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MIXED CROPPING OF LINSEED AND LEGUMES AS A ECOLOGICAL WAY TO EFFECTIVELY INCREASE OIL QUALITY

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ABSTRACT

The aim of the research was the evaluation of the influence linseed-legumes intercropping on linseed yield, fat yield, caloric value of the fat and fatty acids composition. The experiment was performed on experimental field in Kraków (50°07'N 20°05'E). The greatest fat content was found in linseed seeds from mixed cropping with pea (391 kg). Significantly higher calorific value (56.3 GJ) was obtained in seeds of the linseed pure stand. In seeds of linseed predominated unsaturated fatty acids (PUFA, MUFA). A slight increase in PUFA and MUFA content was observed in mixed cropping. The content of alpha-linolenic acid (C18:3n-3) in linseed seeds was significantly influenced by the sowing method and the weather. Linseed in mixed cropping with vetch contained significantly more linoleic acid (18:2n-6) and alpha-linolenic acid (18:3n-3) than in pure stand. There was considerably more linoleic acid (C18:2n-6) in mixed cropping with vetch, but in mixed cropping with pea there was much less of that acid.

Key words: fat, caloric value, linseed, intercropping.
Abbreviations used: SFA - saturated fatty acid; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty; L - linseed, Lp - linseed from mixture with pea; Lv - linseed from mixture with vetch

INTRODUCTION

For many years linseed (*Linum usitatissimum* L.) has been one of the most important crop grown in worldwide (Mikelsone et al., 2013). The main producers of linseed oil are: the European Union (25.5%), the USA (21%), China (21%), and India (10%). Colder countries such as Finland (Sankari, 2000), Ireland (Easson and Molloy, 2000) or Lithuania (Mikelionis and Endriukaitis, 2000) are currently increasing their linseed production. The European Union countries also are increasing the linseed cultivation acreage (20% of the world acreage), treating the solution as an "ecological intermission" in wheat monocultures they are growing, which is beneficial to the quality of soil, biodiversity and landscape diversity (Zając et al., 2002; Mańkowski et al., 2006). Because of adequate climatic conditions, linseed is grown in maritime regions of Europe, in temperate climate, among others in northern France (72%), Belgium (15%), and Holland (4%), and to a smaller extent in Poland (2.6%), the Czech Republic (2.5%), and the Baltic states (2.4%). Despite the growing linseed acreage in Poland (2 thousand hectares) as well as in other European countries, the production cannot fulfil all the needs. Poland, as well as other European countries, is importing linseed from Canada, the main linseed producer in the world (ca. 31% of the world production), in the form of both raw seeds and linseed oil or oil cake (Oomah and Mazza, 1999). Currently the tendency for growth in demand for seeds is observed in the world, in the recent years this demand has reached 700 thousand tons (FAOSTAT, 2014). The fat content in linseed seeds is usually about 38-44%. Besides that, the seeds contain 25-30% protein and 22-24% nitrogen-free extract, as well as ca. 5.5% fiber and 4% ash. They are especially rich in dietary fiber, lignin, phenolic compounds, and unsaturated fatty acids (Shim et al., 2014). The research conducted until now was aimed at increasing the plants' productivity by application of larger doses of nitrogen, and less research was conducted concerning

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different sowing methods. Until now, agricultural methods were researched sporadically in the case of linseed in mixed cropping, both in Poland (Zając, 2004; Zając et al., 2013) and in other countries (Mishra and Masood, 2002; Patra et al., 2004). The increased dosage of nitrogen through mixed cropping with legumes can be an effective agricultural method, in comparison with pure stand .The initial goal of the research, which led to this new, environmentally friendly way of growing linseed, was the evaluation of the influence mixed cropping linseed with legumes on yield, yield components, the fat yield, the caloric value of the fat and fatty acids composition.

MATERIAL AND METHODS

The research on linseed in pure stand and mixed cropping was conducted in the years 2006-2008, based on a one-factorial field experiment, designed by split-plot with 4 replications, and the size of an experimental field was 10 m^2. During the experiment, linseed was sown in pure stand and in mixed cropping (Klimek-Kopyra et al., 2013). In pure stand the seeds were sown in the amounts: linseed (‘Flanders’ – 480 seeds/m^2), pea (‘Ramrod’ – 80 seeds/m^2), and common vetch (‘Ina’ – 200 seeds/m^2). In mixed cropping, the numbers of seeds were split in half between the crops in every stock. In all cases and during all years when the experiment was performed, the forecrop was spring wheat. Before sowing, the total amount of nitrogen fertilization for pure sowing and mixture were as follow: 30 $kg.ha^{-1}$ N (ammonium nitrate - 46% urea) for pure sowing of linseed, 20 kg/ha for mixtures and 20 $kgha^{-1}$ for pure sowing of pea or vetch. When linseed was in shooting stage, 20 kg N was applied for mixtures and linseed in pure sowing, respectively. Differentiated doses of N for legumes and linseed resulted from the fact that legumes utilize nitrogen provided by Rhizobium bacteria. Plants were fertilized with 48 $kgha^{-1}$ phosphorus in the form of triple superphosphate (46%), and 72 $kg.ha^{-1}$ potassium in the form of potassium salt (60%).

