

**Forage Yield, Nutritive Value, and Nitrogen Fixation Rate of Pea-Cereal  
Intercrops for Greenfeed Production in Saskatchewan**

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## ABSTRACT

Intercropping forage pea (*Pisum sativum* L.) with barley (*Hordeum vulgare* L.) or oat (*Avena sativa* L.) is an alternative way of cropping to improve forage yield and quality for greenfeed or silage production compared to monocropping. A two-year (2016-2017) field study was conducted at Melfort, Saskatoon, and Swift Current, Saskatchewan, Canada to evaluate forage production, nutritive value, and biological nitrogen fixation (BNF) of pea-barley and pea-oat intercrops in comparison to pea, barley and oat monocrops with and without nitrogen (N) fertilizer. Pea-cereal intercrops significantly increased forage DM yield ( $P<0.001$ ) compared to pea monocrops at all three experimental sites. The barley and oat were dominant ( $P<0.001$ ) species in the intercrop by accounting for 65-92% of the total DM yield. The lodging resistance of peas improved ( $P<0.001$ ) in pea-cereal intercropping compared to the monocrop peas. Land equivalent ratio (LER), was higher for intercrops ( $P<0.002$ ) than monocrops with values ranging between 1.03 and 1.18, indicating the advantages of pea-barley and pea-oat intercropping for using growth resources over monocrops. Pea-cereal intercrops contained similar forage neutral detergent fiber (NDF), acid detergent fiber (ADF), and starch concentrations compared to monocrops of barley and oat, but they had higher ( $P<0.001$ ) protein yield (PY) and crude protein (CP) concentration. In the intercrop, substantial amount of fixed N transferred from pea to the barley and oat components. The response of monocrops and pea-cereal intercrops to an addition of N fertilizer varied among the experimental sites. At the Melfort site, N fertilizer increased total DM yield ( $P=0.003$ ), CP concentrations ( $P=0.021$ ), and PY ( $P=0.001$ ) of monocrops and intercrops, but it reduced plant lodging resistance by 0.1-2.0 points ( $P=0.003$ ), and percentage of N derived from atmosphere (%Ndfa) by 22-63% ( $P<0.001$ ). At the Swift Current site, %Ndfa was reduced by 35-65% ( $P=0.019$ ) with N fertilization although when other agronomic traits were not affected. There

was no significant N fertilizer effect at the Saskatoon site. Overall, this study indicates that intercropping forage pea with forage barley or oat is an efficient system relative to corresponding monocrops to increase land productivity, forage DM yield, %Ndfa, CP concentration, PY, and to improve pea lodging resistance. Effect of N fertilizer application to intercropping was site-specific, and it reduced %Ndfa of pea at two of three sites in the study.

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## LIST OF ABBREVIATIONS

ADF	Acid Detergent Fiber
AAFC	Agriculture and Agri-Food Canada
AOAC	Association of Official Analytical Chemists
BC	Botanical composition
BNF	Biological nitrogen fixation
CDC	Crop Development Centre
CP	Crude protein
CR	Competitive ratio
cv.	Cultivar
DMY	Dry matter yield
HSD	Honestly Significant Difference
LER	Land equivalent ratio
NDF	Neutral detergent fiber
Ndfa	Nitrogen derived from atmosphere
NS	Non-significant
PY	Protein yield
RCBD	Randomized Complete Block Design
SEM	Standard error of mean

## Chapter 1. Introduction

Intercropping forage pea (*Pisum sativum* L.) with forage barley (*Hordeum vulgare* L.) or forage oat (*Avena sativa* L.) for greenfeed, and silage production is gaining interest among producers in western Canada. The inclusion of forage pea in intercrops with cereals has a number of potential benefits. The pea crop can increase protein concentration when the cereal crop can increase the digestible energy of feed, and possibly increase the total forage yield of intercrops. Compared to monocrops of cereals, pea-cereal intercrops can reduce the cost of N fertilizer, as peas can fix up to 69 kg N ha<sup>-1</sup> atmospheric nitrogen in association with *Rhizobium leguminosarum* bv. viciae bacteria (Hossain et al. 2017). Intercropping legumes and cereals can also suppress weeds compared to pea monocrops, which can reduce the reliance on chemical weed control (Lithourgidis et al. 2011). However, growth environment and agronomic practices such as seeding ratio, cultivar choice, and crop species can affect forage yield and nutritive value of intercrops, and biological N<sub>2</sub> fixation (BNF) efficiency of peas.

There is limited agronomic information available on seeding ratio of pea and cereal in intercropping system in various soil zones of western Canada. Forage pea has a larger seed than cereals, which is likely to increase seed cost of pea-cereal intercrops compared to cereal monocrops. A high seeding ratio of pea in an intercrop may require an extended drying period before reaching 18%, a safe moisture content for baling. Among cereals, oat tends to produce high forage yield in the Black soil zone, while barley produce a high forage yield in the Dark Brown and Brown soil zones (Walton 1975). Thus, it is essential to determine suitable crop kind and optimum seeding ratio for pea-cereal intercrops in the different growth environments. Application of N fertilizer in legume-cereal intercropping is a relatively common practice to increase the yield

of intercrops, but it is unknown if this practice inhibits or reduce BNF of pea, resulting in no net N gain in the system.

The main objective of this research was to evaluate different pea-cereal intercrops for forage production and BNF in the Brown, Dark Brown, and Black soil zones of Saskatchewan, Canada. The hypotheses of this thesis were: 1) high forage yield and high protein concentration will be obtained from pea-cereal intercrops compared to cereal monocrops, but the advantages of intercrops will vary due to different seeding ratios of intercrops; 2) intercrops containing CDC Horizon pea will produce higher forage yield and have higher lodging resistant than 40-10 due to their genetic differences; 3) application of N fertilizer will significantly reduce BNF of pea, and reduction will be less in pea-cereal intercrops compared to pea monocrop because of high N utilization of cereal crops; 4) drying time will be lower in pea-cereal intercrops compared to monocrops of cereals, and the rate will decrease with the increase of pea component in intercrops; 5) the production cost for pea-cereal intercrop will be lower than pea monocrop, because of seed cost differences and potential yield advantages of intercrops.



## Chapter 2. Literature review

### 2.1 Use of annual crop intercrops

Barley, oat, and triticale (*Triticosecale*) are the most popular annual cereals grown for silage, greenfeed, and pasture in Saskatchewan due to their high forage yield and high forage quality (Spring cereals 2016). McCartney et al. (2004) suggested that the old unproductive perennial forage system can be replaced by intercrops of barley or oat with winter cereals to achieve greater yield and quality in northeast Saskatchewan. Barley has better forage quality than oat and triticale (Surber et al. 2003), as it contains lower neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations (McCartney et al. 2004; Khorasani et al. 1997). Barley and oat greenfeed contained approximately 11% of crude protein (CP), 37 and 38% of ADF, 58 and 61% of NDF, 58 and 57% of total digestible nutrient (TDN), respectively (Forage Crop Production Guide 2014). Though forage dry matter (DM) yield of annual cereals varied among the different soil zones of Saskatchewan, barley usually produces a high yield in the Dark Brown and Brown soils, whereas oat yield high in the moist Black and Grey wooded soils (Walton 1975). Average forage DM yield of barley and oat ranged 5-7.5 t ha<sup>-1</sup> and 5.6-9.8 t ha<sup>-1</sup>, respectively, for greenfeed production in the Black soil zone of Saskatchewan (Forage Crop Production Guide 2014).

Intercropping refer to combinations of two or more species grown at the same space and time to obtain greater yield and quality compared to single crop production (Willey 1979). The crops are seeded in the same row, alternating row or strip or in relay depending on row arrangements of component crops (Sullivan 2001). The use of intercropping increased in organic production (Bedoussac et al. 2014), low-input farming (Vlachostergios et al. 2018) and cover crop (Marufu et al. 2007), grain and silage production (Maxin et al. 2017), and swath grazing (Aasen et al. 2004). Chapko et al. (1991) reported that pea-barley or pea-oat intercrops can be used as a

companion crop for alfalfa (*Medicago sativa* L.) establishment without adversely affecting its forage DM yield. In organic farming, Corre-Hellou et al. (2011) highlighted that annual legume-cereal intercrop can be an alternative way to control weed in organic farming. Intercropping can also improve the use of space and reduce the number of disease incidences (Lithourgidis et al. 2011). The competitiveness of intercrop, in particular, the pea component, against weed growth may in part due to complete use of resources, and improved competence of pea due to upright growth supported by cereals (Corre-Hellou et al. 2011). The incidence and severity of seven foliar fungal diseases in pea-cereal intercrops were evaluated in the organic farming system in Lithuania (Kadžiulienė et al. 2008). The severity of diseases varied among crop types with wheat (*Triticum aestivum* L.) or triticale grown in intercrops with peas affected significantly less by septoria leaf blotch (*Septoria* spp.) and brown rust (*Puccinia recondita*) than their respective monocrops.

Advantages of intercropping pea with cereals are to improve the utilization of environmental resources for growth more efficiently due to their different growth patterns, and nutrient requirements as compared to monocrops (Jensen 1996). In a forage trial, pea-barley forage had better economic return than barley monocrop or barley in intercrops with other legumes in Alberta, Canada (Strydhorst et al. 2008). However, Baron et al. (2000) and Begna et al. (2011) reported that legume-cereal intercrops produced lower forage DM yield than the monocrops of cereals. However, the authors suggested that forage DM yield loss in the intercrops can be compensated by the increase of protein concentration. In an agronomic study, pea-barley intercrop increased the stability of grain yield more than monocrop pea in four consecutive years (Jensen 1996).

### **2.1.1 Forage dry matter yield**

Forage DM yield varies among different intercrops. Strydhorst et al. (2008) found pea-barley produced the highest forage DM yield than intercrops of barley with lupin (*Lupinus albus*) or faba bean (*Vicia faba*). Pea-oat intercrop had greater forage DM yield than pea-barley or pea-triticale intercrops, but all three intercrops produced less DM yield than the monocrops of oat or barley according to Berkenkamp and Meeres (1987) and Dordas et al. (2012). Aasen et al. (2004) found forage pea combined with fall rye (*Secale cereal* L.) and ryegrass (*Lolium multiflorum* Lam.) had less forage DM yield than pea in intercrops with either barley or oat. Baron et al. (2000) reported that forage DM yield and grain yield of component crops tend to be lower in intercrops as compared to their respective monocrops. McCartney et al. (2004) found oat and barley forage DM yield decreased in the intercrops with winter wheat, triticale, fall rye, or ryegrass by 290 kg ha<sup>-1</sup> and 540 kg ha<sup>-1</sup>, respectively. One of the factors for yield reduction in intercrops is competition between species for soil nutrient, water, and light, which leads to a slow growth rate of the plant (Dordas et al. 2012). In pea-cereal intercrops, cereal crop can be dominant species and compromise a high percentage of total forage DM yield. Therefore, cereal species may be important for total forage DM yield production (Carr et al. 2004; Strydhorst et al. 2008). However, legume can be main yield contributor in certain growth conditions such as under high soil moisture (Jedel and Helm 1993).

### **2.1.2 Forage nutritive value**

A summary of forage nutritive value of pea-cereal intercrops is shown in Table 2.1. Chapko et al. (1991) found seeding pea with oat and barley has increased the crude protein concentration of forage by 4.4%, and 3.0%, respectively. The increase in CP concentration varied in different

pea-cereal intercrops, depending on cultivar, species ratio, climatic conditions, and agronomic practices (Gill and Omokanye 2018; Mustafa and Seguin 2004; Dordas et al. 2012). The concentration of CP ranged from 63-199 g kg<sup>-1</sup> in pea-barley and 97-186 g kg<sup>-1</sup> in pea-oat intercrops grown in different environments. The high CP concentration in the intercrop was attributed to the legume component and an increased CP of cereals due to the increased N supply from BNF (Jensen 1996). Dordas et al. (2012) found that pea-oat intercrop had a higher chlorophyll concentration compared to monocrops of oat and pea, which is an indicator for better forage quality of intercrops. Haq et al. (2018) found that pea-oat intercrop has a higher protein concentration and a higher leaf-to-stem ratio than oat monocrop.

The acid detergent fiber (ADF) and neutral detergent fiber (NDF) values are important indicators of fiber concentrations in forages. Acid detergent fiber measures total cellulose and lignin, whereas NDF determines hemicellulose in addition to ADF in forages. Feed digestibility will decrease with an increase of ADF concentration in forage. If NDF concentration is high, forage DM intake of the animal will decrease, resulting in poor animal performance (Collins and Fritz 2003). A number of studies have reported that pea-cereal intercrops reduce forage NDF (Aasen et al. 2004; Pereira-Crespo et al. 2010), and ADF (Kocer and Albayrak 2012) concentrations compared to monocrops of cereals. Inclusion of pea in intercrops, Chapko et al. (1991) reported reduction of ADF and NDF by 0.5-1.0% and 6.2-7.1%, respectively. According to the previous studies (Table 2.1), the ADF concentration ranged from 269-387 g kg<sup>-1</sup> in pea-barley, from 285-429 g kg<sup>-1</sup> in pea-oat, and NDF concentration ranged from 418-681 g kg<sup>-1</sup> in pea-barley, from 440-683 g kg<sup>-1</sup> in pea-oat intercrops. The variability in forage fiber concentration is likely reflection of component crop species or ratio and stage of maturity at harvest.

Starch is one of the primary sources of energy for the animal. Starch concentrations of common barley varieties for silage averaged at 210 g kg<sup>-1</sup> in western Canada (Nair et al. 2016), which is used as primary energy concentration of animal feed (Nair et al. 2016). In pea crops, starch is the major component of pea seed and yields of pure starch range from 32.7-33.7% on a total seed basis (Ratnayake et al. 2001). Pea starch degrades more slowly in the rumen compared to those of wheat or barley (Walhain et al. 1992). The starch concentration of pea-cereal, often affected by stage of maturity, has frequently assessed in silage, but it is under-studied in pea-cereal greenfeed production. The starch concentration ranged from 109-123 g kg<sup>-1</sup>, and from 83-148 g kg<sup>-1</sup> in intercrops of pea with wheat at 75:25 and 25:75 seeding ratios, respectively, when were harvested at the milk to the early dough stages of wheat (Adesogan et al. 2002). The starch concentration of pea-wheat silage increased from 66 to 162 g kg<sup>-1</sup> with maturity at harvest (Salawu et al. 2002). At the early to soft dough stage, the starch concentration of pea-oat intercrop was at 199 g kg<sup>-1</sup> DM prior to ensiling (Rondahl et al. 2007).

Pea-cereal intercrops can enhance forage minerals compared to cereals as legumes tend to have greater concentrations of macro and micro minerals. Mineral supplementation is critical to maintaining animal health and productivity. In cereal greenfeed ration, calcium (Ca) supplementation is required to prevent milk fever risk in cows (Beef Cow Rations and Winter Feeding Guidelines 2016). Intercropping pea with barley and oat has almost doubled the forage Ca concentration than the respective cereal monocrops (Carr et al. 2004). Gill and Omokanye (2018) reported that pea intercropped with barley, triticale, and oat had adequate concentrations of Ca and Mg to meet the mineral requirements of lactating beef cows.

Pea-barley intercrops improved total digestible nutrients (TDN) as compared to corresponding cereal monocrops (Carr et al. 2004). The total digestible nutrient is the sum of the

digestible fiber, protein, lipid, and carbohydrate components of animal feed, which is related to the energy concentration of forage. The feed TDN values required by a beef cow is 55% at mid-pregnancy, 60% at late pregnancy, and 65% at after calving (National Research Council 2000). Monocrop pea has as high as 68% of TDN, which can increase the TDN of intercrops as compared to barley or oat monocrop (Kocer and Albayrak 2012). Intercrops of pea with barley and oat were considered as premium forage quality hay with a relative feed value (RFV) of 127-136 (65% pea: 35% cereal) and 120-123 (55% pea: 35% cereal), respectively (Kocer and Albayrak 2012).

**Table 2.1** Concentrations of crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) of pea-barley and pea-oat intercrops in various studies.

Intercrop	Seeding ratio (pea: cereal %)	Seeding rate (pea: cereal)	N fertilizer	CP	ADF	NDF	Source
				g kg <sup>-1</sup>			
Pea-barley	- <sup>a</sup>	43 and 65 seeds m <sup>-2</sup>	+N <sup>b</sup>	106-193	-	-	Chen et al. 2004
	-	120, 160 and 28 kg ha <sup>-1</sup>	-N	137-149	-	-	Jedel and Helm 1993
	-	75 and 210 seeds m <sup>-2</sup>	-N	127	269	418	Strydhorst et al. 2008
	-	108 and 215 seeds m <sup>-1</sup>	+N	162	316	524	Chapko et al. 1991
	1:1	Total of 120 kg ha <sup>-1</sup>	-N	122-129	320-349	518-537	Mustafa and Seguin 2004
	25:75, 50:50 and 75:25	108 and 108 kg ha <sup>-1</sup>	+N	141-199	-	-	Johnston et al. 1978
	25:75 and 50:50	180 kg ha <sup>-1</sup>	+N	63-80	-	-	Ayub et al 2008
	50:50	148 and 296 seeds m <sup>-2</sup>	-N	135	344	508	Carr et al. 2004
	55:45 and 65:35	120 and 200 kg ha <sup>-1</sup>	+N	150-151	289-302	487-495	Kocer and Albayrak 2012
	60:40	130 and 150 kg ha <sup>-1</sup>	+N	111	-	-	Dordas et al. 2012
	75:50	80 and 250 seeds m <sup>-2</sup>	+N	114-125	296-379	429-521	Gill and Omokanye 2018
	80:20	130 and 150 kg ha <sup>-1</sup>	+N	130	-	-	Dordas et al. 2012
	100:25	75 and 300 seeds m <sup>-2</sup>	+N	140-184	308-387	490-681	Aasen et al. 2004
	1:1	Total of 120 kg ha <sup>-1</sup>	-N	112-122	307-338	495-535	Mustafa and Seguin 2004
	-	108 and 215 seeds m <sup>-2</sup>	+N	179	299	457	Chapko et al. 1991
Pea-oat	-	120, 160 and 30 kg ha <sup>-1</sup>	-N	111-127	-	-	Jedel and Helm 1993
	25:75, 50:50 and 75:25	108 and 215 kg ha <sup>-1</sup>	+N	141-186	-	-	Johnston et al. 1978
	-	187, 146 and 165 kg ha <sup>-1</sup>	+N	94-120	310-340	560-590	Begna et al. 2011
	25:75 and 50:75	n/a	-N	119-128	-	-	Walton 1975
	50:50	148 and 296 seeds m <sup>-2</sup>	-N	100	365	552	Carr et al. 2004
	55:45 and 65:35	120 and 200 kg ha <sup>-1</sup>	+N	140-153	285-306	472-505	Kocer and Albayrak 2012
	60:40 and 80:20	130 and 150 kg ha <sup>-1</sup>	+N	115-132	-	-	Dordas et al. 2012
	75:50	80 and 250 seeds m <sup>-2</sup>	+N	97-123	319-429	489-574	Gill and Omokanye 2018
	100:25	75 and 300 seeds m <sup>-2</sup>	+N	129-170	296-400	440-683	Aasen et al. 2004
	80:20	91 and 192 seeds m <sup>-2</sup>	-N	72-108	-	-	Tsialtas et al. 2018

<sup>a</sup> Information not available.

<sup>b</sup> With N fertilizer (+N) and without N fertilizer (-N).

## **2.2 Annual forage species and cultivars**

Species selection is important for the success of intercropping. Therefore component crops should have complementary effects in terms of resource acquisition, growth patterns, and nutrient requirement during a growing season (Jensen 1996).

### **2.2.1 Forage pea (*Pisum sativum* L.)**

Pea is a cool-season legume crop widely adapted across the agro-ecological zones in Saskatchewan (Saskatchewan Pulse Growers 2016). Among annual legumes, the pea is ranked as one of the highest yielding legumes in southern Alberta and northeast Saskatchewan (Fraser et al. 2004). It is highly palatable and nutrient-rich feed for animal, with an estimated DM yield of 89% of corn (*Zea mays*) (Anderson et al. 2014). The CP concentration of pea biomass ranges from 160-260 g kg<sup>-1</sup> DM. In western Canada, pea cultivars of Trapper and 40-10 are two popular old pea cultivars used for forage production. Trapper and 40-10 are late maturing, normal leaf type cultivars, which are prone to lodging, and are susceptible to powdery mildew (*Erysiphe polygoni*). As compared to Trapper, and 40-10, semi-leafless cultivars have smaller seed size, better seed yield, higher lodging resistant, and resistant to powdery mildew (Warkentin et al. 2009 and 2012). The competitiveness differed considerably among forage pea cultivars with different leaf types (Warkentin et al. 2012). For example, normal leaf type cv. 40-10 pea has long vines and three times better weed suppression in comparison to semi-leafless cultivars (Spies et al. 2011). Gill and Omokanye (2018) found that pea-cereal intercrops containing 40-10 pea had greater DM yield than intercrops with semi-leafless pea cultivars such as CDC Tucker and Cooper. In comparison to old pea cultivars, CDC Tucker, CDC Leroy, and CDC Horizon, all semi-leafless type, have lower ADF and NDF concentrations, ranging from 320-350 and 400-440 g kg<sup>-1</sup> DM, respectively.



In a multiple location trial, mean forage DM yield was the highest in CDC Horizon pea, averaged at 11.8 t ha<sup>-1</sup> (Warkentin et al. 2012).

Soto-Navarro et al. (2015) reported that field pea supplementation to medium quality grass hay has increased total feed intake in gestating crossbred beef cows. Salawu et al. (2002) found total DM intake was significantly higher for pea-wheat silage fed by dairy cows compared to the grass silage. The corn silages substituted with pea-wheat-Italian ryegrass silage cut at the pea pod setting resulted in higher organic matter intake in dairy cows compared to corn silage alone (Urbanski and Brzusk 1996).

### **2.2.2 Forage barley (*Hordeum vulgare* L.)**

Barley is widely grown for malting, food, and animal feed in Canada. Total seeded area of barley in Canada was 2.6 million ha in 2018, with 1.25 million ha in Alberta and 1.1 million ha in Saskatchewan (Statistics Canada 2018). In Saskatchewan, barley has been the most popular spring cereal crop seeded for swath grazing, greenfeed, and silage in all soil zones. Barley has higher CP concentration than corn, oat, and triticale (McCartney et al. 2004), and energy concentration of barley is slightly lower than corn (Khorasani et al. 1997). Steers fed barley-based finishing diets had similar carcass quality and yield grades to corn fed steers (Boles et al. 2014). Forage type barley generally has higher biomass yield than grain type of barley cultivars. Two-row barley tends to have, less hull and fiber concentration, improved digestion, resistant to diseases (Elfson 2011), and have a higher yield than the majority of six-row barley cultivars (Gill et al. 2013).

### **2.2.3 Forage oat (*Avena sativa* L.)**

Oat is widely cultivated for human consumption and is also commonly used as feed and forage for livestock. The majority of oat cultivars have selected for high grain yield. In recent

years, a number of forage type of oat cultivars, such as CDC Baler and CDC Haymaker, have been widely used for forage production in western Canada due to its improved forage biomass yield and forage quality. Long-wide leaves and long panicles are distinctive morphological traits of CDC Haymaker oat. In cultivar evaluation trial in Saskatchewan, CDC Haymaker has exhibited good lodging resistance, moderately resistant to smut (*Ustilago avenae*), but it is prone to stem rust (*Puccinia graminis*) and crown rust (*Puccinia coronata*) diseases. The forage DM yield of CDC Haymaker was 8% higher than that of CDC Baler, averaged at 7.5 t ha<sup>-1</sup> (Secan 2016). It contains 340 g kg<sup>-1</sup> DM of ADF, 550 g kg<sup>-1</sup> DM of NDF, and 80 g kg<sup>-1</sup> DM of CP concentrations.

## **2.3 Agronomy and management**

### **2.3.1 Seeding rate and species ratio**

According to the Saskatchewan Ministry of Agriculture report (2016), current recommended seeding rates of annual forage species for forage production are shown in Table 2.2. However, the seeding rate for intercropping has not been well documented in various soil zones of Saskatchewan. A general recommendation for intercropping is full seeding rate of pea with 25-50% monocrop seeding rate of cereals for silage or greenfeed production (Saskatchewan Ministry of Agriculture 2016). The benefits of intercropping can be significant if the pea component crop takes up more than 50% of the harvested forage biomass (Johnston et al. 1998). However, the inclusion of high pea ratio can potentially affect total forage yield, production cost, ease of mechanical harvest, and drying time of greenfeed.

