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Nutrient Composition and Protein Extractability of Oat Forage Harvested at Different Maturity Stages as Compared to Grain

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Abstract

Two oat varieties (CDC Dancer and Lamont) were grown in fields. Plants were harvested at three key stages: seedling (Forage 1), mid-season (Forage 2), and full grain maturity. The mature plants were separated into stover (Forage 3) and Grain. There were differential changes among measured attributes during growth. From seeds to Forage 1, crude protein (CP) and ash contents increased several fold, beta-glucan decreased, starch disappeared, and oil content remained unchanged for both oat varieties examined. As plants grew, CP, ash, oil and beta-glucan decreased, but other carbohydrates increased significantly. The CP content in Forage 1 was 5–fold higher than Forage 3 and 2-fold higher than Grain. Most macro and micro-minerals in forage followed the same changing pattern as ash. Amino acid composition of forage differed significantly from that of Grain. When subjected to aqueous media with a wide range of pH, forage harvested at each stage as well as grain all had their unique protein extractability curves. For forage, the peak value (the maximum extraction) decreased with maturity. Yet, as observed for both varieties, Forage 1 had a peak value half of that from Grain. In conclusion, based on the results from this study, nutrient composition in oat forages harvested at an early stage can be comparable or even superior to oat grains but low protein extractability limits oat forage use as an alternative protein source for non-ruminants.

Keywords: oat, *Avena sativa*, forage, maturity, protein extraction, composition, amino acids, minerals

1. Introduction

Oats (*Avena sativa* L.) rank seventh in world cereal production (about 23 million metric tons in 2013), following maize, rice, wheat, barley, sorghum and millet (FAOSTAT, 2015). They have many uses as food cereal, feed grain, and green or conserved forage, and also in topical skincare products. Livestock grain feed is the primary use, accounting for about 74% of the world's total production. As a food cereal, oats are among most nutritious: high in protein, oil, and beta-glucan (a soluble dietary fiber). As forage for livestock, whole-crop green oats may be grazed, cut-and-carried, ensiled or made into hay for later use (Webster & Wood, 2011). Colloidal oatmeal obtained from whole oats is used in skincare products to help relieve various skin conditions like dry skin, sunburn, and skin irritation (Mahmood et al., 2013).

One of the major factors that affect the nutritional value of oat forage is the stage of maturity at which it is harvested. Earlier workers studied the effects of maturity at advanced stages of growth on oat forage biomass yield and nutrient contents for livestock feed, and they concluded that the best time to harvest oat for forage is in the late milk to early or mid-dough growing stage (Gardner & Wiggans, 1961; Erickson et al., 1977; Derscheid, 1978; Cherney & Marten, 1982). Smith (1960) documented changes in yield and chemical composition of oat forage from early growth (four leaves) all the way to matured stage, and also concluded that the best stage to harvest forage for hay or silage is the early dough stage. However, little is known about amino acid (AA) composition and protein extractability of oat forage as affected by maturity. Although there are reports on

changes of a few micro-minerals in oat forage during growth (Smith, 1960; Erickson et al., 1977; Cherney & Marten, 1982), a study on changes in a complete profile (macro- and micro-minerals) is lacking. Furthermore, there have been no reports for direct comparison of any of the above attributes between oat forage and oat grain.

In another development, increasing cost and limiting availability of animal proteins have created a need to identify alternative protein sources for use as human food and/or feed for non-ruminants (including fish feed). Considerable emphasis has been focused on the use of conventional plant proteins, such as proteins from oilseeds, cereals and their by-products. Yet, an effort has also been made to evaluate unconventional protein sources, such as leaf proteins (Fiorentini & Galoppini, 1983; Olvera-Novoa et al., 1990; Kammes et al., 2011). The objective of the present study was to evaluate the effects of harvesting stages on general composition, mineral contents, amino acid composition, and pH dependent protein extractability of oat forage. Suitability of oat forage as an alternative source of proteins for human and non-ruminants was also examined along with oat grain.