Sowing was performed at the optimal time, using a planter (a Wintersteiger Tool Carrier 2700) in all the years when the experiment was performed, within the first decades of April, and all the species were sown separately. Legumes (pea or vetch) were sowed first, while linseed later. The sowing distance was 15 cm, the sowing depth in the case of pea was 6-8 cm, for vetch it was 5-6 cm, and for linseed 2-3 cm. Directly after the sowing, the land was harrowed to cover the seeds and to flatten the field. The harvest of the experimental crops was conducted each year between 10-20 August using harvester (Seedmaster Universal Hydrostatic).

The quality assessment of linseed seeds was based on the outcome of the chemical analyses performed. The following methods were used: dry matter was determined with the oven-drying method, in the temperature of 105±2^{o}C, maintained for 5 hours according to the Polish standard [PN-A-74108:1996]. Raw fat was extracted from the plant matter by means of the Soxhlet method [PN–71/A–88021]. The fat content determination method [Official Metod of Analysis of AOAC 991.39] entails extraction of fatty substances form the sampled material by the use of hexane and gravimetric determination of the amount of the substances extracted after the solvent evaporates. The chemical composition and percentage of fatty acids in linseed seeds was determined with the use of a Trace GC Ultra gas chromatograph. The gross caloric value of vetch, pea and linseed seeds was determined by the bomb calorimeter method in accord with the ISO norm [PrPN-EN ISO 9831]. Statistical analyses were performed in STATISTICA software, version 10.0. Analyses of variance (ANOVA) with subsequent multiple comparisons of means were performed. Means were separated by least significant differences (HSD-), and the F-test factorial effects on the significance level of $p<0.05$.

Weather conditions

The thermal conditions during April, measured according to the Lorenc classification (2000), were normal in all the years when the experiment was conducted.

Linseed's water demand is greatest in May and in June (Casa et al., 1999). In the first year of the experiment May temperatures were somewhat cold, during the second somewhat warmer than average, and they kept within the average range during the third year. May precipitation in all three years was classified as within average parameters. June 2006 was classified as average, and in the other two years June was warm.

Precipitation conditions were very varied: very humid in 2006, average in 2007, and very dry in 2008. In July and August, when the curves of thermal and precipitation conditions have the most influence on the linseed yield and quality, both thermal and precipitation conditions were highly varied: from very cold to very warm, and from extremely dry to humid. Based on the number of days when precipitation occurred during the linseed growing season, it was noticed that the distribution of precipitation was different each year (Table 1). In 2006 the number of days with precipitation in June and July was lower than the mean annual number, and in the remaining months it was higher than the mean. In 2007, in April and in August the number of days with precipitation was lower than the mean annual number, and in the remaining months it was close to the mean annual number or slightly higher.

Table 1. Basic meteorological characteristics in 2006-2008

Morphological elements	Years	April	May	June	July	August
Temperature	2006	9.2	13.2	17.4	21.6	17.0
	2007	9.4	15.2	18.4	19.4	19.0
	2008	9.1	13.6	18.4	18.7	18.2
Precipitation	2006	45.7	51.9	89.4	14.2	104.1
	2007	15.4	51.7	72.1	74.7	76.3
	2008	35.2	26.8	25.7	142.6	41.6
Number of days with precipitation	2006	12	13	8	4	22
	2007	5	13	11	19	8
	2008	12	6	5	10	13

