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The Promoting Effect and Utilization of Alcohol on Legume and Grass Forage Plants

II. The Chemical Compositions of Alfalfa and Orchardgrass

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草類におけるアルコールの増進効果と利用

II. アルファルファならびにオーチャードグラスの飼料成分

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Introduction

In the first paper of this series (OOHARA *et al.* 1971) it was reported that the application of methyl, ethyl and buthyl alcohol significantly increased the growth and production of alfalfa, while concentrations of 0.15 to 1.25 liter per are of methyl and ethyl alcohol promoted their yields by about 100%, and application of 2.5 liter per are of methyl alcohol, 5.00 liter per are of ethyl alcohol and 0.25 liter per are of buthyl alcohol increased yields of orchard grass by more 50%, as compared with the control non-alcohol-treated plots. The authors are of the opinion that utilization of alcohol for the increased production of various forage crops is not only possible but is also a reliable method.

In this investigation, all the alcohol-treated samples and non-alcohol-treated samples were analysed for crude protein, crude fat, cellulose, lignin, other carbohydrates, crude ash, chlorophyll, carotenoid, organic carbon, nitrogen, phosphorus, potassium, calcium, magnesium, sodium, manganese, iron, zinc, copper, cobalt, and nickel. The chemical compositions in alfalfa and orchardgrass

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grown in each alcohol treatment were compared against each other. Thus significant effects of alcohol on the levels of the chemical compositions were summarized and are recorded in this paper.

Materials and Method

Each sample of alfalfa (Du Puits) and orchardgrass (Mass Hardy) which were examined in the previous investigation (OOHARA *et al.* 1971) were ground in a mill and used for the determination of crude protein, crude fat, cellulose, lignin, other carbohydrates, crude ash, chlorophyll, carotenoid, organic carbon, total nitrogen, phosphorus, potassium, calcium, magnesium, sodium, manganese, iron, zinc, copper, cobalt and nickel.

Total nitrogen was determined by the micro-Kjeldahl method and the amount of crude protein was calculated by multiplying nitrogen by 6.25. Crude fat and ash were estimated by the usual methods. Chemical analyses of cellulose, lignin and other carbohydrates were carried out by the methods of GRAMPTON *et al.* (1938). The contents of chlorophyll and carotenoid were determined by spectrophotometry. Phosphorus was analysed by the molybdenum blue method. Potassium and sodium were determined by flame photometry, and calcium, magnesium, manganese, iron, zinc, copper, cobalt and nickel by atomic absorption spectrophotometry.

The Properties of Soils

Volcanic ash soils from the Attached Farm of Obihiro Zootechnical University were used in the green-house experiments. These soils were air-dried, sieved through a stainless steel 2 mm mesh, and used for chemical analyses. The results for total organic matter, N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, Co and Ni were respectively 9.62%, 0.38%, 0.110%, 0.24%, 0.470%, 0.147%, 0.63 %, 148.0 ppm, 46 ppm, 28.0 ppm, 4.8 ppm, 3.0 ppm and 50.3 ppm, while values of soil pH, available P, exchangable K, Ca, Mg and Na were 5.91, 0.282 ppm, 133.3 ppm, 14.0 ppm, 26.7 ppm and 50.3 ppm.

A basic application of fertilizers which were supplied were 4 kg N, 16 kg P, 10 kg K and 2 kg $Ca(H_2PO_4)_2H_2O$ per ten are at sowing and soil moisture was maintained throughout the experiment, between field capacity and 50% available moisture.

Results and Discussion

Results of the trials are reported and discussed in sections dealing with the effects of alcohol, changes caused by cutting stages, differences of chemical compositions due to grass species and relationship between tops and roots: on crude protein, crude fat, cellulose, lignin and other carbohydrates of alfalfa and orchardgrass; on chlorophyll and carotenoid; on organic C, total N, C/N, P, C/P, K, Ca, Mg and Na; and on the contents of Fe, Mn, Zn, Cu, Co and Ni.

1. Crude Protein, Crude Fat, Cellulose, Lignin and Other Carbohydrates

Results are given in Table 1. For alfalfa and orchardgrass, Table 2 indicates significant difference between alcohol treatments and control by "t" test.

Table 1. Crude protein, crude fat, cellulose, lignin, other carbohydrates and crude ash compositions of alfalfa and orchardgrass tops, and roots non-alcohol-treated and alcohol-treated

Plot	Alcohol-treatment	Cutting times	Crude protein %	Crude fat %	Cellulose %	Lignin %	Other carbohydrate %	Crude ash %
Alfalfa	Control	1st	17.95	4.03	19.05	6.14	46.57	6.26
		2nd	14.08	5.03	18.97	6.15	44.28	11.49
		Root	9.49	2.30	18.38	11.39	50.22	8.22
	Methyl alcohol (uninoculated)	1st	17.67	3.51	20.02	6.39	36.77	15.64
		2nd	14.75	5.11	21.99	6.10	37.12	14.93
		Root	10.48	2.56	19.68	10.53	44.61	11.78
	Methyl alcohol	1st	19.10	3.38	20.48	6.32	35.90	14.82
		2nd	14.65	6.80	20.88	5.82	41.10	10.75
		Root	9.95	2.63	18.70	11.24	47.35	10.13
	Ethyl alcohol	1st	18.64	4.22	22.30	6.32	36.08	12.44
		2nd	14.08	4.93	21.08	6.02	42.01	11.88
		Root	10.22	2.09	19.46	10.28	46.84	11.11
	Buthyl alcohol	1st	17.66	3.06	19.79	6.13	36.83	16.53
		2nd	16.60	4.81	23.68	6.69	37.39	10.83
		Root	9.51	1.91	17.71	9.79	51.41	9.67
Orchard-grass	Control	1st	20.62	6.59	25.56	7.01	26.27	13.99
		2nd	9.83	4.79	25.32	6.68	41.36	12.02
		Root	4.70	1.79	26.32	12.04	24.72	30.43
	Methyl alcohol	1st	17.13	3.95	26.46	6.42	32.21	13.83
		2nd	8.29	4.66	27.86	5.74	38.84	14.61
		Root	4.40	1.03	22.32	11.71	36.36	24.17
	Ethyl alcohol	1st	16.08	5.05	25.89	6.77	31.97	14.24
		2nd	7.82	4.77	25.85	6.09	40.65	13.82
		Root	3.64	0.70	21.05	12.44	38.96	23.21
	Buthyl alcohol	1st	17.29	4.16	24.97	6.07	35.87	11.64
		2nd	7.97	4.43	25.73	6.52	42.13	13.22
		Root	4.30	0.62	22.08	13.64	34.03	25.33

