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CRUDE PROTEIN, AMINO ACID AND ALKALOID CONTENTS OF ANNUAL SWEET LUPIN (*LUPINUS SPP.* L.) FORAGES AND SEEDS GROWN IN ETHIOPIA

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SUMMARY

Though bitter white lupin (*Lupinus albus* L.) is a traditional crop in Ethiopia, sweet lupins are new to the country. As a result, the nutritional value of low-alkaloid lupins has not been evaluated under Ethiopian conditions. Crude protein, amino acid and alkaloid contents of 16 cultivars of three annual lupin species grown in four lupin growing locations (Merawi, Finoteselam, Kossober-1 and Kossober-2) of Ethiopia were evaluated. Location × cultivar interaction was a significant source of variation for all traits ($p < 0.0001$). In all locations, blue entries had either similar ($p \geq 0.0584$) or higher ($p \leq 0.0235$) forage crude protein content than the Local Landrace, white group and yellow entry. Compared with the Local Landrace, white and blue entries, the sole yellow entry had higher ($p \leq 0.0148$) seed crude protein content at all locations except at Kossober-2, where it had similar ($p = 0.8460$) crude protein content as white entries. The Local Landrace had the highest forage and seed alkaloid contents. However, sweet blue Vitabor and Sanabor entries had the lowest forage and seed alkaloid contents, respectively. Low alkaloid and higher crude protein contents of sweet lupins grown in Ethiopia show the possibility to use sweet lupin forage and seeds as cheap home-grown protein source for livestock feed and human food in the country. However, for more reliable information, the laboratory results need to be verified by animal and human evaluations of the crop.

INTRODUCTION

In the highland and mid-altitude areas of Ethiopia, crop and livestock production are the major components of the farming system. Though these components compete for resources, they also may complement each other. In these areas natural pasture and crop residues are the major and widely available feeds. In the highlands of Ethiopia, while use of grazing lands as feed resources declined due to their use for crop production and settlement, use of crop residues has increased (Benin *et al.*, 2003). According to studies conducted in the mixed crop livestock farming system of Ethiopia,

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the total annual contribution of crop residues in livestock feed is between 61% and 76 % of the total annual livestock feed supply (Belay, 2009; Bogale, 2004). However, crop residues, especially cereals, have a very poor feeding value with poor metabolisable energy, negligible available protein and are seriously deficient in mineral and vitamins (Lulseged and Jamal, 1999). In Ethiopia commercial concentrates and industrial by-products are mostly inaccessible and/or quite expensive for the smallholder farmers, but home-grown multipurpose forage legumes could be one option to be used as cheap sources of protein in livestock production in the mixed crop–livestock farming system of Ethiopia.

Lupin is one of the potential multipurpose crops to be utilised as a home-grown cheap protein source in the developing world because of its low agronomic requirement. Even though bitter white lupin is a traditional old crop in Ethiopia, sweet lupins are new for the country. Lupins are known for their high protein value in human food and livestock feed. However, it has limitations associated with its alkaloid content (Wink, 1993, 2008). The major anti-nutritional factors in lupin are quinolizidine alkaloids, which are responsible for the bitter taste in lupin, and human and animal toxicity because they act as neurotoxins. Alkaloids are responsible for the bitter taste, lower palatability and toxicity in lupin seed and forage (Vilarino and Ravetta, 2007; Zulak *et al.*, 2006). In bitter cultivars, the alkaloid contents range between 0.5% and 6% and in sweet cultivars it is less than 0.02% (Wink, 2008).

The potential of a given feed to support a target livestock production type and level can be predicted by determining the chemical composition of that feed (van Soest, 1994). In addition to the beneficiary nutrient fractions, knowing the amount of the alkaloid content of lupins is very important because the chemical composition of crops can be affected by the growing environmental conditions such as soil type, temperature and water availability. An experiment conducted by the proponents of this study on sweet lupins in Ethiopia showed that sweet annual lupins are adaptive and productive in the traditional lupin growing areas of the country (Yeheyis *et al.*, 2012). The same authors reported a forage dry matter (DM) yield of up to 4.5 t/ha from sweet white lupin (*Lupinus albus* L.) and seed yield of up to 5.4 t/ha from sweet blue lupin (*Lupinus angustifolius* L.). However, their nutritional value under Ethiopian conditions was not known. Hence, in addition to studying about the adaptability of sweet lupins, information about the crude protein, amino acid and alkaloid contents is essential. Thus, this study was conducted with the objective of evaluating the crude protein, amino acid and alkaloid contents of different sweet annual lupin cultivars, namely blue lupin, white lupin and yellow lupin (*Lupinus luteus* L.) grown in four different traditional lupin growing locations of Ethiopia.

MATERIALS AND METHODS

Description of growing areas

Lupins were grown in four locations, namely Merawi (11.27°N, 37.56°E), Finoteselam (10.84 °N, 37.36 °E), Kossober-1 and Kossober-2 (10.85 °N, 36.80 °E) in north-western Ethiopia. The altitude for Merawi and Finoteselam testing sites is

2095 and 1935 metres above sea level (m.a.s.l.), respectively, and these sites were assumed to represent the mid-altitude (relatively warm) traditional lupin growing areas. The altitude for Kossober-1 and Kossober-2 testing sites is 2610 m.a.s.l. and were assumed to represent the high-altitude (relatively cold) traditional lupin growing areas. The mean maximum and minimum daily temperature in the mid-altitude testing sites is 29 °C and 11 °C, respectively. The mean maximum and minimum daily temperature in the high-altitude testing sites is 22 °C and 10 °C, respectively. The Food and Agriculture Organisation of the United Nations (FAO) classification soil type for Merawi and Finoteselam was a Nitosol whereas in Kossober it was an Acrisol. The soil pH at Merawi, Finoteselam and Kossober was 4.8, 5.3 and 4.8, respectively. The total annual rainfall (mm) from a 10-year data in Merawi, Finoteselam and Kossober is 1602, 1189 and 2348, respectively.