2. Materials and Methods

2.1 Oat Forage and Grain Production

Two oat varieties, CDC Dancer (hulled) and Lamont (hulless), were selected and sown on two replicate plots for each in early May of 2014 at the University of Idaho Agricultural Experimental Station, Aberdeen, Idaho. A fertillizer consisting of both N and P was applied before planting. During the growing season, the plots were sprinkler irrigated as needed, generally every 4 days at the early stages and once a week in late stages. Forage was sampled at three growing stages, expanding from early growth to maturity: Stage 1, plantlets with 4 to 8 leaves; Stage 2, plants almost ready to flower; and Stage 3, grains hardened and fully matured in the field. These three distinct stages represented: 1) the early seedling stage, 2) the mid-season growing stage, and 3) maturity stage, respectively. Since CDC Dancer grew a little faster than Lamont, the dates for harvesting forage samples at each of three stages differed slightly from each other, as both varieties were sown on the same day. For every stage, sample collection for CDC Dancer was a few days earlier than Lamont, and this difference enlarged as the growth stage advanced. Forage at Stage 1 was sampled by pulling plantlets out of the soil and roots were immediately cut off with scissors. For Stages 2 and 3, only above soil plant samples were used. Plants harvested at Stages 1 and 2 are termed as Forage 1 and Forage 2, respectively. For Stage 3 plants, stover and grain were separated and termed as Forage 3 and Grain, respectively. Two replicate samples were collected from each stage for each variety.

2.2 Oat Forage Drying

Immediately upon collection/harvesting, oat forage and grain samples were weighed and fan dried on a rack in a greenhouse for 4 days and then further dried in a forage-dryer at 32 °C for a week. After drying and reweighing (for calculating initial moisture content), the forage samples were chopped into small pieces, ground into fine powder (to pass 0.5 mm screen) by a Cyclone grinder, and stored in sealed bags at 4 °C before chemical analysis. The grain samples bypassed the chopping step. This low temperature drying was needed to minimize protein denaturation.

2.3 Compositional Analysis

Ground forage and grain samples were analyzed for moisture, oil, protein, ash, beta-glucan, and starch contents. Moisture and ash contents were determined according to published official methods (AOAC, 2012). The protein content was measured by a published combustion method (AOAC, 2012) using a protein analyzer (Model FP-528, Leco Corp. St. Joseph, MI, USA), and a factor of 6.25 was used for converting total nitrogen to crude protein content (i.e. % crude protein = % N × 6.25). Beta-glucan was measured according to Method 995.16 (AOAC, 2012), using the beta-glucan assay kit supplied by Megazyme International Ireland Ltd. (Wicklow, Ireland). The oil content was determined by an Official Procedure (AOCS, 2005), using a fat analyzer (Model XT 10, Ankom Technology, Macedon, NY USA). However, instead of using petroleum ether, hexane was the extracting solvent. Starch was measured according to an enzymatic method described elsewhere (Liu & Han, 2012). Total CHO content was calculated based on the difference between 100% and the sum of contents of protein, oil, and ash, on % dry matter basis. The content of other carbohydrates (CHO), the term referring to the total carbohydrates excluding beta-glucan and starch, was calculated based on the difference between total CHO and sum of beta-glucan and starch. It includes soluble sugars, cellulose, hemicelluloses, and lignin.

Mineral elements were determined using a Perkin-Elmer Optima 3200 ICP-OES (inductively coupled plasma-optical emission spectrometer) to quantify constituents in an aqueous solution following nitric acid digestion of the samples. Standard quality control measures, including blanks, check standards, and reference

materials were used for all chemical analyses. Amino acid composition was analyzed according to an AOAC official method (AOAC, 2012). Briefly, after hydrolysis in 6N HCl/2% phenol at 110 °C for 22 hr, samples were analyzed for individual AA concentrations, using an amino acid analyzer (Model L-8900, Hitachi, Chyoudaku, Japan), with a Hitachi AAA PH column (special analysis, Category #855-4516), and norleucine as an internal standard. Cystine, cysteine, and tryptophan were not analyzed due to low contents.

