Evaluation of Amino Acids, Fatty Acids, Protein, Fat, and Ash in Poultry Carcasses Fermented with *Lactobacillus* Bacteria

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Laboratory studies determined the effect of bacterial fermentation variables on pH, ammonia nitrogen, moisture, protein, fat, and ash in poultry carcasses before and after fermentation with dried whey and cornmeal. Field-produced silage was collected throughout the year to determine the effect of seasonal temperature variation. Amino acid profiles changed little during the 10-week fermentation period, with a slight increase in alanine and decreases in lysine, threonine, and aspartic acid. Increased moisture resulted in decreases in serine, glutamic acid, and proline. The fatty acid profile remained unchanged. Protein, fat, ash, and moisture levels did not change during fermentation. Ammonia nitrogen increased with long-term storage. Lactic acid was the major acid produced.

Keywords: *Poultry carcass; fermentation; composition*

INTRODUCTION

Disposal of poultry carcasses presents significant environmental, biological, and economic problems for the poultry industry. *Lactobacillus* fermentation is a potential method for economically reclaiming dead birds without contaminating the environment and may provide income from the recovery of nutrients (Cai *et al.*, 1994b). Fermented poultry carcasses are suitable for rendering (Cai *et al.*, 1994a). Fermentation of dead birds, poultry offal, and edible food wastes with lactic acid bacteria is very effective in inactivating pathogenic viruses (Wooley *et al.*, 1981) and bacteria (Talkington *et al.*, 1981a,b). In previous studies on direct acidification of poultry offal and carcasses, putrefaction was prevented when silage was maintained below pH 4.5 and volatile nitrogen (NH_3-N) content was less than 0.3% (Cai *et al.*, 1994a).

The ensiled products from poultry viscera, poultry offal, and fish waste have been demonstrated to be useful for incorporation into diets for swine (Tibbetts *et al.*, 1987) and poultry (Hassan and Heath, 1986). The present study was designed to determine whether the fermentation process alters the amino acid and fatty acid composition of such products. Protein, fat, ash, moisture, ammonia nitrogen, and acidity (pH) were also evaluated over 1 year to determine whether seasonal variation affects silage composition. Various acids produced were monitored.

MATERIALS AND METHODS

Laboratory Fermentation. Dead laying hens obtained from a poultry farm in Georgia were chopped with a 20-in. GPR poultry grinding machine and ground again using an Enterprise meat grinder with a 12-mm sizing dice. A 1500-g quantity of ground carcass was mixed with either 5% dried whey (Adell Whey Co., Inc., Adell, WI) and 5% cornmeal, 8% dried whey and 5% cornmeal, or 8% dried whey and 8% cornmeal to create poultry carcass silage. Water, equal to the weight of added carbohydrate, was added to half of the samples. The resulting formulations of carcasses/whey/cornmeal/water by weight were: 90:5:5:0, 90:5:5:10, 87:8:5:0, 87:8:5:13, 84:8:8:0, and 84:8:8:16. Each mixture was stored in an incubator at 32 °C for 70 days. Ammonia nitrogen, moisture, protein, fat, and ash were determined before and after fermentation. Amino acid and fatty acid profiles were determined to detect changes occurring during fermentation. Lactic, acetic, propionic, butyric, and isobutyric acid production was also monitored.

Field Fermentation. Dead carcasses were collected daily from chicken houses on a laying hen farm in Georgia. A fermentation facility composed of a bin containing a mixture of whey and cornmeal (1:1 by weight), a poultry grinding machine, an auger, and a tanker truck was set up on the farm. Each day, about 150–220 carcasses were transported to the site. Five carcasses were chopped at one time in the grinding machine, with carbohydrate mixture added during grinding. The chopped carbohydrate/carcass mixture was augured to the tanker truck for storage.

