Effect of Formic Acid and Urea Phosphate–Calcium Propionate on Amino Acids in Wheat Silage

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Changes in distribution of nitrogenous compounds of chopped wheat plants ensiled during early stages were determined as affected by addition of 0.4% formic acid (FA) or 2.2% urea phosphate–calcium propionate (UP·CaP). Analyses were carried out after an ensiling period of 90 days and after a further aerated silage (AS) period of 7 days. Total amino acid (TAA) content in the dry matter (DM) remained stable during the fermentation period but decreased during aeration of silage in the untreated material (UM). Concentrations of essential amino acids (EAA) decreased during fermentation, this decrease being higher in UM. The free amino acids (FAA) were at low concentration in the fresh material (<10% of the TAA) but were high in ensiled material and reached about 73% (of TAA) in the silage. These levels decreased in AS to 63% in the UM, 54% in the FA-treated, and 67% in the UP·CaP-treated material. The ammonia N content increased during fermentation in UM and especially in the UP·CaP treatments, while this process was depressed by the FA. The concentrations and changes of 20 amino acids (AA) are given. The highest AA concentrations recorded in the fresh material were those of arginine, lysine, alanine, glutamic acid, leucine, aspartic acid, and glycine. The most marked increments in AA as a result of fermentation were ornithine, asparagine, γ-aminobutyric acid, and methionine. Marked decreases in arginine and glutamic acid were also observed. The influence of FA and UP·CaP on amino acids was FA increased mainly tyrosine, arginine, serine, and glutamic acid, whereas γ-aminobutyric acid, glutamine, and methionine were decreased. The UP·CaP increased arginine, tyrosine, and histidine and reduced γ-aminobutyric acid and methionine.

In warm climates where double cropping is possible, wheat for silage is often planted as a winter crop. However, the limited growing time available often necessitates harvesting at an early stage of maturity. At the shooting or flowering stage, however, the wheat is not very suitable for fermentation because of the low dry matter (DM) and water-soluble carbohydrates and is then generally not ensiled. Furthermore, for this reason wheat silage has not been extensively investigated. Silage quality is generally improved as the DM content increases from 20 to 35% (Wilkins et al., 1971). Additives may be employed to enhance the fermentation process and thereby improve the nutritive value and intake. Formic acid (FA) is a well-known additive for this purpose and can be used to reduce the degree of fermentation and to inhibit clostridial activity when added before ensiling to herbage of low water-soluble carbohydrate content (Waldo et al., 1971; Wilkinson et al., 1976). Lancaster et al. (1977) and Barry (1976) reported that FA-treated silage contained more organic matter, less fiber and ammonia, and lower levels of pH and total volatile fatty acids and was of better digestibility and improved daily intake in cows.

Urea and propionic acid are also well-known additives. Several reports (Huber et al., 1968, 1973; Knott et al., 1972; Polan et al., 1968, Sherley et al., 1972) indicate that urea is particularly well utilized by dairy cows when added to corn during ensiling. Huber and Santana (1972) found increased quantities of protein N in urea-treated corn silage. Lessard et al. (1978) reported that in urea-trated silage, the quantities of many individual amino acids (AA) increased considerably, among these being the essential amino acids (EAA) isoleucine, lysine, threonine, and valine. This was in contrast to a decrease in quantity of most AA in the untreated silage. Owens et al. (1981) found that non-protein nitrogen (NPN), when added to corn silage, may increase the content of insoluble potential rumen bypass protein in the silage. Urea phosphate–calcium propionate (UP·CaP) should act as urea and propionic acid, with the technical advantage that the material is granular and thus the propionate ion will be released slowly in the silage.

This study was carried out to determine the changes in the AA concentration of wheat on ensiling and as affected by FA and UP·CaP.

MATERIALS AND METHODS

The wheat plant (variety "Kranich") was harvested at two early stages of maturity: shooting and flowering. The material was chopped and ensiled. To the chopped material was added either 0.4% (w/w) FA or 2.2% (w/w) of a 1:1 mixture of urea phosphate, $CO(NH_2)2{\cdot}H_3PO_4$, and calcium propionate, $Ca(CH_3CH_2COO)_2{\cdot}H_2O$, on a wet basis. (This resulted in a final concentration of approximately 0.8% propioniate ion, 0.21% added P, 0.19% added N, and 0.22% added Ca; produced by Fertilizers and Chemicals Ltd., Haifa Israel.)

