### We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

186,000

200M

Download

154
Countries delivered to

Our authors are among the

**TOP 1%** 

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us? Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



### Reducing of Alkaloid Contents During the Process of Lactic Acid Silaging

Annett Gefrom and Annette Zeyner

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66287

#### **Abstract**

The current study is conducted to investigate whether lupine grains can successfully be ensiled with residual moisture contents of around 65% and alkaloid contents can be reduced during the process of lactic acid formation by native and added lactic acid bacteria, respectively. Based on the fermentation quality of the sampled grain meal silage in the dry mass of 65% and further results, silaging is shown to be a suitable method of preservation. A reduction in the alkaloid content during the silaging cannot be assumed (statistical) due to the irregular dynamic of the observed content.

**Keywords:** lupines, lactic acid moist grain silaging, nutritional facts and alkaloid content, lupine debittering

#### 1. Introduction

At present, soy is the most important protein feed for monogastric farm animals and also of high importance for polygastric species, particularly in face of the ban of feedstuffs of animal origin. To cover the high demand for animal nutrition, the European Union is dependent on the import of soybeans and soy products. The European agricultural policy is focused on alternative protein sources because of the ongoing debate on the import of genetically modified soy. Additional arguments for maximum self-supply with protein from local sources are the goals of sustainable production, the fluctuation of prices in soy bean business, and the future competition between food and energy production.

Legume grains like those from lupine species are valuable feedstuffs in this concern. Beside the remarkable protein content, they impress by a high density of energy.



Due to the uneven ripeness of the stock (**Figure 1**) and particularly if harvested at moist conditions, for example, in late summertime, a cost-intensive artificial drying of the grains is necessary. Storing lupine grains with a residual moisture above 14% might increase the risk for elevated counts of moulds and yeasts.



Figure 1. Uneven ripeness of lupine corns (picture: A. Gefrom, LFA MV).

The chemical conservation with soda lye, propionic acid or feed urea is possible at a moisture of <20–25%, but demands a Hazard Analysis and Critical Control Concept. The material may be storable up to 6 months but might be limited in their use with respect to the target animal species or specific situation in question. Furthermore, chemical conservation of feedstuffs is not permitted for organic farming.

Ensiling lupine grains may be an inexpensive and ecologically advantageous alternative to produce a high-protein feed of local origin that can be used in both conventional and organic farming. Lactic acid fermentation of fully mature legume grains with accordingly high dry matter contents is, however, impossible because the osmotolerance of the vast majority of lactobacilli is overused under such conditions. To create good conditions for silaging, lupine grains can be harvested either fully mature and remoistened or before full ripeness with accordingly high moisture content. According to technical aspects, harvesting before full ripeness and conservation by lactobacilli have the following particular benefits:

- Preservation of the feed can be performed by the farmer himself directly after harvest.
- Independence of harvest date and dry matter content (possible with moisture contents of up to about 35%).
- Early field clearance and therefore effective use of the land.

- Minimisation of nutrient loss (storage and threshing loss).
- Reduced costs through elimination of the artificial drying.
- Cost-effective production of feedstuff with high energy and protein content.
- Providing high-quality feedstuffs rich in protein and energy for farm animals in both conventional and organic farming systems.

The current study was conducted to investigate whether lupine grains can successfully be ensiled with residual moisture contents of around 65%, and alkaloid contents can be reduced during the process of lactic acid formation by native and added lactic acid bacteria, respectively. Hypotheses were that high-quality silages with reduced alkaloid contents can be produced in this way.

Alkaloids are important anti-nutritional factors limiting the use of lupine grains in the nutrition of farm animals. Due to the very effective alkaloids, low dose could induce physiological effects. Alkaloids damage among others the central nervous system, respiratory tract, and liver [1, 2]. Because of that, native bitter lupine grains are not suitable as feedstuff in particular for monogastric animals. For sweet lupine varieties provided as feed, actual guidelines demand a maximum alkaloid content of 0.05% to exclude intoxication [3, 4]. Further, the bitter taste can lead to a reduced acceptance and feed intake thus causing decreased performance. Pigs are particularly sensitive in this concern. However, under the environmental conditions of high temperatures, the accumulation of alkaloids may vary, and the alkaloid content in sweet lupines may be higher than the alkaloid limit for using lupines as feedstuff [5].

On the other hand, alkaloids are part of the protective system of the plant against pathogens. Alkaloids are nitrogenous combinations and attributive to some of the most toxic plant ingredients. Because of the molecular structure, the alkaloids in lupines allocate to chinolizidine alkaloide [6–8]. In corns of domestic lupines appear predominantly main quinolizidine alkaloids like spartein, lupinin, and lupanin in aggregate relevant to the lupine genre [9]. Other alkaloid contents in lupines are under 1%.