When the tank was full or nearly full, which took from 28 to 53 days depending on flock mortality, it was carried to a rendering plant for processing into poultry meal. Throughout the year, when silage was unloaded at the rendering plant, four samples were collected and mixed together for chemical analysis to determine the effect of seasonal temperature variation on fermentation. All samples were ground again with a 3.2-mm sizing dice and then kept at −40 °C if immediate chemical analysis was not performed. Acidity (pH), ammonia nitrogen, moisture, protein, fat, ash, amino acid profile of protein, fatty acid profile of fat, and various acids produced were evaluated each season.

Formulations and storage times varied during the year to prevent putrefaction of the carcass silage. The winter sample, collected in January, was formulated with 90:5:5:20 carcass/whey/cornmeal/water and was stored for 30 days. The spring sample, collected in March, had a 94:3:3:12 formula and 53-day storage time. Three samples were collected during the summer. The June sample had a formulation and storage time of 90:5:5:0 and 32 days, respectively; July and August samples had an 85:7.5:7.5:0 formula and were stored for 37 and 28 days, respectively. The sample collected in the fall putrefied due to an error in carbohydrate administration and, therefore, was not tested.

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Chemical Analysis. Total nitrogen and ammonia (volatile) nitrogen were determined according to AOAC (1984) and APHA (1985) methods, respectively. The crude protein content was calculated by subtracting ammonia nitrogen from total nitrogen and multiplying the results by 6.25. Crude fat, moisture, and ash in samples were analyzed according to AOAC (1984) Methods Sections 24.005a, 24.003b, and 24.009, respectively. Acidity (pH) was measured with a Corning pH meter equipped with a flat surface combination probe electrode. Lactic acid and volatile fatty acids were analyzed by gas chromatography (Cai *et al.*, 1994a).

Fatty acid composition of fat was determined by gas chromatography as follows. Total fat was extracted from 200 mg of freeze-dried powdered sample using 5 mL of 2:1:0.8 methanol/chloroform/water by volume (Bligh and Dyer, 1959). After the extract was filtered through defatted glass wool, 1.3 mL of chloroform and 1.3 mL of water were added. This brought the methanol/chloroform/water solution to 2:2:1.8 by volume to facilitate gravity separation. Fat extract in the chloroform layer was dried and deaerated by purging with N_2 at 38 °C. Fat was then hydrolyzed and methylated using 2 mL of boron trifluoride in methanol at 70 °C for 1 h (Morrison and Smith, 1964). After methylation, 3 mL of water was added. The resultant mixture was then extracted with 4 mL of *n*-hexane. Fatty acid methyl esters in hexane were analyzed with a Perkin-Elmer Sigma 3B gas chromatograph equipped with a flame ionization detector and a glass column (1.8 m × 2 mm i.d.) packed with Chromosorb (W/AW, 100/120 mesh, coated with 10% SP-2330) (Supelco, Inc., Bellefonte, PA). Column temperature was held at 200 °C for 2 min and then increased to 240 °C at 4 °C/min with N_2 as the carrier gas at 65 mL/min. Injector and detector temperatures were 260 °C. For quantification, heneicosanoic acid was used as internal standard. Fatty acids were identified by comparing retention times with those of purified standards. Peak areas and percentages were determined using a Hewlett-Packard 3392A integrator.