Planting and experimental design

Growing of lupins was done from June 2009 to January 2010. For the experiment, a total of 16 annual lupin cultivars of three species (white, blue and yellow lupins) were used. The cultivars used were white lupin cultivars (Local Landrace, Fortuna, Feodora, L-1082, L-1057, AU-Alpha, AU-Homer), blue lupin cultivars (Bora, Boregine, Borlu, Boruta, Haags Blaue, Probor, Sanabor, Vitabor) and yellow lupin cultivar (Bornal). Except white Local Landrace and AU-Homer, all fourteen cultivars were sweet cultivars. White Local was included as a local check and the seed was purchased from local markets of the respective testing sites. Fortuna and Feodora seeds were obtained from Südwestdeutsche Saatzucht, Germany. The seed source for L-1082, L-1057, AU-Alpha, AU-Homer was Auburn University, Alabama, USA. For all blue lupin cultivars and yellow Bornal the seed source was Saatzucht Steinach GmbH, Germany. The 16 cultivars were arranged and planted in a randomised complete block design (RCBD) with three replications in all four testing sites. The plot size was 1.2 × 4 m. Spacing was 7 cm between plants and 30 cm between rows, giving a target plant density of 48 plants/m^2. In all testing sites, planting was done at the beginning of the main rainy season from 2–15 July 2009. Planting was done by hand on a well-prepared seedbed and fertiliser was not applied. Weeding was done manually twice, at seedling and just before flowering stages.

Sampling and sample processing

Each plot was divided in half crosswise with an effective plot size of 1.2 × 2 m. One-half was used for forage sampling and the other for seed sampling. Forage sampling was done when the plants reached around 50% flowering stage and seed sampling at maturity. In both the cases the sampling was done from the middle two rows excluding the border rows. Forage samples were dried in a forced air oven at 65 °C till constant weight for DM determination. The seed samples were air-dried till constant weight. After drying, both the forage and seed samples were ground using a hammer mill to pass through a 1-mm stainless steel sieve. The ground forage and seed samples

were kept in a tightly closed plastic bottle at room temperature for further chemical composition analyses.

Chemical composition analyses

Crude protein (CP) content of the forage and seeds of the different lupin cultivars from the different locations were determined using the Near-Infrared Reflectance Spectroscopy (NIRS) method. During NIRS analyses of both forage and seed samples the measurements were done using NIR-spectrometer model DA7200. Initially spectra were collected from the ground forage and seed samples. During spectra collection each ground sample was put in cups in duplicate and scanned using the spectrometer. The developed spectra of the samples were stored in one file. Based on the collected spectra 40 samples from the forage and 51 samples from the seed were selected for crude protein analysis using wet chemical analyses for calibration and validation according to the procedures described by Association of Official Analytical Chemists (AOAC, 1990). By using the collected spectra and the results of the wet chemical analysis calibration equations were developed and validation was done. Using Unscrambler software a regression model was developed between NIR spectra and the crude protein analysis result. On the basis of this regression, model prediction of the crude protein content of all the forage and seed samples was done.

The alkaloid content of the samples was determined by capillary GLC and GLC-MS according to the procedures described by Wink *et al.* (1995). First the ground samples were homogenised in 0.5 N HCl solution. This homogenate solution was adjusted to pH 12 with 6 N aqueous NaOH solution. Then from this solution the alkaloids were extracted by a solid phase extraction method and analysed by Gas Chromatography-Mass Spectrometry (GLC-MS). Individual quinolizidine alkaloids were identified by their informative mass spectra and authentic reference compounds. Lupanine was used as an external standard for quantification (Wink, 1993; Wink *et al.*, 1995). Analysis of the amino acid profiles was done according to the procedures described by Naumann and Bassler (1997). The analyser used was Biochrom 20 amino acid analyser. During the analysis, hydrolysis was done by diluted HCl and the quantity of the amino acids in the hydrolysate was determined by ion exchange chromatography using amino acid analyser (high pressure liquid chromatography). The values of all chemical composition parameters are expressed on DM basis.

Data analysis

The collected data were subjected to analysis of variance using mixed model procedures as implemented in SAS version 9.2.2 (2003) PROC GLIMMIX, where location, cultivar and the two-way interaction were fixed effects. The sole random effect was block (location). R-side modelling was used to account for heterogeneous variances among species and/or locations and provide for an adequate residual variance structure based on a Corrected Akaike's Information Criterion (AICC). Because the location × cultivar interaction was a significant source of variation for all traits ($p < 0.0001$), the eight contrasts of interests among cultivars were assessed for

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each location using the LSMESTIMATE statement in the above-named procedure; the simulation option ($\alpha = 0.10$) was used to account for the inflation in the Type-I error when making several comparisons from the same body of data. For the contrast analysis, the groups contrasted were Local (Local Landrace only), white (white lupin cultivars Fortuna, Feodora, L-1082, L-1057, AU-Alpha, AU-Homer), blue (blue lupin cultivars Bora, Boregine, Borlu, Boruta, Haags Blaue, Probor, Sanabor, Vitabor), yellow (Bornal only), AU-determinate (L-1082, L-1057), AU-indeterminate (sweet AU-Alpha, bitter AU-Homer) and other-indeterminates (Fortuna, Feodora). Due to lack of enough data, statistical analysis was not done for alkaloid content and amino acid profile results.