The pH dependent protein extractability was carried out as follows: three grams of ground oat forage or oat grain sample were vortexed with 30 mL water in a 50 mL centrifuge tube. The pH of the mix was adjusted to a given value with 2M NaOH or 2M HCl solution. All tubes were placed horizontally in a shaker having a feature of back and forth shaking, and were shaken for 90 min at a speed of 100 cycles (one cycle = one back and forth action) per min. Upon extraction, tubes were centrifuged at 1000 × g for 10 min. The supernatant was discarded. The precipitate (residue) was saved, dried at a forced air oven at 60 °C for 48 hrs, ground into powder and analyzed for protein content. The amount of protein extracted into supernatant was the difference in the amount of protein between the starting sample and the residue.

2.4 Statistical Analysis

Data on chemical composition of forage and grains were analyzed with a JMP software, version 10 (JMP, a Business Unit of SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA) under the factorial model with replicates in order to determine effects of variety, forage stage/grain, and their interactions on measured parameters. The significant level was set at $p < 0.05$.

3. Results and Discussion

3.1 Chemical Composition of Growing Oat Forages and Seeds/Grains (Table 1)

From seeds for planting to seedling (Forage 1) of CDC Dancer, contents of CP, ash, other CHO significantly increased, while starch, beta-glucan and total CHO decreased significantly. Specifically, the CP content almost tripled (from 11.60% to 32.56%), ash increased about 4.5 fold (from 3.40% to 15.19), beta-glucan decreased from 2.53% to 1.59%, and starch almost disappeared (from 45.27% to 0.19%). At the same time, other CHO increased from 29.84% to 45.63%, and total CHO decreased from 80.70% to 48.04%. Oil content remained relatively unchanged.

Table 1. Chemical composition of forage of two oat varieties harvested at three stages, seeds for initial planting, and seeds upon harvest

Variety	Stage	Moisture at harvest	Moisture after dry	Crude protein	Oil	Ash	Starch	Beta-glucan	Other CHO	Total CHO
CDC Dancer	Seed for planting		8.05 b	11.60 g	4.31 c	3.40 d	46.15 b	2.53 c	32.01 d	80.70 a
CDC Dancer	Forage 1	86.47 a	5.28 de	32.56 b	4.21 c	15.19 a	0.19 d	1.59 d	46.26 c	48.04 f
CDC Dancer	Forage 2	82.83 b	3.55 h	17.24 d	2.51 d	11.43 c	0.17 d	0.46 e	68.19 b	68.81 e
CDC Dancer	Forage 3	75.46 c	3.21 h	6.23 h	1.93 d	12.54 b	0.16 d	0.20 e	78.94 a	79.30 b
CDC Dancer	Grain	33.51 d	6.44 c	12.31 f	4.05 c	3.55 d	44.16 c	3.64 a	31.79 d	80.09 ab
Lamont	Seed for planting		9.14 a	17.08 d	7.77 a	2.08 e	52.96 a	3.03 b	17.08 e	73.08 d
Lamont	Forage 1	85.90 a	5.11 ef	34.84 a	4.59 c	15.21 a	0.13 d	1.67 d	43.56 c	45.37 g
Lamont	Forage 2	81.83 b	4.52 g	18.02 c	2.49 d	11.57 c	0.05 d	0.46 e	67.41 b	67.92 e
Lamont	Forage 3	73.91 c	4.77 fg	6.03 h	1.93 d	11.78 c	0.03 d	0.22 e	80.01 a	80.26 ab
Lamont	Grain	28.27 e	5.64 d	16.58 e	6.42 b	2.26 e	53.39 a	3.99 a	17.36 e	74.74 c

Note. Mean of two replicate results, expressed as % dry matter except for the moisture content. CHO, carbohydrates.

Column means with different letters differed significantly at $p < 0.05$.

