SILAGES OF CITRUS PULP-POULTRY LITTER-CORN FORAGE FOR SHEEP

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Citrus pulp was ensiled with the mixture of poultry litter and corn forage (60:40) in the ratio 0:100, 10:90, 20:80, 30:70 and 40:60 (wet basis), respectively. Samples of pre-ensiled mixtures showed the presence of total (3.1×10^5-1.01×10^5) and fecal (1.01×10^4-0.18×10^4) colony forming units (CFU). *Salmonella, Shigella and Proteus* were also present. Ensiling was effective in complete elimination of all pathogens. Dry matter (DM), crude protein (CP), crude fiber (CF) and ash contents decreased ($P<0.05$) linearly with the increase of citrus pulp in the silages (714-559, 167-120, and 292- 251g kg^{-1}, DM basis, respectively). Water soluble carbohydrates (WSC) for pre-ensiled mixtures were, 51, 57, 63, 68 and 74 g kg^{-1} on DM basis, respectively. Following ensiling pH and WSC decreased ($P<0.05$) for all the silages but decrease was higher ($P<0.05$) for the silages containing citrus pulp than the silage without citrus pulp. Lactic acid concentration in the silages increased linearly ($P<0.05$) with the increase of citrus pulp. The nutritive value of silages was evaluated in a digestion trial with 30 wethers. The apparent digestibility of DM, CP and CF for animals fed poultry litter-corn forage alone was 618g.kg^{-1}, 616 g.$kg^{1}$and 510 g.kg^{-1} respectively. However, digestibilities of all the components increased ($P<0.05$) with increase of citrus pulp up to 30% level in the diet then decreased. The diet containing 30% citrus pulp showed higher ($P<0.05$) digestibility of all the components than the diets containing 10, 20 and 40% citrus pulp. The results indicated that citrus pulp can be used safely up to 30% in ruminants' diet as source of digestible carbohydrates with out any adverse effect on the health of animals. Ensiling appeared to be safe and feasible mean of preserving citrus pulp and losses of the nutrients due to the high moisture content of the citrus pulp can be reduced by ensiling it with poultry litter and chopped corn forage.
Keywords: Citrus pulp, poultry litter, corn forage, pathogens, digestibility, ensiling, sheep.

INTRODUCTION

Livestock production in Pakistan is limited by fodder shortage during the dry season. To face this problem, one can apply different strategies such as the utilization of by-products and storing forage from wet season surplus. Feeding by-products of the crop and food processing industries to livestock has two important advantages: i) diminish dependence of livestock on grains and ii) to fill the gap of nutrients deficiency. Ruminant feeding systems based on locally available by-product feedstuffs (BPF) are often a practical alternative because the rumen microbial ecosystem can utilize BPF, which often contain high levels of structural fiber, to meet their nutrient requirements for maintenance, growth, reproduction and production (Arthington *et al.* (2002).

In Pakistan, citrus fruits are grown on the area of 160,000 hectares with production of 1.5 MMT annually (Economic Survey of Pakistan, 2005-6). Fruit processing industry provides juice, concentrate, or canned fruit and wastes including pulp, peel, rag, and seeds. Composition of these wastes varies according to the originating crop and method of production. (Hendrickson and Kesterson, 1965).

Poultry litter consists of bedding material, excreta, wasted feed, feathers, bacterial biomass, and soil that contribute to the variation in litter composition and quality (Goetsch *et al.*, 1998). Its use as a feed has an economic value high enough to be transported for long distances (Harvey *et al.*, 1996). There has been some concern about pathogenic bacteria that may potentially be found in poultry litter. However, ensiling is safe, economical and efficient method of eliminating all types of pathogens present in litter (Chaudhry *et al.* 1993, Fontenot *et al.*, 1990; McCaskey *et al.*, 1985).

Various processing methods applied for citrus pulp to use as feed for ruminants include; mechanical drying, pelleting and making meal are cost effective (Hadjipanayiotou, 1987).

Due to the perishable property of citrus pulp in tropical countries, it would be convenient to develop economical and efficient method of preservation that would enable these plant materials to be utilized as animal feeds for longer periods of time.

The objectives of this study were to: 1) evaluate the preservation of citrus pulp by ensilation and 2) determine fermentation characteristics and nutritive value of ensiled citrus pulp-poultry litter-corn forage when fed to sheep.