RESULTS

Table 2 demonstrates the seed yield, the amount of fat in the crop, oil content and caloric value of the linseed. During the years when the experiment was conducted, it was observed that sowing methods are a significant influence on the seed yield and caloric value of yield. The seed yield was significantly higher in pure stand (2493 kg from ha^{-1}), and lower in the case of linseed in mixed cropping with vetch (1746 kg from ha^{-1}). The water deficit, which occurred in 2008, caused the lowest linseed yield in mixed cropping with pea (1375 $kg.ha^{-1}$). Fat content in seeds was dependent on sowing methods and weather conditions. The highest fat content was observed in linseed seeds from mixed cropping with pea (391 kg), in pure stand it was significantly lower (386 kg). Fat content was significantly different each year. In 2006, the warmest year in the research period, influenced the accumulation of fat in pure stand seeds (424 kg). 2008 in mixed cropping with vetch was the least favourable to fat accumulation (354 kg). Overall fat yield was dependent on cropping and on the fat content in seeds. In pure stand linseed gathered significantly more fat (727 kg) the maximum fat yield from linseed in pure stand was obtained in 2006 and 2007, respectively 1051 and 1055 kg. The smallest fat yield was obtained in linseed grown in mixed cropping with vetch, 519 kg (2007) and with pea, 527 kg (2006). The usability of linseed as animal fodder was influenced by the caloric value of the seed yield, meaning the amount of energy accumulated in the crops. The analysis performed as part of the experiment proves that sowing methods are a significant influence on the energy value of the seeds (Table 2). In pure stand the energetic value of linseed seeds was significantly higher (56.3 GJ) while in mixed cropping with vetch it was significantly lower (40 GJ). The caloric value of the seeds was definitely conditioned by the cropping method. The maximum caloric value of linseed was obtained in pure stand (66.3 GJ), and the minimal in mixed cropping with vetch (30.7 GJ). Habitat conditions of growth and the development of mixed crops of linseed and pea have proven to be insignificant to caloric values of harvests each year.

Table 2. Influence of cropping harvest ($kg\cdot ha^{-1}$) and amount of oil in seeds ($g\cdot kg^{-1}$), as well as the caloric value of the harvest ($GJ\cdot ha^{-1}$) (mean values for the period 2006-2008)

Treatments	Seed yield ($kg\cdot ha^{-1}$)	Fat yield ($kg.ha^{-1}$)	Fat content in seeds ($g\cdot kg^{-1}$)	Caloric value of yield ($GJ.ha^{-1}$)
Lp	1924.5+472.9a	740.2+186.7a	391.9+13.4a	44.8+10.6a
Lv	1746.2+439.8a	638.4+143.6a	374,8+15.3b	40.7+10.5a
L	2493.7+593.4b	944.7+225.3b	386.3+28.2c	56.3+14.6b
Mean	2054.8	774.4	384.3	47.2
Year (Y)	0.310	0.348	0.00**	0.502
Sowing method (S)	0.000**	0.000**	0.00**	0.000**
Y*S	0.000**	0.000**	0.00**	0.000**

p value <0.01**

Linseed oil obtained in the experiment contained 9.6-10% of saturated acids (5-6% palmitic acid, 3-5% stearic acid), and 87-90% of unsaturated acids (52-59% linolenic acid, 17-24% oleic acid) (Table 3). In linseeds, mainly unsaturated fatty acids (PUFA, MUFA) were found, and the amount of saturated fatty acids (SFA) was comparably low. The concentration of fatty acid was on similar level in all crops, however, there was a slight increase in the amount of PUFA and MUFA present in mixed crops Additionaly, it was noticed that sowing method had an equally significant influence on the amount of SFA, PUFA, and MUFA (Table 4).

Chemical analysis of fatty acid profile showed significant differences in the percentage of individual fatty acids, depending on sowing methods (Table 3). The linoleic acid (18:2n-6) and alpha-linolenic acid (18:3n-3) were the most frequent ones of the USFA group, found in the linseed oil. The amount of alpha-linolenic acid (18:3n-3) in linseed seeds was significantly influence by cropping and weather conditions. In mixed cropping with vetch, linseed had significantly more linoleic acid (18:2n-6) and alpha-linolenic acid (18:3n-3) than in pure stand. 2007 was the best year for mixed cropping of linseed (Table 3). Significantly more vaccenic acid (18:1n-7) was found in linseed seeds from mixed cropping with pea, and the amount of this acid was the smallest in seeds from mixed cropping with vetch. The oil, extracted from seeds in 2007 contained significantly more of the acid than in 2008. Therefore, the amount of linolenic acid (18:2n-6) was essentially shaped by cropping. Substantially more of the linolenic acid (18:2n-6) was found in pure stand and in mixed cropping witch vetch, and significantly less in mixed cropping with pea. 2006 was significantly beneficial for the accumulation of the linolenic acid (18:2n-6), and 2008 was definitely unfavourable.

The amount of the myristoleic acid (14:1) and pentadecenoic acid (15:1) was relevantly influenced only by the sowing methods (Table 3). These acids were most prominent in linseed oil from mixed crops with pea. The amount of the palmitoleic acid (16:1n-7) was similar in all crops, mixed or pure. However, a seasonal difference in their presence was observed in seeds, which was the result of changing weather conditions. In a humid year (2006) a tendency to accumulate more of the palmitoleic acid (16:1n-7) was observed, and in an arid or moderately humid year (2008) significantly less acid was accumulated. 2006 was significantly better for the accumulation of palmitoleic acid (16:1n-7).

