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Carotene-Xanthophyll in Field-Wilted and Dehydrated Alfalfa and Coastal Bermuda Grass

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Dehydrated coastal Bermuda grass (CBG) [Cynodon dactylon (L.) Pers.] and alfalfa (ALF) (Medicago sativa L.) are important sources of carotene and xanthophylls for pigmenting broilers and eggs. Recently, commercial dehydrators have resorted to preliminary field wilting to partially dry these crops. Although this practice saves fuel, some carotene and xanthophyll are lost. This study was conducted to compare changes in the carotene-xanthophyll content of fresh cut vs. field-wilted and then mechanically dehydrated CBG and ALF and also to determine if major changes occur in the relative amounts of monohydroxy-, dihydroxy-, and polyoxyxanthophylls due to processing. Field wilting for 6 h resulted in losses of up to 25% carotene and 20% xanthophyll. Field wilting overnight did not decrease total carotene or xanthophyll in CBG; however, ALF lost about 25% carotene and 14% xanthophyll. Although carotene was relatively stable during dehydration, up to 45% xanthophyll was lost. No appreciable changes were found in the relative amounts of the monohydroxy-, dihydroxy-, and polyoxyxanthophylls in the fresh or dehydrated forages.

Dehydrated Coastal Bermuda grass (CBG) [Cynodon dactylon (L) Pers.] and alfalfa (ALF) (Medicago sativa L.) are used in poultry feeds as sources of carotenoids for pigmenting egg yolks and broilers. In the past, both forages were dehydrated immediately following harvest. However, because of the current high cost of fossil fuel, forages processors now resort to field wilting to partially dry the crop. The cut forage is usually field dried for about 6 h during the day; however, when cut in the late afternoon or evening, it may remain in the field overnight and be dehydrated the next morning. Although partial field wilting appreciably reduces the fuel required for mechanical dehydration (Butler et al., 1969), some carotene and xanthophyll are lost. When exposed to heat, light, and air, these labile compounds undergo oxidation and isomerization (Kohler et al., 1967; Livingston et al., 1970; Marusich and Bauernfeind, 1981). As a result, forages that have been field wilted and mechanically dehydrated may vary widely in the relative proportions of monohydroxy-, dihydroxy-, and polyoxyxanthophylls, which differ in pigmentation potency (Marusich and Bauernfeind, 1981; Kuzmicky et al., 1969). Middendorff et al. (1980) reported that the nonepoxide (dihydroxy) xanthophyll content in sun-cured and dehydrated ALF meals varied considerably as did the levels of these substances in the blood serum of poultry.

In fresh forage, there is a positive relationship between carotene and xanthophyll content (Livingston et al., 1968a,b). Because of this, feed ingredient buyers assume that if carotene is high, xanthophyll also will be high. However, this may not be true for dehydrated forage products as field wilting and dehydration may result in differential losses in carotene and xanthophyll. Livingston et al. (1977) reported large losses in carotene and xanthophyll when ALF was field wilted for 4 h and mechanically dehydrated. Compared to ALF, few data have been published on factors that influence carotene-xanthophyll content in sun-cured and dehydrated CBG (Middendorff et al., 1980). In certain studies where CBG and ALF were compared, either the forages differed widely in quality or information was not available on origin, processing conditions, etc. (Dua and Day, 1964; Barnett and Morgan, 1959; Middendorff, 1980). In assessing potential pigmentation potency of different forage species, materials produced under similar conditions should be compared.

The objective of this study was to compare changes in the carotene-xanthophyll content of fresh cut vs. fieldwilted and then mechanically dehydrated CBG and ALF were produced and processed under commercial conditions and also to determine if changes occur in the relative proportions of carotene and the monohydroxy-, dihydroxy-, and polyoxyxanthophylls in these forages that may be related to processing conditions.

MATERIALS AND METHODS

CBG and ALF were harvested from fields managed by a commercial forage processor (dehydrator). Both forages were grown on a Wagram sand (Arenic Paleudult loamy siliceous thermic family) under optimum conditions of pH and fertility. In 1981, the CBG and ALF fields were

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Table I. Moisture in Coastal Bermuda Grass and Alfalfa Forage, 1981–1982

	moisture, %	
	1981	1982
coastal		
\mathbf{fresh}^{b}	70.0	55.0
wilted ^c	65.8	38.2
wilted— N^d	66.1	57.6
alfalfa		
fresh	74.6	69.7
wilted	49.7	47.9
wilted—N	69.1	63.8

^aMeans, triplicate samples, SE < 1.3. ^bFresh cut. ^cWilted. ^dwilted overnight.

harvested Aug 4–5, after 23 and 32 days of regrowth, respectively. In 1982, both forages were harvested Sept 8–9 after 52 days of regrowth. During the 1981 growing period, rainfall was adequate and good growth occurred. During field wilting, ambient temperatures ranged from 21.1 to 34.4 °C. Relative humidity was 49–86% and wind velocity 6.4–16.1 km/h. In 1982, droughty conditions prevailed during the growing period and minimal growth occurred. During field wilting, ambient temperatures were 13.3–30 °C. Relative humidity was 35–73% and wind velocity 4.8–17.7 km/h.