The ensiling was duplicated in special 1.5-L sealed jars with a fermentation lock (J. Weck GMbH u. Co., Wher-Oflinger, West Germany) at 30 °C. After 90 days of fermentation, a further aerated silage (AS) was undertaken at 30 °C for 7 days according to the methods developed by Woolford et al., (1977). According to this method, samples of 3 L are held in a heat-insulated chamber, at constant temperature. Air is passed through the silage being tested and continues through a wash bottle containing KOH solution. The CO_2 engendered by the silage is trapped and can be measured by titration.

The 7-day period of aeration of silage was chosen as a period during which aerobic conditions may be experienced, including air penetration before unloading and until consumption in the barn, and reduces free amino acids in the absence of additives and increasing ammonia. Representative samples were taken for analysis. Duplicate samples of 300 g were held in a freezer at −25 °C until

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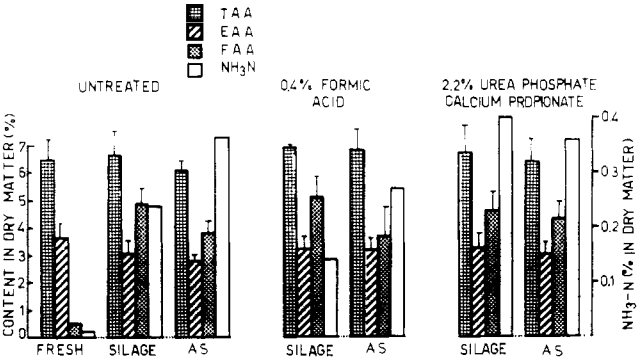

Figure 1. Changes in amino acids and ammonia N during ensiling and after aeration of silage in treated and untreated material compound to fresh wheat forage.

analysis or drying in a freeze-dryer (Typ Epsilon, Martin Christ 3360, Ostenrode am Harz, West Germany). Dry matter was determined by toluene distillation (Dewar and McDonald, 1961). Total N was determined by the Kjeldhal method and protein was calculated by multiplying the N × 6.25. Ammonia N was determined by using an ammonia electrode (Orion Research Inc., Cambridge, MA). In this method, 40 g of silage was mixed with 90 mL of distilled water for 3 min and then filtered. Twenty milliliters of the filtrate was used for analysis. A calibration curve was obtained by using a standard concentration of ammonium chloride. Free amino acids (FAA) were extracted after homogenizing in an ice-cold water mixture of acetone and water (7:3 v/v) and analyzed directly. The bound AA in the remaining material were analyzed after filtering FAA and following hydrolysis (HCl, 6 M, 24 h, 120 °C) of material remaining on the filter paper after the FAA had been filtered. The AA were determined from freeze-dried material by using an amino acid analyzer (Biotronik Wissenschafliche Gerate GmbH, Model L.C. 6000). Total N analysis was also carried out on the solution prepared for use with the AA analyzer. This was done as a check with the AA analysis.

RESULTS

Changes in the AA pattern and ammonia N during ensiling and following the AS in the treated and untreated material (UM) are shown in Figure 1. Total amino acids (TAA) remained relatively stable, with a tendency to increase during ensiling and decrease during the AS, especially in the UM.

The amount of FAA was less than 10% of the TAA in the fresh material and increased to 69–75% in all ensiled material (Figure 1). As a result of the AS, FAA decreased from 72% in the silage to 63% in the UM and to 54% in the FA-treated material but did not decrease in the UP·CaP-treated material. The EAA comprised 56% of the TAA in the fresh material and decreased to 46–48% in the silage and AS. The total amounts of EAA in the silage and AS were higher in the formic acid treated material, compared to UM. Ammonia N content was very low in the fresh material and increased during ensiling and as a result of the AS, although the FA treatment suppressed this tendency. In the FA-treated material there was 44% less ammonia N than in the untreated silage and 25% less for the AS. In contrast, the UP·CaP treatment increased ammonia N up to 164% in the silage in comparison with that of the untreated material.