The amino acid composition of protein was determined by high-performance liquid chromatography (HPLC). Sample preparation for HPLC analysis involved the hydrolysis of freeze-dried defatted (FDD) samples according to Scheme B described by Phillips (1983). Briefly, an FDD sample in acid-washed test tubes was deaerated and hydrolyzed with 6 N HCl containing 0.5% phenol for 24 h at 110 °C. The protein hydrolysate was adjusted to pH 1.6 and filtered through a 0.22-m Teflon filter. The amino acid composition of hydrolysates of each sample was determined by ion-exchange chromatography using a Waters 840 data and chromatography control station (Millipore Corp., Milford, MA). The system was equipped with a Waters system interface module (SIM), two Waters Model 510 HPLC pumps, a Waters intelligent sample processor (WISP) Model 710B, one Eldex reagent pump, one Waters temperature control module, a postcolumn reaction coil, one column oven, one reaction coil oven, and a Waters Model 440 absorbance detector and was controlled by Waters software Baseline 810. The cation exchange amino acid column was from Pickering Laboratories (Mountain View, CA). Amino acids were eluted by a gradient of pH 3.28 sodium citrate eluent to pH 7.40 sodium citrate eluent at a total flow rate of 0.3 mL/min and a column temperature of 50 °C. Ninhydrin was used for postcolumn derivation of amino acids detected at 436 (proline) and 546 nm (all others) by the Waters detector at a reaction coil at 120 °C. Reagents used for HPLC were obtained from Pickering Laboratories. Tryptophan and cysteine were not determined.

Statistical Analysis. Statistical methods of analysis of variance and a general linear model with Duncan's multiple comparison procedures (SAS Institute, 1988) were used to analyze experimental data. Significance was defined as $P \leq 0.05$. No statistical analysis was done on the amino acid or fatty acid composition of the field samples.

RESULTS AND DISCUSSION

Laboratory Fermentation. Typical analysis of carcass silage composition is shown in Table 1. No differences in moisture, protein, fat, and ash in silage occurred before or after fermentation. Long-term storage (70 days) of carcass silage resulted in higher ammonia content. The ammonia nitrogen after storage for 70 days was 1.05% on a dry weight basis, approximately 3 times as much as the initial value before fermentation. Previous experience indicated that carcass silage containing more than 1% ammonia nitrogen produces putrid odors. All carcass silages fermented with 5% whey and 5% cornmeal resulted in putrefaction after storage for 70 days. The other silages were marginal in terms of ammonia content after long-term storage.

Lactic acid and acetic acid in silage increased from 0.48% and 0.38% to 4.95% and 1.32%, respectively, during fermentation of carcasses with 8% whey and 5–8% cornmeal (Table 2). No significant changes in propionic, butyric, and isobutyric acids occurred during fermentation. Thus, the high level of lactic acid was mainly responsible for low silage pH during fermentation. It should be noted that it was not necessary to add silage cultures to enhance fermentation.

The amino acid composition of protein and the fatty acid composition of fat in carcass silage are shown in Tables 3 and 4. Aspartic acid, threonine, and lysine decreased, whereas alanine increased during fermentation (Table 3). When water was added to the carcasses,

Table 1. Composition of Carcass Silage Formulations before and after Long-Term Fermentation

fermentation time (days)	mixture composition (carcass/whey/cornmeal/H_2O)	composition[a] (%)				
		protein	fat	ash	moisture	NH_3-N
0	90:5:5:0	49.0		12.1	59.6	0.44
	90:5:5:10	49.6		10.8	63.4	0.39
	87:8:5:0	42.1	21.9	10.0	55.7	0.30
	87:8:5:13	43.4	23.7	10.0	62.9	0.36
	84:8:8:0	41.7	20.8	9.0	55.4	0.36
	84:8:8:16	43.0	22.2	10.0	61.0	0.35
	average	44.8	22.3	10.3	59.5	0.37
70	90:5:5:0	47.8		12.5	61.7	1.33
	90:5:5:10	48.5		10.4	64.0	1.15
	87:8:5:0	41.0	24.5	10.4	56.7	0.96
	87:8:5:13	42.0	23.6	9.8	60.6	0.96
	84:8:8:0	42.8	22.6	10.0	57.1	0.95
	84:8:8:16	45.1	23.2	10.8	63.0	0.94
	average	44.5	23.5	10.6	60.5	1.05*

[a] All but moisture were figured on a dry weight basis. *Significantly different from initial values ($P \leq 0.05$).

