ENVIRONMENT AND GENETIC EFFECT ON QUALITY OF HEMP GRAIN AND OIL EXTRACTED FROM GRAIN GROWN ACROSS NORTHERN ONTARIO (46TH TO 50TH LATITUDE AND 80TH TO 94TH LONGITUDE) IN 1998 & 1999.

by
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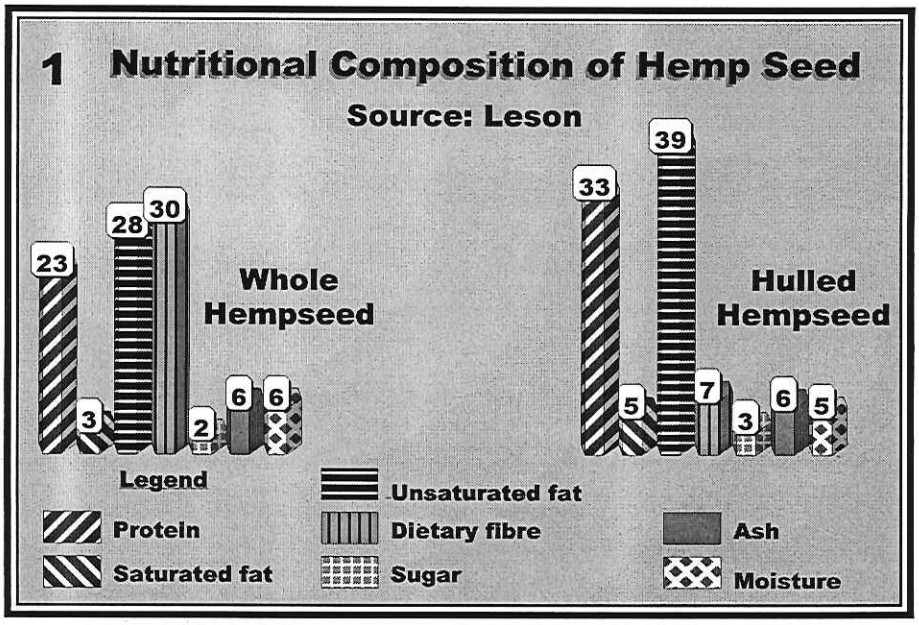
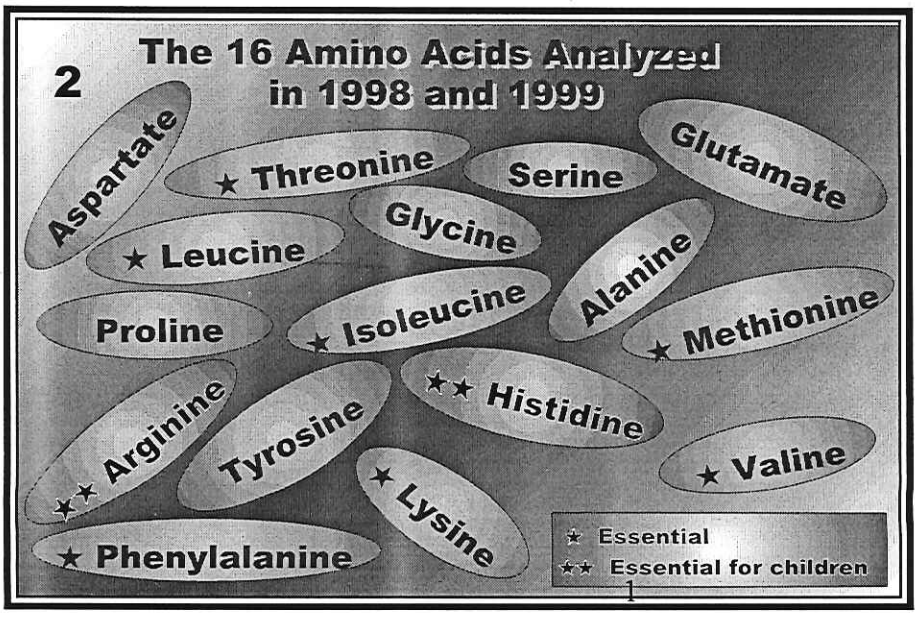
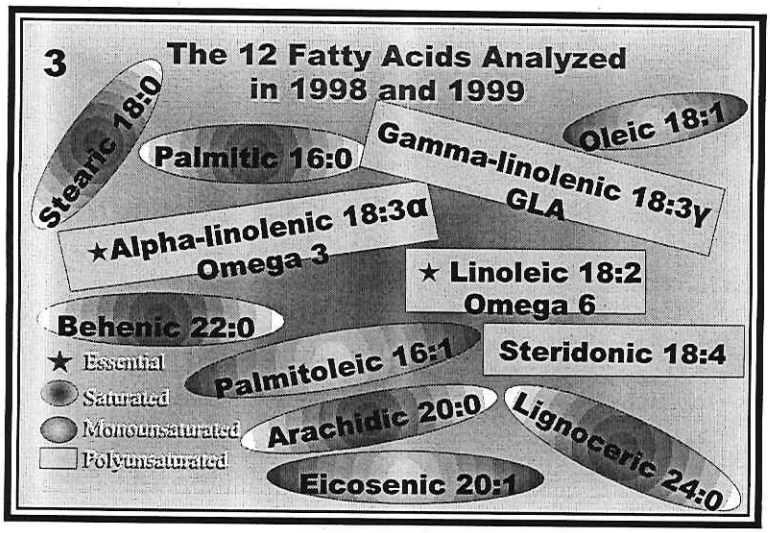
INTRODUCTION

The North American hemp renaissance with focused attention on of using hemp nuts (achene) and processed products as health food was largely brought about by the publishing of "*The Hemp Seed Cookbook*" (Wirtshafter, J.D. and Carol Miller), Dr. Udo Erasmus' promotion of hemp oil as "*the most perfectly balanced, natural EFA-rich oil available" and "nature's most perfectly balanced oil"* in *Fats that Heal, Fats that Kill* (Erasmus) and