Remarks; Percentage on a dry weight basis.

Table 2. Significant levels of some organic compositions of alfalfa and orchardgrass alcohol-treated as compared with control

	Chemical composition	Cutting times	Alfalfa				Orchardgrass		
			Methyl alcohol (uninoculated)	Methyl alcohol	Ethyl alcohol	Buthyl alcohol	Methyl alcohol	Ethyl alcohol	Buthyl alcohol
Control non-alcohol-treated	Crude protein	1st	– (−0.28)	** (1.15)	* (0.29)	– (−0.29)	*** (−3.49)	*** (−4.54)	*** (−3.33)
		2nd	* (0.67)	* (0.57)	– (0.00)	** (2.52)	** (−1.54)	** (−2.01)	** (−1.86)
		Root	* (0.99)	– (0.46)	* (0.73)	– (0.02)	– (−0.30)	* (−1.06)	– (−0.40)
	Crude fat	1st	– (−0.52)	* (−0.65)	– (0.19)	* (−0.97)	** (−2.64)	** (−1.74)	** (−2.43)
		2nd	– (0.08)	** (1.73)	– (−0.10)	– (−0.22)	– (−0.13)	– (0.02)	– (−0.36)
		Root	– (0.26)	– (0.33)	– (−0.21)	– (−0.39)	* (−0.76)	* (−1.09)	* (−1.17)
	Cellulose	1st	* (0.97)	** (1.43)	*** (3.25)	* (0.74)	* (0.90)	– (0.33)	– (−0.59)
		2nd	*** (3.02)	** (1.91)	** (2.11)	*** (4.71)	** (2.54)	– (0.29)	– (0.41)
		Root	* (1.30)	– (0.32)	* (1.08)	– (−0.67)	*** (−4.00)	*** (−5.27)	*** (−4.24)
	Lignin	1st	– (0.25)	– (0.18)	– (0.18)	– (−0.01)	– (−0.59)	– (−0.24)	* (0.94)
		2nd	– (−0.05)	– (−0.33)	– (−0.13)	– (0.54)	* (−0.94)	– (−0.59)	* (0.78)
		Root	* (−0.86)	– (−0.15)	* (−1.11)	* (1.60)	– (−0.33)	– (0.40)	* (1.60)
	Other carbohydrate	1st	*** (−9.80)	*** (−10.67)	*** (−10.49)	*** (−9.74)	*** (5.94)	*** (6.70)	*** (9.60)
		2nd	*** (−7.16)	** (−3.18)	* (−2.27)	*** (−6.89)	* (−2.52)	– (−0.71)	– (0.77)
		Root	*** (−5.61)	* (−2.87)	** (−3.38)	* (1.19)	*** (11.64)	*** (14.24)	*** (9.31)
	Crude ash	1st	*** (9.38)	*** (8.56)	*** (6.18)	*** (10.27)	– (−0.16)	– (0.25)	* (−2.35)
		2nd	** (3.44)	– (−0.74)	– (0.39)	– (−0.66)	* (2.59)	* (1.80)	– (1.20)
		Root	** (3.56)	* (1.91)	** (2.89)	* (1.25)	*** (−6.26)	*** (−7.22)	*** (−5.10)

Remarks; ( ) Mean difference between alcohol-treatment and control
– Not significant * Significant at P=0.05
** Significant at P=0.01 *** Significant at P=0.001.

As compared with non-alcohol-treated control, each alcohol treatment of alfalfa in the 1st and 2nd cutting did not differ significantly in its crude protein and fat contents, except for methyl alcohol treatment. Lignin contents at each cutting time indicated no significant differences. In the 1st cutting, the increase of crude ash in each alcohol treatment showed a higher significance but in the 2nd cutting, no increase was seen, while the cellulose content increased significantly and other carbohydrate decreased (Tables 1 and 2).

Generally speaking, all alcohol treatments of alfalfa gave higher values in cellulose and crude ash contents and in contrast, lower values in other car-

bohydrates than the non-alcohol-treated control. These showed highly significant differences at 5 or 1% levels. This result can be described as a slight effect of alcohol as a carbon source. This may be the result of an equilibrium between organic C and N due to the promoting effect of nodule score and weight as found by the authors (1971).

As shown in Tables 1 and 2, crude protein contents of orchardgrass in each alcohol treatment at the 1st and 2nd cutting were lower than control, while crude fat at the 1st cutting and roots decreased significantly. Cellulose and crude ash in the root alone decreased significantly at a 0.1% level, but lignin in the buthyl alcohol treatment increased significantly at a 5% level. Other carbohydrates at the 1st cutting and root increased significantly at a 0.1% level (Tables 1 and 2).