**Table 2.2** The recommended seeding rate of barley, oat, and pea in Saskatchewan, Canada.

Crop	No. of plant m <sup>-2</sup>	Seeding rate (kg ha <sup>-1</sup> )		
		Brown soil	Dark Brown soil	Black soil
Barley	210-250	99	109	123
Oat	215-320	99	110	148
Pea	85	154	123	197-200

An optimum pea to cereal ratio is important for increasing forage DM yield, and quality of the feed (Pozdíšek et al. 2010). High DM yield of annual intercrops primarily depends upon cereal species and its seeding ratio, whereas the increase of forage quality is determined mainly by legume species. In Turkey, Uzun and Aşık (2012) found that pea to oat seeding ratio of 25:75 produced 1.8-6.5 t ha<sup>-1</sup> greater forage DM yield than a pea to oat ratios of 75:25 and 50:50. Similarly, Kocer and Albayrak (2012) found that forage DM yield decreased with the increase of pea component from 55 to 65% in pea-oat or pea-barley intercrops. In a greenhouse study, Ayub et al. (2008) reported that optimum species ratio for pea-barley intercrop was 50:50 (of sole crop seeding rate) based on forage DM yield and quality with additional 50 kg N ha<sup>-1</sup> fertilizer. In central Alberta, Canada pea-oat intercropped at 25:75 ratio produced less forage DM yield compared to oat monocrop, but it contained greater CP concentration (Walton 1975). In northern Greece, Dordas et al. (2012) found a slight increase of DM yield when pea component increased from 60% to 80% of sole crop seeding rate in pea-oat intercrop. However, Izaurralde (1992) did not find yield advantage when pea component increased from 30% to 100% in pea-barley intercrops in Alberta, Canada.

### 2.3.2 Optimum growth stage for greenfeed production

Crop stage of maturity is vital to optimizing yield and nutritive value of the feed. In western Canada, it is generally recommended to harvest forage barley at the early to the mid-dough stage, and oat at the late milk stage for greenfeed and silage production. In legume-cereal intercrops,

harvesting time is based on the growth stages of cereals, as cereals in general mature earlier than legumes (Johnston et al. 1998). Forage DM of cereal increased by 13-40% with the advance of growth stage from booting to soft dough stage (Khorasani et al. 1997). Salawu et al. (2002) recommended the optimum stage for harvesting pea-wheat intercropping was at the soft dough stage of wheat or at yellow wrinkle pod stage of peas. Begna et al. (2011) revealed that forage DM yield and CP concentration in pea-oat intercrop balanced at a flat pod of pea or anthesis stage of oat. As expected, ADF and NDF concentrations increased with the plant growth stage, which would reduce the digestibility of the harvested forage crops (Khorasani et al. 1997). However, in a recent study, it was recommended to delay harvest stages of oat from the late milk to soft dough stage, and of barley from soft dough to hard dough stage, respectively (Rosser et al. 2013). The authors suggested that it increased the forage DM yield, but it did not negatively affect forage quality, except for a slight reduction in CP.

### **2.3.3 Plant lodging**

Plant lodging has been a concern for seeding pea for forage production because it can reduce total forage DM yield and make harvesting difficult. After studying correlations between lodging and incidence of disease in 20 pea cultivars, Banniza et al. (2005) found that the cultivars prone to lodging were susceptible to disease infection and tended to have a low lignin and cellulose concentration in the stems. Jensen (1986) reported lodging after pod filling significantly reduced N assimilation rate in pea. Inclusion of a small portion of cereals with pea can improve lodging resistance of pea, because upright growth of cereals provides physical support to pea (Cowell et al. 1989; Kontturi et al. 2011). Podgórska-Lesiak and Sobkowicz (2013) reported that pea cultivars with conventional leaves and indeterminate growth benefited more from intercropping with barley

at full maturity than the medium-tall pea cultivars. Other researchers showed that conventional-leaf type pea cultivars were prone to lodging in monocrop system (Uzun and Açıkgöz 1998).

#### **2.3.4 Drying rate of greenfeed**

The moisture content of forage is critical to minimize loss of dry matter and nutrient during baling and storing hay. The initial moisture content of fresh forage is normally around 75-85% of DM (Collins and Owens 2003), but safe moisture content for baling is below 18% (of DM) for greenfeed. If hay is baled >20% moisture contents heat generates from plant tissue respiration combined with bacteria and mold growth. The plant respiration converts plant carbohydrates into water and carbon dioxide, increased ADF and NDF concentrations while decreases the net energy content of the hay (Vough n.d). Excessive heat damage, undesirable changes in vitro DM, and NDF concentration occurred in alfalfa hay stored at higher than 20% moisture content (Collins et al. 1987). Drying rate is affected by both weather conditions, growth stage, crop choice, and agronomic practices (Rotz 1993). If the relative humidity is high, and the level of solar radiation and temperature are low, drying rate will be slow. The moisture content of harvested forage can decrease to 20% within 3 to 5 days under favorable weather conditions. Pea often contains 5-10% higher moisture content than cereals at harvest, therefore a high proportion of pea in intercrops can require extensive drying time (Johnston et al. 1998).

#### **2.4 Biological nitrogen fixation of pea**

Pea fixes atmospheric N<sub>2</sub> through a symbiotic association with *Rhizobium leguminosarum* bv. viciae bacteria. Among the major legume crops, pea has stable BNF capability even under relatively dry conditions, fixing 69 kg ha<sup>-1</sup> N in the semi-arid Canadian Prairie (Hossain et al. 2017). Biological N<sub>2</sub> fixation is most likely to occur at the flowering stage of pea (Jensen 1987).

Intercropping pea and barley regulates the internal N cycle through BNF and can reduce the amount of N fertilizer required for crop growth (Hauggaard-Nielsen et al. 2001). Intercropping pea with cereal triggers more BNF due to interspecific competition between the legume and cereal component (Marufu et al. 2007). Izaurralde et al. (1992) reported increased total N, greater N<sub>2</sub> fixation efficiency, and more N mineralization of root and shoot residues for subsequent crops when forage pea and barley were intercropped. The amount of N<sub>2</sub> fixed by pea in pea-cereal intercrops depended upon species, soil type, climatic conditions, and agronomic factors including crop management and competitive abilities of the component crops (Ofori and Stern 1987). Among different type of peas, determinate-growth type pea cultivars fixed more N than the tall, indeterminate types (Jensen 1986), and normal leaf type pea cultivars have potential to release more N to the soil than semi-leafless cultivars (Lupwayi and Soon 2009). After testing three different inoculant formulations of *Rhizobium* strain with or without N fertilizer, Clayton et al. (2004) found pea crop inoculated with granular soil-inoculants without N fertilizer produced greater root nodule numbers and nodule mass, resulting the high percentage of N derived from atmosphere (%Ndfa). The quantity of BNF by uninoculated peas ranged from 31-107 kg N ha<sup>-1</sup> under normal growth condition, and from 4-37 kg N ha<sup>-1</sup> under drought stress (Carranca et al. 1999). Pea fixed 16, 36, and 61% of its plant N at soil pH levels of 4.4, 5.4 and 6.6, respectively (Rice et al. 2000). Biological N<sub>2</sub> fixation of pea grown with cereals may be stimulated, presumably through competition for nitrate or ammonium from cereals in the rhizosphere (Neumann et al. 2007). Biological N<sub>2</sub> fixation in pea can be inhibited by drought stress (Carranca et al. 1999), the supply of extra N fertilizer (Jensen 1986 and 1987) and repetitive cultivation of pea crops in the same land (Knight 2012). Starter N fertilizer may stimulate pea root development (Jensen 1986), in turn, increased forage DM yield and total N accumulation (Sosulski and Buchan 1978).

However, pea cultivars tend to be more sensitive to the presence of a high concentration of soil nitrate and resulting in a significant decrease of BNF (Chalifour and Nelson 1988).

## **Chapter 3. Effects of seeding ratio and N fertilizer on agronomic performance of pea-cereal intercrops for greenfeed production**

### **3.1 Abstract**

Forage pea (*Pisum sativum* L.) intercropping with barley (*Hordeum vulgare* L.) or oat (*Avena sativa* L.) has the potential to increase forage productivity and reduce N fertilizer input. The objective of the study was to determine forage dry matter (DM) yield, botanical composition, lodging resistance, drying time, land equivalent ratio (LER) and competitive ratio of pea-barley and pea-oat intercrops seeded at two different seeding ratios of 100:30 and 50:50 in comparison to pea, barley and oat monocrops with and without N fertilizer. The field experiment was conducted during the grows season of 2016 and 2017 at Swift Current (Brown soil zone), at Saskatoon (Dark Brown soil zone) and Melfort (Black soil zone), Saskatchewan. Pea-cereal intercrops significantly ( $P<0.001$ ) increased forage DM yield than pea monocrops, but DM yield of intercrops were similar or slightly less than barley and oat monocrops. On average, barley and oat accounted for 89% (50:50%) and 74% (100:30%) of the total DM yield of pea-cereal intercrops. Pea lodging reduced significantly ( $P<0.001$ ) in pea-cereal compared to the monocrop peas. The land equivalent ratio (LER) value ranged from 1.03-1.18 for the pea-cereal intercrops, which was significantly ( $P=0.0002$ ) higher than the LER of monocrops. Partial LER value and competitive ratio indicated that the cereal component was highly dominant in the intercrops in our study. Plant height of ‘CDC Horizon’ and ‘40-10’ peas in intercrops was 12 and 17 cm shorter than in their pea monocrops, respectively. The drying time of monocrops and intercrops in windrow was faster ( $P<0.001$ ) in 2017 (mean=4 days) than in 2016 (mean=13 days). The application of N fertilizer increased forage DM yield ( $P=0.0003$ ) at Melfort site, but it did not affect DM yield at Saskatoon and Swift Current



sites. The pea-barley intercrops had similar net return to the barley monocrop, which was higher than pea monocrops.

### **3.2 Introduction**

Intercropping pea with forage oat or barley can be alternative cropping to increase forage DM yield and protein concentration in comparison to the monocrop system. The inclusion of forage pea in intercrops with forage barley or oat for greenfeed and silage production is gaining interest in western Canada. Legumes in intercrops with cereals can reduce the cost of N fertilizer through biological N<sub>2</sub> fixation (BNF) and increase protein concentration of the feed when cereals become the main source for digestible energy (Lithourgidis et al. 2011). However, agronomic factors such as seeding ratio, cultivar choice, crop species, competition, and application of N fertilizer can affect forage DM yield. Forage pea has a larger seed size than cereals, which likely increases establishment cost for pea-cereal intercrops. In addition, a high percentage of forage pea in intercrop may require an extended drying period before reaching optimum moisture for baling the crop for greenfeed. Among cereals, oat tends to produce high forage DM yield in the moist, Black soil zone, when barley has high forage yield in the Dark Brown and the Brown soil zones of western Canada (Walton 1975).

As a common agronomic practice, addition of N fertilizer application may stimulate pea root development (Jensen 1986), in turn, it may increase forage DM yield and total N accumulation (Sosulski and Buchan 1978). However, pea is sensitive to the presence of a high concentration of soil nitrate and resulting in a significant decrease of N<sub>2</sub> fixation rate (Chalifour and Nelson 1988). Several studies have reported that an increase in total DM yield and protein concentration in pea-cereal intercropping with addition of N fertilizer (Chen et al. 2004; Sahota and Malhi 2012). Nitrogen fertilizer reduces species complementarity and induces interspecific competition among

species in the legume-cereal intercropping (Jensen 1996), in particular, increasing the cereals in intercrop (Ghaley et al. 2005). The land equivalent ratio (LER) of pea-cereal tends to be higher under the low rate of N fertilizer (Ofori and Stern 1987). The application of N fertilizer greater than 40 kg N ha<sup>-1</sup> can reduce the number of effective nodules (Clayton et al. 2004), and too high N fertilizer increases the risk of crop lodging (Podgórska-Lesiak and Sobkowicz 2013).

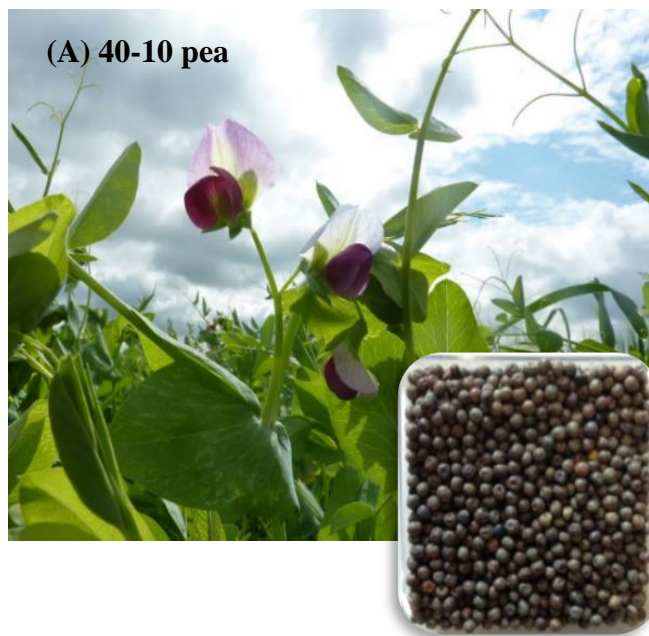
The aim of this study was to 1) evaluate the agronomic performance of forage barley, oat, and pea in monocrops and their binary pea-cereal intercrops with and without N fertilizer in the three different soil zones of Saskatchewan, Canada; 2) calculate economic return of pea-cereal intercrops for greenfeed production in comparison to forage barley or oat monocrops.

### **3.3 Materials and Methodology**

#### **3.3.1 Plant materials**

Forage barley cv. CDC Maverick, forage oat cv. CDC Haymaker, and forage pea cvs. CDC Horizon and 40-10 were used in this study (Figure 1. A-D). CDC Maverick is a smooth-awned, two-row barley cultivar developed for forage production at the Crop Development Centre, University of Saskatchewan (Secan 2015). It is ideally suited for swath grazing, bale grazing, and silage production. The smooth-awn characteristics of barley can reduce the risk of feed sticking in the mouth and throat of livestock. CDC Haymaker was selected for improved forage DM yield with better forage nutritive value. In addition, low lignin in the hull, long-wide leaves, and long panicles are distinctive morphological traits of CDC Haymaker. Pea cultivar 40-10 is widely grown for forage production in western Canada. It is a conventional leaf type cultivar with high biomass yield potential. Forage pea cv. CDC Horizon was developed at the Crop Development

Centre, University of Saskatchewan (Warkentin et al. 2012). CDC Horizon is a semi-leafless leaf type pea with high forage and grain yield, and good resistance to lodging and powdery mildew.



**Figure 3.1** Crop species and cultivars used in the study.

### 3.3.2 Study sites and experimental design

A 2-year (2016-2017) field experiment was conducted at the Agriculture and Agri-Food Canada Research Centres at Saskatoon (52° 07' N, 106° 38' W), Melfort (52° 81' N, 104° 60' W), and Swift Current (50° 28' N, 107° 75' W), Saskatchewan, Canada. Melfort research site is located in the Black soil zone, Saskatoon and Swift Current sites are in the Dark Brown and Brown soil zones of Saskatchewan, Canada, respectively. The soil type is classified as a silt clay (Orthic Thick Black Chernozem) at Melfort (Campbell et al. 1991), Swinton clay loam (Orthic Brown Chernozem soil) at Swift Current (Li et al. 2014) and a Sutherland clay loam (Dark Brown Chernozem soil) at Saskatoon site (Acton and Ellis 1978). The experimental plots were assigned at two different locations in 2016 and 2017 at each experimental sites. The experimental plots were assigned at two different locations in 2016 and 2017 at each experimental sites. At each site, field plots were in a split-plot arrangement in a Randomized Complete Block Design (RCBD) with four replications. The plot size was 2 × 6 m (each plot area 12 m<sup>2</sup>) with four rows at each experimental sites. The row spacing was 30 cm for all sites. Nitrogen fertilizer treatment (with and without N fertilizer addition) was considered as the main plot factor, and forage intercrops in different seeding ratios (100% pea: 30% cereal and 50% pea: 50% cereal) was considered as a sub-plot factor within the main plot. The seeding rates for monocrop pea cultivars, CDC Horizon and 40-10, were 123 and 116 kg ha<sup>-1</sup>, and for monocrops of barley and oat were 159 and 119 kg ha<sup>-1</sup>, respectively (Table 3.1). Prior to seeding, composite soil samples were taken from the three sites at a depth of 0-30 cm in 2016 and 2017 to determine initial soil N, P, and K levels (Table 3.2). The samples were analyzed in the Australian Laboratory Services (ALS) laboratory in Saskatoon, SK. For N fertilizer treatment, to increase the soil N content to 60 kg ha<sup>-1</sup> each site addition of 35, 33

and 25 kg N ha<sup>-1</sup> ammonium nitrate (34-0-0 of NH<sub>4</sub>NO<sub>3</sub>) fertilizer was applied at the time of seeding in 2016 at Melfort, Saskatoon and Swift Current sites, respectively.

**Table 3.1** Seeding rate and component crop seeding ratio of pea-cereal intercrops at Melfort, Saskatoon and Swift Current, SK during the summers of 2016 and 2017.

Treatment	Component crop seeding ratio <sup>a</sup> (%)	Seeding rate (kg ha <sup>-1</sup> )	Targeted number of plant m <sup>-2</sup>
CDC Horizon pea	-	123	86
40-10 pea	-	116	86
Barley	-	159	268
Oat	-	119	268
CDC Horizon: barley	100:30	123:48	86:80
CDC Horizon: barley	50:50	62:80	43:134
CDC Horizon: oat	100:30	123:36	86:80
CDC Horizon: oat	50:50	62:60	43:134
40-10: barley	100:30	116:48	86:80
40-10: barley	50:50	58:80	43:134
40-10: oat	100:30	116:36	86:80
40-10: oat	50:50	58:60	43:134

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

In 2017, again based on soil test result addition of 38, 33 and 23 kg N ha<sup>-1</sup> fertilizer were applied to achieve 60 kg ha<sup>-1</sup> inorganic N in soil soil at Melfort, Saskatoon and Swift Current sites, respectively. Four rows of forage barley were seeded as a buffer zone between the two N treatments to prevent N transfer from adjacent plots at the Melfort and Saskatoon sites. A single row of flax (*Linum usitatissimum*) was seeded at Swift Current site as a buffer. Nitrogen fertilizer was side banded at seeding at Saskatoon site, but it was broadcasted by plots at Melfort and Swift Current sites. The monocrops and pea-cereal intercrops were seeded with Hege 75 plot seeder (Hege Company, Waldenburg, DE) within the same row at each site. At the time of seeding, forage pea seeds were inoculated at the rate of 3 kg ha<sup>-1</sup> using solid core granular inoculant (Nodulator<sup>®</sup> XL SCG) containing 8 x 10<sup>7</sup> living rhizobium strain 1435 (*Rhizobium leguminosarum* bv. viceae) per gram.

**Table 3.2** Soil properties at the 0-30 cm soil profile prior to seeding at three experimental sites.

Year	Site	Texture	pH	EC (mS cm <sup>-1</sup> )	Total inorganic N (kg ha <sup>-1</sup> )	Available P (kg ha <sup>-1</sup> )	Available K (kg ha <sup>-1</sup> )
2016	Melfort	silt clay	6.3	0.2	25	50	673
	Saskatoon	clay loam	8.3	0.2	27	49	1166
	Swift Current	clay loam	6.7	0.1	35	59	929
2017	Melfort	silt clay	6.4	0.2	22	27	1348
	Saskatoon	clay loam	-	-	27	-	-
	Swift Current	clay loam	-	-	37	37	592

### 3.3.3 Seeding and harvesting dates

Seeding and harvesting dates are shown in Table 3.3. The preceding crop was wheat crop in both years at Saskatoon site, while pea and wheat crops were grown at Melfort in 2016 and 2017, respectively. At Swift Current, plots were seeded in summer fallow in 2016 and in corn residue in 2017. In 2017, plots at Saskatoon site were uniformly irrigated to attain the maximum soil moisture due to a drier than normal growth conditions following seeding. Weeds were controlled by spraying glyphosate herbicide (glyphosate 48.8% by weight) at rate of 1.1 kg ha<sup>-1</sup> before seeding at Melfort and Swift Current sites, but all plots were hand weeded using a wheel hoe at Saskatoon site. Harvesting dates for pea-cereal intercrops were determined on the basis of recommended harvesting stages of cereals for greenfeed production. Barley monocrop and pea-barley intercrops were harvested at the hard dough stage of barley, and monocrops of pea and oat, and intercrops of pea-oat were harvested at the soft dough stage of oat. Peas in the monocrop and intercrops were from the flat pod to full pod stages. At Melfort, due to a prolonged period of rainfall in 2016, forage crops were harvested at the beginning of ripening stage of barley, and at the hard dough stage of oat. At Swift Current, one of four replications were not harvested in 2016 due to gopher damage.

**Table 3.3** Seeding and harvesting dates of the field plots at the three experimental sites in 2016 and 2017.

Site and year	Seeding date	Harvesting date	Days after sowing
Melfort			
2016	20 May	17 Aug	92
2017	23 May	14 Aug	83
Saskatoon			
2016	16 May	27 Jul/ 12 Aug <sup>a</sup>	72/88
2017	23 May	10 Aug/ 17 Aug <sup>b</sup>	79/86
Swift Current			
2016	5 May	2 Aug	88
2017	11 May	26 July	76

<sup>a</sup> In 2016, barley intercrops were harvested on July 27, and oat intercrops were harvested on August 12.

<sup>b</sup> In 2017, barley intercrops were harvested on Aug 10, and oat intercrops harvested on August 17.

### 3.4 Data collection

#### 3.4.1 Forage dry matter (DM) yield

Field plots were harvested to a stubble height of 5 cm using a WinterSteiger-forage harvester CiBus F (WinterSteiger, Salt Lake, UT) to determine total fresh forage biomass. At harvest, a handful of sub-sample was taken from each plot in paper bags, weighed fresh and dried at 60°C in a forced-air oven for 48 hrs for forage DM yield determination, which was calculated as follows:

$$DM_{\text{yield}} = \left( \frac{\text{Dry}_{\text{weight}} \times \text{Gross}_{\text{weight}}}{\text{Fresh}_{\text{weight}}} \right) \times \left( \frac{10000}{P} \right) \quad (1)$$

where  $\text{Fresh}_{\text{weight}}$  and  $\text{Dry}_{\text{weight}}$  are sample weights (kg) before and after oven drying;  $\text{Gross}_{\text{weight}}$  is total forage biomass per plot (kg);  $P$  is an experimental plot size (m); 10,000 is a conversion factor for square meter to hectare.



### 3.4.2 Botanical composition

Botanical composition was determined using a dry matter of pea and cereal components in intercrops. Prior to harvest, randomly selected two parallel 50 cm long rows were clipped to 5 cm stubble height from each plot, then the biomass was separated into pea and cereal components. Each component crop was dried at 60°C in a forced-air oven for dry matter determination. The percent composition of the two component crops was calculated using dry weight of the sample.

### 3.4.3 Competitive indices

To evaluate the competition between species in a pea-cereal intercrop, land equivalent ratio (LER) and competitive ratio (CR) were calculated.

#### Land Equivalent Ratio (LER)

The LER for pea-cereal intercrops was calculated according to Willey and Rao (1980):

$$\text{LER} = \text{Partial LER}_{\text{pea}} + \text{Partial LER}_{\text{cereal}} = \left( \frac{\text{Yield}_{\text{IP}}}{\text{Yield}_{\text{MP}}} \right) + \left( \frac{\text{Yield}_{\text{IC}}}{\text{Yield}_{\text{MC}}} \right) \quad (2)$$

where  $\text{Yield}_{\text{MP}}$  is DM yield of pea monocrop, and  $\text{Yield}_{\text{MC}}$  is DM yield of cereal monocrop;  $\text{Yield}_{\text{IP}}$  is DM yield of a pea in intercrop and  $\text{Yield}_{\text{IC}}$  is DM yield of cereal in intercrop. An LER value greater than 1.0 indicates yield advantage for intercrop in terms of growth resource utilization or presence of positive interference among the crop components, and value less than 1.0 indicates negative intraspecific interference compared to monocrops. A partial LER greater than 0.5 indicates a greater yield advantage of individual crops or dominance of individual crop in intercropping.

### Competitive ratio (CR)

Competitive ratio (CR) is used as an indicator to evaluate the competitive ability of different species in intercrop (Willey and Rao 1980) and was expressed as follows:

$$CR_{\text{cereal}} = \left( \frac{\text{Partial LER}_{\text{cereal}}}{\text{Partial LER}_{\text{pea}}} \right) \times \left( \frac{Z_{\text{PR}}}{Z_{\text{CR}}} \right) \quad \text{or} \quad CR_{\text{pea}} = \left( \frac{\text{Partial LER}_{\text{pea}}}{\text{Partial LER}_{\text{cereal}}} \right) \times \left( \frac{Z_{\text{CR}}}{Z_{\text{PR}}} \right) \quad (3)$$

where  $CR_{\text{cereal}}$  is a competitive ratio of cereal relative to pea. If  $CR_{\text{cereal}} > 1.0$ , cereal is more competitive than a pea, if  $CR_{\text{cereal}} < 1.0$ , then cereal is less competitive than a pea in intercrop.  $LER_{\text{cereal}}$  and  $LER_{\text{pea}}$  are the individual LER values for cereal and pea, respectively, and  $Z_{\text{PR}}$  and  $Z_{\text{RP}}$  are the sown proportions of cereal and pea in the intercrop.

#### 3.4.4 Plant lodging score and plant height

Plant lodging was visually scored by each plot prior to forage harvest, using a 9-point scale where 1= completely upright, and 9= completely lodged (Seeding and Variety Guide 2016). Prior to harvest, plant height (cm) of randomly selected one plant was measured for each species per plot using a meter stick in 2017. Plant height was not recorded in 2016.