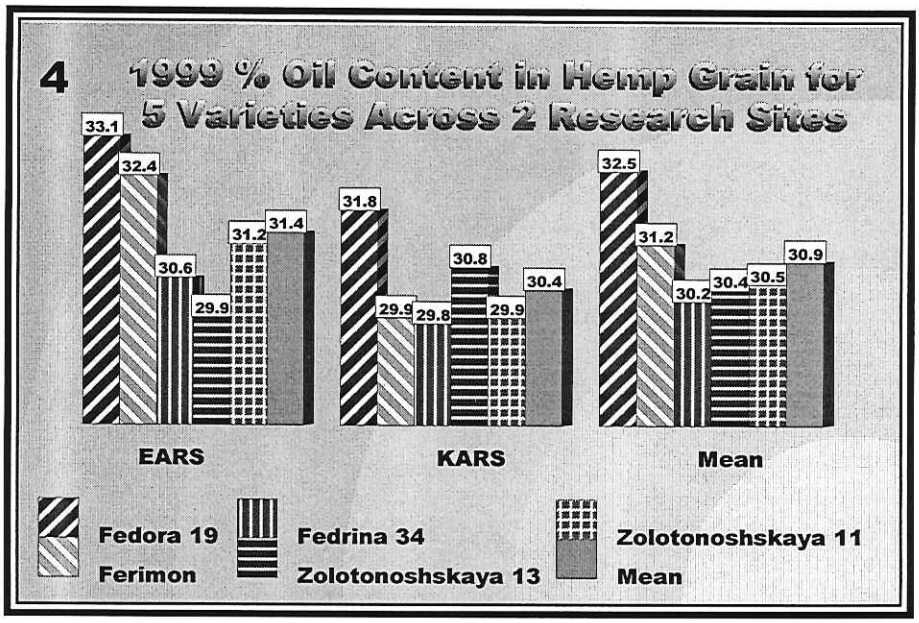


"*Therapeutic Hemp Oil* " *by* Dr. Andrew Weil, MD of University of Arizona College of Medicine (Weil). Hemp seed ranks 4th and 5th amongst essential fatty acids (EFA's)-rich seeds and while it is not the top of it's class, hemp seed is 1st when it comes to balance of EFA's (Osburn). What is unique about hemp seed protein is that 65%



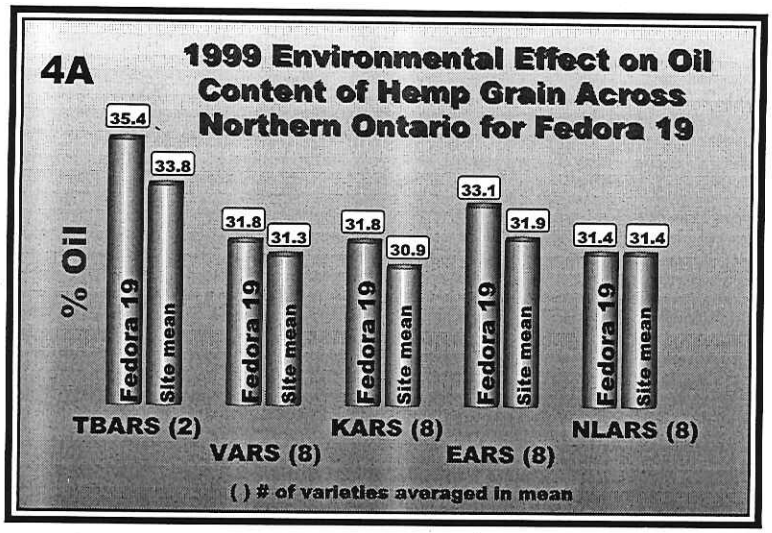
of it is globulin edistin, the highest in the plant kingdom (Osburn). Hemp seed oil is reported to comprise 35% of the total seed weight. It is reported to have the lowest amount of saturated fatty acids (SFA's) at 8%, and the highest amount of the polyunsaturated fatty acids (PUFA's) at 80%, total oil volume (Osburn). Richard Rose (Rose) states that the "*potential for hemp as a food ingredient in industrialized nations, and especially the U.S., matches or exceeds the potential for textiles -- for one simple reason: EFAs*".

Foods can be prepared from hemp seed in 3 general methods : 1) using the whole seed, 2) milling the seed, and 3) using the oil, directly. Industrial hemp is not only being championed as a renewable source of pulp for manufacturing paper and as a superior fibre for making cloth, but also as a new food than can be processed into everything from a milk substitute to tofu and many other types of food products. Much of the Canadian industrial hemp crop grown since 1998 has been for grain production developed for the health food and health care markets. One of the objectives of the northern Ontario industrial hemp research project since 1997 has not only been to establish the feasibility of successfully growing industrial hemp for grain, but also producing a competitive quality hemp grain and oil for the North American market.



Until about 1948, the main use of hemp seed and meal in the U.S. was in feed for poultry, dairy and beef cattle and bird seed (Jones). **Graph 1** demonstrates the classic nutritional composition of the industrial hemp nut and seed (Leson).

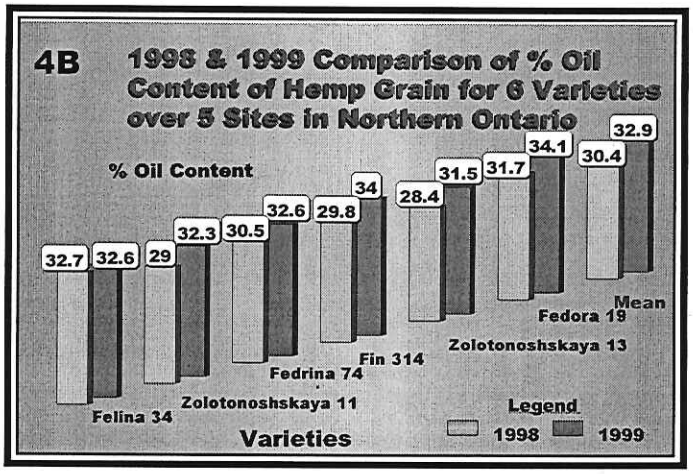
The largest and most comprehensive hemp grain quality research project in Canada was conducted across northern Ontario in 1998 and 1999 from 46th to 50th latitude and 80th to 94th longitude (an east west distance of 2000 km). The performance of 8 hemp grain type cultivars was evaluated for yield and quality. A total of 51 and 44 harvested grain samples were analyzed for oil yield; protein, profile of 16 amino acids (AA's) and fibre of the oil cake; and profile for 12 fatty acids (FA's) of oil in 1998 and 1999 respectively.