In general, the crude protein content of orchardgrass in the alcohol treatments decreased and other carbohydrates increased. The differences between alcohol treatment and control were highly significant at a 1% level. However, other chemical compositions such as crude fat, cellulose, lignin and crude ash showed no significant differences. This result suggests that alcohol is one of the carbon sources.

As a result of this experiment the authors are of the opinion that the response of alfalfa and orchardgrass to applied alcohol can be utilized to increase the yield of nutritions livestock feed and that alcohol may be utilized as a new fertilizer.

The content of crude protein and fat of alfalfa tops were higher than those of roots. The differences were highly significant at a 1% level. Cellulose and crude ash of roots were significantly lowered as compared with the tops. However, lignin and other carbohydrate of roots were higher than those of tops and there were highly significant differences at a 1% level. The differences between chemical compositions of orchardgrass tops and roots showed the same tendency as alfalfa, but the crude ash contents of orchardgrass roots were approximately twice as high as in the tops, while other carbohydrates showed no significant difference. In this experiment, the relationship between chemical compositions of tops and roots showed no significant changes.

A comparison of the chemical compositions between alfalfa and orchardgrass showed that although crude fat, lignin, other carbohydrates and crude ash were similar. The crude protein content of alfalfa and cellulose of orchardgrass were higher than orchardgrass and alfalfa with a significant difference at a 5% level respectively.

Crude protein contents of alfalfa and orchardgrass were higher at the 1st cutting stage of growth and decreased at the 2nd cutting (Table 1). However, other carbohydrate contents were increased in a manner opposite to that of crude protein. The changes of nutrient contents of orchardgrass according to cutting stage were greater than those of alfalfa.

2. Chlorophyll and Carotenoid

The parts per thousand content of chlorophyll and carotenoid of alfalfa and orchardgrass tops non-alcohol-treated and alcohol-treated were given in Table 3. In each cutting, Table 4 indicated significant levels by the "t" test.

Chlorophyll-a content of alfalfa at the 1st and 2nd cutting showed that each alcohol caused a decrease in content while ethyl alcohol did not show a significant difference. In contrast, alcohol caused a rise in chlorophyll-b contents, which did not always reach significant values, and also it was noted that contents higher than control did not differ significantly at the 1st cutting. Each alcohol gave an increase in content of carotenoid of alfalfa at the 1st and 2nd cutting. The differences between each alcohol treatment and control were highly significant at a 1% level (Table 4).

In general, chlorophyll-a content of alfalfa were decreased by the effect of alcohol while carotenoid content increased. Therefore, chlorophyll-a to chloro-

Table 3. Chlorophyll and carotenoid contents of alfalfa and orchardgrass tops non-alcohol-treated and alcohol-treated

Plot	Alcohol-treatment	Cutting times	Chlorophyll			Carotenoid
			a (‰)	b (‰)	a/b	(‰)
Alfalfa	Control	1st	2.98	2.28	1.31	0.350
		2nd	3.00	2.01	1.49	0.374
	Methyl alcohol (uninoculated)	1st	2.45	2.47	0.99	0.405
		2nd	2.72	2.69	1.01	0.490
	Methyl alcohol	1st	2.63	2.39	1.10	0.374
		2nd	2.77	2.36	1.17	0.445
	Ethyl alcohol	1st	2.91	2.52	1.16	0.414
		2nd	2.96	2.30	1.29	0.480
	Buthyl alcohol	1st	2.40	2.33	1.03	0.400
		2nd	2.69	2.32	1.16	0.493
Orchard-grass	Control	1st	3.85	3.33	1.16	0.576
		2nd	2.97	2.28	1.30	0.561
	Methyl alcohol	1st	3.02	3.13	0.97	0.429
		2nd	2.56	2.12	1.21	0.381
	Ethyl alcohol	1st	3.02	3.03	1.00	0.432
		2nd	1.72	1.24	1.39	0.409
	Buthyl alcohol	1st	3.06	3.00	1.02	0.393
		2nd	2.00	1.52	1.32	0.367

Remarks; Parts per thousand on a dry weight basis.

phyll-b ratio (Table 3) of alfalfa in each alcohol treatment was lower than that of control. It is clear from the data in Table 3 and 4 that the yellow green color of alfalfa alcohol-treated (OOHARA *et al.* 1971) is caused by the effect of the decreased chlorophyll-a and increased carotenoid and that each alcohol is also one of the carbon sources.

The data presented in Table 3 give the best estimate of chlorophyll and carotenoid of orchardgrass and the effect of alcohol. The content of chlorophyll and carotenoid in the alcohol treatment was lower than in the control and significant differences were very high at a 1% level. Therefore, it can be said that the value of chlorophyll-a to chlorophyll-b ratio between alcohol treatments and control showed no significant difference. The effects of each alcohol on the contents of chlorophyll and carotenoid of orchardgrass were similar to those in alfalfa.

In the present trial, a significant difference of chlorophyll and carotenoid content between legume and grass was not found. Chlorophyll-a and carotenoid of alfalfa at the 2nd cutting appreciably increased in their contents as compared with the 1st cutting but there was no significant difference in the case of chlorophyll-b content. As regards orchardgrass, chlorophyll and carotenoid at the 2nd cutting decreased significantly in content (Table 3). This may be attributed to nutrient deficiency in the pot soil due to the uptake and utilization of the plant at the 1st cutting stage.