The concentration of 20 AA in the untreated fresh material, silage, and aerated silage is shown in Table I. The concentration of FAA was very low in the fresh material (<10%), except for that of γ-aminobutyric acid

Table I. Amino Acid Nitrogen of Untreated, Ensiled, and Ensiled Aerated Wheat Plants[a]

source material		Arg	Lys	Ala	Glu	Leu	Asp	Gly	Val	Pro	His	Thr	Ser	Ile	Phe	Tyr	γ-amino-butyric acid	Met	Gln	Asn	Orn	residual N
fresh	TAA	1615	1093	821	801	695	654	646	587	554	486	455	454	410	409	202	139	95	93	59	21	615
	±SE	336	122	62	75	75	44	52	52	107	69	50	34	56	58	37	28	20	19	16	2	69
fresh	FAA	34	29	151	22	25	26	18	30	77	18	30	45	16	14	10	116	2	93	59	8	105
	±SE	10	3	9	15	4	5	2	4	45	5	4	5	3	3	2	8	1	19	16	2	11
silage	TAA	299	1024	1004	396	825	666	789	676	596	357	477	342	465	456	91	596	170	7	393	466	1421
	±SE	34	267	113	96	110	163	85	44	125	83	54	159	79	75	27	147	45	2	122	102	174
silage	FAA		691	767	196	614	467	556	514	475	232	358	220	352	331	54	565	142	7	393	382	1051
	±SE		214	89	105	91	146	66	73	116	79	57	165	69	62	26	141	40	2	122	89	57
AS	TAA	458	853	1018	496	790	626	760	662	522	125	457	366	444	413	118	572	40	10	213	293	1353
	±SE	315	120	95	91	85	139	75	75	81	15	69	124	52	46	30	171	30	6	54	162	48
AS	FAA		405	703	183	506	335	462	426	361	72	276	190	280	245	45	542	105	10	213	222	966
	±SE		94	105	73	80	130	29	66	82	23	61	119	51	42	26	167	16	6	54	121	101

[a] Data are the mean of composition obtained from fresh, ensiled, and aerated ensiled material taken at flowering and at shooting stages. Mean ± SE, μg/g amino acid N found at concentrations above 21 μg/g of DM are shown in the table. The remaining acids and ammonia N are included in the residual N.

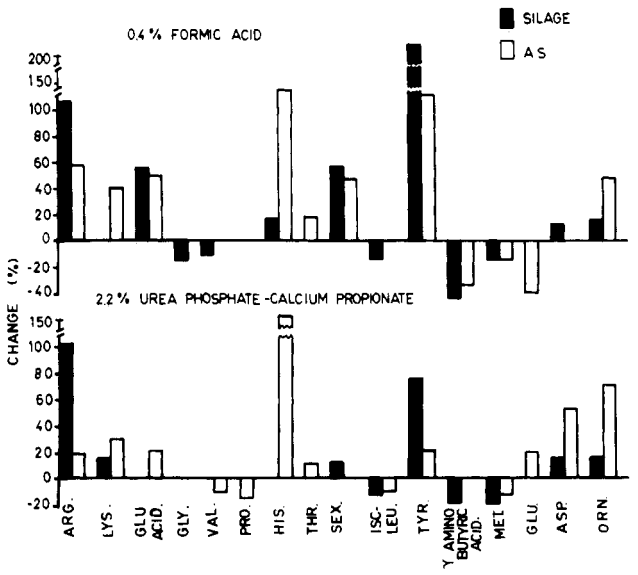

Figure 2. Major changes in amino acids in wheat silage, as affected by the additives 0.4% formic acid and 2.2% urea phosphate–calcium propionate. Amino acid levels in the untreated material were taken as 0%. Those that changed less than 10% are not shown.

(83%) and alanine (18.4%). In the three conditions of material (fresh, silage, and AS), glutamine and asparagine were detected only in the free form.