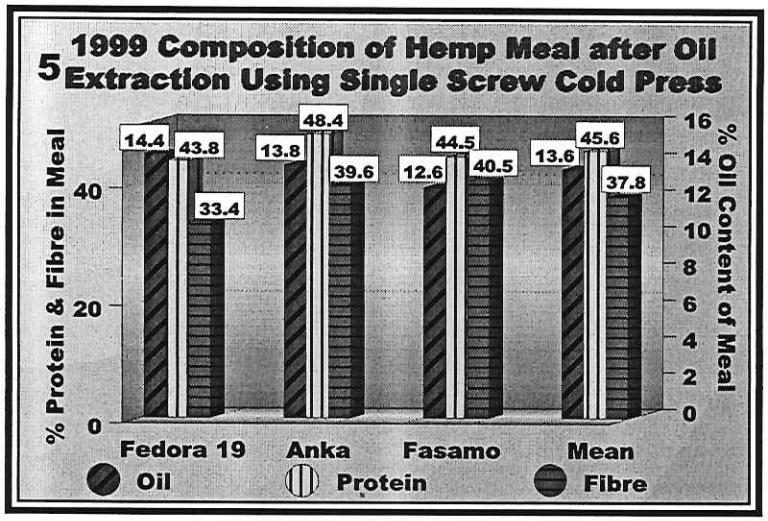


Data were collected from both commercial (28 two-hectare strip sites in 1998) and 5 Kemptville College / University of Guelph agricultural research sites across northern Ontario with replicated research trials in 1998 and 1999. The report will also include and discuss data from southern Ontario. The two years multiple location and variety data addresses the environment x genotype effect on hemp grain, oil cake and oil quality.

ACKNOWLEDGMENTS AND FUNDING

The authors wish to acknowledge the significant efforts of all the research technical staff at the respective research stations across northern Ontario for successfully conducting the industrial hemp trials for grain production. Also, for the support and cooperation of all the participating industrial hemp growers across northern Ontario. The exceptional assistance of Mr. Herb Hinz, summer assistant in 1998 must be recognized. The loan of the Single Screw Komet Oil Press from the Indian Agricultural Program of Ontario was a significant contribution to this project.

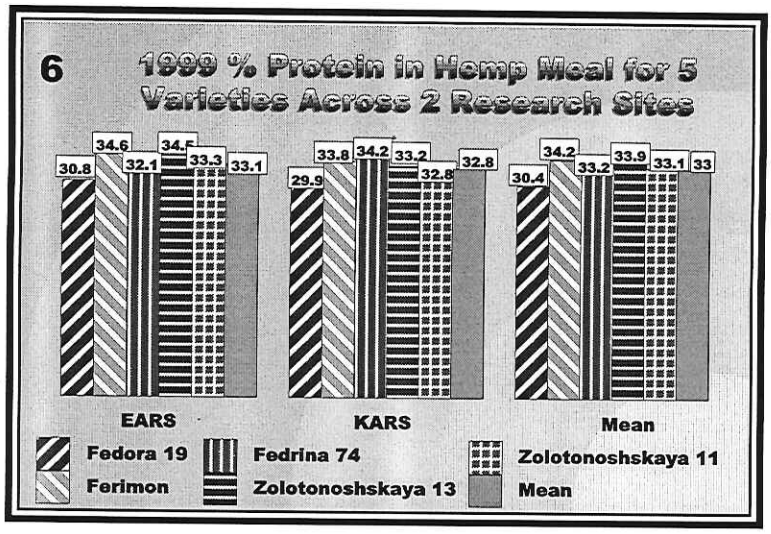




Funding for the discussed research was from Northern Ontario Heritage Fund Corporation, Northern Ontario District Development Corporations, Industrial Research Assistance Program, Thunder Bay Industrial Hemp Grower's Association and Can Adapt. In-kind contributions were from Quetico Centre, Atikokan, Ontario; Northern Ontario Industrial Hemp Growers; Kemptville/College and Ridgetown College, University of Guelph, Ontario, Canada and Lakehead University, Thunder Bay, Ontario, Canada.

METHODOLOGY

Clean grain samples (4% moisture) were analysed for oil content, protein and fibre content in the meal, 16



amino acids in the meal (**Graph 2**). Seven are EAA's and 2 are EAA's for children. The 12 fatty acids in the hemp oil displayed in **Graph 3** are grouped as 2 EFA's, 5 SFA's, 3 monounsaturated fatty acids (MUFA's) and 4 PUFA's. The laboratory analysis was completed by Dr. Roman Przybylski, University of Manitoba, Winnipeg, Manitoba, Canada (co-author). Some samples of hemp grain were extracted for oil and meal using a single spindle Komet cold press at the Thunder Bay Agricultural Research Station (TBARS).

RESULTS AND DISCUSSION

Oil Content of Industrial Hemp Grain.