RESULTS

The locations where the study was conducted had variations in temperature, rainfall and soil type. Thus, the location × cultivar interaction effect was analysed for the crude protein content of the forage and seed samples. The result showed that the interaction effect observed between cultivar and location for the crude protein content was significant ($p \leq 0.0001$).

Forage crude protein content

In terms of the forage crude protein content among all locations, blue entry (Probor) had the highest forage crude protein content at Kossber-2, exceeding the Local Landrace by 60 g/kg (Table 1). At Kossober-1 almost similar difference was observed in the forage crude protein content between blue Vitabor (with the highest crude protein content) and the Local Landrace. However, in the mid-altitude locations the Local Landrace at Finoteselam and white L-1082 at Merawi had the highest crude protein content. Among all locations the forage crude protein content was low for most cultivars at Finoteselam. Compared with the yellow entry and white group, the Local Landrace had similar ($p \geq 0.0986$) forage crude protein content at all locations except at Merawi, where the yellow entry had significantly higher ($p = 0.0445$) crude protein content than the Local Landrace. As a whole, the crude protein content from blue entries was quite good compared with the other entries within location. At all locations the blue entries had either similar ($p \geq 0.0584$) or significantly higher ($p \leq 0.0235$) forage crude protein content than the Local Landrace, white group and yellow entry. The sole yellow entry had similar forage crude protein content as the white group at Finoteselam and Kossober-1 locations but had significantly higher crude protein content at Merawi and the opposite was true at Kossober-2. The forage crude protein content was not significantly different for all determinate and indeterminate groups and their possible pair-wise contrasts at all locations except at Merawi, where L-1082 had the highest crude protein content, which resulted in the AU-determinate group having significantly higher crude protein content than the AU-indeterminate group.

Table 1. Least square means and contrast estimates for forage crude protein (g/kg DM) from laboratory evaluation of seven white, eight blue and one yellow annual lupin accessions at four locations (Merawi, Finoteselam, Kossober-1 and Kossober-2) in Ethiopia.

	Mid-altitude						High-altitude					
	Merawi			Finoteselam			Kossober-1			Kossober-2		
Species, cultivar	Mean	SE	Rank	Mean	SE	Rank	Mean	SE	Rank	Mean	SE	Rank
White, Local	231.6	13.72	12	207.6	10.20	1	252.5	13.72	13	244.6	10.20	16
Blue, Bora	233.8	5.05	11	205.2	10.20	3	285.3	5.05	8	273.0	5.05	10
Blue, Boregine	269.7	5.05	8	207.5	10.20	2	295.7	5.05	5	291.1	5.05	6
Blue, Borlu	267.1	5.05	9	179.0	10.20	13	292.7	5.05	6	284.3	5.05	8
Blue, Boruta	274.9	5.05	5	201.4	10.20	4	289.6	5.05	7	288.4	5.05	7
Blue, Haags Blaue	273.2	5.05	6	197.7	10.20	5	298.4	5.05	3	296.1	5.05	3
Blue, Probor	276.8	5.05	3	180.7	10.20	12	296.7	5.05	4	305.4	5.05	1
Blue, Sanabor	285.6	5.05	2	194.7	10.20	6	302.7	5.05	2	298.5	5.05	2
Blue, Vitabor	275.6	5.05	4	182.7	10.20	10	304.1	5.05	1	292.2	5.05	5
Yellow, Bornal	273.0	5.05	7	172.4	10.20	15	240.5	5.05	15	249.9	5.05	15
White, Feodora	196.2	13.72	15	186.8	10.20	9	203.0	23.68	16	269.7	10.20	12
White, Fortuna	244.7	13.72	10	182.5	10.20	11	260.0	16.78	11	272.0	10.20	11
White, L-1082	302.2	13.72	1	192.3	10.20	8	263.6	16.78	10	275.4	10.20	9
White, L-1057	192.5	13.72	16	158.4	10.20	16	255.8	16.78	12	292.4	12.45	4
White, AU Alpha	198.9	13.72	14	194.5	10.20	7	274.1	13.72	9	265.4	10.20	13
White, AU Homer	199.9	13.72	13	177.5	10.20	14	251.3	13.72	14	251.4	10.20	14
Contrast	MDiff	SE	AdjP	MDiff	SE	AdjP	MDiff	SE	AdjP	MDiff	SE	AdjP
Local vs. white	9.2	14.67	0.9788	25.6	10.81	0.1275	1.2	15.28	1.0000	–26.4	10.88	0.1096
Local vs. blue	–38.0	13.68	0.0584	14.0	10.62	0.6800	–43.2	13.68	0.0235	–46.5	10.14	0.0001
Local vs. yellow	–41.4	14.35	0.0445	35.3	14.15	0.0986	12.0	14.35	0.9248	–5.3	11.03	0.9939
White vs. blue	–47.2	5.78	0.0000	–11.6	5.40	0.2017	–44.4	7.20	0.0000	–20.1	4.56	0.0004
White vs. yellow	–50.6	7.23	0.0000	9.6	10.81	0.9098	10.8	8.41	0.6941	21.2	6.30	0.0093
Blue vs. yellow	–3.4	4.92	0.9673	21.2	10.62	0.2659	55.1	4.92	0.0000	41.3	4.92	0.0000
AU-det vs. AU-indt	48.0	13.58	0.0093	–10.6	10.01	0.8368	–3.0	15.21	0.9999	25.5	10.63	0.1174
AU-indt vs. other-indt	–21.1	13.58	0.5194	1.3	10.01	1.0000	31.2	17.36	0.3736	–12.4	10.01	0.7337

AU-det: AU-determinate; AU-indt: AU-indeterminate; other-indt: other-indeterminates; Mdiff: LS mean difference; SE: standard error.