#### 3.4.5 Drying time of greenfeed

Drying time of pea-cereal intercrop was determined in a field, and in a drying room after harvest in 2016 and 2017. In field, the harvested pea-cereal intercrops were laid in 1 m wide windrows to dry under natural environmental conditions at each site without racking or turning the rows. The moisture concentration of windrow was measured daily between 11:00 AM to 2:00 PM using an AgraTronix™ Windrow 07141 hay moisture probe (AgraTronix, Ohio, USA) for five consecutive days, followed by every other day until moisture concentration reaching 18% of a safe

baling level. For each measurement, three readings were taken, and then the values were averaged for each plot. A total number of days required for drying pea-cereal intercrop was then calculated. Additional sub-samples were taken from each plot at Saskatoon site and dried at 35°C constant temperature. The sub-samples were then weighed daily until the sample reaching constant weight. Moisture concentration of sub-samples was calculated from weight differences between the days.

### **3.4.6 Economic analysis**

A simple economic analysis was conducted to compare production cost and economic return of different pea-cereal intercrops at each experimental site. Production costs included the expenses of seed, fertilizer, chemical (herbicide and inoculant), machinery, and specialized labour (Table 3.4). Seed and chemical costs were based on actual rates applied at each site. The cost of certified seeds was \$0.62 kg<sup>-1</sup> for forage pea (Van Burck Seed Ltd, SK), and \$0.42 kg<sup>-1</sup> for forage barley and \$0.41 kg<sup>-1</sup> for forage oat (Ardell Seeds Ltd, SK). Pea inoculant, Nodulator<sup>®</sup> XL SCG (BASF), was \$9.7 kg<sup>-1</sup> and N fertilizer was \$0.41 kg<sup>-1</sup> (Crop planning guide 2017). Herbicide Roundup WeatherMAX<sup>®</sup> (glyphosate 48.8% by weight) was \$7.4 L<sup>-1</sup>.

**Table 3.4** Summary of selected input cost used for economic analysis.

Input items	Cost	Units
<i>Seed cost</i>		
- Forage peas	0.62	\$ kg <sup>-1</sup>
- Forage barley	0.42	\$ kg <sup>-1</sup>
- Forage oat	0.40	\$ kg <sup>-1</sup>
<i>Fertilizer</i>		
- Ammonium nitrate	0.41	\$ kg <sup>-1</sup>
<i>Chemical</i>		
- Glyphosate	7.40	\$ L <sup>-1</sup>
- Inoculant	9.70	\$ kg <sup>-1</sup>
<i>Machinery operating costs</i>		
- Zero till seeder	37.1	\$ ha <sup>-1</sup>
- Fertilizer application	21.0	\$ ha <sup>-1</sup>
- Spraying	17.3	\$ ha <sup>-1</sup>
- Swathing	19.3	\$ ha <sup>-1</sup>
- Rake	14.8	\$ ha <sup>-1</sup>
- Bale wrap and bailing	38.4	\$ ha <sup>-1</sup>
- Trucking and hauling	15.9	\$ ha <sup>-1</sup>
<i>Custom work and hired labour</i>	43.0	\$ h <sup>-1</sup>

Other variable production costs, such as hired labour and machinery cost (fuel and repair) were based on the average custom rate for 2017 (Crop planning guide 2017). The average market price in 2018 for feed barley, oat and pea were \$192.7, \$168.9 and \$228.2 t<sup>-1</sup> (\$6.21 per bushel), respectively, in Saskatchewan (Source: Saskatchewan Ministry of Agriculture). The economic return was calculated using the following equation adapted from O'Donovan et al. (2001):

$$N = (Y_P P_P + Y_C P_C) - V \text{ or } \text{Economic return} = \text{Total revenue} - \text{Total input} \quad (4)$$

where N is the economic return (\$ ha<sup>-1</sup>), Y<sub>P</sub> is DM yield of pea (t ha<sup>-1</sup>), P<sub>P</sub> is the price of feed pea (\$ t<sup>-1</sup>), Y<sub>C</sub> is DM yield of cereal (t ha<sup>-1</sup>), P<sub>C</sub> is the price of feed cereal (\$ t<sup>-1</sup>), and V is the total production input cost.

### 3.5 Statistical analysis

Data were analyzed as a split-plot arrangement in RCBD using Proc Mixed Model of SAS 9.4 (SAS Institute, Inc. Cary, NC). In Proc Mixed Model, experimental site, N fertilizer treatment, intercrop, and their interactions were treated as fixed effects, when year, replication and replication  $\times$  fertilizer interaction were treated as random effects. Due to the significant site  $\times$  fertilizer and site  $\times$  intercrop analysis of variance (ANOVA) for forage dry matter (DM) yield and economic analysis was performed by each site. In other cases, data were combined unless the effects of the interaction were significant. If the treatment effect was significant at  $P \leq 0.05$ , means were separated by Tukey's Honestly Significant Difference (HSD) test in SAS 9.4 (SAS Institute, Inc. Cary, NC). Analyses of pre-planned orthogonal contrasts were performed to determine significance in the comparison of different groups of treatments.

## **3.6 Results**

### **3.6.1 Climatic conditions**

In 2016, total rainfall between May to August was 124%, 115% and 110% of the long-term average (1981-2010) of 226, 212 and 236 mm in Melfort, Saskatoon, and Swift Current sites, respectively (Table 3.5). At the beginning of the growing season in 2016, it was relatively dry and warm at Melfort site, but more rain accumulated in later in the season. Rainfall was distributed evenly throughout summer 2016 at Saskatoon site. At Swift Current site, monthly rainfall in 2016 was below the long-term average with the exception of two times high rainfall amount in May. Besides, in May 2016, the mean monthly air temperatures were similar to the long-term values at the three sites. In general, it was a favorable condition for plant growth in 2016. In contrast to 2016 and long-term average, the growing season in 2017 was drier and warmer, especially at Swift Current site. Monthly rainfall between May to August was 56%, 76% and 36% of the long-term average in Melfort, Saskatoon and Swift current, respectively in 2017. The monthly mean air temperatures between May to August ranged from 11-19 °C, 12-20°C, and 12-21°C at Melfort, Saskatoon, and Swift Current, respectively.

**Table 3.5** Monthly rainfall and mean monthly air temperature during the growing seasons of 2016 and 2017 at Melfort, Saskatoon, and Swift Current, SK.

Site	Month	Mean Temperature (°C)			Rainfall (mm)		
		2016	2017	Long-term avg. <sup>a</sup>	2016	2017	Long-term avg.
Melfort (ID 4055079) <sup>b</sup>	May	13.6	10.8	10.7	16.8	46.4	42.9
	June	17.0	15.2	15.9	53.2	44.1	54.3
	July	18.1	18.6	17.5	128.7	33.3	76.7
	August	16.3	17.2	16.8	80.8	3.1	52.4
	Mean/Sum	16.3	15.5	15.2	279.5	126.9	226.3
Saskatoon (ID 1513)	May	13.5	11.9	11.2	49.6	55.0	43.0
	June	17.5	15.8	15.8	46.4	47.8	65.8
	July	18.4	19.7	18.5	66.6	28.2	60.3
	August	16.9	18.0	17.6	81.0	30.0	42.6
	Mean/Sum	16.6	16.4	15.8	243.6	161	211.7
Swift Current (ID 4028038)	May	13.1	12.3	10.9	109.9	21.0	51.2
	June	16.8	15.7	15.3	56.1	25.3	77.1
	July	17.6	20.6	18.2	51.0	11.0	60.1
	August	16.7	17.9	17.6	41.5	28.0	47.4
	Mean/Sum	16.1	16.6	15.8	258.5	85.3	235.8

<sup>a</sup> Long-term average from 1981 to 2010.

<sup>b</sup> The Climate ID is a unique identifier assigned by the Meteorological Service of Canada for each location having archived observations.

**Table 3.6** Analysis of variance (ANOVA) for agronomic traits of pea-cereal intercrops at Melfort, Saskatoon and Swift Current, SK in 2016 and 2017.

Source	df <sup>a</sup>	Dry matter yield	Botanical composition	Plant height		Plant lodging	Competitive indices		Drying time	Economic analysis	
				Pea	Cereal		LER	CR		Cost	Return
N fertilizer (F)	1	<0.001	NS	NS	0.032	0.024	0.028	NS	NS	<0.001	0.05
Intercrop (I)	11	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Site (S)	2	<0.001	<0.001	<0.001	<0.001	0.022	<0.001	<0.001	<0.001	NS	<0.001
F × I	11	NS	NS	NS	Ns	NS	NS	NS	NS	<0.001	NS
S × F	2	<0.001	NS	NS	0.036	NS	NS	0.023	NS	NS	<0.001
S × I	22	0.002	NS	<0.001	<0.001	<0.001	NS	NS	<0.001	NS	<0.001
S × F × I	22	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Year (Y)	1	N/a	N/a	N/a	N/a	N/a	N/a	N/a	<0.001	N/a	N/a
S × Y	2	N/a	N/a	N/a	N/a	N/a	N/a	N/a	<0.001	N/a	N/a

LER=land equivalent ratio; CR=competitive ratio. NS, non-significant. N/a, not available.

<sup>a</sup>Degree of freedom.



### 3.6.2 Forage dry matter (DM) yield

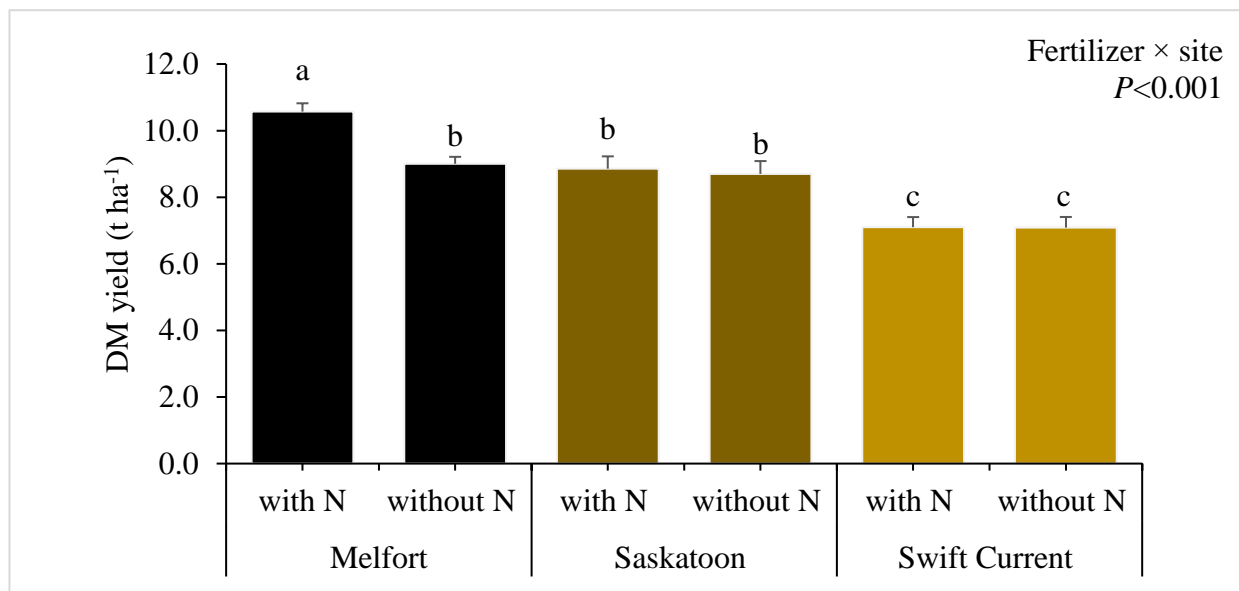
Forage DM yield was significantly affected by the site ( $P<0.001$ ), intercrop ( $P<0.001$ ), N fertilizer ( $P<0.001$ ), and the interactions of site  $\times$  N fertilizer ( $P<0.001$ ) and site  $\times$  intercrop ( $P=0.002$ ) treatments (Table 3.6). The interactions of N fertilizer  $\times$  intercrop and site  $\times$  N fertilizer  $\times$  intercrop had no significant effects on forage DM yield (Table 3.6).

As there were significant effects of site  $\times$  N fertilizer and site  $\times$  intercrop, DM yield data were further analyzed by the site (Table 3.7). Forage DM yield was significantly ( $P<0.001$ ) different among intercrops at all three sites. Nitrogen fertilizer ( $P=0.0003$ ) increased the DM yield only at Melfort site when there was no effect at Saskatoon ( $P=0.59$ ) and Swift Current ( $P=0.93$ ) sites (Table 3.7). Forage DM yield ranged from 4.7-10.6 t ha<sup>-1</sup> at Saskatoon, and from 4.4-8.7 t ha<sup>-1</sup> at Swift Current. At Melfort, forage DM yield was from 6.6-12.4 t ha<sup>-1</sup> and 6.0-10.2 t ha<sup>-1</sup>, respectively, with and without N fertilizer treatments (Table 3.7). Mean forage DM yield was the highest at Melfort (9.8 t ha<sup>-1</sup>), intermediate at Saskatoon (8.8 t ha<sup>-1</sup>), and the lowest at Swift Current (7.2 t ha<sup>-1</sup>).

Compared to the two pea monocrops, pea-cereal intercrops produced significantly greater forage DM yield at the all three sites, and the percent increase was 52-73% in Melfort, 68-118% in Saskatoon and 27-70% in Swift Current, respectively. Within the intercrops, forage DM yield was similar within the sites, except 40-10 pea: oat intercrop (100:30) at Saskatoon and Swift Current, and 40-10 pea: barley intercrop (100:30) at Swift Current had low forage DM yield.

Based on contrast comparison, pea-cereal intercrops seeded at 50:50 ratio produced significantly ( $P=0.0091$ ) higher DM yield (10.7 t ha<sup>-1</sup>) than those seeded at 100:30 ratio (9.9 t ha<sup>-1</sup>) at Melfort, but it was only numerically higher at Saskatoon (9.7 t ha<sup>-1</sup> and 9.4 t ha<sup>-1</sup>) and Swift

Current ( $7.4 \text{ t ha}^{-1}$  and  $6.8 \text{ t ha}^{-1}$ ) sites (Table 3.7). Regardless of seeding ratio, pea-barley intercrops yielded significantly ( $P<0.01$ ) higher ( $10.2 \text{ t ha}^{-1}$ ) than the pea-oat intercrops ( $8.8 \text{ t ha}^{-1}$ ) at Saskatoon site, it was similar at Swift Current ( $7.8$  and  $7.1 \text{ t ha}^{-1}$ ), and almost the same at Melfort ( $10.2$  and  $10.5 \text{ t ha}^{-1}$ ). Forage DM yield increased ( $P<0.001$ ) with the application of an extra N fertilizer at Melfort site, but it did not affect the DM yield at Saskatoon and Swift Current sites.



**Figure 3.2** Effect of N fertilizer on mean forage DM yield of pea-cereal intercrops averaged across years (2016 and 2017) at Melfort, Saskatoon, Swift Current, SK. Means with the same lower case letter were not significant at  $P>0.05$  based on Tukey's HSD test. Error bars are standard errors.

**Table 3.7** Two-year mean (2016-2017) forage dry matter (DM) yield ( $\text{t ha}^{-1}$ ) of forage pea, barley, oat in monocrops and in pea-cereal intercrops with (+N) and without (-N) N fertilizer at Melfort, Saskatoon and Swift Current, SK.

Treatment	Component crop ratio <sup>a</sup> (%)	Melfort		Saskatoon	Swift Current
		-N	+N		
CDC Horizon pea	100	6.3 <i>b</i>	6.8 <i>b</i>	5.2 <i>c</i>	5.7 <i>de</i>
40-10 pea	100	6.0 <i>b</i>	6.6 <i>b</i>	4.7 <i>c</i>	4.4 <i>e</i>
Barley	100	9.5 <i>a</i>	12.4 <i>a</i>	10.6 <i>ab</i>	8.7 <i>a</i>
Oat	100	10.2 <i>a</i>	12.1 <i>a</i>	8.4 <i>ab</i>	7.6 <i>abc</i>
CDC Horizon: barley	100:30	8.9 <i>a</i>	10.8 <i>a</i>	10.8 <i>a</i>	8.0 <i>ab</i>
CDC Horizon: barley	50:50	9.2 <i>a</i>	11.7 <i>a</i>	10.0 <i>ab</i>	8.6 <i>a</i>
CDC Horizon: oat	100:30	9.2 <i>a</i>	11.0 <i>a</i>	8.6 <i>ab</i>	7.3 <i>abc</i>
CDC Horizon: oat	50:50	10.1 <i>a</i>	11.3 <i>a</i>	9.3 <i>ab</i>	7.3 <i>abc</i>
40-10: barley	100:30	9.0 <i>a</i>	10.5 <i>a</i>	10.0 <i>ab</i>	6.8 <i>bcd</i>
40-10: barley	50:50	9.4 <i>a</i>	12.1 <i>a</i>	10.5 <i>ab</i>	7.8 <i>abc</i>
40-10: oat	100:30	9.8 <i>a</i>	10.2 <i>a</i>	8.3 <i>b</i>	6.4 <i>cd</i>
40-10: oat	50:50	10.6 <i>a</i>	11.6 <i>a</i>	9.0 <i>ab</i>	7.4 <i>abc</i>
Mean		9.0	10.6	8.8	7.2
SEM <sup>b</sup>		1.05	0.96	2.53	2.42
ANOVA					
Fertilizer (F)		0.0003		NS	NS
Intercrop (I)		<0.001		<0.001	<0.001
F × I		NS		NS	NS
CONTRAST					
40-10 vs. CDC Horizon		NS		NS	NS
Barley vs. oat		NS		NS	NS
Barley vs. pea-barley		NS		NS	NS
Barley vs. pea-oat		NS		NS	NS
Oat vs. pea-oat		NS		NS	NS
Oat vs. pea-barley		NS		NS	NS
Pea-barley vs. pea-oat		NS		0.015	NS
Pea-cereal (100:30) vs. (50:50)		0.009		NS	NS

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> Standard error of the means.

### 3.6.3 Botanical composition

Botanical composition differed significantly among intercrops ( $P<0.001$ ) and among experimental sites ( $P<0.001$ ), but N fertilizer ( $P=0.59$ ) and interactions N fertilizer  $\times$  intercrop ( $P=0.84$ ), N fertilizer  $\times$  site ( $P=0.14$ ), site  $\times$  intercrop ( $P=0.32$ ) and site  $\times$  N fertilizer  $\times$  intercrop ( $P=0.92$ ) did not affect botanical composition (Table 3.6).

The main component of all intercrops was cereals, accounting for 64.5-92.4% of the total DM yield of pea-cereal intercrops. In addition, cereal component differed among the intercrops and between the three experimental sites ( $P<0.001$ ) (Table 3.8). The pea component in pea-cereal seeded at 50:50 and 100:30 ratios was 11% and 26%, respectively. Among the three experimental sites, pea component at Saskatoon (22.3%) was greater than Swift Current (15.3%), but it was similar to that of Melfort (18.7%) site (Table 3.8). In the intercrops, percentage of cereal component has a tendency to increase with the supply of N fertilizer at Melfort and Saskatoon sites (Data not shown).

**Table 3.8** Botanical composition (% dry matter, DM) of pea-barley and pea-oat intercrops at Melfort, Saskatoon and Swift Current sites during the growing seasons of 2016 and 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Botanical composition (%DM)	
		Pea	Cereal
Intercrop			
CDC Horizon: barley	100:30	22.2 <i>bc</i>	77.8 <i>de</i>
CDC Horizon: barley	50:50	8.8 <i>ef</i>	91.2 <i>ab</i>
CDC Horizon: oat	100:30	19.6 <i>cd</i>	80.4 <i>cd</i>
CDC Horizon: oat	50:50	7.6 <i>f</i>	92.4 <i>a</i>
40-10: barley	100:30	35.5 <i>a</i>	64.5 <i>f</i>
40-10: barley	50:50	16.2 <i>cde</i>	83.8 <i>bcd</i>
40-10: oat	100:30	28.4 <i>ab</i>	71.6 <i>ef</i>
40-10: oat	50:50	12.1 <i>def</i>	87.9 <i>abc</i>
SEM <sup>b</sup>		1.76	1.76
<i>P</i> value		<0.001	<0.001
Site			
Melfort		18.7 <i>ab</i>	81.3 <i>ab</i>
Saskatoon		22.3 <i>a</i>	77.7 <i>b</i>
Swift Current		15.3 <i>b</i>	84.7 <i>a</i>
SEM		1.08	1.08
<i>P</i> value		<0.001	<0.001

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> Standard error of the means.

### 3.6.4 Plant height

There were significant effects of intercrop ( $P<0.001$ ), experimental site ( $P<0.001$ ) and site  $\times$  intercrop interaction ( $P<0.001$ ) on both pea and cereal height. However, N fertilizer ( $P=0.59$ ), and interactions of N fertilizer  $\times$  intercrop ( $P=0.20$ ), N fertilizer  $\times$  site ( $P=0.34$ ), and site  $\times$  N fertilizer  $\times$  intercrop ( $P=0.54$ ) did not affect plant height of pea component crop (Table 3.6). N fertilizer ( $P<0.001$ ) and interaction of site  $\times$  fertilizer ( $P=0.04$ ) increased the plant height of cereal (Table 3.6).

**Table 3.9** Plant height (cm) of pea and cereal in monocrops and in pea-cereal intercrops at Melfort, Saskatoon and Swift Current in 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Plant height	
		Pea	Cereal
Intercrop			
CDC Horizon pea	100	66.7 <i>b</i>	-
40-10 pea	100	86.3 <i>a</i>	-
Barley	100	-	84.9 <i>c</i>
Oat	100	-	85.9 <i>bc</i>
CDC Horizon: barley	100:30	56.5 <i>c</i>	86.6 <i>bc</i>
CDC Horizon: barley	50:50	53.5 <i>c</i>	86.4 <i>bc</i>
CDC Horizon: oat	100:30	57.1 <i>c</i>	92.1 <i>a</i>
CDC Horizon: oat	50:50	52.1 <i>c</i>	91.6 <i>a</i>
40-10: barley	100:30	72.8 <i>b</i>	88.3 <i>abc</i>
40-10: barley	50:50	68.6 <i>b</i>	85.8 <i>bc</i>
40-10: oat	100:30	68.0 <i>b</i>	89.5 <i>abc</i>
40-10: oat	50:50	66.3 <i>b</i>	89.7 <i>ab</i>
SEM <sup>b</sup>		1.47	1.14
<i>P</i> value		<0.001	<0.001
Fertilizer			
-N <sup>c</sup>		65.0 <i>a</i>	86.2 <i>b</i>
+N		65.5 <i>a</i>	89.9 <i>a</i>
SEM		0.66	0.75
<i>P</i> value		NS	0.032
Site			
Melfort		79.5 <i>a</i>	95.1 <i>a</i>
Saskatoon		64.2 <i>b</i>	92.9 <i>b</i>
Swift Current		50.7 <i>c</i>	76.2 <i>c</i>
SEM		0.81	0.74
<i>P</i> value		<0.001	<0.001
CONTRAST			
40-10 vs. CDC Horizon		<0.001	-
40-10 vs. 40-10: cereal		<0.001	-
CDC Horizon vs. CDC Horizon: cereal		<0.001	-
Barley vs. oat		-	NS
Barley vs. pea: barley		-	NS
Oat vs. pea: oat		-	NS

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> Standard error of the means.

<sup>c</sup> -N, without N fertilizer; +N, with N fertilizer.

The height of pea plant was in ascending order of 40-10 pea > 40-10 pea: cereal intercrops > CDC Horizon pea > CDC Horizon pea: cereal intercrops. Intercropping significantly reduced plant height (12 and 17 cm, respectively) of CDC Horizon and 40-10 peas compared to their monocrops. The height of barley and oat did not significantly differ between monocrops and intercrops. Nitrogen fertilizer increased ( $P=0.03$ ) plant height of cereal on average by 3 cm both in monocrops and intercrops. Among the three experimental sites, plant heights of pea and cereal were the tallest at Melfort (pea: 79.5 cm, cereal: 95.1 cm), intermediate (pea: 64.2 cm, cereal: 92.9 cm) at Saskatoon, and the shortest (pea: 50.7 cm, cereal: 76.2 cm) at Swift Current.

#### **3.6.4 Plant lodging score**

Plant lodging score was significantly influenced by intercrop ( $P<0.001$ ), N fertilizer ( $P=0.02$ ), experimental site ( $P=0.02$ ), and site  $\times$  intercrop interaction ( $P=0.001$ ) (Table 3.6), but it was not altered by interactions of N fertilizer  $\times$  intercrop ( $P=0.91$ ), site  $\times$  N fertilizer ( $P=0.34$ ), and site  $\times$  N fertilizer  $\times$  intercrop ( $P=0.96$ ) (Table 3.6).

The data were further analyzed by the site due to a significant site  $\times$  intercrop interaction. Plant lodging score was significantly different among the intercrops ( $P=0.001$ ) at the three sites. Nitrogen fertilizer ( $P=0.0003$ ) had significantly increased the plant lodging at Melfort, but it did not affect lodging at Saskatoon ( $P=0.18$ ) and Swift current ( $P=0.2$ ) sites (Table 3.10). The plant lodging score for pea-cereal intercrops and monocrops ranged from 1.4-8.2 at Melfort, from 1.4-7.0 at Saskatoon and from 1.7-7.4 at Swift Current (Table 3.10). 40-10 pea monocrop had the highest lodging score at all experimental sites. At all sites, intercropping of 40-10 with cereals significantly increased the lodging resistance with lodging score on average reduced by 3.7 points, when it was reduced by 0.7 point for CDC Horizon with cereals.