All treatments were cut with a 3.66 m wide Sperry-New Holland mower-conditioner Model 1495, and left in a 1.22 m wide windrow. The forages were either cut and immediately dehydrated, cut and field wilted for 6 h and then dehydrated, or cut in the late afternoon (about 5 p.m.), allowed to field wilt overnight, and dehydrated the next morning. Each forage treatment was picked up with a Fox, Model 3310, forage harvester equipped for one 1.9-cm theoretical chop. Trucks immediately transported the chopped forage 4.83 km to the dehydrator. Dehydration was accomplished with a triple-pass 85/25 Heil dehydrator rated at 3629 kg/h water evaporating capacity. The processing temperature at the drum discharge end (exit temperature) was regulated by adjustment of the gas feed to the burners. After dehydration, each forage was ground through a 3.97-mm screen in a Ronning hammermill. Pelleting was accomplished with a 149-kW California pellet mill equipped with a 6.35×57.15 mm rotating die. Pellets coming from the mill were cooled to ambient temperature and placed in four 22.7-kg double-walled paper bags.

Three random samples (about 6.8 kg of wet weight) of the fresh cut and field wilted forages were placed in plastic bags and immediately packed in dry ice in insulated containers. Both the fresh frozen and pelleted samples were transported to the laboratory and stored in the dark at -23.3 °C. The fresh frozen material was subsequently freeze-dried. Both the freeze-dried and pelleted material were ground through a 1-mm screen with a Wiley mill.

Moisture. Moisture was determined on the ground forages and pellets by drying duplicate samples in a forced draft oven (130 °C) for 2 h and averaging the difference in weight loss.

Crude Protein. Crude protein $(N \times 6.25)$ was determined in duplicate by the AOAC procedure 2.057 (Horowitz, 1980).

Carotene-Xanthophyll. Carotene and xanthophyll(s) in the freeze-dried forage and pellets were determined in duplicate on all samples by the AOAC (hot saponification) procedure 43.018 (Horowitz, 1980). Monohydroxy pigments (MHP) and dihydroxy pigments (DHP) were separated on adsorbent I and eluted with the MHP and DHP eluents. Polyoxy pigments (POP) were eluted from the same column with hexane-acetone-methanol (80:10:10).



Figure 1. Carotene and total xanthophyll content in fresh-cut Coastal Bermuda grass (CBG) and alfalfa (ALF) (dry basis), 1981–1982.



Figure 2. Carotene-xanthophyll in Coastal Bermuda grass forage and pellets (dry basis), 1981.

RESULTS AND DISCUSSION

Except for the CBG harvested in 1981, field wilting for 6 h during midday reduced moisture 31-33% (Table I). In 1981, rainfall was adequate and good growth occurred. Upon cutting, the large amount of CBG formed a deep, closed windrow that precluded good drying. In contrast, the cut ALF formed a high, open type windrow that facilitated good drying. In 1982, rainfall was lacking and less than optimum growth occurred. When cut, both forages formed a shallow, open type windrow, and during the day, good drying was achieved. Forage cut in the late afternoon and field wilted overnight lost less than 10% moisture. Compared to ALF, CBG contained about 83% more carotene and 34% more xanthophyll. Both forages contained 16-20% crude protein and were of high quality. The lower carotene-xanthophyll values for the fresh-cut CBG and ALF harvested in 1982 compared to 1981 reflects the lack of rainfall and greater maturity of these forages (Figure 1)

Changes in the carotene-xanthophyll content of CBG and ALF forage and pellets produced in 1981 are shown in Figures 2 and 3, respectively. Differences in the standard errors indicate that there was greater variation among the CBG samples than among the ALF samples. This is in agreement with field observations, particularly in 1981 when CBG made abundant growth. Field wilting CBG and ALF during midday resulted in average carotene losses of 8 and 18% and average xanthophyll losses of 9 and 14%, respectively. Field wilting overnight did not



Figure 3. Carotene–xanthophyll in alfalfa forage and pellets (dry basis), 1981.

Table II. Exit Temperatures (°C) during Dehydration of Coastal Bermuda Grass and Alfalfa, 1981–1982

	1981	1982	
coastal			
\mathbf{fresh}^{a}	129	129	
wilted ^b	114	103	
wilterd-N ^c	121	112	
alfalfa			
fresh	131	129	
wilted	121	110	
wilted-N	128	117	

^a Fresh cut. ^b Wilted. ^c Wilted overnight.

decrease total carotene or xanthophyll content in CBG; however, ALF lost about 25% carotene and 14% xanthophyll. The higher carotene-xanthophyll losses exhibited by ALF were possible due to greater oxidative and enzymatic degradation that occurred overnight in the more open type windrow.