Forage alkaloid content

Due to the high cost of analysis, alkaloid analysis was done for few selected forage samples. At the mid-altitude locations among the forage samples analysed, the bitter Local Landrace had the highest forage alkaloid content (10,231 mg/kg DM) and the sweet blue Vitabor entry had the lowest alkaloid content (112 mg/kg DM) (Table 4). Among the sweet entries blue Bornal had the highest forage alkaloid content exceeding the sweet Vitabor entry by 1194 mg/kg DM. Similarly, at the high-altitude locations the Local Landrace and the sweet Vitabor entry had the highest (6153 mg/kg DM) and the lowest (459 mg/kg DM) forage alkaloid content, respectively. The range between the highest and the lowest forage alkaloid content among the sweet entries (357 mg/kg DM) was much lower than the observed range at the mid-altitude locations. Generally, except for the Local Landrace and blue Bornal, the forage

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Table 2. Least square means and contrast estimates for seed crude protein (g/kg DM) from laboratory evaluation of seven white, eight blue and one yellow annual lupin accessions at four locations (Merawi, Finoteselam, Kossober-1 and Kossober-2) in Ethiopia.

	Mid-altitude						High-altitude					
	Merawi			Finoteselam			Kossober-1			Kossober-2		
Species, cultivar	Mean	SE	Rank	Mean	SE	Rank	Mean	SE	Rank	Mean	SE	Rank
White, Local	382.6	6.12	2	360.3	6.12	5	382.2	3.57	2	394.7	6.12	2
Blue, Bora	338.3	6.12	7	295.7	11.00	12	320.3	6.12	8	309.6	6.12	14
Blue, Boregine	328.2	6.12	9	318.3	11.00	10	314.5	6.12	9	295.5	6.12	15
Blue, Borlu	351.3	6.12	5	356.5	11.00	6	325.5	6.12	7	329.3	6.12	11
Blue, Boruta	333.2	6.12	8	350.2	11.00	7	302.3	6.12	11	312.8	6.12	13
Blue, Haags Blaue	277.6	6.12	11	299.2	11.00	11	278.9	6.12	12	274.4	6.12	16
Blue, Probor	358.4	6.12	4	349.6	13.49	8	341.5	6.12	5	344.9	6.12	9
Blue, Sanabor	348.8	6.12	6	373.5	11.00	3	337.6	6.12	6	329.6	6.12	10
Blue, Vitabor	326.0	6.12	10	328.6	11.00	9	305.9	6.12	10	314.0	6.12	12
Yellow, Bornal	415.8	2.80	1	449.4	3.47	1	401.0	2.80	1	390.1	6.12	4
White, Feodora	†			365.3	10.70	4	†			404.8	10.80	1
White, Fortuna	†			†			†			393.1	10.80	3
White, L-1082	364.4	6.12	3	379.8	7.53	2	†			360.1	6.12	8
White, L-1057	†			†			†			373.5	10.80	7
White, AU Alpha	†			†			377.1	5.25	3	383.1	6.12	5
White, AU Homer	†			†			374.6	5.39	4	381.7	6.12	6
Contrast	MDiff	SE	AdjP	MDiff	SE	AdjP	MDiff	SE	AdjP	MDiff	SE	AdjP
Local vs. white	18.2	8.68	0.1605	–12.3	8.95	0.5161	6.3	5.21	0.6274	12.0	7.11	0.4331
Local vs. blue	49.9	6.51	0.0000	26.3	7.33	0.0051	66.4	4.20	0.0000	81.0	6.51	0.0000
Local vs. yellow	–33.2	6.76	0.0004	–89.1	7.06	0.0000	–18.8	4.57	0.0148	4.6	8.68	0.9903
White vs. blue	31.6	6.51	0.0004	38.6	7.66	0.0001	60.1	4.24	0.0000	69.0	4.19	0.0000
White vs. yellow	–51.4	6.76	0.0000	–76.9	7.39	0.0000	–25.1	4.61	0.0029	–7.4	7.11	0.8460
Blue vs. yellow	–83.1	3.56	0.0000	–115.4	5.32	0.0000	–85.2	3.56	0.0000	–76.3	6.51	0.0000
AU-det vs. AU-indt	†			†			†			–15.6	7.58	0.2309
AU-indt vs. other-indt	†			†			†			–16.5	8.90	0.3301

†No data; AU-det: AU-determinate; AU-indt: AU-indeterminate; other-indt: other-indeterminates; Mdiff: LS mean difference; SE: standard error.

alkaloid content was higher for the samples from the high-altitude locations than for those from the mid-altitude locations.

Seed crude protein content

Except at Kossober-2, there was no complete data for all entries of the white group. Thus, this could be the limitation of the contrast analyses involving the white group entries. Seed crude protein content ranged between 274 g/kg and 449 g/kg DM at Kossober-2 from Haags Blaue entry and at Finoteselam from yellow entry, respectively (Table 2). Except at Kossober-2, where Feodora had the highest crude protein content, the sole yellow entry had the highest crude protein content at other three locations. Among all entries, Haags Blaue had the lowest seed crude protein content at all locations. As a group, compared with the Local Landrace, white and blue entries, the sole yellow entry had significantly higher ($p \leq 0.0148$) seed crude protein content at

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Table 3. Amino acid profile (g/kg DM) of the original blue Sanabor seed, and the Ethiopia-grown white Local and blue Sanabor.