**Table 3.10** Lodging score (1-9 scale) of forage pea, barley, oat in monocrop and in pea-cereal intercrops with (+N) and without (-N) N fertilizer at Melfort, Saskatoon and Swift Current during the growth seasons of 2016 and 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Lodging score <sup>b</sup>			
		Melfort		Saskatoon	Swift Current
		-N	+N		
CDC Horizon pea	100	3.1 <i>bcd</i>	3.6 <i>bc</i>	3.1 <i>bcd</i>	2.1 <i>cde</i>
40-10 pea	100	7.0 <i>a</i>	8.2 <i>a</i>	7.0 <i>a</i>	7.4 <i>a</i>
Barley	100	1.4 <i>d</i>	3.3 <i>bc</i>	1.4 <i>d</i>	1.8 <i>de</i>
Oat	100	1.5 <i>d</i>	2.5 <i>bc</i>	1.5 <i>d</i>	2.3 <i>cde</i>
CDC Horizon: barley	100:30	1.6 <i>d</i>	3.3 <i>bc</i>	1.6 <i>d</i>	2.1 <i>cde</i>
CDC Horizon: barley	50:50	1.5 <i>d</i>	3.5 <i>bc</i>	1.5 <i>d</i>	2.4 <i>cde</i>
CDC Horizon: oat	100:30	2.1 <i>d</i>	2.2 <i>c</i>	2.1 <i>d</i>	2.3 <i>cde</i>
CDC Horizon: oat	50:50	2.6 <i>cd</i>	3.1 <i>bc</i>	2.6 <i>cd</i>	1.7 <i>e</i>
40-10: barley	100:30	4.7 <i>b</i>	4.6 <i>b</i>	4.7 <i>b</i>	4.3 <i>b</i>
40-10: barley	50:50	2.9 <i>bcd</i>	3.4 <i>bc</i>	2.9 <i>bcd</i>	3.8 <i>bc</i>
40-10: oat	100:30	4.2 <i>bc</i>	4.3 <i>bc</i>	4.2 <i>bc</i>	3.5 <i>bcd</i>
40-10: oat	50:50	2.7 <i>cd</i>	3.5 <i>bc</i>	2.7 <i>cd</i>	2.1 <i>cde</i>
SEM <sup>c</sup>		0.78	1.72	0.82	1.25
ANOVA					
Fertilizer (F)		0.0003		NS	NS
Intercrop (I)		<0.001		<0.001	<0.001
F × I		NS		NS	NS

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> Lodging score 1= completely upright to 9= completely lodged.

<sup>c</sup> Standard error of the means.

### 3.6.6 Competitive indices

Land equivalent ratio (LER) was significant by intercrop ( $P=0.002$ ), N fertilizer ( $P=0.03$ ), and experimental site ( $P<0.001$ ), but the interactions of N fertilizer × intercrop ( $P=0.98$ ), site × N fertilizer ( $P=0.68$ ), site × intercrop ( $P=0.45$ ) and site × N fertilizer × intercrop ( $P=0.78$ ) were not significant for LER (Table 3.6). Total LER values ranged from 1.03-1.18 for the pea-cereal intercrops, indicating increased use of the efficiency of growth resources by 3-18% in the



intercropping system (Table 3.11). Nitrogen fertilizer significantly ( $P=0.028$ ) decreased total LER values of pea-cereal intercrops by 0.4 points. Among the experimental sites, total LER was highest (1.20) at Saskatoon, followed by Melfort (1.05) and the lowest at Swift Current (1.02). Cereal component was the dominant species with a mean partial LER value of 0.79, whereas peas were a less competitive component having a mean partial LER value of 0.30 (Table 3.11).

Similarly, competitive ratio (CR) was significant by intercrop ( $P<0.001$ ), experimental site ( $P<0.001$ ) and site  $\times$  fertilizer ( $P=0.023$ ), but was unchanged by N fertilizer ( $P=0.07$ ), fertilizer  $\times$  intercrop ( $P=0.46$ ), site  $\times$  intercrop ( $P=0.31$ ) and site  $\times$  fertilizer  $\times$  intercrop ( $P=0.73$ ) (Table 3.6). Based on CR value, the barley and cereal components were the dominant species having a higher range of competitive ratio (7.5-21.6) exceeding 1.0 (Table 3.11). The competitive ratios for peas in the pea-cereal intercrop ranged from 0.13-0.41, which were lower than 1.0.

**Table 3.11** Total and partial land equivalent ratio (LER) values, and competitive ratio (CR) for pea and cereal in the pea-cereal intercrops with (+N) and without (-N) N fertilizer at Melfort, Saskatoon and Swift Current, SK in 2016 and 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Partial LER		Total LER	CR <sup>b</sup>	
		Pea	Cereal		Pea	Cereal
Intercrop						
CDC Horizon: barley	100:30	0.34 <sup>bc</sup>	0.74 <sup>cd</sup>	1.08 <sup>ab</sup>	0.16	11.8
CDC Horizon: barley	50:50	0.12 <sup>de</sup>	0.89 <sup>ab</sup>	1.03 <sup>b</sup>	0.16	16.9
CDC Horizon: oat	100:30	0.27 <sup>c</sup>	0.78 <sup>c</sup>	1.05 <sup>b</sup>	0.13	19.6
CDC Horizon: oat	50:50	0.11 <sup>e</sup>	0.94 <sup>a</sup>	1.06 <sup>b</sup>	0.14	21.6
40-10: barley	100:30	0.58 <sup>a</sup>	0.60 <sup>e</sup>	1.18 <sup>a</sup>	0.41	9.9
40-10: barley	50:50	0.31 <sup>c</sup>	0.82 <sup>bc</sup>	1.11 <sup>ab</sup>	0.40	7.5
40-10: oat	100:30	0.46 <sup>ab</sup>	0.66 <sup>de</sup>	1.11 <sup>ab</sup>	0.24	8.1
40-10: oat	50:50	0.24 <sup>cd</sup>	0.88 <sup>ab</sup>	1.12 <sup>ab</sup>	0.27	9.9
SEM <sup>c</sup>		0.06	0.03	0.04	3.38	0.06
<i>P</i> value		0.001	0.001	0.0002		
Fertilizer						
-N <sup>d</sup>		0.30 <sup>a</sup>	0.81 <sup>a</sup>	1.11 <sup>a</sup>		
+N		0.30 <sup>a</sup>	0.76 <sup>a</sup>	1.07 <sup>b</sup>		
SEM		0.06	0.02	0.03		
<i>P</i> value		ns	0.053	0.028		
Site						
Melfort		0.29 <sup>b</sup>	0.77 <sup>a</sup>	1.05 <sup>b</sup>		
Saskatoon		0.41 <sup>a</sup>	0.79 <sup>a</sup>	1.20 <sup>a</sup>		
Swift Current		0.22 <sup>b</sup>	0.80 <sup>a</sup>	1.02 <sup>b</sup>		
SEM		0.06	0.02	0.03		
<i>P</i> value		<0.001	NS	<0.001		

Means within a column with the same lower case italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. ns, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> CR >1 indicates the component crop is the dominant type in an intercrop.

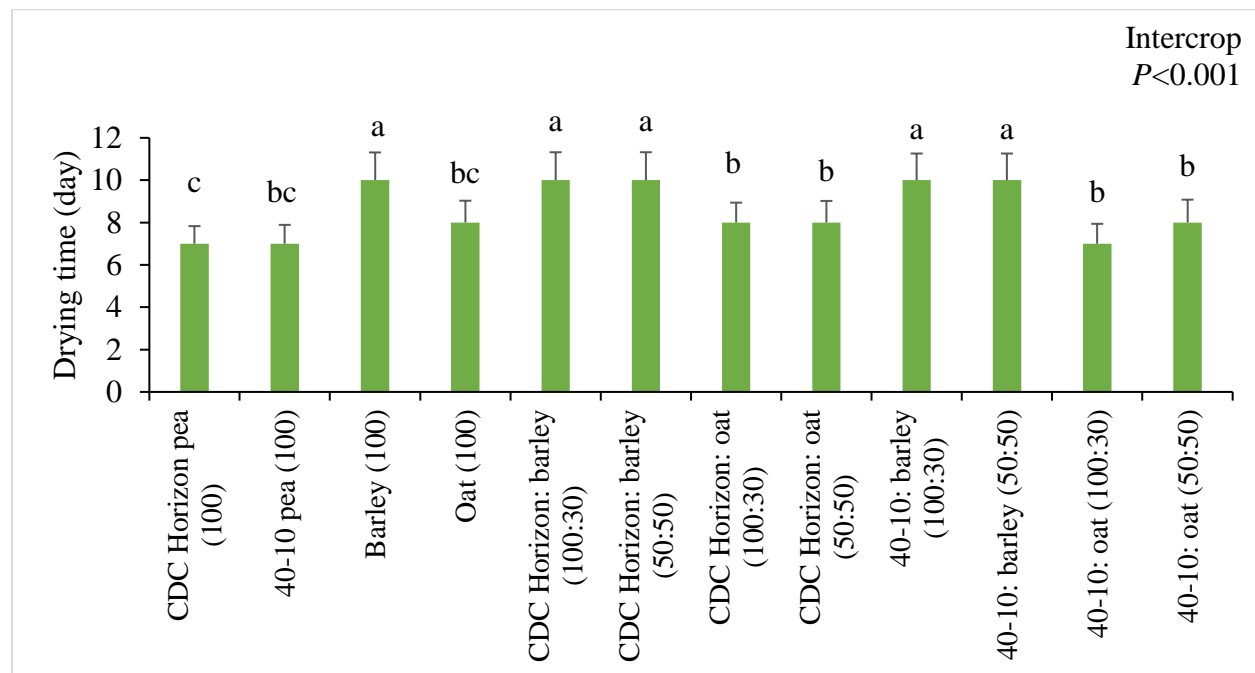
<sup>c</sup> Standard error of the means.

<sup>d</sup> -N, without N fertilizer; +N, with N fertilizer.

### 3.6.7 Drying time of greenfeed

Drying time of greenfeed varied significantly for intercrop ( $P<0.001$ ), experimental site ( $P<0.001$ ), year ( $P<0.001$ ), site  $\times$  year ( $P<0.001$ ) and site  $\times$  intercrop ( $P<0.001$ ). However, N

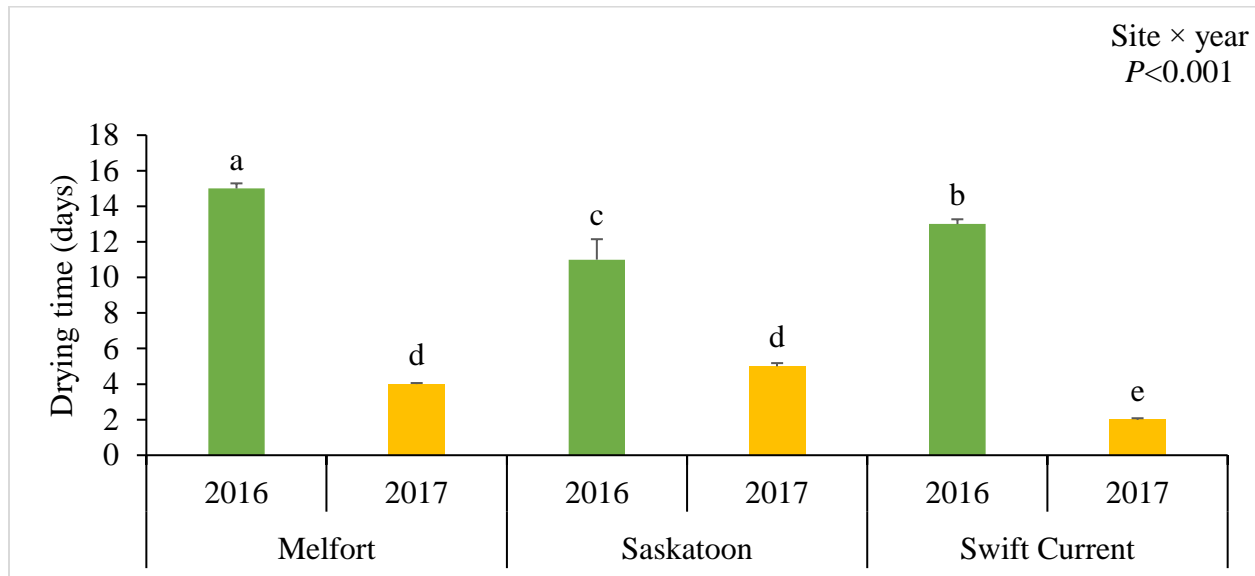
fertilizer ( $P=0.88$ ) and the interactions of N fertilizer  $\times$  intercrop ( $P=0.99$ ), site  $\times$  N fertilizer ( $P=0.85$ ), and site  $\times$  N fertilizer  $\times$  intercrop ( $P=1.00$ ) did not affect drying time (Table 3.6).



**Figure 3.3** Two-year (2016-2017) mean drying time (day) of forage barley, oat, and pea monocrops and pea-cereal intercropped for greenfeed production. Means with the same lowercase letter were not significantly different at the  $P>0.05$  according to Tukey's HSD test. Error bars are standard errors.

The moisture content of the pea-cereal intercropped ranged from 59-68% at harvest (Data not shown). On average, all monocrops and intercropped required 8-10 days to reach 18% safe baling moisture content (Figure 3.3). Barley monocrop and pea-barley intercropped took longer days to reach safe baling moisture level compared to monocrops of a pea, oat, and pea-oat intercropped. In 2016, the average drying time of greenfeed was 13 days compared to the 4 days of 2017 (Figure 3.4). In 2016, average drying time was 15, 13, and 11 days at Melfort, Swift Current, and

Saskatoon. In 2017, the average drying time was 2, 4, and 5 days for Swift Current, Saskatoon, and Melfort, respectively (Figure 3.4).



**Figure 3.4** Total drying time (day) of forage barley, oat, and pea monocrops and pea-cereal intercrops for greenfeed production at Melfort, Saskatoon and Swift Current, SK in 2016 and 2017. Means with the same lower-case letter were not significantly different at the  $P > 0.05$  according to Tukey's HSD test. Error bars are standard errors.

### 3.6.8 Economic analysis

The economic return was significantly affected by intercrop ( $P < 0.001$ ) and N fertilizer ( $P = 0.05$ ), which differed among the three sites ( $P < 0.001$ ). In addition, the interactions of site  $\times$  N fertilizer ( $P < 0.001$ ) and site  $\times$  intercrop ( $P < 0.001$ ) affected the economic return. The fertilizer  $\times$  intercrop ( $P = 0.34$ ) and site  $\times$  fertilizer  $\times$  intercrop ( $P = 0.99$ ) did not affect the economic return. At Melfort, an increased economic return ( $P = 0.0004$ ) was observed with N fertilizer application due to an increased forage DM yield even though the production cost was high. However, N fertilizer did not change the economic returns of monocrops and intercrops at Swift Current ( $P = 0.11$ ) and Saskatoon ( $P = 0.71$ ) sites.

As expected, the production cost was different for intercrops ( $P<0.001$ ) and N fertilizer ( $P<0.001$ ) (Table 3.6). The total production cost was \$282 ha<sup>-1</sup> and \$263 ha<sup>-1</sup> for barley and oat monocrops without N fertilizer and increased to \$334 ha<sup>-1</sup>, and \$315 ha<sup>-1</sup> with N fertilizer, respectively. The costs for monocrops of CDC Horizon and 40-10 peas were \$316 ha<sup>-1</sup> and \$321 ha<sup>-1</sup> without fertilizer and increased to \$369 and \$373 ha<sup>-1</sup> with N fertilizer, respectively. The production costs for the pea-cereal intercrops at 50:50 and 100:30 seeding ratios without N fertilizer were \$296 ha<sup>-1</sup> and \$348 ha<sup>-1</sup> and increased to \$336 ha<sup>-1</sup> and \$388 ha<sup>-1</sup> with additional N input, respectively.

The average seed costs were \$94 ha<sup>-1</sup> and \$71 ha<sup>-1</sup> for pea-barley intercrops seeded in 100:30 and 50:50 ratios, respectively. The average seed cost for pea-oat intercrops at 100:30 and 50:50 ratios was \$89 ha<sup>-1</sup> and \$61 ha<sup>-1</sup>, respectively (Table 3.12). However, the total seed costs were \$48, \$67, \$72, and \$76 ha<sup>-1</sup> for monocrops of oat, barley, 40-10 and CDC Horizon peas, respectively.

At Melfort, pea-barley intercrops and barley monocrop had relatively higher economic return compared with other intercrops. Barley with N fertilizer was the most profitable stand with an economic return of \$2049 ha<sup>-1</sup> or an extra cash value of \$455 ha<sup>-1</sup> compared to the unfertilized stand. This was followed by 40-10 pea: barley intercrop (50:50) and CDC Horizon pea: barley intercrop (50:50) with economic returns of \$2042 and \$1931 ha<sup>-1</sup> or an extra value of \$424 and \$369 ha<sup>-1</sup> with N fertilizer, respectively. Intercrops of 40-10 pea: oat (100:30) and CDC Horizon pea receiving N fertilizer were not profitable with returns of \$19 and \$47 ha<sup>-1</sup>, which could not offset the costs of N fertilizer and its application cost (\$52 ha<sup>-1</sup>). Among pea-oat intercrops, oat seeded with CDC Horizon pea had better economic returns of \$154 and \$271 ha<sup>-1</sup> at 50:50 and 100:30 seeding ratio, respectively, compared to those intercrops combined with 40-10 pea.

The economic return was relatively high for pea-barley intercrops and barley monocrop, followed by pea-oat intercrops and oat monocrop, and low for pea monocrops at Saskatoon and Swift Current sites. The most remunerative stands at Saskatoon were \$1796, \$1750 and \$1735 ha<sup>-1</sup> for intercrops of CDC Horizon pea: barley (100:30), 40-10 pea: barley (50:50) and barley monocrop, respectively. At Swift Current, barley monocrop and CDC Horizon pea: barley intercrop (50:50) had the highest economic returns of \$1358 ha<sup>-1</sup> and \$1356 ha<sup>-1</sup>, respectively. Monocrop of CDC Horizon and 40-10 peas had the lowest economic returns of \$839 ha<sup>-1</sup> and \$740 ha<sup>-1</sup> at Saskatoon, and \$939 ha<sup>-1</sup> and \$656 ha<sup>-1</sup> at Swift Current, respectively.

**Table 3.12** Economic return and production cost (thousand \$ ha<sup>-1</sup>) of forage pea, barley, oat monocrops, and pea-cereal intercrops at Melfort, Swift Current, and Saskatoon, SK in 2016 and 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Total seed cost	Total production cost		Economic return			
					Melfort		Saskatoon	Swift Current
			-N	+N	-N	+N		
CDC Horizon pea	100	76	321	373	1131	1178	839 <sup>de</sup>	939 <sup>d</sup>
40-10 pea	100	72	316	369	1061	1128	740 <sup>e</sup>	656 <sup>e</sup>
Barley	100	67	282	334	1541	2049	1735 <sup>ab</sup>	1358 <sup>a</sup>
Oat	100	48	263	315	1452	1732	1134 <sup>de</sup>	1002 <sup>bcd</sup>
CDC Horizon: barley	100:30	96	341	393	1443	1766	1796 <sup>a</sup>	1228 <sup>ab</sup>
CDC Horizon: barley	50:50	72	302	354	1509	1931	1625 <sup>abc</sup>	1356 <sup>a</sup>
CDC Horizon: oat	100:30	91	335	387	1327	1598	1191 <sup>cde</sup>	967 <sup>cd</sup>
CDC Horizon: oat	50:50	62	292	345	1469	1623	1292 <sup>bcd</sup>	950 <sup>d</sup>
40-10: barley	100:30	92	336	389	1503	1762	1681 <sup>ab</sup>	1031 <sup>bcd</sup>
40-10: barley	50:50	70	299	352	1565	2042	1750 <sup>ab</sup>	1216 <sup>abc</sup>
40-10: oat	100:30	86	331	383	1481	1501	1185 <sup>cde</sup>	836 <sup>de</sup>
40-10: oat	50:50	60	290	342	1577	1696	1274 <sup>bcd</sup>	986 <sup>bcd</sup>
Mean		-	309	361	1422	1667	1354	1044
SEM <sup>b</sup>		-	3.2	3.2	190	173	490	461
ANOVA								
Fertilizer (F)		-	-		<0.001		NS	NS
Intercrop (I)		-	-		<0.001		<0.001	<0.001
F × I		-	-		<0.001		NS	NS

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop. <sup>b</sup> Standard error of the means.

### 3.7 Discussion

Forage DM yield of the pea-cereal intercrops was not greater than forage barley and oat monocrops, but the intercrops had mean of 65% higher DM yield than monocrop peas. The results are consistent with the majority of previous studies on intercropping of pea with different cereals in the Northern Great Plains (Aasen et al. 2004; Begna et al. 2011; Gill and Omokanye 2018). However, Carr et al. (2004) found an increase of DM yield in pea-barley and pea-oat intercrops relative to cereal monocrops under low amounts of soil N ( $<15 \text{ kg N ha}^{-1}$ ). In addition, high pea seeding ratio (100 vs. 50) did not affect the total DM yield of intercrops in our study, because cereals were dominant species, accounting for 65-92% of total DM yield of the intercrops. Similarly, Neugschwandtner and Kaul (2013) reported that oat was a dominant component in the pea-oat intercrops. The yield of intercrops primarily depends on the cereal component, while only small degree on legume species (Staniak et al. 2014). Rapid growth and extensive root system of the cereals can be advantages for accessing more resources for growth over peas in a intercrop (Jensen 1996; Dordas et al. 2012). Nitrogen fertilizer increased forage DM yield at Melfort, but no significant effect was noticed in the other two experimental sites. This may be due to N availability under higher soil moisture at Melfort, compared to Saskatoon and Swift Current. Nuttall et al. (1991) reported that high precipitation maximized the efficiency of fertilizer use in the grass-legume pasture.

Pea-cereal intercrops had total LER value of 1.03-1.18, indicating improved efficiency of resources utilization in this study, which is in agreement with previous studies (Hauggaard-Nielsen and Jensen 2001, Sahota and Malhi 2012). However, N fertilizer reduced total LER by 0.4 points. Ofori and Stern (1987) found total LER reduced 0.5 points at  $120 \text{ kg N ha}^{-1}$  fertilizer. In addition, Yang et al. (2018) found total LER of pea-maize intercrop was reduced by 3.9-16.4% with N

fertilizer application compared with no N treatment. Nitrogen fertilizer may reduce species complementarity and induce interspecific competition among species in the legume-cereal intercropping, and also decrease the N<sub>2</sub> fixation rate (Hauggaard-Nielsen et al. 2001; Jensen 1996).

In this study, lodging score of peas was lower in intercrops with barley and oat than monocrop peas, which may be because of additional physical support provided by cereals. Barillot et al. (2014) reported that lodging of the three pea cultivars was significantly reduced in pea-wheat intercrop. Compared to CDC Horizon, the reduction of lodging was greater for 40-10 in the pea-cereal intercropping. Podgórska-Lesiak and Sobkowicz (2013) found that pea cultivars with conventional leaf type benefited more from physical support of cereal, leading to substantial increases in DM yield in the pea-barley intercrop. At Melfort, N fertilizer increased plant lodging of monocrops and intercrops. This may be primarily attributed to highest plant height and biomass production of monocrops and intercrops with N fertilizer at Melfort site. Zhang et al. (2017) reported that broadcasting N fertilizer to wheat increased the plant lodging significantly by reducing lignin, cellulose, water-soluble carbohydrate to N ratio concentrations of basal internodes.

The shorter plant height of pea in the pea-cereal intercrop than in pea monocrop showed the existence of competition for resources between the pea and cereals in the intercrop, and this effect was significant on the pea. In previous studies, Ghaley et al. (2005) found that legume growth was suppressed by cereal in intercropping because of positive N effect on cereal growth. Ofori and Stern (1987) reported that dense tiller development of cereal under N sufficient condition could cause shading and limiting light interception for a pea in the intercrop.

Mustafa and Seguin (2004) reported that pea-cereal intercrop generally is harvested for silage rather than for hay production due to relatively high moisture content. In this study, on



average, barley and oat accounted for 89% (50:50) and 74% (100:30) of the total DM yield of pea-cereal intercrops. High pea seeding ratio (100% pea: 30% cereal) produced higher pea component than the low seeding ratio, but cereals were still the main component of the intercrops. Therefore, an increase of pea seeding ratio did not affect the drying time in this study. Weather condition after swathings is the factor for drying time of pea-cereal intercrops in the field.

Forage DM yield is an important indicator of economic return for greenfeed production. High DM yield of the intercrops and monocrops at Melfort resulted in high economic returns. In general, barley and pea-barley intercrops seeded at 50:50 ratio had the highest economic returns. Similarly, Sahota and Malhi (2012) obtained a higher net return from the barley-pea intercrop without N fertilizer compared to barley monocrop stand with 80 kg N ha<sup>-1</sup> in Ontario, Canada.

In summary, the results of the present study showed that intercropping pea with barley or oat can produce similar forage DM yield to barley and oat monocrops, which was higher economic returns relative to monocrop peas. On average, the cereal component accounted for 89% (50:50) and 74% (100:30) of the total DM yield of pea-cereal intercrops. The pea-cereal intercrops can use the land resource more efficiently than monocrops based on their LER values. Lodging susceptible pea cv. 40-10 benefited more from pea-cereal intercropping than semi-leafless, lodging resistant cultivar. Pea-oat and pea-barley intercrops seeded at 50:50 ratio was the best intercrop across the soil zones in terms of seed cost, economic return, and DM yield compared to intercrops of 100:30 ratio. Nitrogen fertilization was economically advantageous for pea-cereal intercrops in the Black soil at Melfort, but this was not observed in the Dark Brown soil at Saskatoon and in the Brown soil zones at Swift Current. The drying time of intercrop was more weather dependent than the pea components in the intercrop.