White lupines Lupanin (50–80%), multiflorin (3–10%), 13-hydroxylupanin (5–15%), albin (5–15%)

Blue lupines Lupanin (50–80%), angustifolin (5–20%), 13-hydroxylupanin (10–20%)

Yellow lupines Lupinin (40–70%), spartein (30–50%)

The alkaloids differ in toxicity. Spartein has a lower LD50 (median lethal dose) as lupanin [10]. Chinolizidine alkaloids are located are located in all plant organs, but the concentration and variation differ in texture, seasonal and daytime [9, 11, 12]. They are accumulated in corns and converted to proteins during maturation. They were also used as nitrogen source during germination [13–15].

It is possible that especially at not determinated varieties, green corns of the bastard branch have higher alkaloid content. Under the environmental conditions of high temperatures, the accumulation of alkaloids may vary, and the alkaloid content in sweet lupines may be higher than the alkaloid limit for using lupines as feedstuff [5, 16–18]. A post-harvesting reduction in alkaloids through silaging may allow future cropping decisions basing more on phytosanitary conditions. The aim of this work was to examine whether alkaloids can be reduced in their concentration by fermentation with lactobacilli on the precondition that preservation process per se succeeds.

Alkaloids can be reduced by hydrolytic and oxidative enzymes during germination [13]. From a theoretical point of view, alkaloids may be decomposed throughout silaging under the action of native and supplemented lactic acid bacteria, respectively. A comprehensive action of plant and microbacterial enzymes is possible due to the grinding of the grains, the setting of suitable osmotic conditions, and a sufficiently long fermentation time.

The moisture found in grain meal allows enzymes to process the substances in suspension freed in the fluid phase of fermentation. Phytonutrients exist in the wet phase in soluble form and change through plant enzymes and enzymes of microorganisms occurring during fermentation [19, 20], so that phytonutrients can turn to low molecular weight units and into milk acid [21].

It has been demonstrated that the alkaloid content of lupine grains in milk ripeness can be reduced through silaging [22]. On the contrary, only small success in this concern was reported following silaging of the whole bitter lupine plant where the alkaloids seemed to be fairly stable in the acid milieu of the silage. It is assumed that alkaloids inhibited lactic acid bacteria during the silaging process [23].

Hopper et al. [24, 25] and Santana et al. [20], however, described a possible elimination of bitterness in lupines, since alkaloids serve as energy source for microorganisms. In this way, Toczko [26] and Toczko et al. [27] demonstrated the conversion of lupanin through Pseudomonas sp. These bacteria are representatives for natural epiphytics which are proposed to be particularly important in the first phase of the process of fermentation. Santana and Empis [28] reduced the alkaloid content in flour of white bitter lupine seeds by 50% by incubation with Pseudomonas for 4 days. Camacho et al. [29] also demonstrated a reduction in alkaloids in lupine grains by incubation with *Lactobacillus acidophilus* (B-1910) in a 12% total solids suspension.

Apart from the possible reduction in alkaloids during fermentation, further benefits like physiologically effects of lactic acid silages of moist lupine grains are as follows:

- The positive effects of lactic acid in terms of nutrition physiology, for example, the acidification of distinguished parts of the digestive tract to prevent the proliferation of clostridia and other pathogenic microorganism.
- The improvement in the feeding value due to the reduction in other anti-nutritional factors such as oligosaccharides [30, 31].
- Elevated contents of essential amino acids following proteolysis but only when desmolysis can be prevented [32].
- Improved intake and digestion of the feed [33].

- Improved digestibility of the amino acids (demonstrated for lysine and methionine by Hackl et al. [33]).
- Enabling to elevate the quantity of lupine grains in the daily ration (broiler, 10%; weaned piglets, 12%) and thus, elevated waiver of soy products [34].

#### 2. Reducing of alkaloid contents during the process of lactic acid silaging

#### 2.1. Material and method

The experiments were performed with ripe, storage-dry lupine grains (variety of sweet lupines 'Bora' and bitter lupine 'Azuro'; 2002) as well as with legume grains from different years (2005 and 2006) with a high residual moisture content (~65% DM).