	Mid-altitude		High-altitude		
Amino acid	White Local	Blue Sanabor	White Local	Blue Sanabor	Original seed blue sanabor
Cysteine	5.32	4.84	4.61	4.88	5.14
Asparagine	34.72	32.69	35.33	31.76	33.25
Methionine	2.03	1.95	1.94	1.84	1.91
Threonine	12.41	11.35	12.46	10.76	11.43
Serine	17.78	16.40	18.21	15.90	16.63
Glutamine	66.05	63.08	67.31	63.25	65.74
Glycine	13.71	13.61	13.77	13.33	14.11
Alanine	11.00	11.25	11.51	10.71	11.87
Valine	13.61	12.62	13.82	12.34	13.02
Isoleucine	15.27	14.09	15.86	13.54	14.38
Leucine	24.55	22.71	24.70	22.15	23.30
Tyrosine	15.69	12.67	17.69	11.81	12.63
Phenylalanine	13.24	12.83	13.82	12.49	13.24
Histidine	8.18	9.20	8.22	9.24	9.63
Lysine	16.16	15.82	16.43	15.59	16.68
Arginine	33.37	34.37	35.33	35.64	29.87
Proline	14.65	13.25	14.97	13.12	13.78

all locations except at Kossober-2, where it had similar ($p = 0.8460$) crude protein content as white entries. Similar ($p \geq 0.1605$) seed crude protein content was observed between the Local Landrace and white entries across all locations. Even though the overall seed crude protein content for blue group was consistent at all locations, they had significantly lower ($p \leq 0.0051$) crude protein content than the Local Landrace, white group and yellow entry.

Similar to the alkaloid content, amino acid profile analysis was done for selected lupin samples. The analysis was done for the Local Landrace and blue Sanabor seed samples at both altitudes and for the original blue Sanabor seed (Table 3). The Local Landrace and blue Sanabor seeds grown in Ethiopia had relatively similar amino acid profile except for Tyrosine, where blue Sanabor, including the original seed, had lower Tyrosine than the Local Landrace. The original Sanabor seed and the Ethiopian-grown Local Landrace and blue Sanabor also had similar amino acid profile except for Arginine, where the original Sanabor seed had lower than the Ethiopian-grown seeds.

Seed alkaloid content

Similar to the forage samples, alkaloid analysis was done for selected seed samples. The seed alkaloid content ranged between 178 mg/kg and 16,752 mg/kg DM (Table 4). The bitter Local Landrace had the highest seed alkaloid content at both altitudes. However, the magnitude of the seed alkaloid content was very high at the mid-altitude location (Merawi). The sweet blue entries Boregine at Finoiteselam and Sanabor at Kossober-2 had the lowest seed alkaloid content at the mid- and

Table 4. Forage and seed alkaloid contents (mg/kg DM) from laboratory evaluation of selected white, blue and yellow annual lupin accessions at four locations (Merawi, Finoteselam, Kossober-1 and Kossober-2) in Ethiopia.

	Seed					Forage			
	Mid-altitude		High-altitude			Mid-altitude		High-altitude	
Species, cultivar	M	F	K-1	K-2	Species, cultivar	M	F	K-1	K-2
White, Local	16,752			11,426	White, Local		10,231		6153
Blue, Bora	2261			983	Blue, Borlu	267			607
Blue, Boregine	1365	375		622	Blue, Sanabor	421			816
Blue, Borlu	2292	750		703	Blue, Vitabor	112		459	
Blue, Boruta		653	272	357	Blue, Bornal	1306		542	
Blue, Haags Blaue	651		303	158	White, L-1082		328		542
Blue, Probor		946	365	430					
Blue, Sanabor	524			178					
Blue, Vitabor	452	495	231						
Blue, Bornal			1642						
White, L-1082	769	656		481					

M: Merawi; F: Finoteselam; K-1: Kossober-1; K-2: Kossober-2.

high-altitude locations, respectively. Among all samples analyzed at both altitudes, the sweet blue Sanabor had the lowest (178 mg/kg DM) seed alkaloid content and among the sweet entries at both altitudes, blue Borlu had the highest (2,292 mg/kg DM) seed alkaloid content. Compared with the other sweet seed samples analysed, blue entries Bora (2261 mg/kg DM) and Borlu (2292 mg/kg DM) had exceptionally higher seed alkaloid content. Unlike to the forage alkaloid content, the overall seed alkaloid content was higher at the mid-altitude locations than at the high-altitude locations.

DISCUSSION

Forage crude protein and alkaloid contents

One limitation of this study was that altitude and soil type are confounded. Hence, the observed location × cultivar interaction effect for different variables (forage and seed crude protein contents) may not be necessarily due to the difference in altitude among locations alone. The forage crude protein content in this study from all cultivars at all locations varied between 158 g/kg DM (from white L-1057) and 305 g/kg DM (from blue Probor). The results of this study are much higher than reported by Bruno-Soares and Vaz (1999), who reported the maximum forage crude protein contents of 142 and 167 g/kg DM from white and blue lupins at pod stage sampling, respectively. The discrepancy in these two results could be associated with differences in stage of sampling and cultivars used. Similarly, Bhardwaj *et al.* (2010) reported a mean forage crude protein content of 187 g/kg DM, which is lower than the crude protein content reported in this study from most white entries. However, the forage crude protein content from yellow lupin reported by Bruno-Soares *et al.* (1999) with a range of 180 to 220 g/kg DM is in line with the range of forage crude protein content

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of yellow lupin, 172 to 273 g/kg DM reported in this study. The relatively lower forage crude protein content for most of the cultivars in the mid-altitude testing sites, especially at Finoteselam, could be associated with the relatively lower rainfall and higher temperature of the study sites. According to Norton and Poppi (1995), higher temperature and lower rainfall during vegetative phase generally increase fibre content and decrease digestibility and nutrient contents of the plant parts by largely decreasing the soluble carbohydrate content of the plant tissues.