### **Transition section between Chapter 3 and Chapter 4**

Forage pea-barley, and pea-oat intercrops at two different seeding ratios were evaluated for forage DM yield, botanical composition, compatibility, lodging resistance, drying time and economic analysis in comparison to pea, barley, and oat monocrops with and without N fertilizer in Chapter 3. As the intercrops are used for animal feeding, in Chapter 4, forage nutritive value (concentrations of protein, acid detergent fiber, neutral detergent fiber, starch) of pea-cereal intercrops were compared to monocrops to understand improvement of nutritional values.

## Chapter 4. Effects of seeding ratio and species on forage nutritive value of pea-cereal intercrops

### 4.1 Abstract

Intercropping pea (*Pisum sativum* L.) with barley (*Hordeum vulgare* L.) or oat (*Avena sativa* L.) may increase forage nutritive value, especially the protein concentration. A 2-year (2016-2017) experiment was conducted to evaluate forage nutritive value of pea-barley and pea-oat intercrops seeded at 100:30 and 50:50 ratio in comparison to pea, barley and oat monocrops with and without N fertilization. Protein yield (PY), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and starch concentrations were determined. There was significant variation for all measured variables ( $P<0.001$ ). Intercropping barley and oat with pea increased ( $P<0.001$ ) CP concentration by 8-39% at Melfort, 23-64% at Saskatoon, and 14-32% at Swift Current, SK compared to the barley or oat monocrops. Pea-cereal intercrops increased PY ( $P<0.001$ ) per unit area at all three experimental sites, but an increase in pea ratio did not increase PY of the intercrops. The total starch concentration of pea-cereal intercrops was similar to pea monocrops at all three sites. Forage ADF and NDF concentrations were similar between pea-cereal intercrops and cereal monocrops at Melfort site, but they were lower for pea-cereal intercrops compared to cereals at Swift Current site, and NDF concentration was lower for pea-barley intercrops at Saskatoon site. Nitrogen fertilizer increased both CP concentration (2-44%,  $P=0.02$ ) and PY (17-87%,  $P<0.001$ ) in both monocrops and intercrops at Melfort site, but there was no effect at Saskatoon and Swift Current sites. The study suggests that intercropping barley and oat with pea can improve PY and reduce NDF concentration compared to cereals without the addition of N fertilizer.

## 4.2 Introduction

Intercrops of pea with barley and oat were considered as a premium forage quality hay with a relative feed value (RFV) of 120-136 (Kocer and Albayrak 2012). Forage nutritive value of legume-barley intercrops was comparable to alfalfa harvested at pre-bloom or early-bloom stage (Strydhorst et al. 2008). Intercropping legume with cereals increases (CP) concentration and improve forage nutritive value (Aasen et al. 2004; Begna et al. 2011). Johnston et al. (1998) reported a 3-4% increase of CP concentration of pea-cereal intercrops compared to cereal monocrops. Barley intercropped with pea for forage production had 27% greater CP concentration than barley monocrop (Strydhorst et al. 2008). Increase of protein concentration was significant when pea component was higher than 200 g kg<sup>-1</sup> DM in the intercrops (Salawu et al. 2002). In addition, the choice of cereal component crop also affects CP concentration of intercrop with barley with pea having greater CP concentration than oat with pea intercrops (Chapko et al. 1991; Lauk and Lauk 2008, Dordas et al. 2012). Legume component crop not only increases the protein concentration; it also increases PY calculated from component crop DM yield and CP concentration (Chapko et al. 1991). Gill and Omokanye (2018) reported a higher PY of pea-cereal intercrops than cereal monocrops in Alberta, Canada. Similarly, pea-barley and pea-oat intercrops have produced 32 kg ha<sup>-1</sup> and 37 kg ha<sup>-1</sup> greater PY in comparison to their cereal monocrops (Carr et al. 2004).

Acid detergent fiber (ADF) is an important indicator for forage digestibility, and neutral detergent fiber (NDF) is an indicator of forage intake. Begna et al. (2011) reported pea-oat intercrop reduced ADF and NDF concentrations by 2.3 and 4.7%, respectively, compared to oat monocrop. It is common to find a decrease of NDF in pea-cereal intercrop (Khorasani and Kenelly 1997; Johnston et al. 1998), which may be because of reduction of hemicellulose content

(Pellicanò et al. 2015). However, several studies have reported no changes in ADF and NDF concentrations in pea-cereal intercropping (Aasen et al. 2004; Jedel and Helm 1993). The starch concentration of pea-cereal has frequently evaluated in silage than in greenfeed. The starch concentration ranged from 109-123 g kg<sup>-1</sup> in intercrops of pea with wheat sown in 75:25 seeding ratio at the early milk to the early dough stage (Adesogan et al. 2002). In pea-oat intercrop harvested at the early to soft dough stage, starch content was at 199 g kg DM<sup>-1</sup> (Rondahl et al. 2007).

The objective of this study was to evaluate forage nutritive value of different pea-cereal intercrops in comparison to pea, barley, and oat monocrops with and without N fertilizer.

#### **4.3 Materials and methodology**

The experimental design of the field study was described in Chapter 3. In brief, the study was conducted at AAFC Research Centres at Saskatoon (52° 07' N, 106° 38' W), Melfort (52° 81' N, 104° 60' W), and Swift Current (50° 28' N, 107° 75' W) in 2016 and 2017. Forage pea (*Pisum sativum*) cultivars CDC Horizon and 40-10, forage barley (*Hordeum vulgare* L.) cultivar CDC Maverick, and forage oat (*Avena sativum*) cultivar CDC Haymaker were used in the study. At each site, field plots were arranged in a split-plot with a Randomized Complete Block Design (RCBD) with four replications. Nitrogen fertilizer treatment (with and without N fertilizer) was the main plot factor, and forage monocrops and intercrops (pea, barley, and oat in monocrops and binary intercrops of pea: cereal at 100:30 and 50:50 ratios based on monocrop seeding rate) were considered as a sub-plot factor.

#### 4.4 Forage sampling and chemical analysis

Biomass samples were taken from each plot immediately before the forage harvest for nutritive value analysis. The samples were dried in a forced-air oven at 60°C for 72 hrs in an individual paper bag. Samples were ground sequentially in a Willey mill (Thomas Wiley, Philadelphia, PA) to 2 mm, followed by 1093 Cyclotec mill (Foss, Höganäs, SE) with 1 mm screen for crude protein and forage fiber (ADF and NDF) analysis. Sub-samples were further ground using a Retsch ZM200 mill (Retsch, Haan, DE) to 0.5 mm for analysis of total starch concentration.

##### 4.4.1 Total dry matter determination

To express forage nutrient composition percentage in dry matter (DM) basis, the moisture content in the samples was determined according to the procedure of the Association of Official Analytical Chemists (AOAC) Official Method 967.03 (1990). Approximately ~2 g of 1 mm ground subsamples were weighed and recorded in an aluminum dishes (50 mm diameter) with cover. The dishes then were placed in a forced-air oven at 105°C for 2 hrs and subsequently cooled down in the desiccator at room temperature. The initial and final weights are used to calculate DM percentage of the sample with the following equation:

$$\% \text{ DM} = \frac{W_2 - W_3}{W_1 - W_3} \times 100\% \quad (5)$$

where  $W_1$  is an initial dry weight of the sample in the aluminum dish (with cover) in grams,  $W_2$  is a dried weight of the sample in an aluminum dish (with cover) in grams and  $W_3$  is a tare weight of aluminum dish (with cover) in grams.

#### **4.4.2 Crude protein determination**

Crude protein (CP) concentration of pea-cereal intercrops was determined by LECO 628 Nitrogen/Protein Analyzer (Leco Corporation, St. Joseph, MI). Approximately 1 g dried sample was precisely weighed into a tin foil cup. The samples were then allocated in a tray in LECO 628 Nitrogen/Protein Analyzer for N analysis. At each time, a total of 36 samples was allocated, and each sample analysis took 3-4 min. In the analyzer, the samples combusted with oxygen and generated gases containing nitrogen oxides into a ballast tank. Helium, as a carrier, passes through the combustion gas, and it is then reduced to nitrogen. Water and carbon dioxide in combustion gases were removed when they passed through a tube containing magnesium perchlorate and sodium hydroxide on a silicate carrier. Again, helium is used as a reference in a thermal conductivity detector for measuring nitrogen in the samples. Nitrogen content was converted to crude protein by multiplying by a conversion factor 6.25. Protein yield (PY) ( $\text{kg ha}^{-1}$ ) was calculated by multiplying the percentage of CP by total DM yield per hectare.

#### **4.4.3 Starch determination**

Total starch concentration was determined according to AOAC Official Method 996.11 procedure using the Megazyme ‘Total Starch Assay Procedure’ kit (Megazyme, Bray, Ireland). In this procedure, ~80 mg of ground sample was placed in a 50 ml polypropylene tubes and hydrated with 0.4 ml of aqueous ethanol (80% v/v). Two millimeters of Dimethyl sulphoxide (DMSO) was added immediately to the tube and heated for 5 min in a water bath at 50°C. After adding each solution, the tube content was stirred vigorously on a vortex shaker for removal of lumps, followed by adding 3 ml of thermostable  $\alpha$ -amylase and placed in a boiling water bath for 15 min. During water bath period, the tubes were stirred for 4, 8, and 12 min. Then, 0.1 ml of Amyloglucosidase

was added and incubated again in a water bath at 50°C for 30 min. After the final incubation, the tube content volume was adjusted to 10 ml by adding distilled water and stirred thoroughly on vortex shaker. The tubes were centrifuged at 3,000 rpm for 10 min, and aliquots of 0.1 ml were extracted and diluted in 3 ml of Gopod Reagent (glucose determination reagent) and incubated at 50°C for 20 min. The absorbance reading of individual sample solution at 510 nm was determined using a spectrophotometer (Thermo Scientific Inc. Genesys 840-208100 UV/Vis). In the meantime, D-glucose controls (consist of 0.1 ml of D-glucose standard solution and 3 ml Gopod Reagent) and tubes containing reagent with no substrate (blank) (consist of 0.1 ml distilled water and 3 ml of Gopod Reagent) were prepared in new tubes for calculation of starch. Total starch concentration in pea-cereal intercrops is measured as the glucose derived from hydrolyzed starch and was expressed as a percentage of the total sample weight. The following calculation was obtained from the Megazyme-Total Starch Assay Procedure kit booklet (2018).

$$\% \text{Starch} = \Delta A \times \frac{F}{W} \times \frac{162}{180} \quad (6)$$

where  $\Delta A$  is an absorbance read against the sample blank, F is a factor for the conversion of absorbance values to micrograms of glucose, W is a sample weight in mg, 162/180 is a factor to convert free glucose to anhydroglucose, as occurs in starch.

#### **4.4.4 Fiber determination**

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations in pea-cereal intercrops and monocrops were determined by filter bags technique in ANKOM<sup>2000</sup> automated fiber analyzer (ANKOM Technology Corporation, NY). For ADF determination, a representative of ~0.5 g sample was weighed into individual pre-weighed and labeled ANKOM F57 filter bags, then heat sealed. At each time, a total of 23 filters bagged samples along with one blank bag (for



correction) was allocated to suspender trays in a fiber analyzer. During analysis, the water-soluble cell concentration in samples was dissolved by Acid Detergent Solution, followed by a few cycles of rinse in distilled water and leaving the desired fiber fraction. The bagged samples then were soaked in acetone for 3-5 min and dried in a fume hood for 6 min. Afterward, the samples were dried in an oven at 102°C for 2-4 hrs and placed into plastic bags with a desiccant pouch to cool down for 10-15 min (ANKOM<sup>2000</sup> Operator's Manual).

The sample preparation and analysis of NDF is similar to ADF analysis. Only 20 g of sodium sulfite was manually added to Neutral Detergent Solution. Acid and neutral detergent fiber in samples were calculated by the following equation:

$$\text{ADF or NDF} = \frac{(W_3 - (W_1 \times C_1)) \times 100\%}{W_2} \quad (7)$$

where  $W_1$  is a bag tare weight in grams;  $W_2$  is a sample weight in grams;  $W_3$  is a dried weight of filter bag with fiber in grams after extraction process;  $C_1$  is a blank bag correction (running average of final oven-dried weight divided by original blank bag weight in grams).

#### 4.5 Statistical analysis

Data were analyzed as a split-plot arrangement in RBCD using the Mixed Procedure Model of SAS 9.4 (SAS Institute, Inc. Cary, NC). In the model, experimental site, N fertilizer, intercrop, and their interactions were considered as fixed effects. Year, replication and replication  $\times$  fertilizer interaction were considered as random effects. If there was significant site  $\times$  fertilizer or site  $\times$  intercrops interaction for forage nutritive parameters, ANOVA was also performed for each experimental site. The multiple comparisons were made using the Tukey's Honestly Significant

Difference (HSD) test in SAS 9.4 at  $P \leq 0.05$ . Analyses of orthogonal contrasts were also performed to determine significance in the comparison of different groups of treatments.

## 4.6 Results

**Table 4.1** Analysis of variance (ANOVA) for protein yield, concentrations of crude protein (CP), starch, acid detergent fiber (ADF) and neutral detergent fiber (NDF) of pea-cereal intercrops at Melfort, Saskatoon, and Swift Current, SK in 2016 and 2017.

Source	df <sup>a</sup>	Protein yield	Crude protein	Starch	ADF	NDF
N fertilizer (F)	1	0.014	NS	NS	NS	NS
Intercrop (I)	11	<0.001	<0.001	0.003	<0.001	<0.001
Site (S)	2	<0.001	<0.001	<0.001	<0.001	<0.001
F × I	11	NS	NS	0.018	NS	NS
S × F	2	<0.001	<0.001	0.048	NS	NS
S × I	22	<0.001	<0.001	0.002	<0.001	<0.001
S × F × I	22	NS	NS	NS	NS	NS

<sup>a</sup> Degree of freedom. NS, non-significant.

### 4.6.1 Crude protein concentration

The analysis of variation showed that the effects of intercrop ( $P < 0.001$ ), experimental site ( $P < 0.001$ ), interactions of site × intercrop ( $P < 0.001$ ) and site × N fertilizer ( $P < 0.001$ ) on CP concentration were significant (Table 4.1). However, N fertilizer ( $P = 0.12$ ) and interactions of fertilizer × intercrop ( $P = 0.38$ ) and site × fertilizer × intercrop ( $P = 0.12$ ) had no effect on CP concentration (Table 4.1).

Due to significant site × intercrop ( $P < 0.001$ ) and site × N fertilizer effects, data were further analyzed by individual site. At Melfort, CP concentration for monocrops and intercrops varied from 61-137 g kg<sup>-1</sup> (Table 4.2). Pea-cereal intercrops had significantly ( $P < 0.001$ ) higher CP concentration compared to the barley and oat monocrops. The pea-oat intercrops had significantly ( $P = 0.002$ ) higher CP concentration (94 g kg<sup>-1</sup> DM) than the oat monocrop (74 g kg<sup>-1</sup> DM), but

there was no difference ( $P=0.12$ ) between the pea-barley intercrop and the barley monocrop. Among cereal species, pea-barley and pea-oat intercrops did not differ significantly ( $P=0.11$ ) in CP concentration. Crude protein concentration was significantly ( $P=0.006$ ) higher for pea-cereal intercrops seeded at 100:30 (96 g kg<sup>-1</sup> DM) than at 50:50 (86 g kg<sup>-1</sup> DM) ratio. As expected, pea-cereal intercrops (91 g kg<sup>-1</sup> DM) had significantly ( $P<0.001$ ) lower CP concentration than the pea monocrops (128 g kg<sup>-1</sup> DM). At Melfort, N fertilizer significantly ( $P=0.02$ ) increased CP concentration for all monocrops and pea-cereal intercrops (Table 4.2).

At Saskatoon, CP concentration ranged from 68-173 g kg<sup>-1</sup> DM for the monocrops and intercrops (Table 4.3). The pea-barley (106 g kg<sup>-1</sup> DM) and pea-oat (94 g kg<sup>-1</sup> DM) intercrops had significantly ( $P<0.001$ ) higher CP than the barley (79 g kg<sup>-1</sup> DM) and oat (70 g kg<sup>-1</sup> DM) monocrops. The pea-barley intercrops (106 g kg<sup>-1</sup> DM) had significantly higher ( $P<0.001$ ) CP concentration than the pea-oat intercrops (94 g kg<sup>-1</sup> DM). The pea-cereal intercrops of 100:30 ratio produced significantly higher ( $P=0.0004$ ) CP concentration (about 11 g kg<sup>-1</sup> DM) than those seeded at 50:50 ratio. As expected, all the pea-cereal intercrops (100 g kg<sup>-1</sup> DM) had significantly ( $P<0.001$ ) lower CP concentrations than the pea monocrops (161 g kg<sup>-1</sup> DM). Application of N fertilizer at Saskatoon increased ( $P=0.002$ ) CP concentration in some pea-cereal intercrops.

At Swift Current, CP concentrations ranged from 69-125 g kg<sup>-1</sup> DM for pea-cereal intercrops and monocrops (Table 4.4). The pea-barley intercrops (93 g kg<sup>-1</sup> DM) had significantly ( $P<0.001$ ) higher CP concentration than the barley monocrop (70 g kg<sup>-1</sup> DM), but there were no significant differences ( $P=0.22$ ) between the pea-oat intercrops and the oat monocrop and between the pea-barley and the pea-oat intercrops ( $P=0.58$ ). In addition, there was no significant ( $P=0.18$ ) difference of CP concentration between two seeding ratios of intercrops. Similar to the results of other two experimental sites, all the pea-cereal intercrops (91 g kg<sup>-1</sup> DM) produced significantly

( $P < 0.001$ ) lower CP concentrations than pea monocrops ( $125 \text{ g kg}^{-1} \text{ DM}$ ). Nitrogen fertilizer did not increase ( $P = 0.28$ ) the CP concentration.

**Table 4.2** Protein yield (PY), crude protein, starch, acid detergent fiber (ADF), and neutral detergent fiber (NDF) concentrations of pea, barley, oat monocrops and pea-cereal intercrops with (+N) and without (-N) N fertilizer during the growing seasons of 2016 and 2017 at Melfort, SK.

Treatment	Component crop ratio <sup>a</sup> (%)	PY		Crude protein		Starch	ADF	NDF
		-N	+N	-N	+N			
			— kg ha <sup>-1</sup> —		g kg <sup>-1</sup> DM			
CDC Horizon pea	100	710 <i>a</i>	930 <i>a</i>	111 <i>ab</i>	137 <i>a</i>	34 <i>b</i>	346 <i>a</i>	517 <i>ab</i>
40-10 pea	100	780 <i>a</i>	910 <i>a</i>	129 <i>a</i>	137 <i>a</i>	44 <i>b</i>	334 <i>ab</i>	483 <i>b</i>
Barley	100	620 <i>a</i>	1160 <i>a</i>	66 <i>de</i>	92 <i>b</i>	96 <i>a</i>	303 <i>b</i>	527 <i>ab</i>
Oat	100	610 <i>a</i>	1090 <i>a</i>	61 <i>e</i>	88 <i>b</i>	65 <i>ab</i>	314 <i>ab</i>	523 <i>ab</i>
CDC Horizon: barley	100:30	750 <i>a</i>	1010 <i>a</i>	85 <i>bcde</i>	93 <i>b</i>	64 <i>ab</i>	327 <i>ab</i>	538 <i>a</i>
CDC Horizon: barley	50:50	680 <i>a</i>	1100 <i>a</i>	74 <i>de</i>	92 <i>b</i>	50 <i>b</i>	340 <i>a</i>	556 <i>a</i>
CDC Horizon: oat	100:30	770 <i>a</i>	1140 <i>a</i>	86 <i>bcde</i>	103 <i>b</i>	42 <i>b</i>	343 <i>a</i>	546 <i>a</i>
CDC Horizon: oat	50:50	760 <i>a</i>	1090 <i>a</i>	76 <i>cde</i>	96 <i>b</i>	60 <i>ab</i>	335 <i>ab</i>	558 <i>a</i>
40-10: barley	100:30	840 <i>a</i>	980 <i>a</i>	92 <i>bcd</i>	94 <i>b</i>	49 <i>b</i>	323 <i>ab</i>	532 <i>a</i>
40-10: barley	50:50	760 <i>a</i>	1080 <i>a</i>	83 <i>cde</i>	90 <i>b</i>	46 <i>b</i>	325 <i>ab</i>	549 <i>a</i>
40-10: oat	100:30	960 <i>a</i>	1120 <i>a</i>	103 <i>abc</i>	111 <i>b</i>	44 <i>b</i>	325 <i>ab</i>	523 <i>ab</i>
40-10: oat	50:50	800 <i>a</i>	1130 <i>a</i>	77 <i>cde</i>	96 <i>b</i>	54 <i>b</i>	332 <i>ab</i>	526 <i>ab</i>
Mean		753	1062	87	102	54	329	531
SEM <sup>b</sup>		81.5	154.1	15.7	6.4	18.8	48.4	38.6
ANOVA								
Fertilizer (F)		<0.001		0.021		NS	NS	NS
Intercrop (I)		NS		<0.001		<0.001	0.008	<0.001
F × I		NS		NS		NS	NS	NS
CONTRAST								
CDC Horizon vs. 40-10		NS		NS		NS	NS	NS
Pea vs. pea-cereal		NS		<0.001		NS	NS	<0.001
Pea vs. pea-cereal (100:30)		0.036		<0.001		NS	NS	0.008
Pea vs. pea-cereal (50:50)		NS		<0.001		NS	NS	<0.001
Pea-cereal (100:30) vs. (50:50)		NS		0.006		NS	NS	NS
Pea-barley vs. pea-oat		NS		NS		NS	NS	NS
Pea-barley vs. barley		NS		NS		<0.001	NS	NS
Pea-oat vs. oat		NS		0.002		NS	NS	NS

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> Standard error of the means.

#### 4.6.2 Protein yield

Protein yield was significantly affected by intercrop ( $P<0.001$ ), N fertilizer ( $P=0.01$ ), experimental site ( $P<0.001$ ), site  $\times$  N fertilizer ( $P<0.001$ ) and site  $\times$  intercrop ( $P<0.001$ ), but interactions of N fertilizer  $\times$  intercrop ( $P=0.54$ ) and site  $\times$  N fertilizer  $\times$  intercrop ( $P=0.95$ ) were not significant (Table 4.1). Due to the significant effect of site  $\times$  intercrop and site  $\times$  N fertilizer, data were further analyzed by each site. Protein yield was significantly different among the intercrops at Saskatoon ( $P<0.001$ ) and Swift Current ( $P<0.01$ ), but not at Melfort site ( $P=0.40$ ) (Table 4.2; 4.3 and 4.4). Protein yield ranged from 610-1160 kg ha<sup>-1</sup>, from 598-1159 kg ha<sup>-1</sup>, and from 537-797 kg ha<sup>-1</sup> for the monocrops and the pea-cereal intercrops at Melfort, Saskatoon, and Swift Current, respectively (Table 4.2; 4.3 and 4.4).

Based on contrast comparison, the pea-cereal intercrops seeded at 100:30 ratio (946 kg ha<sup>-1</sup>) had significantly ( $P=0.04$ ) higher PY than the pea monocrops (834 kg ha<sup>-1</sup>) at Melfort (Table 4.2). The pea-cereal intercrops at 50:50 ratio (925 kg ha<sup>-1</sup>) had similar PY ( $P=0.17$ ) to that of pea monocrops. However, PY did not ( $P=0.67$ ) differ among the pea-cereal intercrops seeded at the two seeding ratios. Protein yield did not differ either between the pea-barley and the pea-oat intercrops ( $P=0.15$ ) or between the barley ( $P=0.54$ ) and the oat ( $P=0.22$ ) monocrops. Nitrogen fertilizer significantly ( $P<0.001$ ) increased PY by 17-87% in monocrops and intercrops at Melfort site.

At Saskatoon, PY of pea-cereal intercrops in both seeding ratios (939 kg ha<sup>-1</sup>) were significantly ( $P=0.038$ ) higher than the pea monocrops (793 kg ha<sup>-1</sup>) (Table 4.3), but there was no difference ( $P=0.88$ ) between pea-cereal intercrops seeded at 100:30 (903 kg ha<sup>-1</sup>) and 50:50 (912 kg ha<sup>-1</sup>) ratios. The pea-oat intercrops (831 kg ha<sup>-1</sup>) had significantly ( $P<0.001$ ) lower PY than the

pea-barley intercrops ( $1050 \text{ kg ha}^{-1}$ ), but it had higher PY ( $P<0.001$ ) than the oat monocrops ( $598 \text{ kg ha}^{-1}$ ). Protein yield did not differ ( $P=0.07$ ) between the pea-barley intercrops ( $1050 \text{ kg ha}^{-1}$ ) and the barley monocrops ( $830 \text{ kg ha}^{-1}$ ) at Saskatoon. Protein yield did not ( $P=0.38$ ) increase with the addition of N fertilizer at Saskatoon.