During subsequent dehydration of CBG and ALF, carotene appeared to undergo little additional loss (Figures 2 and 3). In fact, in some cases, the dehydrated forages exhibited higher carotene values than the fresh material. Such increase may result from combustion during dehydration of dry, dead grass residues unavoidably picked up during harvesting (Butler et al., 1969). Compared to carotene, xanthophyll appeared to be much less stable to high heat as shown by the greater losses that occurred during dehydration. When CBG that was fresh cut or field wilted overnight was dehydrated, xanthophyll losses averaged 41 and 30%, respectively. However, when CBG was field wilted during midday, only 9% xanthophyll was lost during subsequent dehydration. These losses appeared to be related to the temperature of the dehydrator. It is generally recognized that fresh-cut forage and that cut late in the day and wilted overnight contain higher moisture than forage wilted during midday. Also, ALF contains more moisture than CBG. Accordingly, dehydrator operators usually run the wetter materials at higher temperature to maintain constant production rates. In 1981, the CBG and ALF forages that were fresh cut or field wilted overnight were dehydrated at higher temperatures (121-131 °C) than the forages wilted during midday (114-121 °C) (Table II). Coastal Bermuda grass field dried for 6 h and run at 114 °C only lost about 9% total xanthophyll. However, when either CBG of ALF was dehydrated at 121-131 °C, 30-45% of the total xanthophyll was lost.

Changes in the carotene-xanthophyll content of CBG and ALF forage and pellets produced in 1982 are shown in Figures 4 and 5, respectively. Fresh cut CBG and ALF



Figure 4. Cartoene-xanthophyll in Coastal Bermuda Grass forage and pellets (dry basis), 1982.



Figure 5. Carotene–xanthophyll in alfalfa forage and pellets (dry basis), 1982.

that was field wilted during midday lost an average of 22 and 25% carotene and 22 and 20% xanthophyll, respectively. The preceding growth period was unusually dry. Also, in 1982, conditions for field drying were better than in 1981. As a result, the wilted forages were sufficiently dry to dehydrate at temperatures below 121 °C (Table II). As in 1981, ALF wilted overnight lost more carotene and xanthophyll than the CBG. Also, neither forage lost additional carotene during mechanical dehydration. However, upon dehydration, the fresh-cut CBG and ALF lost 31 and 28% xanthophyll, respectively. In contrast, when the CBG and ALF forages that were wilted during midday or overnight were dehydrated, less than 12% xanthophyll was lost. The lack of appreciable loss in xanthophyll during dehydration of these field wilted forages is believed due to their being dehydrated at 103-117 °C compared to 129 °C for the fresh cut material (Table II).

In an earlier study, Livingston et al. (1977) reported that, when ALF was field wilted for up to 23 h, xanthophyll was more stable than carotene and that, during dehydration, stability of carotene and xanthophyll was about equal. Our results indicate that both carotene and xanthophyll were degraded during field wilting and that conditions during field drying influenced these losses.

In 1981, when the humidity was higher and drying conditions not as favorable (particularly for CBG), carotene and xanthophyll losses were less compared to 1982 when humidity was lower and drying conditions more favorable. Our results also indicate that the temperature during dehydration greatly influences carotene and xanthophyll stability. When the fresh-cut forage was dehydrated at 129–131 °C, xanthophyll losses greatly exceeded those of

Table III. Distribution of Component Xanthophylls in Coastal Bermuda Grass and Alfalfa Forage and Pellets. 1981-1982

MHP,ª %	DHP, ^b %	POP,° %	
0.9 - 1.2	91.0-92.2	6.7-8.1	
0.9-2.0	88.7-91.0	7.0-9.9	
1.3 - 2.2	90.1-95.3	3.4-7.6	
2.1 - 2.7	87. 9- 90.3	7.1-9.7	
1.0-1.6	86.6-88.5	10.4-11.8	
1.2-1.9	86.9-88.7	9.6 - 11.2	
1.2 - 2.2	88.5-89.7	9.1-10.1	
2.1 - 2.5	87.6-89.4	8.5 - 10.3	
	MHP, ^a % 0.9–1.2 0.9–2.0 1.3–2.2 2.1–2.7 1.0–1.6 1.2–1.9 1.2–2.2 2.1–2.5	MHP,* DHP,* % 0.9-1.2 91.0-92.2 0.9-2.0 88.7-91.0 1.3-2.2 90.1-95.3 2.1-2.7 87.9-90.3 1.0-1.6 86.6-88.5 1.2-1.9 86.9-88.7 1.2-2.2 88.5-89.7 2.1-2.5 87.6-89.4	MHP, ^a DHP, ^b POP, ^c % 0.9-1.2 91.0-92.2 6.7-8.1 0.9-2.0 88.7-91.0 7.0-9.9 1.3-2.2 90.1-95.3 3.4-7.6 2.1-2.7 87.9-90.3 7.1-9.7 1.0-1.6 86.6-88.5 10.4-11.8 1.2-1.9 86.9-88.7 9.6-11.2 1.2-2.2 88.5-89.7 9.1-10.1 2.1-2.5 87.6-89.4 8.5-10.3