The forage alkaloid content of the bitter Local Landrace found in this study was much higher than the one report by Vilarino *et al.* (2005) (2700 mg/kg DM). However, the range values of the forage alkaloid content observed for the sweet blue, white and yellow entries was in line with other similar studies (Bruno-Soares and Vaz, 1999; Bruno-Soares *et al.*, 1999; Maknickiene and Asakaviciute, 2010).

Seed crude protein, amino acid and alkaloid contents

The crude protein content is the most important nutrient component in lupin species because the crop is valued for its high crude protein content. In this study yellow lupin had the highest seed crude protein content among the four entry groups followed by all white entries, including the Local Landrace. Blue entries had the lower seed crude protein content. Wasilewko and Buraczewska (1999) in their experiment on these three lupin species reported that yellow lupin had the highest crude protein content followed by white and blue lupin. Gross (1988) also reported similar order in the seed crude protein content among the three annual lupin species. In addition to the trend, the crude protein content of each species in this study was similar with the reports by Bruno-Soares *et al.* (1999), Erbas *et al.* (2005), Flis *et al.* (1999), Roth-Maier (1999) and Sujak *et al.* (2006). Though blue entries had lower crude protein content, the lowest seed crude protein content obtained in this study (274 g/kg DM) from blue Haags Blaue is still favourable for use as a protein supplement in livestock feed and human food. In addition to this, the higher seed-yielding potential of most blue entries, according to the study conducted by the proponents of this study, coupled with their reasonably good seed crude protein content makes the sweet blue species appropriate for further production and use in the study area.

The amount of the individual amino acids in both species in this study was in line with other similar studies (Campos-Andrada *et al.*, 1999; Gilbert and Acamovic, 1999). In addition, the amount of the essential amino acids profile in this study fulfils the requirements of the ideal protein. According to Cole and Van Lunen (1994), the appropriate balance of essential amino acids in the ideal protein would be as follows: lysine, 100; methionine + cysteine, 50; threonine, 65–67; tryptophan, 18; isoleucine, 50; leucine, 100; histidine, 33; phenylalanine + tyrosine, 100 and valine, 70. The relatively good balance of the essential amino acids is very important for the use of sweet lupin seeds as home-grown protein supplement feed in poultry production in Ethiopia.

Just as important as the crude protein content in lupin seeds is the alkaloid content because it limits use of the crop as livestock feed and/or human food. The alkaloid

content presented in this study might not be conclusive due to lack of replications. Nevertheless, the results show the difference in alkaloid content between bitter and sweet cultivars and the variations within sweet cultivars. The seed alkaloid content for the Local Landrace in this study was in agreement with the report by the proponents of this study (Yeheyis *et al.*, 2011), who reported an alkaloid content of 11,700 mg/kg and 14,300 mg/kg DM from the Local Landrace seeds sampled from mid- and high-altitude lupin growing areas, respectively. The seed alkaloid contents of most sweet entries in this study were in agreement with similar studies (Bruno-Soares *et al.*, 1999; Gdala *et al*, 1999). However, the maximum alkaloid content from sweet lupins in this study (2292 mg/kg DM) was much higher than the maximum alkaloid content (720 mg/kg DM) reported by the same authors. The overall mean seed alkaloid content was higher at the mid-altitude (2067 mg/kg DM) than the high-altitude (1297 mg/kg DM). A similar result was obtained with bitter lupins from the Rocky Mountain lupin (*L. argenteus* Pursh), in which alkaloid contents were negatively correlated with altitude (Carey and Wink, 1994). In addition, it could be associated with differences in the amount of rainfall and length of growing season in the two altitude areas. According to Christiansen *et al.* (1997), moisture stress during the vegetative phase increases seed alkaloid content in lupin. In this study the high-altitude study areas (Kossober-1 and Kossober-2) receive larger annual rainfall and have longer growing season than the mid-altitude study areas (Merawi and Finoteselam).

In general, compared with the bitter varieties, sweet annual lupin varieties are sensitive to biotic factors because of their relatively low alkaloid content, which serves as one of their defense mechanisms. Alkaloids are very important for the well being of lupin plant by serving as chemical defence against herbivores and pests. Some alkaloids also have antibacterial, antiviral and antifungal behaviours (Wink, 2008). The successful establishment, growth and yield performance of sweet lupins in Ethiopia (Yeheyis *et al.*, 2012), where the biotic and abiotic stresses on crops are relatively high, shows the wide adaptation potential of sweet lupins and the possibility to use these crops as protein source in Ethiopia. However, as this study is the first on sweet lupins in Ethiopia, their susceptibility to insects and pests in further production process in the country has to be assessed. In addition, there has to be a further study on the stability of alkaloid contents of sweet lupins under different soil types of the traditional lupin growing areas in Ethiopia. According to Gremigni *et al.* (2003) phosphorus and potassium deficiency and their interaction in the soil has an impact on the seed alkaloid content in narrow-leafed lupins.