Table 4. Significant levels of chlorophyll and carotenoid content in alfalfa and orchardgrass alcohol-treated as compared with control

	Chlorophyll & carotenoid	Cutting times	Alfalfa				Orchardgrass		
			Methyl alcohol (uninoculated)	Methyl alcohol	Ethyl alcohol	Buthyl alcohol	Methyl alcohol	Ethyl alcohol	Buthyl alcohol
Control non-alcohol-treated	Chlorophyll-a	1st	** (−0.53)	** (−0.35)	− (−0.07)	** (−0.58)	*** (−0.83)	*** (−0.83)	*** (−0.79)
		2nd	* (−0.28)	* (−0.23)	− (−0.04)	** (−0.31)	* (−0.41)	*** (−1.25)	*** (−0.97)
		Total	** (−0.81)	** (−0.58)	− (−0.11)	** (−0.89)	*** (−1.24)	*** (−2.08)	*** (−1.76)
	Chlorophyll-b	1st	* (0.19)	− (0.11)	* (0.24)	− (0.05)	* (−0.20)	** (−0.30)	** (−0.33)
		2nd	** (0.68)	* (0.35)	* (0.29)	* (0.31)	* (−0.16)	− (−0.04)	*** (−0.76)
		Total	** (0.87)	* (0.46)	* (0.53)	* (0.36)	* (−0.36)	* (−0.34)	*** (−1.09)
	Carotenoid	1st	** (0.055)	* (0.024)	*** (0.064)	** (0.050)	*** (−0.147)	*** (−0.144)	*** (−0.183)
		2nd	*** (0.116)	*** (0.071)	*** (0.106)	*** (0.119)	*** (−0.180)	*** (−0.152)	*** (−0.194)
		Total	*** (0.171)	** (0.095)	*** (0.170)	*** (0.169)	*** (−0.317)	*** (−0.296)	*** (−0.377)

Remarks; ( ) Mean difference between alcohol-treatment and control
− Not significant * Significant at P=0.05
** Significant at P=0.01 *** Significant at P=0.001.

3. Major Elements

The levels of organic C, total N, C/N ratio, P, C/P ratio, K, Ca, Mg and Na in samples of alfalfa and orchardgrass at each cutting and for roots were shown in Tables 5 and 7. The data shown in the tables were analysed for statistical significances by "t" test to compare the results of alcohol treatment

Table 5. Organic C, total N, C/N ratio, P and C/P ratio levels of alfalfa and orchardgrass tops, and roots non-alcohol-treated and alcohol-treated

Plot	Alcohol-treatment	Cutting time	Organic C %	Total N %	C/N ratio	P %	C/P ratio
Alfalfa	Control	1st	47.58	2.87	16.58	0.188	253.3
		2nd	51.46	2.25	22.92	0.147	350.1
		Root	53.24	1.54	35.14	0.126	423.6
	Methyl alcohol (uninoculated)	1st	49.09	2.82	17.40	0.235	209.0
		2nd	49.66	2.36	21.00	0.182	271.9
		Root	51.29	1.73	29.61	0.172	298.0
	Methyl alcohol	1st	49.52	3.04	16.27	0.475	104.2
		2nd	51.83	2.34	22.22	0.184	282.7
		Root	52.24	1.59	32.90	0.144	362.2
	Ethyl alcohol	1st	50.90	2.88	17.65	0.244	208.9
		2nd	51.23	2.69	19.04	0.172	297.7
		Root	51.67	1.52	34.07	0.184	280.5
	Buthyl alcohol	1st	48.52	2.91	16.69	0.199	244.2
		2nd	51.51	2.22	23.17	0.178	289.6
		Root	52.51	1.63	32.25	0.177	295.9
Orchard-grass	Control	1st	50.01	3.08	15.17	0.229	218.4
		2nd	51.14	1.57	32.55	0.292	175.3
		Root	40.47	0.74	54.55	0.105	385.4
	Methyl alcohol	1st	50.09	2.73	18.32	0.242	207.1
		2nd	49.65	1.32	37.70	0.304	163.1
		Root	43.90	0.70	62.47	0.122	360.4
	Ethyl alcohol	1st	50.09	2.57	19.46	0.235	213.3
		2nd	50.09	1.24	40.25	0.269	186.3
		Root	42.91	0.55	77.54	0.187	229.8
	Buthyl alcohol	1st	51.12	2.74	18.64	0.243	210.6
		2nd	50.46	1.27	39.82	0.310	162.7
		Root	45.19	0.68	66.07	0.122	369.2

Remarks; Percentage on a dry weight basis.

with that of the control the results were given in Tables 6 and 8.

A comparison of major element contents in alcohol treatments and control showed that total N of alfalfa in alcohol treatment were not significant but those of orchardgrass decreased with a significant difference at each cutting stage and as for the root, while organic C content was not significant. Thus, the levels of C/N ratio were found to be significantly high in orchardgrass and this is considered to be the increased carbon due to the applied alcohol. Phosphorus in the alcohol treatment was high in content in alfalfa and orchardgrass but their differences did not always reach significant values in the case of orchardgrass. According to this result, it may be said that the levels of C/P ratio decreased significantly. This means that the P compounds such as DNA, RNA, phospholipid and various nucleotides increased significantly. The contents

Table 6. Significant levels of organic C, total N, C/N ratio, P and C/P ratio levels of alfalfa and orchardgrass tops and roots non-alcohol-treated and alcohol-treated