MATERIALS AND METHODS

Citrus pulp was collected in metal drums from local juice factory and wood shaving broiler litter was purchased from commercial broiler house. Corn forage harvested at mature stage having about 40% dry matter was chopped. First a mixture of poultry litter and chopped corn forage was prepared in the ratio of 60:40 (dry matter basis), respectively then citrus pulp was mixed in the mixture (poultry litter-corn forage) in the ratios 0:100, 10:90, 20:80, 30:70, and 40:60 respectively. Each mixture was prepared by weighing the exact amount of each component and respective mixtures were allowed to blend in horizontal mixer for 30 min. The silages were prepared in metal drums (200-litre capacity) lined with double layer of polyethylene. Packing was done by trampling on the drums. Samples of corn, poultry litter, and initial mixture were taken and frozen in polyethylene bags for later analysis.

After 60 days, each silo was opened, examined and samples were taken for chemical and biological analysis. After taking the samples, the bags were immediately sealed. One silo from each treatment was reopened at the time of digestion trial. Samples were taken at each feeding and composited for later analysis. During digestion trial, samples of about 100 g from each silo were taken at every feeding and frozen individually. Frozen silos samples were composited periodically after every five days to give six samples of each material. Samples of microbial study and fermentation characteristic were processed immediately while other samples were frozen for later analysis.

Biological Analysis

Total (Anonymous, 1967) and fecal (Millipore Corp., 1973) coliform were determined on initial samples of each component, initial mixtures and ensiled mixtures. The aseptic samples were collected for microbial analyses and samples were prepared by homogenizing 25 g of sample with 225 ml of distilled water in a blender at full speed for 1 min.

Chemical Analysis

Pre- and post-ensiled materials were analyzed for nitrogen on wet samples (AOA 1984 method). Dry matter was determined by drying in duplicate, 200 g samples of each material in forced draft oven at a maximum of 60 °C for 48 h. Following equilibration with atmospheric moisture, the duplicate dried samples were composited, ground to pass a 1 mm sieve and subjected to analysis for dry matter (DM), ether extract (EE, AOAC, 1984) and crude fiber (CF, Whitehouse *et al.* 1954). Nitrogen on fecal samples was determined on dry samples (AOAC, 1984).

Extracts of poultry litter, corn forage, initial and ensiled mixtures were prepared by homogenizing 25 g of sample with 225 ml of distilled water in a blender for 1 min. The homogenate was filtered through four layers of cheesecloth and the filtrate was used for determining pH (electrometrically), lactic acid (Baker and Summerson, 1941), as modified by Pennington and Sutherland (1956) and water-soluble carbohydrates (Dubois *et al.*, 1956) as adapted by Johnson *et al.* (1966).

Digestion Trial

Thirty wethers weighing 28-33 kg were assigned to six blocks of five animals each, based on weight. Sheep within each block were randomly allotted to five silages: broiler-litter-corn mixture ensiled alone or with citrus pulp in the ratios of 0:100, 10:90, 30:70 and 40:60, respectively. Diets were given at 20 g dry matter kg^{-1} body weight per day. Experimental diets were given for 45 days, and faeces collected during last 10 days in canvas bags held by harness, as described by Fontenot and Hopkins (1965). The conduct of digestion experiment followed the recommendations of Schieman (1981).

Faeces were collected at each morning and dried in forced draft oven at a maximum of 60°C for minimum of 24 h. At the end of the trial, faecal composites were weighed, mixed and sub sampled.

Statistical Procedure

Data was treated by analysis of variance using general linear model procedure of SAS (1982). For ensiling and digestibility trial, treatment and block were included in the model. Linear quadratic and cubic contrasts were used to test the treatment effect.

RESULTS AND DISCUSSION

Chemical Composition and Fermentation Characteristics

Chemical composition of the ingredients (Table 1) showed that citrus pulp has 195, 68, 16, and 188 $g.kg^{-1}$ DM, crude protein (CP), ether extract (EE) and crude fiber (CF), respectively. Our findings for the chemical composition are lower than the findings of Bueno, *et al.* 2002) and higher than the values reported by Migwi, *et* al. (2001). The difference in composition may be contributed by factors such as growing conditions, maturity, rootstock, variety, climate and processing method (Kale and Adsule, 1995).