Though there are inter- and intra-species variations in nutrient composition, sweet lupin entries in general had relatively high nutritive values at all locations. The relatively good nutrient balance, reasonably higher yield performance (Yeheyis *et al.*, 2012) and higher crude protein content of sweet lupins grown in Ethiopia show the possibility to use sweet lupin forage and seeds as cheap home-grown protein source for the small-scale livestock producers in the country. In addition, sweet lupin seeds could be used as protein source ingredients for the feed industry in the country. However, laboratory evaluation of nutrient composition alone cannot be an adequate indicator of nutritive value, since the availability of different nutrients to the animal body is

affected by the extent of digestibility of the nutrients in the animal body. Thus, for more reliable information the laboratory results in this study need to be supported by animal evaluation studies using the forage and seeds of sweet lupins in the traditional lupin growing areas of Ethiopia.

CONCLUSIONS AND RECOMMENDATIONS

According to the results of this study, sweet lupin cultivars evaluated in this study had very good nutritional value. There were variations in chemical composition among entries within and across locations. The very lower alkaloid content of sweet lupins coupled with their relatively better forage and seed crude protein content gives them advantages over bitter genotypes in the study area for further production and use in livestock feed and human food. The big within location alkaloid content difference observed among sweet entries depicts the need for further in-depth study on alkaloid content of sweet lupins. Based on the laboratory evaluation, sweet lupin forage and seed can be used as home-grown protein source in livestock feed and human food. However, for more reliable information the laboratory results need to be verified by animal and human evaluations of the crop.

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REFERENCES

AOAC (1990). *Official Methods of Chemical Analysis*, 15th edn. Arlington, TX, USA: Association of Official Analytical Chemists.

Belay, S. M. (2009). *Feed Resources Availability, Cattle Fattening Practices and Marketing System in Bure Woreda, Amhara Region, Ethiopia*. M.Sc. thesis, Mekelle University, Mekelle, Ethiopia, 105 pp.

Benin, S., Ehui, S. and Pender, J. (2003). Policies for livestock development in the Ethiopian highlands. *Environment, Development and Sustainability* 5:491–510.

Bhardwaj, H. L., Starner, D. E. and van Santen, E. (2010). Preliminary evaluation of white lupin (*Lupinus albus* L.) as a forage crop in the Mid-Atlantic regions of the United States of America. *Journal of Agricultural Science* 2(4):13–17.

Bogale, S. (2004). *Assessment of livestock production systems and feed resource base in Sinana Dinsho District of Bale Highlands, Southeast Oromia*. M.Sc. thesis, Alemaya University of Agriculture, Alemaya, Ethiopia, 141 pp.

Bruno-Soares, A. M., Campos-Andrada, M. P., Falcao, E. and Cunha, L. (1999). An evaluation of the chemical composition and rumen degradability of a new Portuguese *Lupinus luteus* L. cultivar. In *Towards the 21st century.*

Proceedings of the 8th International Lupin Conference, Asilomar, CA, USA, 11–16 May, 115–119 (Ed. G. D. Hill), Canterbury, New Zealand: International Lupin Association.

Bruno-Soares, A. M. and Vaz, M. M. M. (1999). Nutritive value of white and blue lupin forages at different stages of plant growth and the relationship to alkaloid and saponin contents. In *Lupin, An Ancient Crop for the New Mellenium. Proceedings of the 9th International Lupin Conference*, Klink/Muritz, Germany, 20–24 June, 411–413 (Eds. E. van Santen, M. Wink, S. Weissmann and P. Roemer). Canterbury, New Zealand: International Lupin Association, ISBN 0-86476-123-6.

Campos-Andrada, M. P., Santana, F. M. C., Felgueiras, I., Mimoso, M. J. and Empis, J. M. A. (1999). Nutritional value of *Lupinus angustifolius* and *L. cosentinii* accessions with diverse genetic origin. In *Lupin, an Ancient Crop for the New Mellenium. Proceedings of the 9th International Lupin Conference*, Klink/Muritz, Germany, 20–24 June, 414–418 (Eds. E. van Santen, M. Wink, S. Weissmann and P. Roemer). Canterbury, New Zealand: International Lupin Association, ISBN 0-86476-123-6.

Carey, D. B. and Wink, M. (1994). Elevational variation of quinolizidine alkaloid contents in a lupin (Lupinus argenteus) of the rocky mountains. *Journal of Chemical Ecology* 20:849–857.

Christiansen, J. L., Jornsgard, B., Buskov, S. and Olsen, C. E. (1997). Effect of drought stress on content and composition of seed alkaloids in narrow-leafed lupin *Lupinus angustifolius* L. *European Journal of Agronomy* 7:307–314.

Cole, D. J. A. and Van Lunen, T. A. (1994). Ideal amino acid patterns. In *Amino Acids in Farm Animal Nutrition* (Ed. J. P. F. D'Mello). Wallingford, UK: CAB International.

Erbas, M., Certel, M. and Uslu, M. K. (2005). Some chemical properties of white lupin seeds (*Lupinus albus* L.). *Journal of Food Chemistry* 89:341–345.

Flis, M., Zdunczyk, Z. and Juskiewicz, J. (1999). Composition of whole and dehulled seeds of yellow and white lupin. In *Lupin, An Ancient Crop for the New Mellenium. Proceedings of the 9th International Lupin Conference*, Klink/Muritz, Germany, 20–24 June, 422–424 (Eds. E. van Santen, M. Wink, S. Weissmann and P. Roemer). Canterbury, New Zealand: International Lupin Association, ISBN 0-86476-123-6.