T1 **Hemp Meal Chemical Analysis from Kent County**
Laboratory Services Division University of Guelph

Protein	20.3	(%w/w)
Fibre	58.9	"
Ash	5.5	"
Calcium	!62.0	mg/100g
Copper	2.0	"
Iron	13.0	"
Potassium	764.0	"
Manganese	6.3	"
Magnesium	464.0	"
Sodium	18.0	"
Phosphorus	970.0	"
Zinc	7.3	"

The 1999 data for oil content of the hemp grain for 5 varieties (Fedora 19, Ferimon, Fedrina 74, Zolotonoshskaya (Zolo) 13 and Zolotonoshskaya (Zolo) 11) grown at the two research sites of Emo Agricultural Research Station (EARS) and Kapuskasing Agricultural Research Station (KARS) are summarized in **Graph 4** (Scheifele 1999A). Varieties demonstrated differences in oil content at both sites ranging from 33.1% to 29.8%. Fedora 19 had the highest oil content at EARS (33.1%) and KARS (31.8%), whereas Ferimon was the second highest at EARS (32.4%) and second lowest at KARS (29.9%). Zolo 13 had the lowest oil content at EARS (29.9%) and the second highest at KARS (30.8%). The mean values across the two sites for Fedora 19, Ferimon, Fedrina 74, Zolo 13 and Zolo 11were 32.5%, 31.2%, 30.2%, 30.4% and 30.6% respectively with a total mean of 30.9%. EARS and KARS had overall mean values of 31.4% and 30.4% respectively.

T1A **Amino Acid Composition of Hemp Seed Meal from Northern Ontario**
Percentage in hemp meal after oil removal

Aspartate	3.47	Methionine	0.73
Threonine	1.17	Isoleucine	0.82
Serine	1.64	Leucine	1.85
Glutamate	5.40	Tyrosine	0.76
Proline	1.36	Phenylalanine	1.28
Glycine	1.37	Histidine	0.71
Alanine	1.33	Lysine	1.18
Valine	1.12	Arginine	3/37

The data in **Graph 4A** demonstrates site effect in 1999 on oil content of Fedora 19 and site mean across the northern Ontario research sites in 1999. The ( ) value represents the number of varieties in the mean. Thunder Bay (TB) site produced the highest oil content (33.8%) with Verner Agricultural

T2 Feed Analysis of Various Products

TEST	HEMP DRY BASIS	SOYA 44%	LINSEED MEAL	CANOLA MEAL	COTTON SEED MEAL
Dry Matter %	91.10	89.00	90.00	89.00	92.70
Protein% (Nx6.25) Fibres	32.55	42.54	32.00	38.00	41.40
Acid Detergent Fibre %	49.20	7.50	11.25	13.88	13.63
NDF%	***	0.00	25.00	0.00	28.00
Minerals:					
- Calcium %	0.15	0.30	0.37	0.68	0.19
- Phosphorus %	0.87	0.52	0.73	1.17	1.09
- Potassium %	0.80	2.08	1.18	1.29	1.25
- Magnesium %	0.46	0.28	0.51	0.64	0.49
- Sodium & Energy	0.01	0.03	0.15	0.10	0.05
TDN (Estimated) %	51.05	72.09	69.00	64.00	73.60
Net Enegy (LAC) MCAL/Kg	1.07	0.87	0.78	0.83	0.80
Net Energy (GAIN) MCAL/Kg	0.43	0.61	0.52	0.45	0.54
Net Energy (MAINT) MCAL/Kg	0.98	0.92	0.81	0.73	0.83
CA:P Ratio	.17:1	.57:1	.51:1	.58:1	.17:1

Research Station (VARS), KARS, EARS and New Liskeard Agricultural Research Station (NLARS) having 31.3%, 30.9%, 31.9% and 31.4% respectively. Fedora 19 had the highest oil content at TB (35.4%) and the lowest at NLARS (31.4%). Fedora 19 had a mean value of 32.5% compared to the overall mean of 32.0%.

Graph 4B demonstrates the year effect from 1998 and 1999 for Felina 34, Fedrina 74, Fedora 19, Zolo 11, Zolo 13 and FIN 314 averaged over the same 5 research sites for both years (Scheifele 1999A, Hinz & Scheifele 1998). All varieties had higher levels of oil in 1999 (mean = 32.9%) than in 1998 (mean = 30.4%). Zolo 13 had the overall lowest oil content in both years.

Composition of Industrial Hemp Meal.

Graph 5 summarizes the 1999 data for hemp meal composition for oil, protein and fibre for Fedora 19, Anka and Fasamo. It is noted that the oil was extracted from the hemp grain using the Single Screw Komet Oil Press. The remaining oil content in the hemp meal was 14.4% to 12.6% (Scheifele 1999). The protein levels were 43.8%, 48.4% and 44.5% for Fedora 19, Anka and Fasamo respectively. The fibre levels were 33.4%, 39.6% and 40.5% respectively. The mean oil, protein and fibre levels were 13.6%, 45.6% and 37.8% respectively. Anka had the highest protein content and Fedora 19 was the lowest.

The percent protein in the hemp meal in 1999 for 5 varieties (Fedora 19, Ferimon, Fedrina 74, Zolo 13 and Zolo 11) for EARS and KARS is summarized in **Graph 6**. Ferimon had the highest protein content at EARS 34.5%) and Fedrina 74 had the highest at KARS (34.2%). Ferimon had the highest content averaged across both sites (34.2%). Fedora 19 was the lowest at both sites (30.4%). The mean protein content for all five varieties across the two sites was 33.0%.