The significant differences in CP, ash, starch, beta-glucan, other CHO, and total CHO contents between Forage 1 and seed for planting confirm that transition from seeds to seedling is featured by a drastic and rapid change in biochemical composition. The most significant changes were seen with starch and protein contents with a net negative change of 99.6% and a net positive change of 280%, respectively, going from seeds to seedlings. Higher ash content in seedlings over seeds is indicative of higher level of micro-nutrients present and are discussed in

more detail later in the text.

From Forage 1 to Forage 3, CDC Dancer showed a decreasing trend for almost all attributes except for carbohydrates and starch (Table 1); e.g. the moisture content at the time of harvest decreased from 86.47 to 75.46%, CP content from 32.56 to 6.23%, oil content from 4.21 to 1.93%, ash content from 15.19 to 12.54%, and beta-glucan from 1.59 to 0.20%. Starch was almost absent (< 0.2%) with no significant changes in all three forage samples. In contrast, other CHO increased from 46.26 to 78.94%, and total CHO increased from 48.04 to 79.30%.

Although Forage 3 and harvested seeds samples came from the same plants of the matured stage, the two had very distinct chemical composition profiles. This confirms that chemical composition is tissue specific. Specifically, for CDC Dancer, the grain was about 2 times higher than Forage 3 in CP (12.31% vs. 6.23%) and oil (4.05% vs. 1.93%) contents, but 3.5 times less in ash and 2.4 times less in other CHO. The moisture content upon harvesting also exhibited decreasing trend from Forage 3 to Grain. Total CHO content remained same in both Forage 3 and grain. Starch and beta-glucan contents were present at a significantly higher level in grains. Note that compositional data on measured attributes of seeds for planting and of harvested grain changed slightly in both directions, reflecting some environmental and growing year effects.

Though Lamont is a hulless variety, the changing trends in composition of forages and grain, were very similar to those found with CDC Dancer. Interestingly, though the grain composition between the two varieties differed significantly, the composition of forage harvested at the same stage varied little between the two varieties. The larger compositional difference of grain between the two varieties over their forage could be attributed to the presence of hulls in grain of CDC Dancer.

Smith (1960) showed that the contents of CP, oil, and ash declined from early growth to maturity. Erickson et al. (1977) reported that protein and ash decreased with advancing maturity of oat forage, but the fiber level remained unchanged. Cherney and Marten (1982) noticed that with maturation, four small grains (barley, oat, wheat, and triticale) all had a decreasing CP content and increasing cell wall constituents. The findings in current study on compositional changes of growing forages were consistent with all the previous reports (Smith, 1960; Erickson et al., 1977; Cherney & Marten, 1980). However, the previous investigators did not measure contents of starch and beta-glucan, nor did they compare composition of growing forage to oat grain. Such additional information provided from this study is valuable with respect to oat biochemistry and value added utilization of oat forage. For example, results from the current study showed that unlike starch, which disappeared even from Forage 1, beta-glucan content decreased gradually to a very low level during the entire growing season. Thus beta-glucan was less tissue specific than starch since the latter was synthesized only in oat grains.

3.2 Macro- and Micro-Mineral Composition of Growing Oat Forages and Matured Grains (Table 2)

From oat sown seeds to seedling (Forage 1), there was over 4-fold increase in ash content (Table 1). As the seedling grew into Forage 2, the ash content decreased, but it remained high in maturing forage. In order to know the changing patterns of individual minerals, samples were also measured for mineral composition. Data (Table 2) show that forage and grain samples contained 6 macro minerals, Ca, Mg, P, K, Na, and S, and as many as 8 detectable micro minerals, Al, Ba, Cu, Fe, Mn, Ni, Si, and Zn. The mineral profile of Forage 1-3 within a variety had unique patterns. Growing from Forage 1 to Forage 3, CDC Dancer showed decreasing contents of eight minerals (Mg, P, K, S, Cu, Mn, Ni, and Zn). The contents of Ca, Al, Ba, and Fe decreased from Forage 1 to Forage 2, and then either slightly increased or stayed the same to Forage 3. The contents of Na and Si increased from Forage 1 to Forage 3, and this was true for both varieties. Most of the mineral profiles and changes during forage growth described above for CDC Dancer were the same in the Lamont variety.