	Elements	Cutting times	Alfalfa				Orchardgrass		
			Methyl alcohol (uninoculated)	Methyl alcohol	Ethyl alcohol	Buthyl alcohol	Methyl alcohol	Ethyl alcohol	Buthyl alcohol
Control non-alcohol-treated	Organic C	1st	(1.51) −	(1.94) −	(3.32) *	(0.94) −	(0.08) −	(0.08) −	(1.11) −
		2nd	(−1.80) −	(0.37) −	(−0.23) −	(0.01) −	(−1.49) −	(−1.05) −	(−0.68) −
		Root	(1.95) −	(−1.00) −	(−1.57) −	(−0.73) −	(3.43) *	(2.44) *	(4.72) **
	Total N	1st	(−0.05) −	(0.17) *	(0.01) −	(0.04) −	(−0.35) ***	(−0.51) ***	(−0.34) ***
		2nd	(0.11) *	(0.09) −	(0.44) ***	(−0.03) −	(−0.25) *	(−0.33) **	(−0.30) **
		Root	(0.19) *	(0.05) −	(−0.02) −	(0.09) −	(−0.04) −	(−0.19) *	(−0.06) −
	C/N	1st	(0.82) −	(−0.31) −	(1.07) −	(0.11) −	(3.15) *	(4.47) **	(3.47) *
		2nd	(−1.92) −	(−0.70) −	(−3.88) *	(0.25) −	(5.15) **	(7.70) ***	(7.27) **
		Root	(−5.53) **	(−2.24) *	(−1.07) −	(−2.89) *	(7.92) ***	(22.95) ***	(11.52) ***
	P	1st	(0.047) ***	(0.287) ***	(0.056) ***	(0.011) −	(0.013) −	(0.006) −	(0.014) −
		2nd	(0.035) **	(0.037) **	(0.025) *	(0.031) **	(0.012) −	(−0.023) *	(0.018) *
		Root	(0.046) ***	(0.018) *	(0.058) ***	(0.051) ***	(0.017) *	(0.010) −	(0.015) *
	C/P	1st	(−44.3) **	(−149.1) ***	(−44.4) **	(−9.1) −	(−11.3) −	(−5.1) −	(−7.8) −
		2nd	(−78.2) ***	(−67.4) ***	(−52.4) **	(−60.5) ***	(−12.2) −	(11.0) −	(−0.4) −
		Root	(−125.6) ***	(−61.4) ***	(−143.1) ***	(−127.7) ***	(−25.0) *	(−155.6) ***	(8.8) −

Remarks; ( ) Mean difference between alcohol-treatment and control
− Not significant * Significant at P=0.05
** Significant at P=0.01 *** Significant at P=0.001.

of K, Ca and Na in the alcohol treatment were similar to that of the control. Values of Mg in the control were higher at each cutting stage and for the root but a part of these showed no significant difference (Table 8). This decrease of Mg is in agreement with the effect of chlorophyll.

In general, there were no obvious differences of the contents of organic C,

Table 7. Contents of K, Ca, Mg and Na of alfalfa and orchardgrass tops and roots non-alcohol-treated and alcohol-treated

Plot	Alcohol-treatment	Cutting times	K %	Ca %	Mg %	Na ‰
Alfalfa	Control	1st	1.60	1.47	0.399	0.85
		2nd	1.52	1.03	0.236	0.64
		Root	0.61	0.68	0.147	0.71
	Methyl alcohol (uninoculated)	1st	1.55	1.32	0.388	0.78
		2nd	1.69	0.98	0.211	0.58
		Root	0.73	0.63	0.149	0.67
	Methyl alcohol	1st	1.58	1.20	0.317	0.65
		2nd	1.60	0.93	0.178	0.64
		Root	0.59	0.68	0.146	1.16
	Ethyl alcohol	1st	1.62	1.26	0.398	0.94
		2nd	1.50	0.98	0.197	0.54
		Root	0.82	0.61	0.171	1.02
	Buthyl alcohol	1st	1.53	1.18	0.365	0.65
		2nd	1.65	0.96	0.235	0.63
		Root	0.81	0.60	0.175	0.92
Orchard-grass	Control	1st	2.77	0.42	0.284	0.54
		2nd	2.04	0.45	0.217	0.42
		Root	0.32	0.79	0.092	1.56
	Methyl alcohol	1st	2.37	0.42	0.209	0.21
		2nd	2.00	0.37	0.205	0.49
		Root	0.39	0.80	0.090	1.36
	Ethyl alcohol	1st	2.52	0.43	0.212	0.32
		2nd	1.89	0.38	0.194	0.31
		Root	0.27	0.81	0.089	1.44
	Buthyl alcohol	1st	2.55	0.40	0.238	0.51
		2nd	1.90	0.43	0.223	0.30
		Root	0.38	0.79	0.106	1.48

Remarks; Percentage and parts per thousand on a dry weight basis.

Table 8. Significant levels of the contents of K, Ca, Mg and Na of alfalfa and orchardgrass tops and roots non-alcohol-treated and alcohol-treated