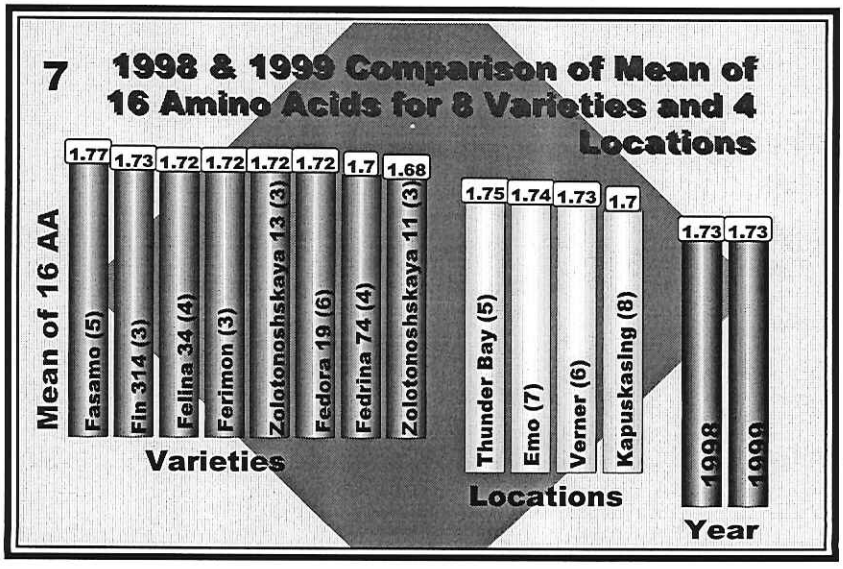
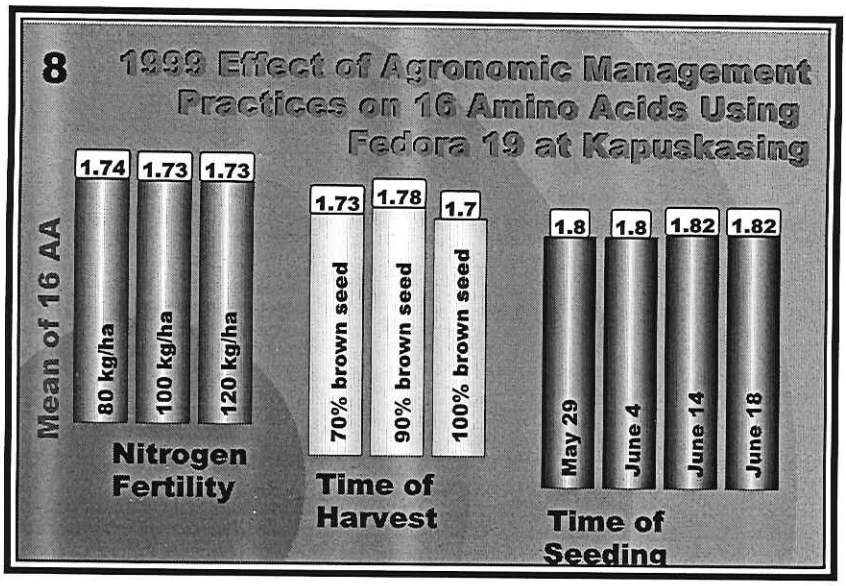
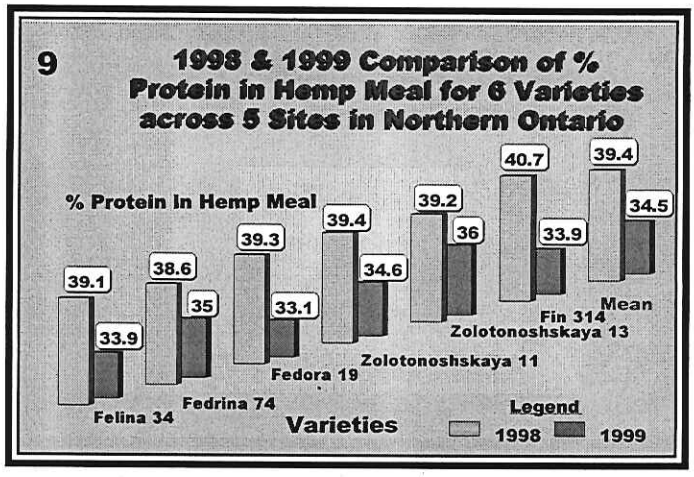


Table 1 summarizes the chemical and mineral analysis of hemp meal from Kent county Ontario, Canada (Dragla 1999). **Table 1A** summarizes the 16 amino acid profile on dry matter basis from northern Ontario (Scheifele 1999A). **Table 2** summarizes the feed analysis for hemp meal from Thunder Bay, northern Ontario compared on dry basis to soya 44%, linseed meal, canola meal and



cotton seed meal. The different products are compared for protein (%), ADF, NDF, minerals, TDN, NE (Lac), NE (Gain) and NE (Maint).

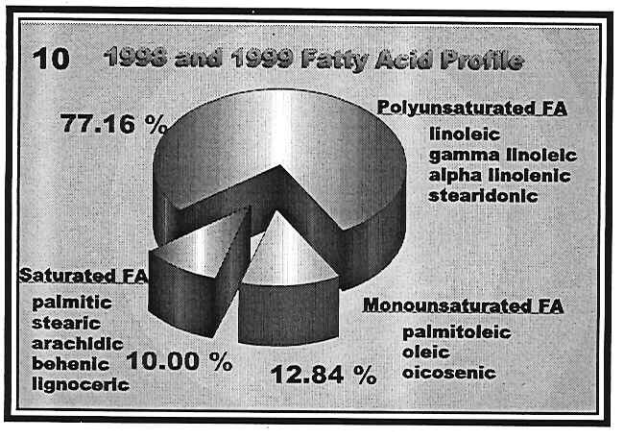
The eight varieties were evaluated for AA variability across all 5 research sites tested in 1999 using a regression analysis. The correlations ranged from 99.2% to 99.9% and the slopes were from 0.98 to 1.02. This demonstrates the varieties to be very stable and consistent for the 16 AA's analysed. The comparison of 1998 to 1999 for the 8 varieties across the 5 research sites showed very little variation due to year effect. Likewise there was very little location effect across the 5 research sites. **Graph 7** compares the mean value for all of the 16 AA's in 1999 for 8 varieties and 4 research sites. Fasamo had the highest mean value (1.77) and Zolo 11 the lowest (1.68). TBARS had the highest mean site value (1.75) and KARS had the lowest (1.70). The mean values for 1998 and 1999 were the same (1.73%).