The differences in the contents of all minerals between Forage 3 and harvest grain followed a pattern which was consistent for both varieties. Data in Table 2 show increasing contents of four minerals, P, S, Ni, and Zn and no change in content of Cu in grain, as compared to Forage 3. All the other minerals showed a lower content in grain than Forage 3. A few additional observations are worth noting here. Grain and Forage 1 both had the highest P content. As forage matured, P content decreased significantly from Forage 1 to 3 and then accumulated at higher levels in grain. Na showed a pattern opposite to that P.

Mineral changes during oat forage growth have been investigated by several previous studies, but none documented as many minerals as the current study. Smith (1960) found that the content of P, Ca, and K in oat forage declined from early growth to maturity. Erickson et al. (1977) reported that P and K decreased with advancing maturity of oat forage, but Ca and Mg levels were not affected by growth stage. Cherney and Marten (1982) showed that all four small grain species (barley, oat, wheat, and triticale) followed a general decrease in concentration of four macro-minerals, namely, Ca, Mg, K, and P. The current study agreed with findings of

Smith (1960) and Cherney and Marten (1982) on changes in macro minerals during oat forage growth. Again, none of the previous studies compared the mineral profile of growing forage directly to oat grain, nor profiled contents of micro-minerals. The findings on the concentrations and changes of macro and micro minerals in oat forage during growth can be useful for animal and also for human nutritional applications.

Table 2. Mineral composition of oat forage harvested at different stages and of seeds upon new harvest

Maturity	Ca	Mg	P	K	Na	S	Al	Ba	Cu	Fe	Mn	Ni	Si	Zn
	----------mg/g----------						----------μg/g----------							
CDC Dancer														
Forage 1	5.55b	3.05b	4.95b	58.00a	1.55e	4.40b	490a	8b	17a	410a	150 a	7b	35ab	79b
Forage 2	3.45e	2.00d	2.40e	38.50c	6.55b	2.50c	170c	2c	12b	110c	130b	5d	41ab	52c
Forage 3	4.25d	1.70e	0.75f	29.00d	8.95a	1.75e	170c	10a	8 d	115c	120c	<2e	73a	37f
Grain	0.87f	1.40f	4.05c	4.85e	<0.2g	1.75e	37f	1d	8d	9e	57e	7b	26ab	40e
Lamont														
Forage 1	7.00a	3.55a	5.30a	59.00a	0.99f	4.80a	425b	8b	17a	380b	150a	0	28ab	83a
Forage 2	4.95c	2.45c	2.75d	43.00b	2.75d	2.40cd	110e	3c	10c	88d	130b	5d	38ab	46d
Forage 3	4.50d	2.05d	0.65f	32.50d	5.90c	1.55e	125d	0	7d	98d	90 d	<2e	59ab	21g
Grain	0.82f	1.65e	5.05ab	4.90e	<0.2g	2.10d	<2g	<1d	6e	10e	51f	6c	13b	39ef

Note. Mean of two replicate results, expressed as a dry matter basis. Column means with different letters differed significantly at $p < 0.05$.