Chemical composition of poultry litter presented in table 1 showed crude protein content of the litter (24%, DM basis), higher than our earlier findings (Alrokayan et al. 1998), but lower than the values reported by Harmon et al. (1975[ab]). Ash value of the poultry litter was similar to the values reported by Casewell et al. (1975) but lower than the values reported by Flachowsky and Henning (1990). These differences in composition could be due to differences in bedding material, the number of batches of birds housed on the litter, broiler house management, method of litter removal, and moisture content (Fontenot et al. 1990). Corn forage was similar in CP, ash and crude fiber reported by Chaudhry et al. (1993) and Harmon et al. (1975[a, b]).

Composition of initial and ensiled mixtures (Table 2&3) was almost similar except that dry matter and crude fiber contents of the ensiled mixture decreased (P<0.05) after ensiling, it may be due to the respiration of plant cell and action of microbes in the ensiled mixture (Ridla and Ushida 1999[a]). Following ensiling decrease in cell wall constituents may be due to action of bacterial enzyme to hydrolyze cell wall components, especially for more digestible constituent of plant cell wall. Inclusion of citrus pulp caused linear decrease (P<0.05) in dry matter (DM), crude protein (CP), crude fiber (CF) and ash contents of the mixtures. However, the values for water soluble carbohydrates and nitrogen free extract increased linearly (P<0.05) with increase of citrus pulp in the mixture. Decrease in DM, CP, CF and ash contents and increase in WSC and NFE values are mainly attributed by the difference in chemical composition of citrus pulp. Similar results have been reported by the (Migwi, et al. 2001) when they ensiled fresh citrus pulp with high dry matter agro-industrial waste.

Fermentation characteristics of the silages are presented in table 4. Water soluble carbohydrates decreased (P<0.05) and lactic acid production increased (P<0.05) in all the silages, indicated that desirable fermentation was achieved. soluble carbohydrates present in plants such as corn and fruit products are capable to produce sufficiently high levels of acids especially lactic acid and acetic acid needed to preserve non-fermentable materials (Ariza et al. 2001). Buffering effect of poultry litter mainly due to production of ammonia by hydrolysis of uric acid has been shown to increase the concentration of lactic acid (Harmon et al. 1975[a]). Values of pH for all the mixtures decreased following ensiling and ranges from 4.58 to 3.97, similar to the values reported by Alrokayan et al. (1998) when sorghum was ensiled with poultry litter. Values for pH decreased with the increase of citrus pulp in the mixture, mainly due to higher levels of fermentable carbohydrates present in citrus pulp. Similar values (pH 4.01) have been reported by Migwi et al. (2001) when citrus pulp was ensiled with the mixtures of poultry litter and wheat straw. Chaudhry et al. (1993) observed pH values of 4.32 and 3.78 for corn forage ensiled alone or with poultry litter. The close agreement of the values obtained in present study with the previously reported values indicates that desirable ensiling has occurred.

Bacterial Determination

Higher counts of total and fecal colony forming units (CFU) were found in initial mixtures containing poultry litter. *Salmonella, Shigella* and *Proteus* were present in the pre-ensiled mixtures (table 5). Following ensiling, all mixtures were tested negative for total and fecal CFU, *Salmonella, Shigella* and *Proteus*. Ensiling process completely eliminates microorganisms has been reported by Casewell et al. (1975) when poultry litter was ensiled with 40% moisture. Similar findings have been reported by McCaskey et al. (1985) and Chaudhry et al. (1993). Some *lactobacilli* species produce sufficient hydrogen peroxide to inhibit coliform and *Salmonella* organisms (Martin, and Bozoglu, 1996; Dahyia and Speck, 1969). Moreover, some lactobacilli produce sufficient hydrogen peroxide to inhibit coliform and *Salmonellae* organisms (Dahiya and Speck, 1969). Chung and Geopfert (1970) reported that heat production during anaerobic fermentation also contribute to the inhibition of coliform organisms.