As a result of ensiling process in all treatments, a large decrease in AA concentrations was found in glutamine (90%), arginine (80%) detected only in the bound fraction, tyrosine (50%), glutamic acid (50%), and histidine (25%) and a smaller decrease in serine and lysine. The concentration of ornithine, asparagine, and γ-aminobutyric acid increased considerably upon ensiling (2220, 666, and 430%, respectively), and methionine, alanine, leucine, valine, isoleucine, and phenylalanine increased somewhat (10–90%). In the AS in both treatments, the main differences in AA concentrations, compared with those in silage, were a reduction of 65% in histidine, 46% in asparagine, 37% in ornithine, 17% in methionine and lysine, and 12% in proline and an increase of 25% in glutamic acid, 30% in tyrosine, and 53% in arginine.

Major changes in the AA as a result of the additives (0.4% FA, 2.2% UP·CaP) are given in Figure 2; the AA that differed by 10% (or less than the value of the UM) are not shown.

Thus, after addition of FA, there were increases (in order of magnitude) in tyrosine, arginine, glutamic acid, and serine in silage and in histidine, tyrosine, arginine, ornithine, serine, and lysine in the AS. There was also a decrease in γ-aminobutyric acid, glutamine, and methionine in silage and as a result of the AS. The addition of UP·CaP resulted in an increase of tyrosine, histidine, asparagine, lysine, and ornithine, particularly in silage, and an increase in histidine, lysine, tyrosine, and arginine was observed in the aerated silage. Reduced γ-aminobutyric acid and methionine were recorded in the silage and reduced glutamic acid and methionine in the AS.

DISCUSSION

Changes in amino acid distribution in the early stages of maturation of the wheat plant, and the effect of addition of FA or UP·CaP on silage and the AS on this distribution, have not been discussed extensively in the literature.

McDonald et al. (1960) reported that the amount of ammonia N in fresh herbage is usually less than 1% of total N (TN). In this study the ammonia N was only 0.58%. Wilson and Tilly (1965) examined five grasses and six samples of lucerne and showed that the highest concentrations of AA were those of arginine, which, together with lysine, aspartic acid, glutamic acid, alanine, leucine and glycine, accounted for 63% of the TAA nitrogen, whereas in the present study the figure is 60.6% of TAA nitrogen, with arginine being present at the highest concentration in the fresh material. Although the TAA remained relatively stable as a result of ensiling and the AS, the EAA was reduced to 83% in the silage and to 77% as a result of AS. The FAA increased 8.8 and 7.9 times and the ammonia N increased 24 and 36 times in the silage and AS, respectively.

The FA may act by retarding the fermentation process. In the experiments described here, FA reduced ammonia N production during ensiling and in the AS. This is in agreement with the findings of Derbyshire et al. (1976) and Lancaster et al. (1977) that FA increased energy recovery, reduced fermentation acids, and decreased protein degradation. Crawshaw et al. (1980) reported the inhibitory effect of FA on aerobic deterioration in the AS and on ammonia N production. A similar decrease in ammonia N production was observed in this study.

The UP·CaP additive was intended to produce a dual effect, due to the addition of the propionate ion and urea. However, the anticipated results were not obtained: the UP·CaP treatment increased EAA and ammonia N in silage and the AS, while the TAA remained stable. It appeared that in this case the ammonia N increase was derived from urea and not due to degradation of the AA. Pahlow (1979) reported that addition of 0.5% urea to fresh materials inhibits growth of lactic acid bacteria and yeasts, inhibits heat production, and leaves the pH relatively stable. Lessard et al. (1978) reported that urea-treated silage contained more true protein FAA and more of the EAA isoleucine, lysine, threonine, and valine. However, in this study, the major effect of UP·CaP was to increase ammonia N, the AA arginine and tyrosine in the silage, and histidine, ornithine and lysine, in the AS. The UP·CaP additive was less beneficial than expected; however, this study confirms the inhibitory effect of FA on ammonia N production in wheat silage.

Registry No. FA, 64-18-6; UP·CaP, 89232-82-6; ammonia, 7664-41-7.