The 1999 effects of agronomic management practices on the 16 AA's for Fedora 19 at KARS are summarized in **Graph 8**. The values were generated in a regression analysis of all 16 AA's regressed over the environmental means. The three management practices evaluated were: nitrogen fertility levels, time of seeding and time of harvest. The lower nitrogen fertility level (80 kg/ha) gave the highest mean AA level (1.74%), lowest standard deviation (1.25%) and lowest slope (0.98) in regression analysis. The harvest date of 90% brown seed produced the highest mean AA level of 1.78% and the later dates of seeding (June 14 & 18) gave the highest mean AA levels (1.82%). The correlation values for all 3 management practices ranged from 99.43% to 99.84% and the slopes ranged from 0.94 to I.04. This demonstrates a very stable and consistent response of the 16 AA's to management.

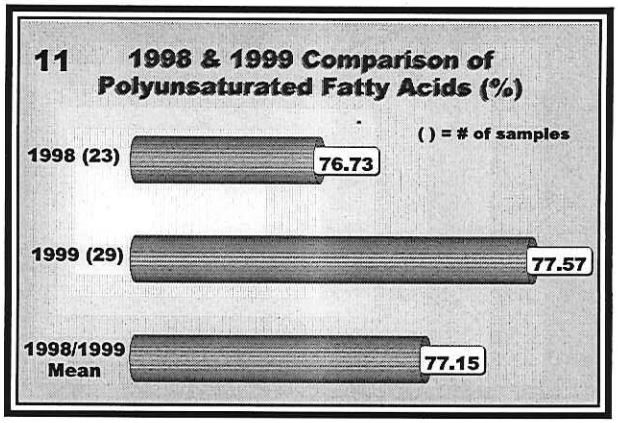
T3 Essential Fatty Acid Profile in Hemp Oil (Anka)

	Percent
C-16 Palmitic	7.0
C18:1 Palmitoleic	0.1
C18 Stearic	2.4
C18:1 Oleic	11.7
C18:2 Linoleic	52.3
C18:3 Linolenic	15.2
C18:3 Gamma Linolenic	3.0
C20 Arachidic	0.8
C20:1 cis	0.4
C22 Behenic	0.3
C24 Lignoceric	0.1

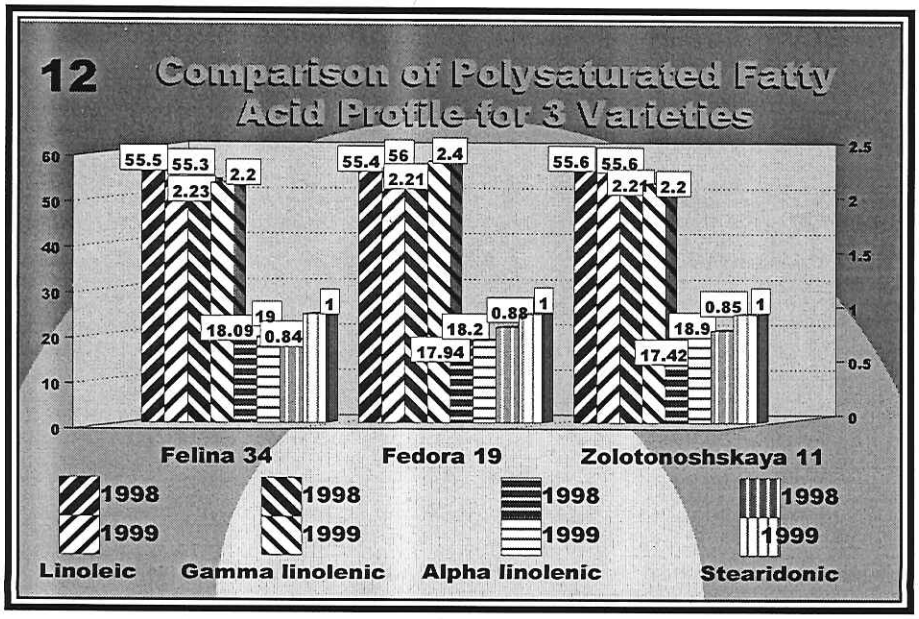


The data summarized in **Graph 9** compares the percent protein in hemp meal for 6 varieties (Felina 34, Fedrina 74, Fedora 19, Zolo 11, Zolo 13 and FIN 314 across 5 research sites for 1998 and 1999. FIN 314 had the highest percent in 1998 (40.7%) and Zolo 13 in 1999 (36.0%). The 1998 percent protein levels were consistently higher (mean = 39.4%) than 1999 (mean = 34.5%). **Graph 4B** demonstrates the higher mean oil content to have been in 1999.

Fatty Acid Profile of Industrial Hemp Oil.
The 12 FA's from 1999 were analysed using regression analysis, regressing each FA against the environment mean which for the variety analysis consisted of 8 variety mean. The Variety means ranged from 8.32 to 8.34. The slopes ranged from 0.99 to 1.01 and the correlations were 99.9% to 100%. The 12 FA's appeared to be very consistent and stable for the 8 varieties across the 5 research sites.

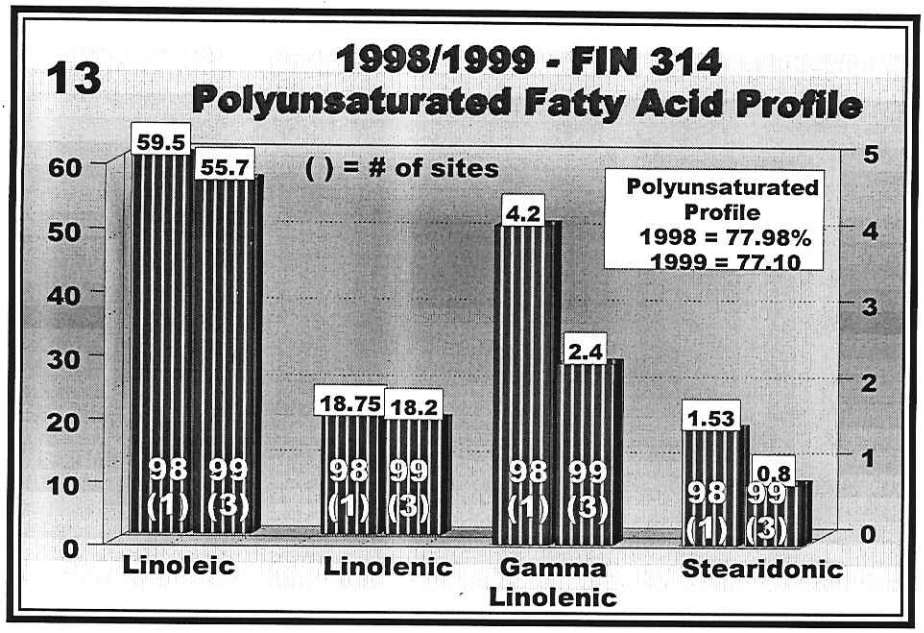


Similarly the 12 FA's were regressed over the environmental mean for each of the 5 research sites. The environmental mean consisted of 29 samples. The research site means ranged from 8.28 to 8.35, a larger variation than the variety effect. The slopes ranged from 0.994 to 1.011 and the correlations were 99.9% to 100%.