At Swift Current, all pea-cereal intercrops ( $656 \text{ kg ha}^{-1}$ ) had greater PY than the pea monocrops ( $569 \text{ kg ha}^{-1}$ ), but there was no significant ( $P=0.12$ ) difference between the two seeding ratios (Table 4.4). Protein yield of the pea-barley intercrops ( $697 \text{ kg ha}^{-1}$ ) was numerically greater than the pea-oat intercrops ( $623 \text{ kg ha}^{-1}$ ) and the barley monocrop ( $548 \text{ kg ha}^{-1}$ ), but the differences were not significant ( $P=0.15$  and  $P=0.06$ , respectively). Protein yield of the pea-oat intercrops ( $623 \text{ kg ha}^{-1}$ ) was similar ( $P=0.69$ ) to that of the oat monocrop ( $656 \text{ kg ha}^{-1}$ ). Protein yield was not significantly differed ( $P=0.64$ ) for pea-cereal intercrops seeded at 100:30 ( $647 \text{ kg ha}^{-1}$ ) and 50:50 ( $672 \text{ kg ha}^{-1}$ ) ratios. Protein yield did not ( $P=0.32$ ) increase with the addition of N fertilizer at Swift Current.

**Table 4.3** Protein yield (PY), crude protein, starch, acid detergent fiber (ADF), and neutral detergent fiber (NDF) concentrations of pea, barley, oat monocrops and pea-cereal intercrops with (+N) and without (-N) N fertilizer during the growing seasons of 2016 and 2017 at Saskatoon, SK.

Treatment	Component crop ratio <sup>a</sup> (%)	PY	Crude protein		Starch	ADF	NDF
			-N	+N			
		-kg ha <sup>-1</sup> -	g kg <sup>-1</sup> DM				
CDC Horizon pea	100	815 <sup>bc</sup>	156 <sup>a</sup>	158 <sup>a</sup>	34 <sup>ab</sup>	268 <sup>c</sup>	480 <sup>de</sup>
40-10 pea	100	799 <sup>bc</sup>	173 <sup>a</sup>	156 <sup>a</sup>	29 <sup>abc</sup>	300 <sup>ab</sup>	475 <sup>e</sup>
Barley	100	830 <sup>bc</sup>	77 <sup>de</sup>	78 <sup>de</sup>	34 <sup>ab</sup>	267 <sup>c</sup>	522 <sup>abcd</sup>
Oat	100	598 <sup>c</sup>	68 <sup>e</sup>	72 <sup>e</sup>	27 <sup>abc</sup>	323 <sup>a</sup>	556 <sup>a</sup>
CDC Horizon: barley	100:30	1159 <sup>a</sup>	112 <sup>bc</sup>	107 <sup>bc</sup>	30 <sup>abc</sup>	280 <sup>bc</sup>	499 <sup>cde</sup>
CDC Horizon: barley	50:50	896 <sup>ab</sup>	99 <sup>cd</sup>	90 <sup>bcde</sup>	33 <sup>ab</sup>	264 <sup>c</sup>	508 <sup>bcde</sup>
CDC Horizon: oat	100:30	781 <sup>bc</sup>	91 <sup>cd</sup>	94 <sup>bcde</sup>	20 <sup>c</sup>	312 <sup>a</sup>	545 <sup>abc</sup>
CDC Horizon: oat	50:50	851 <sup>bc</sup>	95 <sup>cd</sup>	87 <sup>cde</sup>	23 <sup>bc</sup>	305 <sup>ab</sup>	540 <sup>abc</sup>
40-10: barley	100:30	1155 <sup>a</sup>	129 <sup>b</sup>	113 <sup>b</sup>	38 <sup>a</sup>	258 <sup>c</sup>	482 <sup>de</sup>
40-10: barley	50:50	1011 <sup>ab</sup>	100 <sup>cd</sup>	98 <sup>bcd</sup>	31 <sup>abc</sup>	266 <sup>c</sup>	502 <sup>bcde</sup>
40-10: oat	100:30	830 <sup>bc</sup>	109 <sup>bc</sup>	90 <sup>bcde</sup>	19 <sup>c</sup>	315 <sup>a</sup>	541 <sup>abc</sup>
40-10: oat	50:50	862 <sup>bc</sup>	98 <sup>cd</sup>	91 <sup>bcde</sup>	25 <sup>bc</sup>	305 <sup>ab</sup>	545 <sup>ab</sup>
Mean		882	109	103	29	289	516
SEM <sup>b</sup>		224	6.6	5.5	8.0	8.9	11.6
ANOVA							
Fertilizer (F)		NS	0.002		NS	NS	NS
Intercrop (I)		<0.001	<0.001		<0.001	<0.001	<0.001
F × I		NS	NS		NS	NS	NS
CONTRAST							
CDC Horizon vs. 40-10		NS	NS		NS	NS	NS
Pea vs pea-cereal		0.038	<0.001		NS	NS	<0.001
Pea vs. pea-cereal (100:30)		0.020	<0.001		NS	NS	<0.001
Pea vs. pea-cereal (50:50)		NS	<0.001		NS	NS	<0.001
Pea-cereal (100:30) vs. (50:50)		NS	0.0004		NS	NS	NS
Pea-barley vs. pea-oat		<0.001	<0.001		0.002	<0.001	<0.001
Pea-barley vs. barley		NS	<0.001		NS	NS	0.046
Pea-oat vs. oat		<0.001	<0.001		NS	NS	NS

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> Standard error of the means.



#### 4.6.3 Total starch concentration

The effects of intercrop ( $P=0.003$ ), experimental site ( $P<0.001$ ), interactions of N fertilizer  $\times$  intercrop ( $P<0.001$ ), site  $\times$  N fertilizer ( $P=0.05$ ) and site  $\times$  intercrop ( $P=0.002$ ) were significant for total starch concentration. However, N fertilizer ( $P=0.50$ ) and interaction of site  $\times$  N fertilizer  $\times$  intercrop ( $P=0.41$ ) had no effect on total starch concentration. Due to significant site  $\times$  intercrop, data were further analyzed by each site (Table 4.1). Intercrops significant varied for total starch concentration at Melfort ( $P<0.001$ ) and Saskatoon ( $P<0.001$ ) sites, but there was no difference ( $P=0.39$ ) at Swift Current (Table 4.2; 4.3 and 4.4).

Total starch concentrations ranged from 34-96 g kg<sup>-1</sup> DM at Melfort, from 19-38g kg<sup>-1</sup> DM at Saskatoon, and from 24-64 g kg<sup>-1</sup> DM at Swift Current. Total starch concentration did not differ significantly ( $P=0.07$ ) between pea monocrops (39 g kg<sup>-1</sup> DM) and pea-cereal intercrops (49 g kg<sup>-1</sup> DM) at Melfort site (Table 4.2). The barley monocrop had significantly ( $P<0.001$ ) higher total starch concentration (100 g kg<sup>-1</sup> DM) than the pea-barley intercrops (53 g kg<sup>-1</sup> DM). The concentration of starch in the monocrop oat (49 g kg<sup>-1</sup> DM) was similar to ( $P=0.086$ ) the pea-oat intercrops (67 g kg<sup>-1</sup> DM). Total starch concentrations of the pea-barley intercrops (53 g kg<sup>-1</sup> DM) were similar to ( $P=0.52$ ) the pea-oat intercrops (49 g kg<sup>-1</sup> DM).

At Saskatoon site, no significant differences ( $P=0.33$ ) were found in total starch concentration among the pea-cereal intercrops (28 g kg<sup>-1</sup> DM) and the pea monocrops (31 g kg<sup>-1</sup> DM) as shown in Table 4.3. Total starch concentration was similar ( $P=0.83$ ) between the pea-barley intercrops (32 g kg<sup>-1</sup> DM) and the barley monocrop (34 g kg<sup>-1</sup> DM), and between ( $P=0.26$ ) the pea-oat intercrops (23 g kg<sup>-1</sup> DM) and the oat monocrop (28 g kg<sup>-1</sup> DM). The pea-barley intercrops (32 g kg<sup>-1</sup> DM) had higher ( $P=0.002$ ) starch concentration than in the pea-oat intercrops

(23 g kg<sup>-1</sup> DM). At Swift Current site, no significant differences were observed between or among the monocrops and the intercrops for total starch concentration (Table 4.4).

#### **4.6.4 Forage fiber concentration**

Intercrop ( $P=0.001$ ), experimental site ( $P=0.001$ ) and interactions of site  $\times$  intercrop ( $P=0.001$ ) were significant for acid detergent fiber (ADF) concentration. However, N fertilizer ( $P=0.76$ ), interactions of N fertilizer  $\times$  intercrop ( $P=0.38$ ), site  $\times$  N fertilizer ( $P=0.97$ ) and site  $\times$  N fertilizer  $\times$  intercrop ( $P=0.96$ ) were not significant for ADF concentration (Table 4.1).

The effects of intercrop ( $P=0.001$ ), experimental site ( $P=0.001$ ), and interactions of site  $\times$  intercrop ( $P=0.001$ ) were significant for neutral detergent fiber (NDF), but N fertilizer ( $P=0.77$ ), interactions of N fertilizer  $\times$  intercrop ( $P=0.53$ ), site  $\times$  N fertilizer ( $P=0.68$ ) and site  $\times$  N fertilizer  $\times$  intercrop ( $P=0.29$ ) had no significant effects on NDF concentration (Table 4.1).

The concentrations of both ADF ( $P=0.008$ ) and NDF ( $P<0.001$ ) were significantly different for the intercrops at Melfort and Saskatoon, but only NDF was significantly different ( $P<0.001$ ) at Swift Current. Nitrogen fertilizer and interaction of N fertilizer  $\times$  intercrop did not have significant effects on ADF and NDF at all three sites (Table 4.2; 4.3 and 4.4). The ADF concentration for monocrops and pea-cereal intercrops ranged from 303-346 g kg<sup>-1</sup> DM at Melfort, from 258-323 g kg<sup>-1</sup> DM at Saskatoon and from 319-343 g kg<sup>-1</sup> DM at Swift Current (Table 4.2, 4.3, and 4.4). The NDF concentration for monocrops and pea-cereal intercrops ranged from 483-558 g kg<sup>-1</sup> DM at Melfort, from 475-556 g kg<sup>-1</sup> DM at Saskatoon and from 451-591 g kg<sup>-1</sup> DM at Swift Current (Table 4.2, 4.3, and 4.4). The NDF concentration was significantly higher ( $P<0.001$ ) for pea-cereal intercrops than the pea monocrops at the three experimental sites, but there was no

differences for ADF concentration among the monocrops of pea, barley, oat and their intercrops at any experimental site (Table 4.2, 4.3, and 4.4).

Based on contrast comparison, the pea monocrops ( $500 \text{ g kg}^{-1} \text{ DM}$ ) had significantly lower NDF concentration ( $P<0.001$ ) than the pea-cereal intercrops ( $541 \text{ g kg}^{-1} \text{ DM}$ ) at Melfort (Table 4.2). The ADF concentration did not differ ( $P=0.46$ ) among the pea monocrops and the pea-cereal intercrops. The barley monocrops did not differ to corresponding the pea-barley intercrops in ADF ( $P=0.16$ ) and NDF ( $P=0.35$ ) concentrations. The oat monocrops did not differ from the pea-oat intercrops in ADF ( $P=0.07$ ) and NDF ( $P=0.19$ ) concentrations. The pea-barley intercrops and the pea-oat intercrops did not differ significantly in ADF ( $P=0.61$ ) and NDF ( $P=0.59$ ) concentration. Differences were also not found in the pea-cereal intercropped in 50:50 and 100:30 ratio for ADF ( $P=0.73$ ) and NDF ( $P=0.22$ ) concentrations.

At Saskatoon, pea monocrops ( $476 \text{ g kg}^{-1} \text{ DM}$ ) had significantly lower NDF concentration ( $P<0.001$ ) than the pea-cereal intercrops ( $520 \text{ g kg}^{-1} \text{ DM}$ ) (Table 4.3). The ADF concentrations ( $P=0.51$ ) was similar between the pea monocrops and the pea-cereal intercrops. The differences between the oat monocrop and the pea-oat intercrops in concentrations of ADF ( $P=0.08$ ) and NDF ( $P=0.15$ ) were not significant. The barley monocrop and the pea-barley intercrops also did not differ significantly in concentrations of ADF ( $P=0.99$ ) and NDF ( $P=0.05$ ). The pea-barley intercrops ( $267$  and  $499 \text{ g kg}^{-1} \text{ DM}$ , respectively) had significantly lower ADF ( $P<0.001$ ) and NDF ( $P<0.001$ ) concentrations than the pea-oat intercrops ( $309$  and  $543 \text{ g kg}^{-1} \text{ DM}$ , respectively). Pea-cereal intercrops in both seeding ratios of 100:30 and 50:50 had similar concentrations of ADF ( $P=0.31$ ) and NDF ( $P=0.36$ ).

**Table.4.4** Protein yield (PY), crude protein, starch, acid detergent fiber (ADF), and neutral detergent fiber (NDF) concentrations of pea, barley, oat monocrops and pea-cereal intercrops with (+N) and without (-N) N fertilizer during the growing seasons of 2016 and 2017 at Swift Current, SK.

Treatment	Component crop ratio <sup>a</sup> (%)	PY	Crude protein	Starch	ADF	NDF
		-kg ha <sup>-1</sup> -	g kg <sup>-1</sup> DM			
CDC Horizon pea	100	667 <sup>ab</sup>	125 <sup>a</sup>	37 <sup>a</sup>	330 <sup>a</sup>	461 <sup>d</sup>
40-10 pea	100	537 <sup>b</sup>	124 <sup>ab</sup>	38 <sup>a</sup>	319 <sup>a</sup>	451 <sup>d</sup>
Barley	100	574 <sup>b</sup>	69 <sup>d</sup>	33 <sup>a</sup>	333 <sup>a</sup>	591 <sup>a</sup>
Oat	100	667 <sup>ab</sup>	87 <sup>cd</sup>	33 <sup>a</sup>	336 <sup>a</sup>	568 <sup>ab</sup>
CDC Horizon: barley	100:30	716 <sup>ab</sup>	89 <sup>cd</sup>	37 <sup>a</sup>	333 <sup>a</sup>	547 <sup>abc</sup>
CDC Horizon: barley	50:50	797 <sup>a</sup>	90 <sup>cd</sup>	46 <sup>a</sup>	321 <sup>a</sup>	552 <sup>abc</sup>
CDC Horizon: oat	100:30	650 <sup>ab</sup>	91 <sup>c</sup>	34 <sup>a</sup>	343 <sup>a</sup>	553 <sup>abc</sup>
CDC Horizon: oat	50:50	658 <sup>ab</sup>	93 <sup>c</sup>	36 <sup>a</sup>	338 <sup>a</sup>	558 <sup>abc</sup>
40-10: barley	100:30	675 <sup>ab</sup>	99 <sup>c</sup>	44 <sup>a</sup>	324 <sup>a</sup>	522 <sup>c</sup>
40-10: barley	50:50	703 <sup>ab</sup>	91 <sup>cd</sup>	48 <sup>a</sup>	320 <sup>a</sup>	526 <sup>bc</sup>
40-10: oat	100:30	654 <sup>ab</sup>	103 <sup>bc</sup>	34 <sup>a</sup>	329 <sup>a</sup>	534 <sup>bc</sup>
40-10: oat	50:50	634 <sup>ab</sup>	90 <sup>cd</sup>	43 <sup>a</sup>	330 <sup>a</sup>	558 <sup>abc</sup>
Mean		661	96	39	330	535
SEM <sup>b</sup>		206	5.8	8.4	45	16
ANOVA						
Fertilizer (F)		NS	NS	NS	NS	NS
Intercrop (I)		0.012	<0.001	NS	NS	<0.001
F × I		NS	NS	0.031	NS	NS
CONTRAST						
CDC Horizon vs. 40-10		NS	NS	NS	NS	NS
Pea vs pea-cereal		NS	<0.001	NS	NS	<0.001
Pea vs. pea-cereal (100:30)		NS	<0.001	NS	NS	<0.001
Pea vs. pea-cereal (50:50)		NS	<0.001	NS	NS	<0.001
Pea-cereal (100:30) vs. (50:50)		NS	NS	NS	NS	NS
Pea-barley vs. pea-oat		NS	NS	NS	NS	NS
Pea-barley vs. barley		NS	<0.001	NS	NS	<0.001
Pea-oat vs. oat		NS	NS	NS	NS	0.026

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> Standard error of the means. Ns, non-significant.

At Swift Current, the pea monocrops ( $454 \text{ g kg}^{-1} \text{ DM}$ ) had significantly ( $P<0.001$ ) lower NDF concentration than the pea-cereal intercrops ( $549 \text{ g kg}^{-1} \text{ DM}$ ), but had similar ( $P=0.55$ ) ADF concentrations (Table 4.4). The NDF concentrations of the pea-barley ( $P<0.001$ ,  $535 \text{ g kg}^{-1} \text{ DM}$ ) and the pea-oat ( $P=0.03$ ,  $549 \text{ g kg}^{-1} \text{ DM}$ ) intercrops were significantly lower than that of barley ( $589 \text{ g kg}^{-1} \text{ DM}$ ) and oat ( $566 \text{ g kg}^{-1} \text{ DM}$ ) monocrops, respectively. However, the barley ( $P=0.65$ ) and oat ( $P=0.93$ ) monocrops had similar ADF concentration to the pea-barley and pea-oat intercrops. The pea-cereal intercrops seeded in 100:30 and 50:50 ratios had similar concentrations of ADF ( $P=0.62$ ) and NDF ( $P=0.19$ ).

#### **4.7 Discussion**

The study was conducted to evaluate forage nutritive value of different pea-cereal intercrops at the three soil zones of Saskatchewan, Canada. The main advantage of the pea-cereal intercrop is to improve CP concentration. As expected, the concentration of CP increased with the increase of pea proportion in the pea-cereal intercrops in this study, which is in agreement with the findings of the majority of studies (Hauggaard-Nielsen et al. 2001; Carr et al. 2004; Dordas et al. 2012; Uzun and Asik 2012). Beside high CP of legume in the intercrops, Mohsenabadi et al. (2008) found that barley CP concentration in the barley-vetch intercrops was higher compared to barley monocrop. Pellicanò et al. (2015) reported that cereals grown in intercrop with pea had higher N concentration in straw compared to their monocrops. This may be because of N transfer from legume to cereals in the legume-cereal intercropping system (Jensen 1996).

The CP concentration was not significantly affected by the type of cereal component in intercrops, ranging from  $90\text{-}103 \text{ g kg}^{-1} \text{ DM}$  for pea-oat intercrops and from  $89\text{-}105 \text{ g kg}^{-1} \text{ DM}$  for pea-barley intercrops, respectively. Similarly, Mustafa and Seguin (2004) reported that the

difference in CP concentration was small among the cereal components of pea-barley, pea-oat, and pea-wheat silages in eastern Canada. In contrast, Jedel and Helm (1993) reported higher CP concentration for pea-barley than for pea-oat intercrops at Lacombe, Alberta. The mean CP concentration in pea-oat intercrops in this study ( $94 \text{ g kg}^{-1}$ ) was lower than the studies conducted at Wisconsin, USA ( $179 \text{ g kg}^{-1}$ ) (Chapko et al. 1991), southwestern North Dakota, USA ( $100 \text{ g kg}^{-1}$ ) (Carr et al. 2004) and northwest Alberta, Canada ( $112 \text{ g kg}^{-1}$ ) (Gill and Omokanye 2018). The mean CP concentration of pea-barley intercrops ( $96 \text{ g kg}^{-1}$ ) in this study was also lower than those studies conducted in Northern Great Plains reported by Strydhorst et al. 2008 ( $127 \text{ g kg}^{-1}$ ), Carr et al. 2004 ( $135 \text{ g kg}^{-1}$ ) and Gill and Omokanye 2018 ( $97\text{-}117 \text{ g kg}^{-1}$ ). The differences in CP may be in part due to variation in soil nutrient and soil type, growth conditions, and agronomic management, especially the harvest stage of the crops. The impact of growth stage on CP concentration of pea-cereal intercrops often is greater than the impact of the crop species and cultivars (McElroy and Gervais 1983). In general, the majority of plots in our study were harvested at later growth stage (i.e., pea-barley intercrops at the hard dough, pea-oat intercrops at the late soft dough), which could result in lower CP concentrations.

The animal in different stages of production requires different nutritional uptake (NRC 2000). Beef cows in middle pregnancy period requires dietary CP of  $70 \text{ g kg}^{-1}$  and increases to  $90 \text{ g kg}^{-1}$  in late pregnancy (NRC 2000). Lactating cows, depending on gain goals (900-1500 lbs) and prediction of daily milk (10-30 lbs/day), requires CP of  $85\text{-}106 \text{ g kg}^{-1}$ . The replacement heifers need between  $89\text{-}96 \text{ g kg}^{-1}$  of CP to reach the potential of 1000 to 1400 lbs mature weight. Across all the three sites, monocrops of CDC Horizon ( $135 \text{ g kg}^{-1}$ ) and 40-10 peas ( $140 \text{ g kg}^{-1} \text{ DM}$ ) exceeded the CP requirements for gestating and lactating cows and replacement heifers. All forage

pea-barley and pea-oat intercrops had adequate CP concentration to meet gestating beef cows and replacement beef heifers (National Research Council 2000).

Advantage of intercropping pea-cereal increases protein yield per area compared to cereal monocrops (Berkenkamp and Meeres 1987; Aasen et al. 2004; Strydhorst et al. 2008). In our study, pea-cereal intercrops at 100:30 seeding ratio did not significantly differ in PY from those at 50:50 seeding ratio across all experimental sites. Because increasing protein yield per unit, areas requires a significant proportion (i.e 200 g per kg) of the legume DM (Carr et al. 1998) and high total forage DM (Strydhorst et al. 2008). An increase in PY with the addition of N fertilizer was found only at Melfort site due to an increase in both DM yield and CP concentration after adding N fertilizer. This increase of DM yield at Melfort may be due to its high soil moisture level than the other two sites in our study. Campbell et al. (1991) found that the grain protein concentration of spring wheat tended to increase with the increase in N fertility under wet conditions.

The total starch concentration of pea-cereal intercrops in our study was similar to the pea and cereal monocrops. Mustafa and Seguin (2004) did not find significant differences in starch concentration between pea-cereal intercrops and pea monocrop silages when harvested at the booting stage, but the difference increased when the crops were harvested at the milk stage. Salawu et al. (2002) reported that starch concentrations of pea-wheat silage were increased from 66 g kg<sup>-1</sup> at the early milk to 162 g kg<sup>-1</sup> at hard dough stage of wheat.

In this study, intercropping pea with barley or oat did not affect ADF concentration compared to cereal monocrops, which was similar to previous studies of intercropping cereal with pea in the Northern Great Plains (Carr et al. 2004; Chen et al. 2004; Johnston et al. 1998). Forage NDF concentration reduced in the pea-cereal intercrops in our study. Chapko et al. (1991) reported a decrease in NDF concentration by 6% and 7% in pea-barley and pea-oat intercrops in Wisconsin,

USA. Khorasani and Kennely (1997) reported a 4-5% reduction in NDF concentration in pea-barley mixtures compared to barley monocrop in Alberta, Canada. Nitrogen fertilizer, however, did not affect the ADF and NDF concentrations in our study in agreement with the findings of Dirienzo et al. (1991).

In summary, this study showed that the inclusion of forage pea in intercrops with barley or oat increased CP concentration and PY in comparison with the cereal monocrops. Pea-cereal at 100:30 ratio had higher CP concentration to 50:50 ratio, but produced similar PY, which was greater than cereal monocrops. Total starch, forage ADF concentrations were unaffected by intercropping except NDF was lower in some intercrops than monocrops. Nitrogen fertilizer was effective to increase CP and PY in the pea-cereal intercrops in moist Melfort site, but there was no effect in the drier regions at Saskatoon and Swift Current sites. The study also indicates that the pea-barley/oat intercrops were sufficient to meet minimum CP requirements of beef cows at different gestation periods.



### **Transition section between Chapter 4 and Chapter 5**

In Chapter 4, forage nutritive values, i.e., crude protein, protein yield, starch, acid detergent fiber, and neutral detergent fiber concentrations of pea-cereal intercrops were described. In Chapter 4, the present study showed an increase of crude protein concentration and total protein yield in pea-cereal intercrops even though the forage DM yield was similar or slightly lower than cereal monocrops in Chapter 3 experiment. Besides forage DM yield, nutritional value of pea in pea-cereal intercrops, BNF is also an important factor for including pea in the cropping system. Thus, the experiment in Chapter 5 was designed to evaluate BNF of pea in monocrop and in pea-cereal intercrop with and without N fertilizer.