Apparent Digestibility

Differences in composition of diets containing different proportions of citrus pulp reflected the composition of silages. Diets without citrus pulp were readily accepted by lambs but animals took longer time to adapt to the diets containing citrus pulp due to distinctive smell and taste. Bath et al. (1980) reported that dehydrated citrus pulp or silages containing citrus pulp should be introduced gradually into rations allowing animals to take sufficient time to become accustomed to its distinctive smell and taste. Digestibility values for all nutrients were higher (P<0.05) for diets containing citrus pulp than the diet without citrus pulp. Among citrus pulp containing diets highest (P<0.05) digestibility values for DM, CP, and CF were found for the diet containing 30% citrus pulp than those containing 10, 20, or 40 % citrus pulp quadratic effect. Reason for higher digestibility values of OM, DM, CP, and CF for the diets containing citrus pulp is two fold; i) presence of more digestible carbohydrates (pectin and cellulose) in citrus pulp secondly ii) higher buffering capacity of citrus pulp resist lowering of rumen pH thus positively altered rumen fermentation Migwi *et al.* (2001). Chen *et al.* (1981) and Wing *et al.* (1988) found decreased molar concentration of acetic acid and isovaleric acid and increased molar concentration of propionic acid and valeric acid, that resulted lower acetic:propionic acid ratio when corn grain was replaced with dried citrus pulp in lamb diet. In present study higher ($P<0.05$) CF digestibility coefficient was found for diets containing 30% citrus pulp. Similar results have been reported by Ben-Ghedalia *et al.* (1989) and Barrios *et al.* (2003). They also found higher NDF and ADF digestibility coefficients when starchy feed was replaced by citrus pulp. Citrus pulp improves the utilization of other dietary NDF, possibly due to positive effects on rumen microflora (Barrios *et al.*, 2003). Migwi *et al.* (2001) and Scerra *et al.* (2001) found higher digestibility values of OM, DM, CP, and CF for diets containing citrus pulp-wheat straw silage. Further they suggested that citrus pulp could safely be fed to lamb up to 200g/kg of the diet.

It is concluded from the study that to limit the losses due to high moisture content of the citrus pulp, it can successfully be preserved by ensiling with poultry litter and chopped corn forage for use as feed for ruminants. Moreover, ensiling is safe and feasible mean of preserving high moisture citrus pulp when ensiled with high DM containing Agro-industrial wastes (poultry litter and corn forage) and converting these into a palatable and nutritious feed for ruminants. Results indicated that citrus pulp-poultry litter-corn silage can be important components of ruminant feeding systems because silage of citrus pulp-poultry litter-corn forage contains energy substrates for ruminal microbes, including both soluble carbohydrates and rapidly digested NDF. It is recommended that citrus pulp can be used as a high energy feed in ruminant's rations that can support growth and lactation in ruminants.

Table 1. Chemical Composition of citrus pulp, poultry litter and corn forage[ab]

Items	Citrus Pulp	Poultry litter	Corn
DM g kg^{-1}	195	903	432
CP g kg^{-1}	68	224	815
CF g kg^{-1}	188	313	261
EF g kg^{-1}	16	14.3	21
Ash g kg^{-1}	8.5	198	47.8
WSC g kg^{-1}	85.6	23.5	93.1
NFE g kg^{-1}	720	252	589

DM= dry matter, CP= crude protein, CF=crude fiber, EE= Ether extract, NFE= nitrogen free extract, WSC= Water soluble carbohydrates.
[a]Each value represents the mean of six samples.
[b]Dry matter basis.

Table 2. Chemical Composition of pre-ensiled citrus pulp- poultry litter-corn forage mixture[ab]

Items	Citrus pulp : poultry litter-corn forage mixture					
	0:100	10:90	20:80	30:70	40:60	SEM
DM[cdef] g kg^{-1}	714	663	611	559	507	1.09
CP [cdef]g kg^{-1}	167	157	147	137	129	1.32
CF[cdef]g kg^{-1}	292	281	274	267	251	1.97
EF g kg^{-1}	18	17	17	17	17	-
Ash [cdef]g kg^{-1}	138	125	112	99	86	1.97
WSC [cdef]g kg^{-1}	51	57	63	68	74	3.32
NFE [cdef]g kg^{-1}	387	420	453	487	521	4.09

SEM= standard error of means, DM= dry matter, CP= crude protein, CF=crude fiber, EE= Ether extract, NFE=nitrogen free extract, WSC= Water soluble carbohydrates.
[a]Each value represents the mean of six samples.
[b]Dry matter basis.
[c]Linear effect ($P<0.05$).
[d]Citrus pulp containing mixtures vs mixtures with out citrus pulp differ ($P<0.05$).
[e]Mixtures containing 10% citrus pulp vs mixtures containing 20%, 30% and 40 % citrus pulp differ ($P<0.05$).
[f]Mixtures containing 30% citrus pulp vs mixtures containing 40 % citrus pulp differ ($P<0.05$).