	Elements	Cutting times	Alfalfa				Orchardgrass		
			Methyl alcohol (uninoculated)	Methyl alcohol	Ethyl alcohol	Buthyl alcohol	Methyl alcohol	Ethyl alcohol	Buthyl alcohol
Control non-alcohol-treated	K	1st	(−0.05)$^{-}$	(−0.02)$^{-}$	(0.02)$^{-}$	(−0.07)$^{-}$	(−0.40)***	(−0.25)**	(−0.22)**
		2nd	(0.17)*	(0.08)$^{-}$	(−0.02)$^{-}$	(0.13)*	(−0.04)$^{-}$	(−0.15)*	(−0.14)*
		Root	(0.12)*	(−0.02)$^{-}$	(0.21)**	(0.20)**	(0.07)$^{-}$	(−0.05)$^{-}$	(0.06)$^{-}$
	Ca	1st	(−0.15)*	(−0.27)*	(−0.21)*	(−0.29)*	(0.00)$^{-}$	(0.01)$^{-}$	(−0.02)$^{-}$
		2nd	(−0.05)$^{-}$	(−0.10)$^{-}$	(−0.05)$^{-}$	(−0.07)$^{-}$	(−0.08)$^{-}$	(−0.07)$^{-}$	(−0.02)$^{-}$
		Root	(−0.05)$^{-}$	(0.00)$^{-}$	(−0.07)$^{-}$	(−0.08)$^{-}$	(0.01)$^{-}$	(0.02)$^{-}$	(0.00)$^{-}$
	Mg	1st	(−0.011)*	(−0.082)***	(−0.001)$^{-}$	(−0.034)**	(−0.075)***	(−0.072)***	(−0.046)***
		2nd	(−0.025)**	(−0.058)***	(−0.039)**	(−0.001)$^{-}$	(−0.012)*	(−0.023)**	(0.006)$^{-}$
		Root	(0.002)$^{-}$	(−0.001)$^{-}$	(0.024)**	(0.029)**	(−0.002)$^{-}$	(−0.003)$^{-}$	(0.014)*
	Na	1st	(−0.07)*	(−0.20)**	(0.09)*	(−0.20)**	(−0.23)**	(−0.22)**	(−0.03)$^{-}$
		2nd	(−0.06)$^{-}$	(0.00)$^{-}$	(−0.10)*	(−0.01)$^{-}$	(0.07)$^{-}$	(−0.11)*	(−0.12)*
		Root	(−0.04)$^{-}$	(0.42)***	(0.31)***	(0.21)**	(−0.20)**	(0.12)*	(−0.08)$^{-}$

Remarks; ( ) Mean difference between alcohol-treatment and control
− Not significant * Significant at P=0.05
** Significant at P=0.01 *** Significant at P=0.001.

K, Ca and Na in alfalfa and orchardgrass in alcohol treatments as compared with that of the control. However, alcohol significantly depressed the Mg content in alfalfa and orchardgrass, reduced N content in orchardgrass, and raised P content in alfalfa and orchardgrass.

For the changes of major elements due to the cutting stage, total N, K, Ca, Mg and Na were higher at the 1st cutting than at 2nd cutting. OOHARA *et al.* (1963) reported that in their field experiments the mineral contents changed considerably with the various conditions at the cutting stage. Organic C contents in alfalfa were high at the 1st cutting but the P content was low. The contents in orchardgrass were in a manner opposite to alfalfa.

Total N, P and Mg contents in roots of alfalfa and orchardgrass were lower than those in the tops, while organic C and Ca in the roots of orchardgrass were higher than those in the tops while those in alfalfa were lower. On an average the K content in alfalfa tops and orchardgrass were twice as high or higher than those in roots but Na showed opposite results to K. This seems to be due to the interaction between Na and K.

In particular, alfalfa contained more N, Ca, Mg and Na than did orchardgrass but the K content in alfalfa was lower than those in orchardgrass, while organic C and P showed no significant difference between alfalfa and orchardgrass. OOHARA *et al.* (1963) reported that the contents of N, Ca, P and Na in legumes were higher than those in orchardgrass, and the contents of K in orchardgrass were higher than those in alfalfa. This suggests that in mineral contents, there were significant differences between legumes and grasses.

4. Trace Elements

The mean content of trace elements were determined in alfalfa and orchardgrass samples from all treatments at each cutting. Statistical analyses to detect a response of Fe, Mn, Zn, Cu, Co and Ni to alcohol applied to alfalfa and orchardgrass were carried out and the results were summarised in Table 10.

As shown in Table 10, each content of minor elements at each cutting stage and in roots increased or decreased significantly and was similar to results seen in the control. Thus it may be said that there were no appreciable effects of the alcohol application on the contents of these minor elements. This suggests that there are no changes of the contents of minor elements in alfalfa and orchardgrass due to application of alcohol.

Mean values of Fe, Mn and Cu in the roots of alfalfa and orchardgrass were much higher than those in the tops but the Co and Ni contents in the roots were lower than those in the tops. The roots of alfalfa contained lower levels of Zn than the tops.

The contents of Fe in orchardgrass increased significantly at the 2nd cutting and in alfalfa a decrease was seen. In the case of Zn and Cu, values at the 1st cutting tended to be lower in alfalfa and orchardgrass than those at the 2nd cutting. The results of chemical analysis (Table 9) confirmed that Zn was not deficient for the growth of alfalfa. According to WIDDOSON (1966) and RICEMAN and JONES (1958), most clovers with less than 15 ppm of Zn in leaves can be suspected of Zn deficiency. In this experiment, the tops of alfalfa contained 12.1 to 70.2 ppm of Zn. The contents of Co and Ni were much or slightly higher at the 1st cutting respectively than at the 2nd cutting. The tendency of Co was in agreement with the result, in which where all species are taken together, the mean Co concentration for the winter period was significantly higher than those for the other three seasons, as reported by ANDREWS (1966).

In the same properties of soils, trace element contents were compared between alfalfa and orchardgrass. The contents of Fe and Mn were higher in orchardgrass than in alfalfa but those of Zn, Cu, Co and Ni were higher in alfalfa than in orchardgrass. In the case of Co, a number of workers (ASKEW and DIXON 1937, MITCHEL 1945, LEE 1951, BEESON and MCDONALD 1951, ANDREW 1966) have reported that in general, legumes contain higher concentrations of Co than do grasses. MCNAUGHT and DOROFAEFF (1968) have reported

that on the effect of magnesium fertilizers, the contents of Zn, Cu and Fe were higher in white clover than in grasses but Mn was higher in grasses than in white clover.