Table 2. Concentration of Acids Produced in Carcass Silage before and after Long-Term Fermentation

fermentation time (days)	mixture composition (carcass/whey/cornmeal/H_2O)	acid (% on a wet weight basis)				
		lactic	acetic	propionic	butyric	isobutyric
0	87:8:5:0	0.40	0.31	0.10	0.08	0.12
	87:8:5:13	0.74	0.41	0.12	0.12	0.16
	84:8:8:0	0.52	0.46	0.19	0.12	0.26
	84:8:8:16	0.26	0.36	0.10	0.07	0.13
	average	0.48	0.38	0.13	0.17	0.10
70	87:8:5:0	5.48	1.60	0.25	0.26	0.27
	87:8:5:13	3.36	0.64	0.11	0.11	0.09
	84:8:8:0	5.42	1.60	0.29	0.28	0.28
	84:8:8:16	5.54	1.46	0.29	0.24	0.19
	average	4.95*	1.32*	0.22	0.21	0.22

* Significantly different from initial value ($P \leq 0.05$).

Table 3. Changes in Amino Acid Composition (Milligrams per Gram of Dry Matter) as a Result of Long-Term Fermentation of Protein Carcasses (84% Carcass:8% Whey:8% Cornmeal) Formulated with and without Water

	no water added fermentation		16% water added fermentation	
amino acid	0 days	70 days	0 days	70 days
aspartic	26.4	12.2*	29.7	9.9*
threonine	13.1	6.0*	14.5	4.6*
serine	18.0	12.2	25.0	9.4*
glutamic	38.3	18.7	42.7	14.7*
proline	22.5	15.1	29.7	13.0*
glycine	25.7	23.3	31.4	22.0
alanine	21.4	32.0*	22.0	30.8*
valine	18.1	15.5	21.5	16.3
methionine	5.5	4.1	5.9	3.3
isoleucine	14.3	11.8	16.4	10.0
leucine	24.3	16.5	28.2	18.7
tyrosine	9.1	4.2	10.2	3.1*
phenylalanine	12.9	7.8	15.6	7.1*
lysine	15.4	6.2*	15.0	3.8*
histidine	5.9	6.0	5.9	3.8
arginine	19.1	23.3	23.3	16.2
cystine		5.6	5.5	5.3
total	290.0	220.5	342.5	192.0

* Significantly different from initial value ($P \leq 0.05$).

Table 4. Average Composition of Fatty Acids in Carcass Silages (Formulations 87:8:5:0, 87:8:5:13, 84:8:8:0, and 84:8:8:16 by Weight of Carcass/Whey/Cornmeal/Water) before and after Long-Term Fermentation

fermentation	fatty acid (mg/g of dry matter)						
time (days)	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
0	1.19	41.3	7.77	13.0	74.3	25.7	0.43
70	0.83	39.7	7.36	13.6	70.7	34.6	0.94

Table 5. Composition of Carcass Silage after Long-Term Storage Seasonally

silage	composition[a] (%)					
batch	protein	fat	ash	moisture	NH_3-N	pH
January	51.0*	24.4	10.9	68.2**	0.66	4.95
March	57.4***	28.1	11.3	69.0**	1.03*	5.70*
June	49.1*	27.9	11.0	63.1*	1.38***	6.10**
July	47.0	28.5	9.3	56.1	1.05*	5.70*
August	53.6**	28.6	12.6*	55.9	1.25**	5.55*

[a] All but moisture were figured on a dry weight basis. *,**,***, Significantly different from other values ($P \leq 0.05$).

Table 6. Acids Produced in Carcass Silage after Long-Term Storage Seasonally

silage	acid (% on a net weight basis)				
batch	lactic	acetic	propionic	butyric	isobutyric
January	4.28	0.48	0.16	0.12	0.06
March	2.03	0.75	0.30	0.18	0.13
July	4.81	1.48	0.30	0.89	0.16
August	4.40	1.80	0.27	0.67	0.16

concentrations of serine, glutamic acid, and proline were also lower after fermentation. No differences in glycine, valine, methionine, isoleucine, leucine, histidine, arginine, and cystine levels were detected before and after fermentation. Fermentation did not have a significant effect on fatty acid profiles (Table 4).

