at least for 48 h. It could be added in the broiler diet as a replacement of protein concentrate up to 5.0% for cost-effective broiler production.

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