All those results confirmed that the response of alfalfa and orchardgrass to applied alcohol could be utilized for an increased yield.

Table 9. Contents of Fe, Mn, Zn, Cu, Co and Ni of alfalfa and orchardgrass tops and roots non-alcohol-treated and alcohol-treated

Plot	Alcohol-treatment	Cutting time	Fe ppm	Mn ppm	Zn ppm	Cu ppm	Co ppm	Ni ppm
Alfalfa	Control	1st	141.2	57.6	49.8	11.24	0.274	0.052
		2nd	80.2	58.2	18.5	8.84	0.348	0.066
		Root	311.0	64.5	40.1	9.00	0.140	0.028
	Methyl alcohol (uninoculated)	1st	120.6	43.3	70.2	9.79	0.280	0.049
		2nd	93.4	59.8	18.4	12.83	0.432	0.062
		Root	419.6	88.3	45.3	10.33	0.244	0.026
	Methyl alcohol	1st	116.7	51.3	45.3	9.84	0.219	0.069
		2nd	123.2	59.3	15.6	9.00	0.393	0.038
		Root	459.1	85.7	26.5	9.23	0.272	0.026
	Ethyl alcohol	1st	166.9	51.7	41.6	10.06	0.360	0.086
		2nd	135.7	63.5	12.1	7.22	0.433	0.052
		Root	378.9	85.9	29.2	12.37	0.146	0.028
	Buthyl alcohol	1st	126.3	56.9	56.9	9.53	0.236	0.056
		2nd	116.3	59.4	18.9	7.46	0.271	0.100
		Root	379.0	93.3	39.9	9.29	0.149	0.031
Orchard-grass	Control	1st	124.7	42.9	28.8	9.00	0.165	0.036
		2nd	272.1	56.1	22.4	7.58	0.219	0.043
		Root	599.3	115.3	21.8	9.70	0.095	0.023
	Methyl alcohol	1st	117.5	52.5	13.6	7.37	0.195	0.026
		2nd	149.0	60.3	12.2	6.95	0.194	0.028
		Root	599.7	89.6	34.0	8.78	0.100	0.021
	Ethyl alcohol	1st	112.9	50.2	13.1	7.62	0.141	0.023
		2nd	116.8	41.8	13.3	7.22	0.217	0.022
		Root	613.5	132.6	27.9	8.95	0.087	0.017
	Buthyl alcohol	1st	107.3	58.4	14.0	7.59	0.169	0.026
		2nd	124.7	49.5	10.3	7.49	0.242	0.031
		Root	561.3	139.8	31.4	10.50	0.106	0.023

Remarks; Parts per million on a dry weight basis.

Table 10. Significant levels of contents of Fe, Mn, Zn, Cu, Co and Ni of alfalfa and orchardgrass tops and roots non-alcohol-treated and alcohol-treated

	Elements	Cutting time	Alfalfa				Orchardgrass		
			Methyl alcohol (uninoculated)	Methyl alcohol	Ethyl alcohol	Buthyl alcohol	Methyl alcohol	Ethyl alcohol	Buthyl alcohol
Control non-alcohol-treated	Fe	1st	*(−20.6)	*(−24.5)	*(25.7)	−(14.9)	−(−7.2)	−(−11.8)	−(−17.4)
		2nd	−(13.2)	**(43.0)	**(55.5)	*(36.1)	***(−123.1)	***(−155.3)	***(−147.4)
		Root	***(108.6)	***(148.1)	***(67.9)	**(68.0)	−(0.4)	−(14.2)	*(−38.0)
	Mn	1st	**(−14.3)	*(−6.3)	*(−5.9)	−(−0.7)	*(9.6)	*(7.3)	*(15.5)
		2nd	−(1.6)	−(1.1)	*(5.3)	−(1.2)	−(4.2)	**(−14.3)	*(−6.6)
		Root	**(23.8)	**(21.2)	**(21.4)	***(28.8)	***(−25.7)	**(17.3)	**(24.5)
	Zn	1st	***(20.4)	−(−4.3)	*(−8.2)	*(7.1)	**(−15.2)	**(−15.7)	**(−14.8)
		2nd	−(−0.1)	−(−2.9)	*(−6.4)	−(0.4)	**(−10.2)	*(−9.1)	**(−12.1)
		Root	*(5.2)	**(−13.6)	**(−10.9)	−(−0.2)	**(13.0)	*(6.1)	*(9.6)
	Cu	1st	*(−1.45)	*(−1.40)	*(−1.18)	*(−1.71)	*(−1.63)	*(−1.38)	*(−1.41)
		2nd	***(3.99)	−(0.16)	*(−1.62)	*(−1.38)	−(−0.63)	−(−0.36)	−(−0.09)
		Root	*(1.33)	−(0.23)	***(3.37)	−(0.29)	*(−0.92)	−(−0.75)	−(0.80)
	Co	1st	−(0.006)	**(−0.055)	**(0.086)	*(−0.038)	*(0.030)	*(−0.024)	−(0.004)
		2nd	**(0.084)	**(0.045)	**(0.085)	**(−0.077)	*(−0.025)	−(−0.002)	*(0.023)
		Root	***(0.104)	***(0.132)	−(0.006)	−(0.009)	−(0.005)	−(−0.008)	−(0.011)
	Ni	1st	−(−0.003)	**(0.017)	**(0.034)	−(0.004)	*(−0.010)	*(−0.013)	*(−0.010)
		2nd	−(−0.004)	**(−0.028)	*(−0.014)	**(0.034)	*(−0.015)	**(−0.023)	*(−0.012)
		Root	−(−0.002)	−(−0.002)	−(0.000)	−(0.003)	−(−0.002)	−(−0.006)	−(0.000)

Remarks; ( ) Mean difference between alcohol-treatment and control
− Not significant * Significant at P=0.05
** Significant at P=0.01 *** Significant at P=0.001.

summary

In this paper, the response of crude protein, crude fat, cellulose, lignin, other carbohydrates, crude ash, chlorophyll, carotenoid, organic carbon, total N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, Co and Ni to applied methyl, ethyl and buthyl alcohol were examined in alfalfa and orchardgrass. As compared with control non-alcohol-treated, the significant main effects of alcohol on the contents of those chemical compositions were summarized and are reported as

follows.