Among the saturated acids (SFA), palmitic acid (16:0) and stearic acid (18:0) were in the highest concentration. The most palmitic acid (16:0) was found in the pure stand linseed, however, in 2006 the most significant differentiation in the amount of the acid in crops was observed. The presence of stearic acid (18:0) was substantially influenced by the cropping and weather conditions. Significantly more stearic acid (18:0) was accumulated by linseed in mixed cropping with pea, and 2006 was the optimal year. In the case of myristic acid (14:0), weather conditions and the interaction between cropping and weather were the only influences. Significantly more myristic acid was found in mixed cropping with pea in 2006 (Table 3).

Table 3. The concentration of fatty acids in linseed oil depending on sowing methods

Treatments	14:1	15:1	16:1n-9	16:1n-7	18:1n-9	18:1n-7	18:2n-6	18:3n-3	14:0	15:0	16:0	18:0
Lp	0.007b	0.008c	0.034a	0.068a	21.3a	0.766	12.23a	55.24a	0.061b	0.031b	5.78	3.93a
Lv	0.004a	0.007b	0.033a	0.061b	20.2b	0.744	12.84b	56.21b	0.055a	0.027a	5.79	3.58b
L	0.004a	0.005a	0.036a	0.068a	20.9c	0.643	12.53c	55.48c	0.058ab	0.029ab	5.89	3.88c
Mean	0.005	0.007	0.035	0.066	20.8	0.718	12.54	55.64	0.058	0.029	5.82	3.79
Year (Y)	0.000*	0.376	0.00*	0.000*	0.00*	0.00*	0.00*	0.00*	0.000**	0.00*	0.000**	0.000**
Sowing method (S)	0.000*	0.000*	0.220	0.002*	0.00*	0.06	0.00*	0.00*	0.00**	0.01*	0.062	0.000**
Y*S	0.014*	0.001*	0.005*	0.000*	0.00*	0.03	0.00*	0.00*	0.010**	0.01*	0.000**	0.000**

p value <0.05*, 0.01**. Lp = linseed from mixture with pea; Lv = linseed from mixture with vetch; L = linseed.

Table 4. Percentage of saturated and unsaturated acids in linseed oil, depending on the cropping and weather conditions

Treatments	Year	% Fat content	SFA	MUFA	PUFA	USFA	PUFA n-6	PUFA n-3	n-6:n-3
Lp	2006	40.4	11.68	21.96	65.99	87.95	11.97	54.02	0.22
	2007	42.8	8.88	19.14	71.95	91.09	12.90	59.05	0.22
	2008	39.2	9.44	25.93	64.48	90.41	11.84	52.64	0.22
	Mean	40.8	10.0	22.30	67.50	89.81	12.20	55.20	0.22
Lv	2006	38.4	9.51	21.20	69.17	90.34	14.17	55.00	0.26
	2007	41.6	9.56	18.68	71.66	90.33	12.25	59.41	0.21
	2008	37.2	9.82	23.78	66.34	90.12	12.09	54.25	0.22
	Mean	39.1	9.64	21.20	69.00	90.27	12.80	56.20	0.23
L	2006	42.4	11.6	21.63	66.67	88.30	14.17	54.63	0.26
	2007	39.0	9.14	20.15	70.66	90.81	12.25	57.14	0.21
	2008	39.2	9.42	23.49	66.69	90.18	12.09	54.66	0.22
	Mean	40.2	10.05	21.80	68.00	89.83	12.50	55.50	0.23