Gdala, J., Smulikowska, S., Zdunczyk, Z. and Pastuszewska, B. (1999). Nutrients and anti-nutrients in three lupin species grown in Poland. In *Lupin, An Ancient Crop for the New Mellenium. Proceedings of the 9th International Lupin Conference*, Klink/Muritz, Germany, 20–24 June, 425–431 (Eds. E. van Santen, M. Wink, S. Weissmann and P. Roemer). Canterbury, New Zealand: International Lupin Association, ISBN 0-86476-123-6.

Gilbert, C. and Acamovic, T. (1999). The nutritional value of two British grown cultivars of *Lupinus albus* for broiler chicken. In *Lupin, An Ancient Crop for the New Mellenium. Proceedings of the 9th International Lupin Conference*, Klink/Muritz, Germany, 20–24 June, 400–402 (Eds. E. van Santen, M. Wink, S. Weissmann and P. Roemer). Canterbury, New Zealand: International Lupin Association, ISBN 0-86476-123-6.

Gremigni, P., Hamblin, J., Harris, D. and Cowling, W. A. (2003). The interaction of phosphorous and potassium with seed alkaloid concentrations, yield and mineral content in narrow-leafed lupin (*Lupinus angustifolius* L.). *Plant and Soil* 253:413–427.

Gross, R. (1988). Lupins in human nutrition. In *Proceedings of the 5th International Lupin Conference*, 5–8 July, 51–63 (Ed. T. Twardowski). Poland: Poznan.

Lulseged, G. and Jamal, M. (1999). The potential of crop residues, particularly wheat straw, as livestock feed in Ethiopia. In *Farmers, Their Animals and the Environment* (ILRI on disc). Nairobi, Kenya: FAO/ILRI.

Maknickiene, Z. and Asakaviciute, R. (2010). Research on low-alkaloid concentration in cultivars of lupin (*Lupinus sp.*) in Lithuania. *General and Applied Plant Physiology* 36(3–4):204–211.

Naumann, C. and Bassler, R. (1997). *Die Chemiche Untersuchung von Futtermitteln. Methodenbuch*, Band III, Ergänzungen 1993 (The Chemical Analysis of Feeds. Book of Methods, Vol. 3). Darmstadt, Germany: VDLUFA, Verlag.

Norton, B. W. and Poppi, D. P. (1995). Composition and nutritional attributes of pasture legumes. In *Tropical Legumes in Animal Nutrition*, 23–46 (Eds. J. P. F. D'Mello and C. Devendra). Wallingford, UK: CAB International.

Roth-Maier, D. A. (1999). Utilization of lupin in animal nutrition. In *Lupin, An Ancient Crop for the New Mellenium. Proceedings of the 9th International Lupin Conference*, Klink/Muritz, Germany, 20–24 June, 394–399 (Eds. E. van Santen, M. Wink, S. Weissmann and P. Roemer). Canterbury, New Zealand: International Lupin Association, ISBN 0-86476-123-6.

SAS (2003). *Statistical Analysis System, Version 9.2.2*. Cary, NC, USA: SAS Institute.

Sujak, A., Kotlarz, A. and Strobel, W. (2006). Compositional and nutritional evaluation of several lupin seeds. *Food Chemistry* 98:711–719.

Van Soest, P. J. (1994). *Nutritional Ecology of Ruminants*, 2nd edn. Ethaca, NY, USA: Cornell University Press.

Vilarino, M. del. P. and Ravetta, D. A. (2007). Tolerance to herbivory in lupin genotypes with different alkaloid concentration: interspecific differences between *Lupinus albus* L. and *L. angustifolius* L. *Environmental and Experimental Botany* 63:130–136.

Vilarino, M. del P., Maregianni, G., Grass, M. Y., Leicach, S. R. and Ravetta, D. A. (2005). Post-damage alkaloid concentration in sweet and bitter varieties and its effect on subsequent herbivory. *Journal of Applied Entomology* 129(5):233–238.

Wasilewko, J. and Buraczewska, L. (1999). Chemical composition including contents of aminoacids, minerals and alkaloids in seeds of three lupin species cultivated in Poland. *Journal of Animal Feed Science* 8:1–12.

Wink, M. (1993). Quinolizidine alkaloids. In *Methods in Plant Biochemistry*, 197–239 (Ed. P. Waterman). London: Academic Press.

Wink, M. (2008). Ecological roles of alkaloids. In *Modern Alkaloids; Structure, Isolation, Synthesis and Biology*, 3–24 (Eds E. Fattorusso and O. Taglialatela-Scafati). KGaA, Weinheim, Germany: Wiley-WCH Verlag GmbH.

Wink, M., Meißner, C. and Witte, L. (1995). Patterns of quinolizidine alkaloids in 56 species of the genus Lupinus. *Phytochemistry* (Oxf) 38:139–153.

Yeheyis, L., Kijora, C., Wink, M. and Peters, K. J. (2011). Effect of a traditional processing method on chemical composition of local white lupin (*Lupinus albus* L.) seed in North-Western Ethiopia. *Zeitschrift für Naturforschung* 66c (in press).

Yeheyis, L., Kijora, C., van Santen, E. and Peters, K. J. (2012). Sweet annual lupins (*Lupinus spp.*); their adaptability and productivity in different agro-ecological zones of Ethiopia. *Journal of Animal Science Advances* 2(2): 201–215.

Zulak, K. G., Liscombe, D. K., Ashihara, H. and Facchini, P. J. (2006). Alkaloids. In *Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet*, 102–136 (Eds. A. Crozier, M. N. Clifford and H. Ashihara). Oxford, UK: Blackwell.