1. Cellulose and crude ash contents of alfalfa were increased significantly by application of alcohol but other carbohydrates decreased, while crude protein, crude fat and lignin contents showed no significant differences.

2. Crude protein contents of orchardgrass in the alcohol treatments decreased and other carbohydrates increased. However, other chemical composition such as crude fat, cellulose, lignin and crude ash showed no significant differences.

3. The contents of crude protein, crude fat and cellulose of alfalfa and orchardgrass tops were significantly higher than those of the roots but the lignin content of the tops were lower than those of the roots. Other carbohydrate contents were lower in the tops of alfalfa than in the roots but in the case of orchardgrass, there were no significant differences between the tops and roots. Crude ash contents were lower in the roots of alfalfa and in the tops of orchardgrass than in the tops and in roots respectively.

4. Chlorophyll-a contents of alfalfa were decreased by the effect of alcohol but carotenoid was increased significantly. Alcohol caused a rise in chlorophyll-b content, but did not always reach significant values.

5. The contents of chlorophyll and carotenoid in the alcohol treatment of orchardgrass were lower than those in the control non-alcohol-treated and significant differences were very high at a 1% level.

6. There were no obvious differences of contents of organic C, K, Ca and Na in alfalfa and orchardgrass in the alcohol treatments. However, alcohol significantly depressed the Mg contents in alfalfa and orchardgrass, reduced N content in orchardgrass, and raised P content in alfalfa and orchardgrass.

7. The contents of Fe, Mn, Zn ,Cu, Co and Ni in alfalfa and orchardgrass were not affected significantly by the applied alcohol.

8. Mean values of Fe, Mn and Cu in roots of alfalfa and orchardgrass were much higher than those in the tops but Co and Ni contents in the roots were lower than those in the tops. The roots of alfalfa contained lower levels of Zn than the tops.

9. In the pot cultured under the same properties of soils, the Fe and Mn were higher in orchardgrass than in alfalfa but those of Zn, Cu, Co and Ni were much higher in alfalfa than in orchardgrass.

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要　　約

アルファルファならびにオーチャードグラスにおけるメチル，エチルおよびブチルアルコール施用に対する各種化学成分に及ぼす影響について検討した。これらの結果を要約すると次のごとくである。

1. アルファルファのセルローズおよび粗灰分はアルコール施用によって増加するが，炭水化物は減少し粗蛋白質，粗脂肪，リグニンは変動がみられない。

2. アルコール施用区ではオーチャードグラスの粗蛋白質は減少するが，炭水化物は増加した。粗脂肪，セルローズ，リグニン，粗灰分の変動はなかった。

3. アルファルファ，オーチャードグラスの地上部の粗蛋白質，粗脂肪，セルローズは，根部よりそれらの含量が高いが，リグニンは根部のほうが高い。その他の炭水化物はアルファルファでは地上部より根部が高い。

4. アルファルファのクロロフィルaは，アルコール施用区で減少し，カロチノイドは増加する。クロロフィルbについては有意性は認められないが減少する傾向がある。

5. オーチャードグラスのクロロフィルaおよびb，カロチノイド含量は，アルコール施用によって減少する。

6. アルコール施用における草体中の有機炭素，K, Ca, Na は対照区との有意差が認められないが，Mg はやや減少し，P は増加した。N 含量はオーチャードグラスで減少した。

7. Fe, Mn, Zn, Cu, Co, Ni 含量はアルコール施用においては変動がない。

8. Fe, Mn, Cu は根部が多量に含有し，Co, Ni は地上部のほうが高い。

9. 同一土壌条件で栽培した場合，Fe, Mn はオーチャードグラス，Zn, Cu, Co, Ni はアルファルファがそれぞれ含量が高い。

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妊娠早期の乳腺分泌液に関する研究

V. 蛋白質および非蛋白態窒素化合物の組成について

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Studies on Secretory Fluid from the Mammary Gland of Pregnant Heifers

Part V. On the Composition of Protein and Nonprotein Nitrogen

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緒　　言

第2報で，われわれ[1]はウシの妊娠早期の分泌液（プレミルク）の化学的組成について報告した。その際，蛋白質含量は正常乳のそれと比較して，非常に高い値であることを認めたが，その組成を明らかにすることはできなかった。今回は，各種蛋白質組成と，あわせて非蛋白態窒素化合物の組成を調べたので報告する。

実　験　方　法

1. 実験材料

試料として用いたプレミルクは，昭和44年度中にホルスタイン種未経産牛から採取した妊娠216日から284日目のもの，および分娩後3日目までの初乳である。使用時まで，トルオール1滴を加えて凍結保存した。採取法は，第1報[2]に記したとおりである。

2. 各態蛋白質の定量法

常法[3]に従い，全蛋白，非カゼイン態，非蛋白態，プロテオース・ペプトン・非蛋白態，およびグロブリン態各窒素を，ミクロキエルダール法により求めた。ついでカゼイン態，アルブ

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