DISCUSSION

The sowing method of linseed cv. 'Flanders' had significant influence on the seed yield, fat yield, caloric value of seeds and on the amount of fatty acids profile, however it was a weak influence on morphological characteristics of the plants. Khan et al. (2005) have proven that the number of branches with capsules in linseed is genetically conditioned, however density of the plants in crop is able to modify it stronger. Our research has not validated these claims. The morphological characteristics of linseed were not much influenced by sowing methods. Weather conditions had more influence on morphological characteristics of the plants, and consequently, on the seed yield and its quality. Varied reactions of linseed to water conditions during growing period is confirmed by Diepenbrock & Iwersen (1989), who found that more humidity of soil during June and July prolongs the growing and maturing stages of linseed growing, which is beneficial to the accumulation of oil in seeds and to its quality. Our own research has shown that weather conditions can be a negative influence on the fat yield. The results of Greven et al. (2004) confirm this observation. In particular, their study indicated that temperature during growth and development of linseed has significant influence on the quality of seeds. Mirshehari et al. (2012) have proven that irrigation has significant influence on fat yield. During drought the fat yield was 50% less on the average, that is, it went down from 616 kg/ha to 338 kg/ha. According to Zając et al. (2002) the amount of fat in seeds and the percentage of oleic acid in oil is fixed in linseed varieties and does not change significantly under the influence of habitat conditions. Aufhammer et al. (2000) have achieved different results in their research, where fat yield in seed harvests was different throughout the years, dependent on the use of fertilizers and amounted to 860 up to 1006 $kg \cdot ha^{-1}$. The aforementioned researchers have also proven that the characteristic can vary in some of the Canadian varieties. Our own research has confirmed what Aufhammer et al. (2000) have stated, indicating that fat yield was different, depending on the agrotechnological conditions. In pure stand linseed fertility was much higher, and fat content was significantly higher. The smallest fat yield was obtained in mixed cropping with vetch.

Rahimi and Bahrami (2011) have researched the influence of fertilizers on harvest, fat yield, and the quality of fat in linseed. They have proven that increasing nitrogen fertilizing from 50 to 100 kg has significantly increased the harvest of seeds (from 584 kg/ha to 1518 kg/ha), as well as fat yield (from 181 kg/ha to 426 kg/ha). On the other hand, the percentage of fat in seeds was lowered from 30% to 27.7%. What is more, the researchers have proven that the increase in nitrogen fertilizing of linseed influenced the increase in the presence of linolenic acid from 51.1% to 52.21%, of linolenic acid from 14.68% to 15.09%, and the decrease of oleic acid presence from 20.29% to 19.79%. Our own research brought similar results. Mixed cropping of linseed and pea caused an increase of nitrogen level in the soil (N-fixation process). In the case of linseed fertilization in pure stand (72 kg/ha) the amount of unsaturated fatty acids was significantly lower than in mixed cropping with pea or vetch. Mixed cropping of linseed was a significant influence on the fatty acid profile. Significantly more PUFAs were found in linseed from mixed cropping with vetch, while mixed cropping with pea was significantly better for MUFAs accumulation. Pure stand only resulted in a significant increase in palmitic, saturated, acid presence. Different results were obtained by Vinogradov et al. (2012), who researched the influence of intense mineral fertilizing (90 kg/ha) on the harvest, fat yield, and presence of individual fatty acids. His research has shown that fertilizing with large doses of nitrogen had no influence on the characteristics investigated. Opposite observation was noticed by Klimek-Kopyra et al. (2013). Klimek-Kopyra et al. (2013) claimed that the most beneficial effect of nutrients on the yield and fat content was exerted by increasing the mineral fertilization from 90 to 180 kg NPK/ha. In presented studies we applied 50 kg N for linseed in pure sowing, while 40 kg in mixture with legumes. From our observations indicated that mix cropping was more suitable for fat content in seeds. It was noticed higher level of fat content in linseed cultivated with pea, than in pure sowing. However, lower linseed density in mixture caused lower seed yield and lower fat yield as well. As seen in an analysis of data from written sources about linseed agrotechnical issue (Zając et al., 2013; Klimek-Kopyra et al., 2013), none of the authors have described their yield in the terms of energetic value. In our own research, the caloric value of seeds was significantly changed both by sowing methods and by the weather conditions.

Cultivation linseed in pure stand was more effective, in the sense of more caloric value of seeds per unit area. Significantly more caloric value per acreage unit was obtained from linseed seeds from pure stand (56 GJ/ha) in comparison with mixed cropping seeds. In mixed cropping the caloric value of seeds was much lower, amounting to 40-44 (GJ/ha).

In conclusion, the amount of fat in seeds was significantly dependant on cropping and the weather conditions. The most fat was found in linseed seeds from mixed cropping with fat. In pure stand, the caloric value of the harvest was significantly higher. In linseed seeds, mostly unsaturated fatty acids (PUFA and MUFA) were found. In mixed cropping with vetch, the linseed contained significantly more of the linoleic acid (18:2n-6) and alpha-linolenic acid (18:3n-3) than in pure stand. The linoleic acid (18:2n-6) content was significantly higher in pure stand and in mixed cropping with vetch, and lower in mixed cropping with pea. The myristoleic acid (14:1) and pentadecenoic acid (15:1) content in linseed oil was the highest in mixed cropping with pea. A proper admixture of some species of legumes can have a positive influence on the content of an individual fatty acid in linseed seeds, but this often happens at the cost of lowering the content of another fatty acid.

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