## **Chapter 5. Biological nitrogen fixation of pea in pea-cereal intercrops under different growth conditions**

### **5.1 Abstract**

Biological N<sub>2</sub> fixation (BNF) of pea in pea-cereal intercrops may vary due to component crop type, soil and climatic conditions. A two-year (2016-2017) field experiment was conducted at Melfort, Saskatoon, and Swift Current, Saskatchewan to evaluate BNF of pea in pea-barley and pea-oat intercrops compared to pea monocrops with and without N fertilizer. Biological N<sub>2</sub> fixation varied significantly among experimental sites ( $P<0.001$ ) and intercrops ( $P<0.001$ ). The percentage of N in aboveground biomass of pea derived from atmosphere (%Nd<sub>fa</sub>) ranged from 27-71% at Melfort, from 52-82% at Saskatoon, and from 48-71% at Swift Current. The mean amount of N<sub>2</sub> fixed in pea was the highest at Melfort (59 kg N ha<sup>-1</sup>) followed by Saskatoon (50 kg N ha<sup>-1</sup>) and the lowest at Swift Current (18 kg N ha<sup>-1</sup>). With the exception of 40-10 pea monocrops, N fertilizer reduced the %Nd<sub>fa</sub> value of pea in monocrop and intercrops by 22-63% at Melfort, and by 35-65% at Swift Current, but no reduction occurred at Saskatoon ( $P=0.29$ ) site. The study showed that 60 kg of N in the soil would likely reduce %Nd<sub>fa</sub> of pea in intercrop. There was a significant %N and amount of N transfer from pea to cereal in the pea-cereal intercrops.

## 5.2 Introduction

Intercropping pea with cereal crops can reduce the reliance of synthetic N fertilizer, as pea fixes atmospheric N<sub>2</sub> through a symbiotic relationship with rhizobia bacteria (*Rhizobium leguminosarum* bv. *viciae*) (Hauggaard-Nielsen et al. 2006). The amount of N<sub>2</sub> fixed by pea in pea-cereal intercrops depended upon species, soil type, climatic conditions, and agronomic factors including crop management and competitive abilities of the component crops (Ofori and Stern 1987). Izaurralde et al. (1992) had reported that improved BNF efficiency when pea and barley were intercropped. Biological N<sub>2</sub> fixation capacity of pea crop grown with cereals may be stimulated, presumably through competition for nitrate or ammonium from cereals in the rhizosphere (Neumann et al. 2007).

Biological N<sub>2</sub> fixation in pea can be inhibited by drought stress (Carranca et al. 1999), the supply of extra N fertilizer (Jensen 1986 and 1987) and repetitive cultivation of pea crops in the same field (Knight 2012). Small amounts of N fertilizers at the beginning of growth may stimulate pea root development (Jensen 1986), in turn, increased forage DM yield and total N accumulation (Sosulski and Buchan 1978). McKenzie et al. (2001) have reported that the application of starter N fertilizer at the rate of 20 kg ha<sup>-1</sup> can increase pea seed yield in western Canada. However, pea is sensitive to the presence of a high concentration of soil nitrate, resulting in a significant decrease of BNF (Chalifour and Nelson 1988). Malhi and Leach (2011) have reported an increase of grain yield for barley with an application rate in between 40 and 80 kg N ha<sup>-1</sup>, but it reduced seed yield of pea in pea-barley intercrop seeded in either same or alternate rows. Similarly, Cowell and van Kessel (1988) have found a greater increase of oat grain yield than pea in the pea-oat intercropping after applying additional N fertilizer. Nitrogen fertilizer application rate greater than 55 kg ha<sup>-1</sup> can inhibit nodule formation and BNF (Dry pea, Saskatchewan Ministry of Agriculture). Even though

BNF is beneficial to component crop in the legume-cereal intercropping, there is limited information available on BNF of pea in pea-cereal intercropping, and N transfer from pea to the component crop with N fertilizer application. Therefore, the objectives of this study were 1) to investigate the effect of intercropping forage pea with forage barley or forage oat on BNF, 2) transfer of fixed N from pea to barley or oat, and 3) to determine effect of N fertilizer on BNF and forage yield of different pea, cereal monocrops and pea-cereal intercrops under different growth environments.

### **5.3 Materials and methodology**

A 2-year (2016-2017) field experiment was conducted at the AAFC at Saskatoon (52° 07' N, 106° 38' W), Melfort (52° 81' N, 104° 60' W), and Swift Current (50° 28' N, 107° 75' W), Saskatchewan, Canada. The experimental design, treatment arrangement, and plot management are described in the M&M session of Chapter 3.

#### **5.3.1 $^{15}\text{N}$ isotope dilution method**

The  $^{15}\text{N}$  enriched isotope dilution method was used to estimate biological N fixation in pea (Hardarson and Danso 1990). The  $^{15}\text{N}$  isotope treatment was carried out after 3-5 weeks of pea seeding (Table 5.3). Ammonium nitrate fertilizer enriched with  $^{15}\text{N}$  isotope ( $^{15}\text{N}$  enriched  $\text{NH}_4\text{-NO}_3$ ) 11 atom % excess was applied at a rate of 6 kg ha<sup>-1</sup> (equivalent to 1 g  $^{15}\text{N}$  m<sup>-2</sup>) to the two center, 1-meter parallel rows of peas plot and reference crops. To increase the coverage of isotope application to enhance isotope absorption by plants,  $^{15}\text{N}$  enriched  $\text{NH}_4\text{-NO}_3$  was diluted in 500 ml of distilled water prior to application. The non-N fixing reference crop was oat monocrop in 2016, and barley and oat in the monocrop and in the intercrops were used as reference crops in 2017.

The rows received  $^{15}\text{N}$  enriched  $\text{NH}_4\text{-NO}_3$  were marked using wooden sticks after isotope application for sample collection (Figure 5.1).



**Figure 5.1**  $^{15}\text{N}$  isotope treated pea-cereal plots in May 2016 at Saskatoon site, SK.

Prior to forage harvest, all aboveground biomass, including seed pods, were hand-harvested with a sickle at the soil surface from the isotope treated rows per plot. To minimize edge effect, only central 0.5 of 1 m long treated rows were sampled. After sampling, the whole plot was harvested for total forage yield determination using Wintersteiger plot forage harvester Cibus F (Wintersteiger, Salt Lake, UT). The corresponding date for sub-sampling aboveground biomass for determination of BNF are shown in Table. 5.3. The samples were separated into pea, oat, and barley components by hand, and fresh biomass was dried in a forced-air oven at  $50^{\circ}\text{C}$  for 48 hrs. Dried sub-samples were passed through a Willey mill (Thomas Scientific, Swedesboro, NJ) to 2

mm, followed by 1093 Cyclotec™ mill (Foss Tecator AB, Höganäs, SE) with 1 mm screen. The ground samples were subsequently re-ground using a rotary ball-bearing mill for fine grinding. Approximately 5 g of sample was placed in a grinding steel jar with grinding media (7-10 cm of chrome steel balls) and placed on the rotary ball.

**Table 5.1** Dates for plot seeding, isotope treatment, sample collection, and forage yield harvest during the summers of 2016 and 2017 at the three experimental sites.

Year/site	Seeding	<sup>15</sup> N isotopic treatment	Sample collection	BNF period (weeks)	Forage yield determination
2016					
Melfort	20 May	9 Jun (3 wks.)	15 Aug	9.4	17 Aug
Saskatoon	16 May	6 Jun (3 wks.)	26 Jul/ 5 Aug <sup>a</sup>	7.1/8.4 <sup>a</sup>	27 Jul/ 12 Aug <sup>a</sup>
Swift Current	5 May	1 Jun (4 wks.)	29 Jul	8.2	2 Aug
2017					
Melfort	23 May	26 Jun (5 wks.)	11 Aug	6.4	14 Aug
Saskatoon	23 May	23 Jun (4 wks.)	10 Aug	6.6	10 Aug/ 17 Aug <sup>a</sup>
Swift Current	11 May	19 Jun (5 wks.)	24 Jul	5.0	Jul 26

<sup>a</sup> Pea-barley and pea-oat intercrops were harvested at two different times of cereal growth stage.

The ground samples were then weighed  $\sim 2.2 \pm 0.5$  mg using a micro-balance (Sartorius Microbalance, CPA2P, USA), and encapsulated in  $8 \times 5$  mm tin capsules (EA Consumables, Inc., NJ, USA). The encapsulated samples were analyzed for atom % <sup>15</sup>N excess and %N concentration with a Costech ECS4010 Elemental Combustion Analyzer (Costech Analytical Technologies Inc., Valencia, CA) coupled to a Delta V Advantage Mass Spectrometer with ConFlo IV interface (Thermo Scientific Inc., Bremen, DE) at the Department of Soil Science of the University of Saskatchewan. Finely ground field pea seed with an atom % <sup>15</sup>N of 0.3675 was used as the standard for the spectrometry measurement.

### 5.3.2 Calculation of biological nitrogen fixation

The percentage of N derived from atmosphere (%Ndfa) in pea was calculated according to Hardarson and Danso (1990):

$$\%Ndfa = \left(1 - \frac{\text{atom\% } ^{15}\text{N excess in pea}}{\text{atom\% } ^{15}\text{N excess in reference crop}}\right) \times 100\% \quad (8)$$

Atom%  $^{15}\text{N}$  excess was calculated by subtracting the atom%  $^{15}\text{N}$  natural abundance (0.3663 atom%) from the corresponding atom%  $^{15}\text{N}$  excess in pea monocrop, pea in pea-cereal intercrop, and reference crops. The amount of  $\text{N}_2$  fixed ( $\text{kg ha}^{-1}$ ) was calculated according to Hardarson and Danso (1990):

$$\text{N}_2 \text{ fixed } (\text{kg ha}^{-1}) = \frac{\%Ndfa \times \text{total N in pea}}{100\%} \quad (9)$$

$$\text{Total N content } (\text{kg ha}^{-1}) = \frac{\%N \times \text{DW}}{100\%} \quad (10)$$

where %N is the percentage of N content in plant, and DW is total dry matter weight ( $\text{kg ha}^{-1}$ ) of plant.

The percentage of N transfer (%N transfer) from pea to barley or oat was calculated in two different methods according to equation (Eq.11) of Ta and Faris (1987) and equation (Eq.12-13) of Johansen and Jensen (1996). The calculation method of Ta and Faris (1987) quantify transfer of soil derived and fixed N in pea to component crops. The difference between the two methods are estimating transfer of N from pea to cereal with and without including the  $^{15}\text{N}$  enriched atom% excess of cereal monocrop. It has been contradictory to use of  $^{15}\text{N}$  enriched cereal in monocrop stand as a reference crop for estimating transfer of fixed N in legume-cereal intercropping system (Chalk and Smith 1994). The root depth and N uptake patterns of cereal may be different in the

intercrop and monocrop, which may cause the ratio of non-labelled to labelled soil N to be different in the two cropping system (Jensen 1996).

$$\%N \text{ transfer} = \left( \frac{\text{atom}\% \text{ } ^{15}\text{N excess}_{\text{MC}} - \text{atom}\% \text{ } ^{15}\text{N excess}_{\text{IC}}}{\text{atom}\% \text{ } ^{15}\text{N excess}_{\text{MC}} - \text{atom}\% \text{ } ^{15}\text{N excess}_{\text{IP}}} \right) \times 100\% \quad (11)$$

where  $\text{atom}\% \text{ } ^{15}\text{N excess}_{\text{MC}}$  is a cereal monocrop,  $\text{atom}\% \text{ } ^{15}\text{N excess}_{\text{IC}}$  is a cereal in intercrop, and  $\text{atom}\% \text{ } ^{15}\text{N excess}_{\text{IP}}$  is a pea in intercrop.

$$\%N \text{ transfer} = \frac{^{15}\text{N content}_{\text{receiver}} \times 100\%}{^{15}\text{N content}_{\text{receiver}} + ^{15}\text{N content}_{\text{donor}}} \quad (12)$$

where cereal is a receiver and pea is a donor in pea-cereal intercrops.

$$^{15}\text{N content}_{\text{plant}} = \frac{\text{atom}\% \text{ } ^{15}\text{N excess}_{\text{plant}} \times \%N_{\text{plant}}}{\text{atom}\% \text{ } ^{15}\text{N excess labelled N}} \quad (13)$$

The amount of N transferred ( $\text{kg ha}^{-1}$ ) from pea to oat or barley in the pea-cereal intercrop was calculated as:

$$\text{N transferred (kg ha}^{-1}\text{)} = \frac{\%N \text{ transfer} \times \text{total N content}_{\text{IC}}}{100\%} \quad (14)$$

## 5.4 Statistical analysis

Data were analyzed as a split-plot arrangement in RCBD using the Proc Mixed Model procedure of SAS 9.4 (SAS Institute, Inc. Cary, NC). Nitrogen fertilizer, experimental site, intercrop, and their two-way and three-way interactions were treated as fixed effects, and year, replication and replication  $\times$  fertilizer interaction were considered as random effects in the model. Where interactions of experimental site, fertilizer and intercrops were significant, analysis of variance (ANOVA) was performed by each experimental site. The multiple comparisons were made using the Tukey's Honestly Significant Difference (HSD) test in SAS 9.4 at  $P \leq 0.05$ .



Analyses of orthogonal contrasts were also performed to determine significance in the comparison of different groups of treatments.

## 5.5 Results

### 5.5.1 Biological nitrogen fixation (BNF)

Analysis of Variance (ANOVA) showed that %Ndfa in pea was significantly affected by intercrop ( $P<0.001$ ), N fertilizer ( $P=0.02$ ), site ( $P<0.001$ ) and N fertilizer  $\times$  site interaction ( $P<0.001$ ) (Table 5.2). However, interactions effects of N fertilizer  $\times$  intercrop ( $P=0.16$ ), site  $\times$  intercrop ( $P=0.08$ ), and site  $\times$  N fertilizer  $\times$  intercrop ( $P=0.94$ ) were not significant for %Ndfa. Due to significant site  $\times$  N fertilizer effect, data were further analyzed by the site. The %Ndfa significantly differed among intercrops at each site ( $P<0.001$ ) (Table 5.3). The %Ndfa in pea was significantly reduced by N fertilizer at Melfort ( $P<0.001$ ) and Swift current ( $P=0.02$ ) sites, but it was not affected at Saskatoon ( $P=0.29$ ) (Table 5.3).

**Table 5.2** Analysis of variance (ANOVA) for percentage of N derived from atmosphere (%Ndfa), amount of N<sub>2</sub> fixed, and fixed N transfer in pea-cereal intercrops at Melfort, Saskatoon, and Swift Current, SK during the growth season of 2016 and 2017.

Source	df <sup>a</sup>	%Ndfa	Amount of N <sub>2</sub> fixed	%N transfer		Amount of N transferred	
				Eq. 11	Eq. 12	Eq. 11	Eq. 12
N fertilizer (F)	1	0.017	NS	NS	NS	NS	NS
Intercrop (I)	9	<0.001	<0.001	0.049	<.0001	NS	<0.001
Site (S)	2	<0.001	<0.001	NS	0.001	NS	<0.001
F $\times$ I	9	NS	NS	NS	NS	NS	NS
S $\times$ F	2	<0.001	NS	NS	0.025	0.043	<0.001
S $\times$ I	18	NS	<0.001	NS	0.008	NS	0.001
S $\times$ F $\times$ I	18	NS	NS	NS	NS	NS	NS

<sup>a</sup> Degree of freedom. NS, non-significant. Six site-year data for (%Ndfa), and three site-year data for N transferred.

The amount of N<sub>2</sub> fixed in pea varied significantly among the intercrop ( $P<0.001$ ), experimental site ( $P<0.001$ ) and intercrop  $\times$  site ( $P<0.001$ ) (Table 5.2). Nitrogen fertilizer ( $P=0.19$ ), and interactions of fertilizer  $\times$  intercrop ( $P=0.55$ ), site  $\times$  fertilizer ( $P=0.75$ ), site  $\times$  fertilizer  $\times$  intercrop ( $P=0.85$ ) had no significant effect on the amount of N<sub>2</sub> fixed. Amount of N<sub>2</sub> fixed varied significantly ( $P<0.001$ ) among the intercrops at each of three sites, but the effects of N fertilizer ( $P=0.64$ ) and fertilizer  $\times$  intercrops interaction ( $P=0.95$ ) were not significant at Melfort ( $P=0.64$  and  $P=0.95$ , respectively), Saskatoon ( $P=0.73$  and  $P=0.27$ , respectively) and Swift Current ( $P=0.17$  and  $P=0.31$ , respectively) (Table 5.4).

**Table 5.3** Percentage of N derived from atmosphere (%Ndfa) in the aboveground biomass of pea in the monocrops and in the pea-cereal intercrops with (+N) and without (-N) N fertilizer at Melfort, Saskatoon and Swift Current, SK in 2016 and 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Saskatoon		Melfort		Swift Current	
		-N	+N	-N	+N	-N	+N
CDC Horizon pea	100	56	63	46 <sup>bc</sup>	32 <sup>bc</sup>	66 <sup>ab</sup>	40 <sup>ab</sup>
40-10 pea	100	47	57	45 <sup>bc</sup>	45 <sup>abc</sup>	53 <sup>b</sup>	54 <sup>ab</sup>
CDC Horizon: barley	100:30	42	61	31 <sup>c</sup>	22 <sup>c</sup>	56 <sup>ab</sup>	42 <sup>ab</sup>
CDC Horizon: barley	50:50	69	74	46 <sup>bc</sup>	24 <sup>c</sup>	60 <sup>ab</sup>	35 <sup>b</sup>
CDC Horizon: oat	100:30	74	68	57 <sup>abc</sup>	41 <sup>abc</sup>	69 <sup>ab</sup>	53 <sup>ab</sup>
CDC Horizon: oat	50:50	64	73	57 <sup>abc</sup>	37 <sup>abc</sup>	66 <sup>ab</sup>	50 <sup>ab</sup>
40-10: barley	100:30	59	67	56 <sup>abc</sup>	31 <sup>bc</sup>	62 <sup>ab</sup>	58 <sup>ab</sup>
40-10: barley	50:50	78	72	70 <sup>ab</sup>	40 <sup>abc</sup>	77 <sup>a</sup>	61 <sup>ab</sup>
40-10: oat	100:30	81	75	76 <sup>a</sup>	56 <sup>ab</sup>	73 <sup>ab</sup>	53 <sup>ab</sup>
40-10: oat	50:50	80	84	79 <sup>a</sup>	63 <sup>a</sup>	78 <sup>a</sup>	65 <sup>a</sup>
Mean		65	69	56	39	66	51
SEM <sup>b</sup>		8.9	7.5	17.4	23.9	5.5	10.1
ANOVA							
Fertilizer (F)			NS		<0.001		0.019
Intercrop (I)			<0.001		<0.001		0.0002
F × I			NS		NS		NS
CONTRAST							
40-10 vs. CDC Horizon			NS		NS		NS
40-10 vs. 40-10: cereal			<0.001		NS		0.012
CDC Horizon vs. CDC Horizon: cereal			NS		NS		NS
CDC Horizon: cereal vs. 40-10: cereal			0.015		<0.001		<0.001
Pea vs. pea: cereal			NS		NS		NS
Pea-oat vs. pea: barley			0.008		0.003		NS
Pea-cereal (100:30) vs. (50:50)			0.014		NS		NS

Means within a column with the same lower case italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate.

<sup>b</sup> Standard error of the means.

Different ANOVA results were observed in the percentage of N transfer (%N transfer) calculated with the Eq.11 and Eq.12. The %N transfer calculated with the Eq. 11 varied significantly ( $P=0.05$ ) among the intercrops (Table 5.2). The effects of N fertilizer ( $P=0.99$ ), experimental sites ( $P=0.73$ ), and the interactions of fertilizer  $\times$  intercrop ( $P=0.89$ ), experimental site  $\times$  fertilizer ( $P=0.25$ ), experimental site  $\times$  intercrop ( $P=0.76$ ) and experimental site  $\times$  fertilizer  $\times$  intercrop ( $P=0.76$ ) were not significant for %N transfer. The %N transfer calculated with the Eq. 12 varied significantly ( $P<0.001$ ) among the intercrops, experimental sites ( $P<0.001$ ), interactions of experimental site  $\times$  fertilizer ( $P=0.025$ ), experimental site  $\times$  intercrop ( $P=0.008$ ) (Table 5.2). The effects of N fertilizer ( $P=0.18$ ), the interactions of fertilizer  $\times$  intercrop ( $P=0.36$ ), experimental site  $\times$  fertilizer  $\times$  intercrop ( $P=0.25$ ) were not significant for %N transfer calculated with Eq.12.

The amount of N transferred from pea to barley or oat in the pea-cereal intercrops was affected only by experimental site  $\times$  fertilizer interaction ( $P=0.04$ ) according to Eq.11 (Table 5.2). Fertilizer ( $P=0.23$ ), intercrop ( $P=0.11$ ), experimental sites ( $P=0.17$ ), and the interactions of fertilizer  $\times$  intercrop ( $P=0.55$ ), experimental site  $\times$  intercrop ( $P=0.48$ ) and experimental site  $\times$  fertilizer  $\times$  intercrop ( $P=0.75$ ) had no effect on the amount of N transferred. According to Eq.12 significant effect of intercrop ( $P<0.001$ ), experimental sites ( $P<0.001$ ), and the interactions of experimental site  $\times$  fertilizer ( $P<0.001$ ) and experimental site  $\times$  intercrop ( $P<0.001$ ) were observed for amount of N transferred (Table 5.2). Nitrogen fertilizer ( $P=0.10$ ), interactions of fertilizer  $\times$  intercrop ( $P=0.84$ ) and experimental site  $\times$  fertilizer  $\times$  intercrop ( $P=0.92$ ) had no effect on the amount of N transfer calculated with Eq.12.

The percentage of N derived from atmosphere (%Ndfa) for monocrop pea, and pea-cereal intercrops ranged from 52 to 82% at Saskatoon, from 27 to 71% at Melfort and from 48 to 71% at Swift Current, respectively (Table 5.3). On average, %Ndfa in intercrops was 26, 17 and 13% higher at Saskatoon, Melfort, and Swift Current, respectively compared to the pea monocrops. Among the intercrops, 40-10 pea: cereal intercrops had greater %Ndfa than CDC Horizon pea: cereal intercrops at all three experimental sites. The pea-oat intercrops had a significantly higher %Ndfa than the pea-barley intercrops at Saskatoon ( $P=0.008$ ) and Melfort ( $P=0.003$ ), but the two cropping systems did not ( $P>0.05$ ) differ at Swift Current. With the exception of 40-10 pea monocrop, addition of N fertilizer significantly reduced %Ndfa by 22-63% at Melfort ( $P<0.001$ ) and by 35-65% at Swift Current sites ( $P=0.002$ ), but did not affect the parameter at Saskatoon site ( $P=0.29$ ).

The amount of fixed  $N_2$  in aboveground biomass of pea was the highest at Melfort site (59 kg N ha<sup>-1</sup>) followed by Saskatoon (50 kg N ha<sup>-1</sup>), and the lowest at Swift Current (18 kg N ha<sup>-1</sup>) (Table 5.4). Based on pre-planned contrast comparison, the total amount of  $N_2$  fixed was significantly higher for pea in intercrop than in monocrop at all three sites ( $P<0.001$ ). On average, pea monocrops fixed 110 kg N ha<sup>-1</sup> at Melfort, 82 kg N ha<sup>-1</sup> at Saskatoon and 40 kg N ha<sup>-1</sup> at Swift Current, whereas peas in pea-cereal intercrop fixed 46, 41 and 13 kg N ha<sup>-1</sup>, respectively. Amount of  $N_2$  fixed was higher for pea-cereal intercropped at 100:30 than at 50:50 seeding ratio at Melfort ( $P=0.002$ ) and Saskatoon ( $P=0.003$ ), but no difference was found at Swift Current site ( $P=0.52$ ). Pea-oat intercrops (48 kg N ha<sup>-1</sup>) had a higher amount of  $N_2$  fixed than pea-barley intercrops (34 kg N ha<sup>-1</sup>) at Saskatoon ( $P=0.015$ ), but no difference was found at Melfort ( $P=0.68$ ) and Swift Current ( $P=0.15$ ).

**Table 5.4** Amount of N<sub>2</sub> fixed (kg ha<sup>-1</sup>) in pea in the monocrops and in the pea-cereal intercrops at Melfort, Saskatoon and Swift Current, SK in summer 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Saskatoon	Melfort	Swift Current
CDC Horizon pea	100	90 <i>a</i>	98 <i>ab</i>	43 <i>a</i>
40-10 pea	100	76 <i>ab</i>	120 <i>a</i>	37 <i>a</i>
CDC Horizon: barley	100:30	32 <i>de</i>	53 <i>cd</i>	13 <i>b</i>
CDC Horizon: barley	50:50	26 <i>e</i>	32 <i>cd</i>	10 <i>b</i>
CDC Horizon: oat	100:30	66 <i>abc</i>	51 <i>cd</i>	18 <i>b</i>
CDC Horizon: oat	50:50	34 <i>cde</i>	36 <i>cd</i>	14 <i>b</i>
40-10: barley	100:30	45 <i>cde</i>	60 <i>bcd</i>	10 <i>b</i>
40-10: barley	50:50	33 <i>de</i>	46 <i>cd</i>	10 <i>b</i>
40-10: oat	100:30	57 <i>bcd</i>	68 <i>bc</i>	12 <i>b</i>
40-10: oat	50:50	40 <i>cde</i>	25 <i>d</i>	12 <i>b</i>
Mean		50	59	18
SEM <sup>b</sup>		8.0	8.8	3.0
ANOVA				
Fertilizer (F)		NS	NS	NS
Intercrop (I)		<0.001	<0.001	<0.001
F × I		NS	NS	NS
CONTRAST				
40-10 vs. CDC Horizon		NS	NS	NS
40-10 vs. 40-10: cereal		<0.001	<0.001	<0.001
CDC Horizon vs. CDC Horizon: cereal		<0.001	<0.001	<0.001
CDC Horizon: cereal vs. 40-10: cereal		NS	NS	NS
Pea vs. pea: cereal		<0.001	<0.001	<0.001
Pea-oat vs. pea: barley		0.015	NS	NS
Pea-cereal (100:30) vs. (50:50)		0.003	0.002	NS
Pea vs. pea-cereal (100:30)		<0.001	<0.001	<0.001
Pea vs. pea-cereal (50:50)		<0.001	<0.001	<0.001

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate.