^aMHP = monohydroxy pigments. ^bDHP = dihydroxy pig-ments. ^cPOP = polyoxy pigments. ^dF = forage. ^eP = pellets.

carotene. However, when the exit temperature of the dehydrator was reduced below 121 °C, xanthophyll losses decreased appreciably (Table II and Figures 4 and 5). Butler et al. (1969) stated that exhaust temperatures of 146-149 °C are normally required when dehydrating fresh CBG. They reported carotene and xanthophyll losses of 10 and 26%, respectively. However, in one run when the dehydrator temperature was lowered to 121-127 °C, only 1 and 4% carotene and xanthophyll were lost, respectively. In our study, we found that an additional 16–23% carotene and 12–27% xanthophyll were lost when the dehydrator temperature was increased from 129 to 146 °C. Livingston et al. (1966) presented data that showed that when the exit temperature of the dehydrator was increased from 104 to 160 °C, average xanthophyll losses for ALF were increased from 8 to 55%.

The distribution of the component xanthophylls (MHP, DHP, and POP) in the CBG and ALF forgaes and pellets is presented in Table III. The DHP (lutein and zeaxanthin) are the main pigmentors, the MHP (zeinoxanthin, cryptoxanthin) are low in pigmentation potency, and the POP are ineffective. This latter group consists largely of oxdiative products of naturally occurring carotenoids (Livingston et al., 1966). These investigators reported that POP are not present in fresh ALF but arise during dehydration. However, in a subsequent study (Livingston et al., 1968a,b), POP were found in both fresh alfalfa and the dehydrated meal. We found that the dihydroxy pigments constitute 86-95%, the monohydroxy pigments 1-3%, and the polyoxy pigments 3-12% of the total xanthophylls in both CBG and ALF. Our data indicate that polyoxy pigments are also present in both fresh and wilted forages, thus confirming the findings of Livingston et al. (1968a,b).

From the range in values shown, the more mature CBG and ALF (1982) contained slightly less DHP and more POP than the less mature forages (1981). Also, by comparing values for the fresh and dehydrated forages, it appears that dehydration may cause a slight increase in the MHP. Except for these differences, no appreciable changes were found in the relative proportions of component xanthophylls as a result of field wilting or dehydration. This was unexpected as we thought that decreases in total xanthophyll as a result of mechanical dehydration would be reflected in a decrease in dihydroxy xanthophylls and an increase in the polyoxyxanthophylls. Although total xanthophyll may be lost during dehydration, our results show little change in the relative proportions of the MHP, DHP, and POP between the fresh or wilted forages and the dehydrated material. While no appreciable change was found between groups of xanthophylls due to processing conditions, the relative proportions of individual compounds within groups may vary. For example, Livingston et al. (1968a,b) point out that for the POP, greater

losses in neoxanthin and violaxanthin occurring during dehydration are offset by increases in the polyoxidation products of lutein and zeaxanthin. Loss in total xanthophyll, however, is probably more important when considering the pigmentation potency of commercially dehydrated meals.

CONCLUSIONS

When CBG and ALF were field wilted for 6 h under good drying conditions, 31-33% moisture was lost. Less than 10% moisture was lost when these forages were field wilted overnight. During field wilting, up to 25% carotene and 20% xanthophyll were lost depending upon drying conditions. While carotene was relatively stable during mechanical dehydration, up to 45% xanthophyll was lost with actual losses dependent upon the temperature of the dehydrator. For maximum recovery of xanthophyll, exit temperatures of dehydrators should be maintained below 115 °C. Although field wilting prior to mechanical dehydration saves fuel and reduces processing costs, this practice has to be weighed against losses in carotene and xanthophyll that can be appreciable if processing conditions are not carefully controlled. Compared to fresh forage, there were no major changes in the relative percentages of monohydroxy-, dihydroxy-, and polyoxyxanthophylls when CBG and ALF was field wilted or mechanically dehydrated. Accordingly, the pigmentation potency of the field-wilted dehydrated forage products should compare favorably to that of the fresh-cut, dehydrated forage products if levels fed supply the same amount of total xanthophyll.

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