Field Fermentation. The chemical composition of seasonally collected farm silage is shown in Tables 5–8. Differences in protein content were due partially to variations in the formulation of the silage mixture before fermentation. With the same carbohydrate formulation for the January and June batches, the January silage had lower ammonia levels (Table 5), indicating the winter temperature affected fermentation. This agrees with our previous results (Cai *et al.*, 1994b) which indicated that higher temperatures resulted in a higher pH and higher ammonia in silage when carbohydrate content was not adequate for fermentation. The high pH and high ammonia content in July and August silages indicated that the proportions of 7.5% whey and 7.5% cornmeal may not have been adequate to stabilize carcasses on the farm, although the same treatment could preserve carcasses for 1–2 months in a laboratory setting. Differences in results between laboratory and field studies may be attributed to the extent of mixing of ground carcasses with carbohydrate. Mixing was thorough in the laboratory but may not have been adequate on the poultry farm since mixing equipment was not provided. To solve this problem, farms may need a good mixer. If mixing equipment is not available, addition of a liquid culture of lactic acid bacteria will enhance fermentation (Cai *et al.*, 1994b).

Table 7. Amino Acid Profiles (Milligrams per Gram of Dry Matter) of Protein in Carcass Silage after Long-Term Storage Seasonally

	silage batch			
amino acid	January	March	July	August
aspartic	28.8	29.9	16.1*	20.0*
threonine	13.2	14.6	8.7*	9.4*
serine	18.8	22.3	16.0	17.2
glutamic	40.3	43.1	23.1*	27.7*
proline	28.4	28.6	18.8*	25.5
glycine	35.2	35.7	35.2	22.6*
alanine	29.4**	34.9*	25.8**	41.2
valine	19.8	24.6	17.0	21.5
methionine	6.3	6.7	4.2	5.3
isoleucine	16.6	19.1	16.8	13.2
leucine	28.0	31.1	21.8	26.6
tyrosine	9.7	10.7	5.7*	6.2*
phenylalanine	14.8	16.9	11.4	13.2
lysine	18.9	17.4	6.9*	7.9*
histidine	6.7	6.2	2.9*	4.1*
arginine	23.1	23.5	15.6*	12.7*
cystine		5.4	5.8	6.3
total	338.0	370.7	251.8	280.6

*,**,***, Significantly different from other values ($P \leq 0.05$).

Table 8. Fatty Acid Profile of Fat in Carcass Silage after Long-Term Storage Seasonally

silage	fatty acid (mg/g of dry matter)						
batch	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
January	1.52	33.8	6.66	10.9	52.2	19.9	0.20
March	1.50	41.3	7.88	14.1	57.5	17.6	0.29
July	2.07	37.0	7.32	12.1	59.7	23.8	0.30
August	2.23	41.0	7.41	13.6	72.9	41.6	0.49

Lactic acid was the predominant acid produced in farm silage (Table 6). It appeared that the higher the carbohydrate proportion, the more lactic acid was produced. Amino acid profiles in winter silages overall were higher than in summer silages (Table 7). This is probably due in part to the higher protein contents in the winter silage resulting from different silage formulation before fermentation. Summer silage contained less aspartic acid, threonine, glutamic acid, tyrosine, lysine, and histidine than winter silage (Table 7). Palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids remained the major components of fat in farm silage (Table 8). This is similar to the composition of fat in silages analyzed before fermentation in the laboratory study (Table 4).

On the basis of these findings and the small volume of poultry meat byproduct used in poultry feeds, it is feasible to expect fermented poultry carcasses to serve as an adequate source of additional fat and protein. Additional studies are required to determine the level of fermented poultry carcasses that could be used in feeds and its effect on weight gain and feed conversion.

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