3.3 Amino Acid Composition of Growing Oat Forages and Matured Grains (Table 3)

The amino acid (AA) composition is one of the most commonly used criteria to judge the nutritional value of a protein. When AA composition is expressed as % of total amino acids recovered, this value becomes independent of the protein content in samples. This makes comparisons among studies possible. In Table 3, the AA compositions of forage and grain from two oat varieties for the present study are shown, along with those of alfalfa leaves, orchard grass, and whole oat meal adapted from previous reports (Fiorentini & Galoppini, 1983; Kammes et al., 2011; Draper, 1973), all expressed as % of total AA recovered for easy comparison. Results indicate that AA composition of oat forage changed with growing stages and differed from that of grain. The changing patterns as well as the extent of differences varied with each individual AA. For example, Arg was lowest in Forage 2 but highest in grain, while His, Met, and Val varied slightly among the 4 samples. Asp was highest in Forage 2, while Glu was highest in grain. All forage samples had higher Lys than the grain sample, but Forage 1 had the highest Lys value. Some individual non-essential AA exhibited much larger differences between forage and grain, exemplified by Ala, Asp, Glu and Pro. Specifically, grain contained significantly less Ala, Asp, and Pro, but overwhelmingly more Glu than forage samples. Pro content in Forage 2 of CDC Dancer and Forage 3 of Lamont was twice as large as that in respective grain. Furthermore, Forage 1 of CDC Dancer contained the highest amount of essential AA (47.14%), while Forage 2 gave the lowest value (40.73%) and the trend was the reverse for Lamont. All these changes and differences in AA composition among samples were consistent between the two oat varieties studied. Thus, results with one variety were confirmed by another variety.

Although information on AA composition of oat forage is limited, there are quite a few reports on AA composition of leaf proteins from various other species (Gerloff et al., 1965; Fiorentini & Galoppini, 1983; Kammes et al., 2011). Comparing AA composition of oat forage from the present study with that of leaf proteins reported previously can be made since oat forage consists mainly of leaves. Gerloff et al. (1965) analyzed AA composition of leaf protein concentrates extracted from green leaves. They found that samples from nine plant species harvested under different conditions of fertilization and maturity did not show large variations in amino acid content (% relative to total amino acid recovered). In the present study, although a few AA in oat forage were found to change with growing stages, the majority of others remained relatively consistent or had only slight changes. Larger difference were found only between forage and grain samples for some individual AA. By a comparison, oat forage had a very similar AA composition to alfalfa leaves and orchard grass reported by

previous investigators (Table 3). This further confirms that AA composition of leaf proteins is generally conserved regardless of species. Note that the AA composition of oat grains reported in the present study generally agreed with that of whole oat meal reported by Draper (1973) (Table 3).

Table 3. Amino acid composition of oats materials of two varieties, along with alfalfa leaf, orchard grass, and oat meal from previous reports

Amino Acid	CDC Dancer				Lamont				Alfalfa leaf[1]	Orchard grass[2]	Oat meal[3]
	Forage1	Forage 2	Forage 3	Grain	Forage1	Forage 2	Forage 3	Grain			
Essential											
Arg	6.11	4.36	5.24	7.18	6.08	4.09	4.77	7.33	6.17	6.30	7.00
His	2.36	2.36	1.96	2.36	2.38	2.25	2.09	2.25	2.45	2.20	1.80
Ile	4.57	4.22	4.38	4.06	4.71	4.46	4.12	3.94	5.24	5.57	3.70
Leu	8.42	7.48	9.08	8.58	8.67	6.57	8.24	8.49	8.92	10.12	7.00
Lys	6.63	4.66	5.29	4.45	6.69	4.87	5.07	3.58	6.31	5.57	4.40
Met	2.01	1.53	1.67	1.75	2.00	1.70	1.57	1.41	3.27	1.98	1.50
Phe	5.87	5.54	5.49	5.81	6.04	5.93	5.46	5.94	10.36	6.60	5.10
Thr	5.20	4.85	5.64	3.92	5.29	4.81	5.29	3.90	5.01	5.21	3.40
Val	5.97	5.71	5.91	5.57	6.17	6.45	5.83	5.53	6.41	7.04	4.70
Subtotal	47.14	40.73	44.66	43.67	48.03	41.11	42.44	42.36		50.59	38.60
Non-essential											
Ala	7.21	6.90	8.57	5.42	7.54	8.08	8.70	5.29	6.07	7.92	5.30
Asp	12.68	14.39	10.93	9.19	11.48	18.60	9.92	10.05	10.44	10.12	8.90
Glu	13.25	11.72	13.82	21.61	13.24	11.41	14.10	23.82	11.18	11.66	21.80
Gly	5.52	4.57	5.58	5.71	5.64	4.07	5.19	5.31	5.21	6.52	5.50
Pro	5.26	13.74	7.62	5.25	5.19	8.58	11.09	4.43	4.69	5.43	6.90
Ser	5.01	4.82	5.20	5.33	5.00	4.82	5.07	5.27	4.42	4.03	5.40
Tyr	3.95	3.13	3.63	3.83	3.88	3.33	3.49	3.48		3.74	4.10
Subtotal	52.86	59.27	55.34	56.33	51.97	58.89	57.56	57.64		49.42	57.90