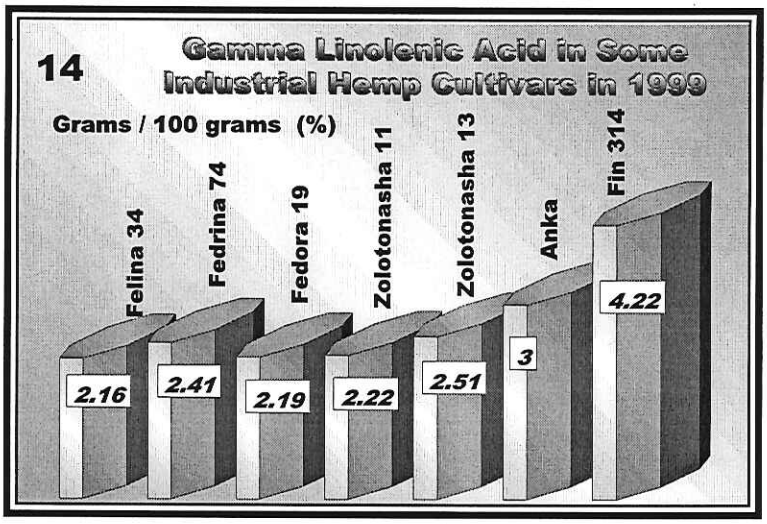
The effect of nitrogen fertility on the 12 FA's was not detectable.

Table 3 summarizes 11 fatty acids for Anka grown in Kent county (Dragla 1999). Anka is the first monoecious early grain variety of industrial hemp developed in north America and registered in Canada (Dragla 199). The Gamma Linolenic Acid (GLA) was 3.0%. Note that the Linoleic (Omega 6) ratio to Alpha-Linolenic (Omega 3) ratio is 1:3.4. The 3 polyunsaturated FA;s



(PUFA) totalled 70.5%. The oil from Anka was not analysed for Stearidonic acid (the 4th PUFA). Stearidonic acid data are provided and discussed for northern Ontario later in the text.

Graph 10 summarizes the 1998 and 1999 FA profile for northern Ontario for 8 varieties across the 5 research sites (23 and 29 samples respectively). The mean PUFA, SFA and MUFA were 77.16%, 10.00% and 12.84% respectively. Leson (Leson) reports 9-11% total SFAs and a total of 89-91% UFA's compared to 10% and 90% for northern Ontario respectively.**Graph 11**summarizes the 1998/1999 the PUFA means. The 1998 PUFA mean for 23 samples was 76.73% and 1999 for 29 samples was 77.57%. The regression analysis showed no year to year effect regressing all 12 FA's against the environmental mean (mean of 1998 (23 samples) and 1999 (29 samples). The two year mean for northern Ontario was 77.15%.
Leson (Leson) reports a range for the PUFAs from 69.4 -91%.

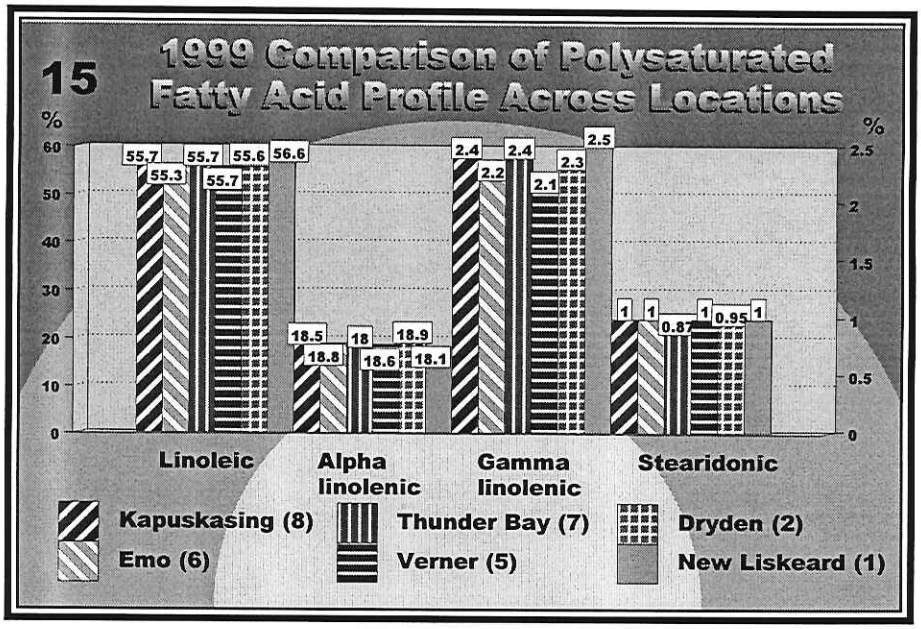


Graph 12 demonstrates the PUFA profile for Felina 34, Fedora 19 and Zolo 11 for 1998/1999 across northern Ontario. Alpha-Linolenic acid (18:3 omega 3) and Stearidonic acid (18:4 omega 3) showed the greatest variation from 1998 to 1999 with higher levels for both in 1999 contributing to the higher 1999 PUFA level in **Graph 11**. There was no measurable variation for the PUFA's between the varieties.