Table 3. Chemical Composition of post-ensiled citrus pulp- poultry litter-corn forage mixture[ab]

Items	Citrus pulp : poultry litter-corn forage mixture					
	0:100	10:90	20:80	30:70	40:60	SEM
DM[cdef] g kg^{-1}	698	639	595	538	487	1.12
CP[cdef] g kg^{-1}	169	160	151	139	127	1.67
CF [cdef] g kg^{-1}	284	278	269	256	248	2.09
EF g kg^{-1}	17	17	17	17	17	-
Ash [cdef] g kg^{-1} g kg^{-1}	139	126	113	100	87	3.14
NFE [cdef] g kg^{-1}	391	419	450	488	521	4.15

SEM= standard error of means, DM= dry matter, CP= crude protein, CF=crude fiber, EE= Ether extract, NFE=nitrogen free extract, WSC= Water soluble carbohydrates.

[a]Each value represents the mean of six samples.

[b]Dry matter basis.

[c]Linear effect (P<0.05).

[d]Citrus pulp containing mixtures vs mixtures with out citrus pulp differ (P<0.05).

[e]Mixtures containing 10% citrus pulp vs mixtures containing 20%, 30% and 40 % citrus pulp differ (P<0.05).

[f]Mixtures containing 30% citrus pulp vs mixtures containing 40 % citrus pulp differ (P<0.05).

Table 4. Total and colony forming units (CFU), *Salmonella, Shigella and Proteus* of initial and ensiled mixtures[a].

Pathogens	Citrus pulp: poultry litter-corn forage mixture				
	0:100	10:90	20:80	30:70	40:60
Total CFU[b] (10^5)					
Pre-ensiled	3.1	2.76	2.25	1.99	1.01
Post ensiled	-	-	-	-	-
Fecal CFU[b] (10^4)					
Pre-ensiled	1.0	0.47	0.39	0.35	0.18
Post-ensiled	-	-	-	-	-
Salmonella[c]					
Pre-ensiled	+	+	+	+	+
Post-ensiled	-	-	-	-	-
Shegella[c]					
Pre-ensiled	+	+	+	+	+
Post-ensiled	-	-	-	-	-
Proteus[d]					
Pre-ensiled	+	+	+	+	+
Post-ensiled	-	-	-	-	-

[a]Six samples per treatments.

[b]CFU, g^{-1} dry basis.

[c]Qualitative study; (+) indicates presence. (-) indicates absence of respective organisms.

Table 5. Fermentation characteristic of pre and post-ensiled mixtures[ab]

Items	Citrus pulp : poultry litter-corn forage mixture					
	0:100	10:90	20:80	30:70	40:60	SEM
pH						
Pre-ensiled[c]	6.5	6.85	6.9	7.0	7.0	0.02
Post-ensiled[c]	4.58	4.41	4.25	4.15	3.97	1.01
Lactic acid, g kg^{-1}						
Pre-ensiled	0.0	0.0	0.0	0.0	0.0	0.0
Post-ensiled[c]	3.25	6.51	7.15	9.15	11.24	1.16
WSC g kg^{-1}						
Pre-ensiled[c]	51	57	63	68	74	1.21
Post-ensiled[c]	3.1	3.3	2.6	1.5	1.4	2.15

SEM=standard error of means
[a]Each value represents the mean of six samples.
[b]Dry matter basis.
[c]Linear effect ($P<0.05$).

Table 6. Digestibility of poultry litter-corn forage-citrus pulp silages when fed to sheep[ab].

Items	Citrus pulp : poultry litter-corn forage mixture					
	0:100	10:90	20:80	30:70	40:60	SEM
DM[cdef] g kg^{-1}	618	639	664	689	653	1.21
CP[cdef] g kg^{-1}	616	637	657	679	651	1.08
CF[cdef] g kg^{-1}	510	535	555	581	560	1.52
EF[cdef] g kg^{-1}	650	660	670	680	668	2.18
Ash[cdef] g kg^{-1}	520	550	580	590	551	1.39

DM= dry matter, CP= crude protein, CF=crude fiber, EE= Ether extract, NFE= nitrogen free extract, WSC= Water soluble carbohydrates, SEM= standard error of means.
[a]Each value represents the mean of six samples.
[b]Dry matter basis.
[c]Quadratic effect ($P<0.05$).
[d]Citrus pulp containing diets vs diets with out citrus pulp differ ($P<0.05$).
[e]Diets containing 10% citrus pulp vs diets containing 20%, 30% and 40 % citrus pulp differ ($P<0.05$).
[f]Diets containing 30% citrus pulp vs diets containing 40 % citrus pulp differ ($P<0.05$).

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