Note. Data are expressed as % relative to total amino acids, means of two replicates for the current study.

[1] Data adapted from Fiorentini and Galoppini (1983). For this column, Met = Met + Cys; Phe = Phe + Tyr.

[2] Data adapted from Kammes et al. (2011). [3] Data adapted from Draper (1973).

Based on AA composition, Gerloff et al. (1965) concluded that leaf protein concentrate should be a well-balanced source of dietary protein if supplemented with synthetic methionine. Similarly, Fiorentini and Galoppini (1983) stated that AA composition of leaf proteins can be comparable to that of animal proteins. Because the present study showed that oat forage had similar AA composition as leaves of other plants it can be concluded that oat forage protein can be as nutritious as proteins of animal origin.

3.4 Protein Extractability from Growing Oat Forages and Matured Grains (Figure 1)

The observation that oat forage harvested at early and middle stages of growth had significantly higher total protein content than oat grain (Table 1) implies that these forages may be able to serve as an alternative source of proteins for human food or non-ruminant feed. In order to demonstrate this potential, we extracted protein from forages harvested at three stages as well as mature grain with an aqueous medium having a wide range of pH. The pH-dependent protein extractability was compared among samples. As shown in Figure 1, protein extractability from oat grain of CDC Dancer was lowest at pH between 3 and 6, with about 16% protein extractability. As pH decreased from 3 to 2 or increased from 6 to 13, significantly more protein was extracted. The curve started to approach a plateau at the higher pH region. The maximum protein extractability was slightly over 80% at a pH of > 9 (Figure 1).

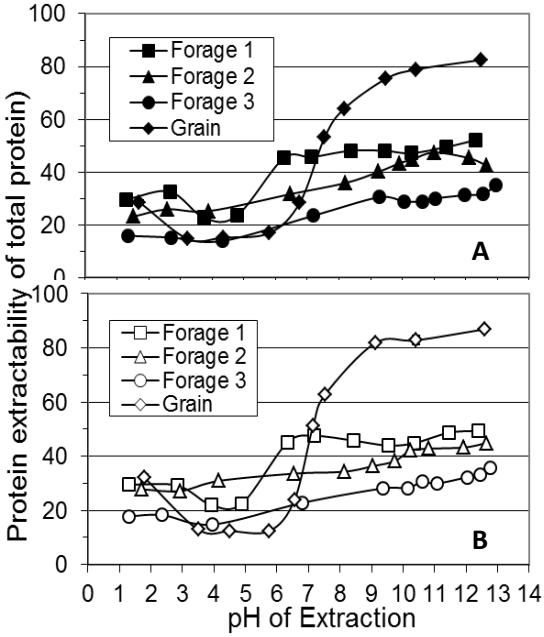

Figure 1. Protein extractability (% of total protein in the original raw material) of oat forage harvested at three different growing stages, as a function of aqueous medium pH, as compared to that of oat grain. A, CDC Dancer variety; B, Lamont variety