Graph 13 gives the PUFA profile for FIN 314 for 1998/1999 across northern Ontario. The () values indicate the number of sites represented by each mean. The (GLA) and Stearidonic acids levels were significantly lower for 1999 compared to 1998 (4.2% to 2.4% and 1.53% to 0.8% respectively. The total mean PUFA levels for 1998 were 77.98% compared to 77.10% for 1999.

The GLA profiles for 7 varieties (Felina 34, Fedrina 74, Fedora 19, Zolo 11, Zolo 13, Anka and FIN 314) grown in Kent county in 1999 are summarized in **Graph 14** for 1999 (Dragla 1999). The GLA values for

Anka and FIN 314 were 3.0% and 4.2% respectively. The range of values for the rest of the varieties was 2.16% to 2.50%. There is no explanation for the lower GLA and Stearidonic Acid values in FIN 314 for northern Ontario in 1999. The values were consistent across all 3 sites measured.



The data in **Graph 15** demonstrates the variability of the PUFA's across 6 locations in northern Ontario for 1999. The ( ) value represents the number of varieties tested at each site. The range of values for Linoleic acid, Alpha Linolenic acid, GLA and Stearidonic acid was 55.3-55.7%, 18.0-18.8%, 2.1-2.5% and 0.82-1.00% respectively. The mean value for the Linoleic Acid was 55.8% and Alpha-Linolenic Acid was 18.7% giving a 1:3 ratio. The location effect on the variation of the PUFA's appears to have been slight but not significant.

SUMMARY AND CONCLUSION

Industrial hemp grain yields (adjusted to 12% moisture) averaged 1062 to 1297 kg/ha across northern Ontario in 1998 and 1999 respectively. Fedora 19 and Fasamo averaged 1100 kg/ha (combine harvested and cleaned) from a 4 ha commercial field in Thunder Bay in 1999.

Fedora 19 had consistently higher oil content and lower protein content across locations and 1998 - 1999 (31.7% and 34.1% respectively for oil content). The oil contents for all varieties and sites in 1999 were higher (2.5%) than for 1998. The five research sites across northern Ontario averaged oil contents ranging from 30.9% to 33.8%. Agronomic management practices of time of seeding affecting length of growing season and time of harvest effecting the ripening impacted oil content, protein and fibre levels, whereas nitrogen fertility levels did not impact these components. The variety Anka had the highest protein content. The protein and oil content were in a reverse relationship. 1998 had higher protein and lower oil content whereas 1999 had the reverse. The 1998/1999 oil and protein relationship had a 79% correlation with a negative slope of -1.03 (Scheifele 1999A). The residual oil content in commercial hemp meal after oil extraction reported by industry ranges from 4-6% (Crew). The AA profile of the hemp meal demonstrates it's quality as livestock, fish and pet food supplement as well as a non-gluten flour for human consumption.

The AA composition of the industrial hemp meal was fairly stable across varieties, sites and years. Fasamo had the highest mean value for all 12 AA's, whereas Zolo 11 had the lowest mean value. TBARS had the highest mean site value and KARS had the lowest value. The 1998 and 1999 mean values were the same. The management practice of "time of harvest" at 90% brown seed had the highest mean value, whereas the 100% brown seed was the lowest.

The profile of the 12 FA's evaluated in the hemp oil demonstrated very consistent and stable qualities for variety, site and year effect. The PUFA's, Alpha-Linolenic acid, GLA and Stearidonic acid, demonstrated the greatest variability for varieties (Anka and FIN 314) and year effect (1999 higher levels). Anka had 3.0% GLA grown in southern Ontario. The lower levels of GLA and Stearidonic acid in FIN 314 in 1999 across northern Ontario cannot be explained. The 1999 seed source for FIN 314 is the greatest suspect. The 1998 level for FIN 314 was 1.53% in northern Ontario similar to that reported by Callaway (Callaway). The composition of PUFA's, SFA's and MUFA's

from northern Ontario was equivalent to the mean of the range reported in literature (Leson). The northern Ontario mean PUFA level ranged from 76.7% to 77.6% for 1998 and 1999 respectively. Leson (Leson) reports a range of 69.4 to 91%. The omega 6:omega 3 ratio for northern Ontario was 1:3 which is the balanced profile required for human nutrition (Erasmus). The author was not able to demonstrate the environmental effect on FA composition as reported by Molleken (Molleken). The FA profile reported for northern Ontario 1999 (Scheifele 1999A) compared to that reported by industry (Crew) was improved for GLA (2.3% compared to 1.7%), Arachidic acid (0.82% compared to 0.5%) and Lignoceric acid (0.15% compared to 0.3%). Only Palmitoleic acid was lower for northern Ontario (0.18%) than that reported by Crew (0.3%).

Rose et al. (Rose et al.) reports that examination of the effect of seed maturity on the FA composition showed that the more mature the seeds are, the higher the percentage of USFA's and the lower the percentage of SFA's. The author did not separate green seed from brown seed as did Rose, however comparing 90% brown seed lots to 70% seed lots, the 90% brown seed had slightly higher percentage of USFA's (90.2%) than 70% brown seed (89.8%). Actual brown seed needs to be separated from the green seed and evaluated separately to establish this fact.

Przybylski et al. reported a significant effect of Manitoba, Canada on the shift in FA composition of hemp oils for varieties grown in Manitoba over two years compared to the original seed (Przybylski). The author used freshly purchased seed from Europe each year. Hence the geographical origin of the seed was considered the same. The difference in geographical seed source of FIN 314 in 1999 might explain the reduced levels of GLA and Stearidonic acid compared to 1998 and that reported by Callaway. The author does recognize that climatic changes have the potential to impact the hemp plant to genetically shift the oil composition for FA profile by adaptation in more northern latitudes..

The reported research supports the fact that industrial hemp grain and resulting processed raw products of meal and oil produced in northern Ontario are consistent in composition of protein, AA's and FA's combined with and excellent profile of PUFA's for the health food and food market.

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