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Mineral Composition of the Edible Muscle Tissue of Seven Species of Fish from the Northeast Pacific

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The edible muscle tissue of Pacific cod, Dover sole, walleye pollock, Pacific whiting, pomfret, Atka mackerel, and American shad was analyzed for its mineral content. Of 25 elements determined, 10 elements—Ag, B, Cd, Co, Mo, Ni, Pb, Sc, V, and Y—were below quantifiable levels and 15 elements—Al, Ba, Ca, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Na, P, Sr, and Zn—were at or above quantifiable levels. Iron and copper concentrations in Atka mackerel and American shad were at least 2 times higher than in any of the other five species. Manganese level in walleye pollock from Shelikof Strait was at least 5 times greater than that in pollock from the Bering Sea or Cape Ommaney, AK. Fish held in refrigerated seawater had significantly higher Na content than fish held in ice. The nape sections of Pacific cod had slightly higher levels of Ca, Hg, Mg, and Mn and slightly lower levels of Cu, Fe, and Zn than the tail sections; no difference was observed for Al, Ba, Cr, K, Li, Na, P, and Sr. The data extend the available information of the mineral composition of the various species of fish from both a nutritional and a health hazard point of view.

In recent years, there has been much interest in the study of trace metal composition of fish, especially after the discovery of widespread mercury pollution in the marine environment (Holden, 1973). It was suspected that other potentially toxic metals may similarly be widespread environmental contaminants. Because of a lack of information on the trace metal content of fish and fishery products intended for human consumption, the National Marine Fisheries Service initiated a series of surveys to determine the trace element content (toxic and essential) in fishery resources. The most comprehensive of these surveys covered 15 elements in 204 species of finfish, mollusca, and crustacea taken from 198 sites around the coastal United States including Alaska and Hawaii (Hall et al., 1978). To date, 20 elements are known to be essential in human/animal nutrition (NRC, 1980), and seafoods are a good dietary source of many of them (Stansby and Hall, 1967).

The study reported here extends the available information on the amount of Al, Ba, Ca, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Na, P, Sr, and Zn in the edible muscle tissue of seven species of fish, Pacific cod (*Gadus macrocephalus*), Dover sole (*Microstomus pacificus*), walleye pollock (*Theragra chalcogramma*), Pacific whiting (*Merluccius productus*), pomfret (*Brama japonica*), Atka mackerel (*Pleurogrammus monopterygius*), and American shad (*Alosa sapidissima*), caught in various locations in the northeast Pacific Ocean. These species are currently under study for expansion of our domestic food fisheries; thus, it is important to know their mineral composition from both a nutritional and a health hazard point of view.

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northwest and Alaska Fisheries Center, Utilization Research Division, Seattle, Washington 98112.

MATERIALS AND METHODS

The fish were obtained from fish-processing companies and research vessels of the National Oceanic and Atmospheric Administration. Most specimens were caught in nearshore waters off the states of Alaska, Washington, Oregon, and California. Pomfret, a pelagic species, were caught in the Gulf of Alaska.

The specimens were washed briefly with tap water to remove slime or ice, drained, weighed, and then filleted by hand with stainless steel knives. Analytical samples consisted of the entire fillets of each fish with the exception of the fillets used for the distribution study, where only the nape and tail sections of the fillets were utilized. The muscle tissue was ground in a Waring blender, packed in plastic containers, and stored at 0 °C until analyzed, normally within 2–3 days.

For all elements except mercury, tissue samples of approximately 10 g each were weighed into acid-washed Vycor crucibles and placed under infrared heat lamps for about 4 h to dry and char. The crucibles were transferred to a muffle furnace, which was allowed to gradually rise (no less than 2 h) to 450 °C, and allowed to remain in the furnace for 24–48 h to obtain a white ash. After cooling, 10 mL of 4% nitric acid was added to each crucible and the crucibles were covered with watch glasses and heated at low heat to near boiling. After cooling to room temperature, each sample was filtered through Whatman No. 40 filter paper into a 25-mL volumetric flask, followed by several rinsings of both the crucible and the filter paper with 4% nitric acid, and made to volume with 4% nitric acid.

The various elements, excluding Hg, were determined by emission spectroscopy (Jarrell-Ash Model 975 inductively coupled argon plasma emission spectrometer). Calibration of the instrument and analysis of the samples were performed with background and interelement corrections. Total mercury was determined by the Official