<sup>b</sup> Standard error of the means.

Two different equations used to calculate the transfer of N from pea to companion cereal provided different values (Table 5.5). The equation (Eq.12) of Johansen and Jensen (1996) gave considerably higher %N transfer in comparison to equation (Eq.11) of Ta and Faris (1987). The percentage of N transferred (%N transfer) from pea to barley or oat in the intercrops ranged from

17.3-43.4% and amount of N transferred was between 12.9-30.6 kg ha<sup>-1</sup> according to the Eq.11. In contrast, the %N transfer and amount of N transferred calculated according to Eq.12 ranged from 53.6-74.5% and 35.8-58.2 kg ha<sup>-1</sup>.

**Table 5.5** Transfer of fixed N from pea to cereal in the pea-cereal intercrops at Melfort, Saskatoon and Swift Current, SK in summer of 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	%N transfer		N transferred (kg ha <sup>-1</sup> )	
		Eq.11	Eq.12	Eq.11	Eq.12
CDC Horizon: barley	100:30	43.4 <sub>a</sub>	53.6 <sub>c</sub>	30.6 <sub>a</sub>	35.8 <sub>e</sub>
CDC Horizon: barley	50:50	32.7 <sub>ab</sub>	60.7 <sub>bc</sub>	27.2 <sub>a</sub>	46.8 <sub>bcd</sub>
CDC Horizon: oat	100:30	24 <sub>ab</sub>	71.6 <sub>bc</sub>	19.3 <sub>a</sub>	51.5 <sub>abc</sub>
CDC Horizon: oat	50:50	29.4 <sub>ab</sub>	70.9 <sub>ab</sub>	26.2 <sub>a</sub>	58.2 <sub>a</sub>
40-10: barley	100:30	33.6 <sub>ab</sub>	59.8 <sub>bc</sub>	24.6 <sub>a</sub>	39.1 <sub>de</sub>
40-10: barley	50:50	21.5 <sub>ab</sub>	67 <sub>ab</sub>	18.2 <sub>a</sub>	49.5 <sub>abcd</sub>
40-10: oat	100:30	23.6 <sub>ab</sub>	71.5 <sub>a</sub>	14.5 <sub>a</sub>	44.5 <sub>cde</sub>
40-10: oat	50:50	17.3 <sub>b</sub>	75.4 <sub>a</sub>	12.9 <sub>a</sub>	57.9 <sub>ab</sub>
SEM <sup>b</sup>		6.1	2.4	5.3	2.8
P value		0.049	<.0001	NS	<0.001

Means within a column with the same lowercase italic letter were not significantly different ( $P>0.05$ ) based on Tukey's HSD test. NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate.

<sup>b</sup> Standard error of the means. Ns, non-significant.

## 5.6 Discussion

This study was designed to examine amount of BNF of pea grown in monocrop and in intercrops, and fixed N transfer from pea to cereals in pea-cereal intercrops under N fertilized field in comparison to the unfertilized field. In this study, the amount of N<sub>2</sub> fixed and percentage of N derived from atmosphere (%Nd<sub>fa</sub>) in the aboveground biomass of pea in monocrop and in pea-cereal intercrops varied among the three experimental sites. On average, %Nd<sub>fa</sub> in pea monocrop (39-59%) in our study was similar to Hossain et al. (2017) and Chapagain and Riseman (2014) in western Canada, which was lower than the values (60-66%) reported by Izaurralde et al. (1992).

The %Ndfa in pea intercropped with barley or oat (35-82%) in our study was similar to the results of other reports (Hauggaard-Nielsen et al. 2009; Tsialtas et al. 2018).

The %Ndfa was greater when peas intercropped with barley or oat compared to pea monocrops, which is in agreement with the previous study on pea-barley intercrops (Jensen 1996). Izaurralde et al. (1992) found that pea grown in pea-barley intercrops had 39% more %Ndfa than in pea monocrops. The high %Ndfa value in pea-cereal intercrops compared to pea monocrop may be due to high competitive ability of cereals for available soil N, which promote legume to fix more N (Cowell et al. 1989; Hauggaard-Nielsen et al. 2006). Jensen (1996) reported that it is simply due to the complementary use of soil inorganic and atmospheric N sources by pea when intercropped with barley. In addition, Chapagain and Riseman (2014) reported intercropped pea had more nodules than monocropped peas, which trigger more N derived from atmosphere.

Up to 20 kg N ha<sup>-1</sup> of starter N fertilizer is recommended for pea to rapidly develop a root system before producing its own N by BNF (Dry pea, Saskatchewan Ministry of Agriculture). In our study, 60 kg of inorganic N in the soil significantly reduced %Ndfa of pea both in monocrop and intercrop at Melfort and Swift Current. Peoples and Herridge (1990) reported that a high soil mineral N content has an adverse effect on BNF of legume by inhibiting nodule formation and nitrogenase activity. Sosulski and Buchan (1977) found that application of 106 kg N ha<sup>-1</sup> application severely depressed BNF even though increased seed yield and protein yield in western Canada. Similarly, Waterer et al. (1994) found a reduction in nitrogenase activity in pea-mustard intercropping with the increase of N fertilizer rates. In addition, symbiotic rhizobia bacteria require a large amount of energy (16 moles of adenosine triphosphate (ATP) to reduce 17 g of NH<sub>3</sub>) to fix atmospheric N<sub>2</sub> (Hubbell and Kidder 2009), therefore host plant uses the available soil N before fixing atmospheric N<sub>2</sub>.



Even though the %Nd<sub>fa</sub> was higher for intercrops, the amount of N<sub>2</sub> fixed by pea was lower for intercrop than in monocrop at all experimental sites. This may be because of the low proportion of the pea DM (8-35%) in intercrop (Cowell et al. 1989; Peoples and Herridge 1990). In addition, the growth environment may also affect the total amount of N<sub>2</sub> fixed by the pea crop. In this study, the amounts of N<sub>2</sub> fixed (59 kg N ha<sup>-1</sup>) was the highest at Melfort, followed by Saskatoon (50 kg N ha<sup>-1</sup>) and the lowest at Swift Current (18 kg N ha<sup>-1</sup>). Similarly, the soil moisture condition was the highest at the Melfort, intermediate at Saskatoon, and the lowest at Brown soil during the study. Water deficit inhibits the establishment and activity of nodule and restricts nitrogenase activity (Tsialtas et al. 2018). Carranca et al. (1999) found that the amount of N<sub>2</sub> fixed by uninoculated peas ranged 31-107 kg N ha<sup>-1</sup> under normal condition, but it reduced to 4-37 kg N ha<sup>-1</sup> under drought stress.

In legume-cereal intercropping system, cereal component may receive some of the required N from legume component crop in intercrop (Fujita et al. 1992) or vice versa (He et al. 2009). Up to 80% of N transfer from N-fixing plant to non-fixing plant, whereas <10% of N transfers in reverse from non-fixing plant to N-fixing plant (He et al. 2009). In our study, a substantial amount of fixed N was transferred from pea to barley or oat components in the pea-cereal intercrops according to the calculation methods of Ta and Farris (1987), and Johansen and Jensen (1996). The Johansen and Jensen (1996) calculation method gave higher value of %N transfer and amount of N transferred from pea to component cereal than those calculated by Ta and Faris (1987) method. Ta and Faris (1987) method has low resolution because the amount of N transferred from pea, being mainly non-labelled fixed N, is small compared to the amount of N-labelled soil N taken up by cereal (Jensen 1996).

The amount of N transferred from pea to the component barley and oat in the intercrops in this study was higher than the values (1-6 kg N ha<sup>-1</sup>) reported by Chapagain and Riseman (2014). The high amount of N transfer from legume to cereal is may due to an increase in BNF and the size of the transferable N source of legume and higher demand of cereal for N in the intercrop (Tomm 1993). The component crop densities in intercropping system also can affect the amount of N transferred between two crops. Fujita et al. (1990) reported that increase in amount of N transferred from soybean to sorghum in soybean-sorghum intercropping when two plants were planted closer in adjacent rows of 12.5 × 12.5 cm compared with the 50 × 50 cm row spacing. Jensen (1996) reported the amount of N transferred increased with increased root contact of pea and barley in mixed stand. In our study, pea and cereal were intercropped completely within the same row which may resulted in higher amount of N transferred than some other studies. Nitrogen transfer may occur on the below-ground indirectly through root to root excretion, root and nodule tissue decomposition (Ta and Farris 1987) or directly mycorrhizal colonization between barley and pea in the intercropping (Johansen and Jensen 1996). In mycorrhizal meditated transfer, mycorrhizal hyphae interconnecting the root systems of the pea and cereal and the fixed N move by diffusion from pea to cereal directly even before exuding in soil (Thilakarathna et al. 2016).

In summary, %Nd<sub>fa</sub> was greater for pea-cereal intercrop than peas monocrop across the experimental sites, but N fertilizer reduced the %Nd<sub>fa</sub> at Melfort and Swift Current. In addition, even though the amount of N<sub>2</sub> fixed was not high, a significant amount of it was transferred from pea to cereal in the intercrops.

## General Discussion

This study evaluated agronomic performance, biological N<sub>2</sub> fixation, and nutritive value of different intercrops of pea-barley and pea-oat in comparison to monocrops of pea, barley, and oat at three research sites at Melfort, Saskatoon, and Swift Current, SK in 2016 and 2017. Forage DM yield, botanical composition, plant lodging resistance, plant height, LER, competitive ratio, drying time, forage nutritive value (PY, CP, NDF, ADF, and starch) and BNF of pea were measured. The results of this study showed that intercropping barley and oat with pea produced higher forage DM yield than pea monocrop, which was similar or slightly lower than barley and oat monocrops. Lower DM yield of legume-cereal intercrops compared to cereal monocrops has been reported in previous studies (Dordas et al. 2012, Aasen et al. 2004; Begna et al. 2011; Gill and Omokanye 2018). However, pea intercropped with barley or oat appeared to be more efficient in growth resource utilization than monocrops of barley, oat, and pea based upon the LER. The value of the LER for forage DM yield was greater than 1.0 for all the pea-cereal intercrops in this study. Similarly, Chen et al. (2004) and Ofori and Stern (1987) reported an improved efficiency of growth resources by pea-cereal intercropping compared to monocrops. This may be due to the complementarity relationship between legume and cereals (Nielsen et al. 2001), because shallow (pea) and deep (cereal) root systems possibly uptake nutrient and water from different soil layers, especially in a resource limited environment. Jensen (1996) stated that barley used more growth resources in its early stages when pea needed them in its later growth stages. In addition, pea-cereal intercrops in our study produced higher CP concentration and PY than barley and oat monocrops, which can offset the cost and forage DM yield loss over cereal monocrops (Gill and Omokanye 2018). In general, pea-barley and pea-oat intercrops produced adequate CP concentrations (i.e., 89 to 105 g DM kg<sup>-1</sup>) to meet protein requirement (70-90 g DM kg<sup>-1</sup>) of

gestating beef cows and replacement beef heifers (National Research Council 2000). However, other macro and micronutrients along with nitrate accumulation level should be examined to fully understand nutritive values of pea-cereal intercrops. The results obtained here partially supported our first hypothesis that intercrops may increase forage DM yield over cereal monocrops.

Pea crop was more effective for fixing atmospheric  $N_2$  in intercrop than in pea monocrop system, which was consistent at the all three experimental sites. In pea-cereal intercrop, cereals may stimulate BNF of legumes, presumably through competition for nitrate or ammonium in the rhizosphere (Neumann et al. 2007). In addition, it is interesting to find that substantial fixed N by pea was transferred directly to barley and oat in the pea-barley or pea-oat intercrops in our study. The amount of N transferred from pea to cereal was between 13 and 31 kg N ha<sup>-1</sup>. This is somewhat unexpected as legume crops generally are able to fix 80% of its own required N through BNF.

Forage DM yield was similar between pea-cereal seeding ratios of 100:30 and 50:50 at Saskatoon and Swift Current sites, but the former had higher PY per area. However, pea-cereal intercrops seeded in 50:50 ratio had higher DM yield than 100:30 when they did not differ in PY. In general, the proportion of pea in intercrops was less than expected, which was mainly due to inter-species competition between the component crops. Similarly, Carr et al. (1998) reported that barley and oat accounted more than pea for total DM yields of pea-barley and pea-oat intercrops resulting in no seeding ratios effects. Pea-cereal intercrops seeded both in 100:30 and 50:50 ratios had little impact on drying time, possibly due to less pea portion presented during the harvested forage. Therefore, weather condition, especially continues rainfall after harvesting, was the most significant factor affecting the drying of pea-cereal intercrop in the field. Our hypothesis “drying time will be lower in pea-cereal intercrops compared to monocrops of cereals, and time will be decreased with the increase of pea component in intercrops” was partially accepted.

High seeding ratio of peas in the intercrops increased seed cost for the pea-cereal intercrops partially due to their large seed size. Even though higher seeding cost for barley monocrop and pea-barley intercrops was noticeable, seeding ratio of 50:50 was still a profitable combination due to higher feed value and demand in the current market. The selection of compatible plant species and cultivars is important to factor to get benefit from the legume-cereal intercropping system (Jensen 1996). Pea-barley intercrop was more suitable at Saskatoon site because of its higher DM yield, PY, total starch, and lower ADF and NDF concentrations than pea-oat intercrop, but there was not difference between the two different intercrops at Melfort and Swift Current sites. This implies that the choice of cereal species perhaps is not crucial than seeding ratio for improving forage DM yield and nutritive values.

The performance of pea cultivars used in this study did not differ significantly in terms of DM yield, forage nutritive value, drying time, economic return, and BNF. CDC Horizon pea showed improved lodging resistance compared to 40-10 pea, which may be due to their difference in their genetics. The extent of lodging for 40-10 pea reduced significantly in the intercrops, which may help make harvesting easier and minimize foliar diseases and yield loss. Podgorska-Lesiak and Sobkowicz (2013) reported that conventional, leafy pea cultivars benefited more to mechanical support provided by cereals and led to substantial increases in DM yield in pea-barley intercrop. This study supported our hypothesis that CDC Horizon would have higher lodging resistance than that of 40-10 peas.

The effect of N fertilizer on intercrops and monocrops varied among the soil zones. In general, the intercrops LER was affected by N fertilizer. Nitrogen fertilizer increased DM yield, CP and PY of monocrops and pea-cereal intercrops at Melfort site, but it reduced lodging resistance, %Ndfa, and increased input cost. Nitrogen fertilizer had no agronomic and economic

advantages on monocrops and intercrops at Saskatoon and Swift Current sites. Nitrogen fertilizer caused a reduction of %Ndfa in monocropped and intercropped peas, but it has no impact on DM yield, CP, and PY in those sites. This indicated that the N fertilizer is not critical as pea can fix more atmospheric N in the pea-cereal intercropping system without N fertilizer. Our hypothesis, therefore, “application of N fertilizer will reduce BNF of a pea, and the reduction will be less in pea-cereal intercrops compared to pea monocrop” was accepted. The pea-barley and pea-oat combinations in the present study showed that they can be a desirable option for greenfeed or silage for producers in western Canada compared to barley or oat monocrops.

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## **Appendix A. Atom% $^{15}\text{N}$ Excess of Monocropped and Intercropped Pea, Barley and Oat**

In 2016, mean (n=4) values of atom%  $^{15}\text{N}$  excess in aboveground biomass of monocropped oat was used as the reference crop for determining BNF in monocropped and intercropped peas at all experimental sites (Table A.1). In 2017, mean (n=4) value of atom%  $^{15}\text{N}$  excess of monocropped barley was used as the reference plant for measuring BNF of the monocropped pea, whereas the intercropped barley and oat as used as the reference plants in the corresponding pea-barley/oat intercrops. In 2016, atom%  $^{15}\text{N}$  excess in oat monocrop with N was 0.139 and 0.163 at Melfort and Swift Current, respectively whereas without N fertilizer it was 0.215 and 0.200 at Melfort and Swift Current site, respectively. At Saskatoon, atom%  $^{15}\text{N}$  excess of 0.233 in oat was used for determining BNF in pea-barley intercrops without N, and 0.281 in oat was used for pea monocrops and pea-oat intercrops without N fertilizer. Atom%  $^{15}\text{N}$  excess 0.278 and 0.316 in oat was used to determining the corresponding pea-barley and pea monocrops and pea-oat intercrops with N fertilizer. In 2017, atom%  $^{15}\text{N}$  excess in barley and oat in monocrop and in intercrops at Saskatoon ranged from 0.217 to 0.536 without N fertilizer and from 0.241 to 0.494 with N fertilizer. At Melfort ranged from 0.164 to 0.359 without N and from 0.128 to 0.249 with N fertilizer. At Swift Current ranged from 0.144 to 0.228 without N and from 0.125 to 0.210 with N fertilizer. Intercropped cereal did not differ in atom%  $^{15}\text{N}$  excess than their monocropped counterparts, but atom%  $^{15}\text{N}$  was slightly greater for cereals with than without N fertilizer applied at Melfort and Swift Current sites.

**Table A.1** Atom%  $^{15}\text{N}$  excess in aboveground biomass of oat and barley (reference crops) with (+N) and without (-N) N fertilizer at Saskatoon, Melfort, and Swift Current, Saskatchewan in 2016 and 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Saskatoon		Melfort		Swift Current	
		-N	+N	-N	+N	-N	+N
2016							
Oat	100	0.233/ 0.281 <sup>b</sup>	0.278/ 0.316	0.215	0.139	0.200	0.163
2017							
Barley	100	0.309	0.361	0.232	0.246	0.208	0.153
Oat	100	0.536	0.494	0.346	0.150	0.228	0.188
CDC Horizon: barley	100:30	0.217	0.276	0.164	0.128	0.144	0.125
CDC Horizon: barley	50:50	0.287	0.277	0.239	0.162	0.177	0.137
CDC Horizon: oat	100:30	0.382	0.318	0.274	0.219	0.186	0.166
CDC Horizon: oat	50:50	0.331	0.392	0.288	0.167	0.175	0.136
40-10: barley	100:30	0.274	0.241	0.197	0.143	0.161	0.210
40-10: barley	50:50	0.302	0.273	0.250	0.249	0.177	0.160
40-10: oat	100:30	0.379	0.420	0.273	0.190	0.184	0.162
40-10: oat	50:50	0.354	0.463	0.359	0.249	0.178	0.208
2017 ANOVA							
Fertilizer (F)		NS		NS		NS	
Intercrop (I)		0.01		ns		0.051	
F × I		NS		NS		NS	

NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> Due to different timing of harvest for pea-barley and pea-oat intercrops at Saskatoon in 2016, two different references were used.

The values of atom%  $^{15}\text{N}$  excess in monocropped and intercropped peas were generally lower than those in reference crops, which indicates that the  $^{15}\text{N}$  uptake from soil was diluted significantly by atmospheric  $\text{N}_2$ . Two-year mean (2016-2017) values of atom%  $^{15}\text{N}$  excess in the aboveground of pea in monocrop and in intercrops ranged from 0.062 to 0.154 without N and from 0.056 to 0.143 with N fertilizer at Saskatoon site (Table A.2). At Melfort, atom%  $^{15}\text{N}$  excess in pea without N ranged from 0.052 to 0.138 and from 0.067 to 0.162 with N fertilizer, whereas at Swift Current site it ranged from 0.039 to 0.096 without N and 0.059 to 0.099 with N fertilizer.

**Table A.2** Atom%  $^{15}\text{N}$  excess in the aboveground biomass of pea in the monocrop and in the pea-cereal intercrops with (+N) and without (-N) N fertilizer at Saskatoon, Melfort and Swift Current, Saskatchewan in 2016 and 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Saskatoon		Melfort		Swift Current	
		-N	+N	-N	+N	-N	+N
CDC Horizon pea	100	0.127	0.123	0.124	0.123	0.069	0.098
40-10 pea	100	0.154	0.143	0.122	0.107	0.096	0.072
CDC Horizon: barley	100:30	0.117	0.092	0.138	0.129	0.070	0.080
CDC Horizon: barley	50:50	0.062	0.069	0.123	0.162	0.073	0.099
CDC Horizon: oat	100:30	0.080	0.095	0.100	0.102	0.058	0.074
CDC Horizon: oat	50:50	0.137	0.089	0.098	0.088	0.066	0.072
40-10: barley	100:30	0.093	0.080	0.090	0.099	0.066	0.078
40-10: barley	50:50	0.056	0.073	0.067	0.106	0.043	0.060
40-10: oat	100:30	0.063	0.082	0.057	0.067	0.051	0.075
40-10: oat	50:50	0.061	0.056	0.052	0.069	0.039	0.059
ANOVA							
Fertilizer (F)		NS		NS		0.028	
Intercrop (I)		<0.001		<0.001		0.0003	
F × I		NS		NS		NS	

NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

## **Appendix B. Nitrogen Content of Pea in Monocrops and in Intercrops**

Intercrop ( $P<0.001$ ), experimental site ( $P<0.001$ ) and interactions of site  $\times$  intercrop ( $P=0.001$ ) and site  $\times$  fertilizer ( $P=0.03$ ) had a significant effect on N content of pea in monocrops and in intercrops (Data not shown). Fertilizer ( $P=0.20$ ) and interactions of fertilizer  $\times$  intercrop ( $P=0.87$ ) and site  $\times$  fertilizer  $\times$  intercrop ( $P=0.99$ ) had no significant effect on N content.

Nitrogen content of peas was significantly affected by intercrop at Saskatoon ( $P=0.03$ ), Melfort ( $P=0.0002$ ) and Swift Current sites ( $P<0.001$ ) (Table B.1). Fertilizer had a significant effect on N content of peas only at Melfort site ( $P<0.001$ ), but not at Saskatoon ( $P=0.29$ ) and Swift Current ( $P=0.93$ ). The interaction of fertilizer  $\times$  intercrop was not significant for N content of peas at Saskatoon ( $P=0.63$ ), Melfort ( $P=0.91$ ) and Swift Current ( $P=0.81$ ).

The percentage of N content in pea in the monocrops and in the intercrops ranged from 2.2-2.5% at Saskatoon, 1.9-2.4% at Melfort and 1.9-2.5 at Swift Current site. Nitrogen fertilizer increased percentage of N content in pea by 3-16% at Melfort site only.

**Table B.1** Percentage of N content in the aboveground biomass of pea in the monocrop and in the pea-cereal intercrops with (+N) and without (-N) N fertilizer at Saskatoon, Melfort and Swift Current, Saskatchewan in 2016 and 2017.

Treatment	Component crop ratio <sup>a</sup> (%)	Saskatoon		Melfort		Swift Current	
		-N	+N	-N	+N	-N	+N
CDC Horizon pea	100	2.4	2.4	1.9	2.2	2.3	2.3
40-10 pea	100	2.5	2.5	2.2	2.6	2.4	2.6
CDC Horizon: barley	100:30	2.3	2.3	2.0	2.1	2.0	2.0
CDC Horizon: barley	50:50	2.3	2.3	1.9	2.0	2.0	2.0
CDC Horizon: oat	100:30	2.3	2.2	2.0	2.2	2.2	2.3
CDC Horizon: oat	50:50	2.3	2.2	2.0	2.2	2.3	2.2
40-10: barley	100:30	2.3	2.3	2.0	2.1	2.0	2.1
40-10: barley	50:50	2.4	2.4	2.1	2.1	2.0	1.9
40-10: oat	100:30	2.5	2.1	2.1	2.3	2.4	2.4
40-10: oat	50:50	2.4	2.5	2.0	2.3	2.5	2.3
Mean		2.4	2.3	2.0	2.2	2.2	2.2
SEM <sup>b</sup>		0.09	0.13	0.24	0.11	0.23	0.21
ANOVA							
Fertilizer (F)		NS		<0.001		NS	
Intercrop (I)		0.039		0.0002		<0.001	
F × I		NS		NS		NS	

NS, non-significant.

<sup>a</sup> Component crop seeding ratio was calculated as a percentage of full seeding rate of individual monocrop.

<sup>b</sup> Standard error of the means.



## Appendix C. Forage Pea-Cereal Intercrops



**Appendix C.1** Forage 40-10 pea: oat intercropped in 50:50% of full seeding rate at Saskatoon site on 26 July 2017



**Appendix C.2** Forage CDC Horizon pea: barley intercropped in 50:50% of full seeding rate at Saskatoon site on 26 July 2017





**Appendix C.3** Forage CDC Horizon pea: barley intercropped in 100:30% of full seeding rate at Saskatoon site on 26 July 2017



**Appendix C.4** Forage 40-10 pea: oat intercropped in 100:30% of full seeding rate at Saskatoon site on 26 July 2017





**Appendix C.5** Forage CDC Horizon pea: barley intercropped in 100:30% of full seeding rate at Swift Current site on 24 July 2017



**Appendix C.6** Forage CDC Horizon pea: oat intercropped in 100:30% of full seeding rate at Swift Current site on 24 July 2017





**Appendix C.7** Forage CDC Horizon pea: barley intercropped in 100:30% of full seeding rate at Melfort site on 11 August 2017



**Appendix C.8** Forage CDC Horizon pea: oat intercropped in 100:30% of full seeding rate at Melfort site on 11 August 2017