The pH dependent curves of forages differed significantly from not only that of grain but also among forage samples harvested at different growing stages. Unlike the curve for protein extractability from grain, which was a reversed bell shape across the pH range, the pH dependent curves for Forage 2 and 3 were basically linear (Figure 1). With increasing pH, the protein extraction increased almost linearly for Forage 2 and Forage 3. Since the slope for the two lines was relatively flat, the pH effect on the two forages was much less significant than grains. Interestingly, Forage 1 exhibited a hybrid curve between grain and Forage 2 or 3. The plateau for the Forage 1 curve was only about half of grain and was reached at the neutral pH as compared to the alkaline pH for grain. Within forage samples, this plateau decreased with maturity. Thus, the maximum protein extractability fron Forage 1 was highest among three forage samples, but this value was only about half of that from grain. Among the three forage samples, at the neutral and alkaline regions, protein extractability decreased significantly with maturation, paralleling the increasing content of other CHO in these samples. Yet, at the acidic range (pH 3.5-5.0), because of the difference in curve shapes, change in protein extractability did not follow growth stage. Instead, it followed the order: Forage 2 > Forage 1 > Forage 3 (Figure 1). The three forage samples had a similar difference between the maximum protein extractability at an alkaline pH and the minimum protein extractability at an acidic pH. This difference for forage proteins was significantly shorter than that for grain protein.

Again, Lamont showed similar pH-dependent protein extractability curves (Figure 1B) as those of CDC Dancer (Figure 1A) for grain and three stages of forage samples. The range (the difference between maximum and minimum) in protein extraction rate from the grain of Lamont was slightly higher than that of CDC Dancer. This variation can be attributed to the presence of hulls in CDC Dancer grain, which interfered with protein extraction.

The sharp difference in protein extraction curves between oat forage and grain is mainly determined by different types of proteins between the two types of tissues. Seed proteins are mostly storage proteins with globulin as the primary one (Webster & Wood, 2011), while the majority of forage proteins are leaf proteins which consist mainly of lamellar proteins (insoluble) of chloroplasts and soluble cytoplasmatic proteins that carry out enzymatic functions essential to photosynthesis (Fiorentini & Galoppini, 1983). Furthermore, it is known that plant tissues (such as oat forage) usually contain high levels of proteases and secondary metabolites, such as phenolic compounds, organic acids, terpenes, and pigments. These compounds can cause protein precipitation when the tissues are disrupted, negatively impacting protein extraction.

Just like AA composition, although information on protein extractability from oat forage is lacking, there are

numerous reports on extractability of leaf proteins from various other species over the years. Betschart and Kinsella (1973) reported that the extractability of total nitrogen from soybean leaves was markedly influenced by pH: minimum amount (about 10%) at pH 3.7 and 4.2 and maximum quantities (30-35%) at neutral to alkaline pH. Based on their extractability profile, they suggested that extraction at pH 7.0-8.0 would be quite effective for soybean leaves. The enhanced extractability of leaf protein with increasing alkalinity has also been observed by Fernandez et al. (1999), while protein extraction yield was found to be relatively low, varying from about 15% to about 60% of total protein, depending on species and processing methods (Devi et al., 1965).

Leaf protein has long been regarded as an additional protein source (Devi et al., 1965). Yet, its applications are severely hindered by low protein extractability, which translates to low yield of protein production (Devi et al., 1965; Betschart & Kinsella, 1973; Fernandez et al., 1999). Similarly, as shown in this study, although oat forage has been used as a valuable feed for ruminants, its use as an alternative protein for other animals is limited by its low protein extractability.

4. Summary and Conclusion

There were differential changes among nutrients in oat forage during growth. The fastest changes occurred during transition from seeds to Forage 1, with CP and ash contents increasing several fold and starch disappearing. As plants grew, protein, ash, oil and beta-glucan decreased, but other CHO increased significantly. Most macro and micro-minerals in forage followed the same changing pattern as ash. AA composition of forage differed significantly from grains. Mature grain and forages harvested at the three stages each had a characteristic curve of pH dependent protein extractability. Among forage samples, Forage 1 had the highest peak value (the maximum extraction) but this peak value was only about half of that from grain. Nutrient composition in oat forage harvested at an early stage can be comparable or even superior to grain, but, just like leaf of other species, low protein extractability of oat forage limits its use as an alternative protein source for humans and non-ruminants.

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