In crushed seeds (sieve 3 mm; **Figure 2**) of ripe, storage-dry lupine grains and at ~65% DM harvested *Lupinus angustifolius* L. (variety of sweet lupines 'Bora', 'Borlu', and bitter lupine 'Azuro') were determined the nutrient contents according to the Weende feed analysis [35]. Crude starch and water-soluble carbohydrates (fructose, glucose, sucrose, and galactose) were analysed by HPLC (Shimadzu-Germany GmbH) [36].



Figure 2. Crushed lupine seeds (picture: A. Gefrom, LFA MV).

The material was ground by ball mill (swing mill 'MM 200' Retsch GmbH & Co. KG, 42781 Germany, grain size 0.01 mm, 5 min with frequency of 30/s) for analysis of starch and alkaloids. The total alkaloid content (quinolizidine alkaloids) was determined by GC-MS [37]:

Preparation To weigh ca. 0.5 g sample in centrifuge tubes, add 20 ml 0.5 N salt acid homogenize and store for 16 h at room temperatures; centrifugalize by 4000 U/min for 30 min and by 20°C; pipette overlap (determine volume); add 6 N NaOH for adjust pH-Wert of 12 in overlap (mix); alkaloids were determinated with solid phase extraction with extrelut column (Merck) and dichlormethan as eluent: add 18 ml to extrelut column (determine volume); wait 15 min; elute with 3 × 20 ml dichlormethan; solvent was evaporated and rest is absorbed with 1 ml methanol; sample could be stored by -20°C

Analysis

Alkaloids were analysed by GC-MS (Shimadzu-Germany GmbH, GC-MS QP 2010), type of column: BP-1 (length: 30 m; diameter: 0.25 mm; thickness: 0.25 µm); split proportion: 50; carrier gas: helium; passage: 94 ml/min; start temperature:120°C; end temperature: 260°C; ion source: 230°C; interface temperature: 300°C; externer standard: lupanin and spartein

The preparation of model silage (ROMOS—Rostock model silages [38]; **Figure 3a** and **b**) with 65% DM (moistened ripe, storage-dry lupines meal and ~65% DM harvested lupine corns) followed using various biological silage additives (600 g material per plastic bag (three repetitions per variation)):





Figure 3. (a and b) Vacuum sealer and ROMOS-Rostock model silages (picture: A. Gefrom, LFA MV).

- Control (without additive)
- Lactic acid bacteria (LAB, homofermentative, Lactobacillus plantarum; 3 × 10<sup>5</sup> cfu/g FM; DSM 8862, 8866)

By using a vacuum sealer, the bags got evacuated and sealed and afterwards stored at 20°C for 90 days (**Figure 4**). The incubation was followed by the pH-measurement (WTW MultiCal pH 526, precision: 0.01), the analysis of ammonia nitrogen and the analysis of organic acids and alcohol by HPLC and gas chromatograph (Shimadzu) in the silage extracts (50 g silage +200 ml aqua dest.; 15 h at 5°C). The contents of lactic acid, fatty acid, and alcohol were analysed by HPLC. Gas chromatograph with standard was used for analysis of acetic acid, butyric acid, propionic acid, and alcohol (ethanol, propanol, butanol, butandiol). Parameter settings of HPLC and gas cromatograph are as follows:

HPLC: Shimadzu-Germany GmbH; separation column: Aminex HPX-87H, Biorad, Hercules, USA; flux  $10 \text{ mM H}_2\text{SO}_4$ , flow rate: 0.6 ml/min, temperature:  $60^\circ\text{C}$  and detektion: UV index by 210 nm Gas chromatograph: Dose 0.5 µl with split; capillary column: FFAP-DF-0.25;  $25 \text{ m} \times 0.32 \text{ mm}$ ; carrier gas:  $N_2 \text{ purity}$ ; P1 Shimadzu with  $= 0.75 \text{ kp/cm}^2$ ;  $P_2 = 1 \text{ kp/cm}^2$ ; hydrogen  $= 0.6 \text{ kp/cm}^2$ ; air  $= 0.5 \text{ kp/cm}^2$ ; temperature program:  $1.5 \text{ min by } 110^\circ\text{C}$  constant, heating phase:  $12^\circ\text{C/min by } 170^\circ\text{C}$ ; 3 min by  $170^\circ\text{C}$  constant; injector chromatpac (GC14A) temperature:  $190^\circ\text{C}$ ; detektion: FID (Shimadzu), temperature:  $190^\circ\text{C}$ ; detector sensitivity:  $10^2$ ; split ratio: 1:50-1:70; inside standards: iso-capronic acid for volatile fatty acids, n-pentanol for alcohol



Figure 4. Model silages after 90 days (picture: A. Gefrom, LFA MV).

Evaluation of parameters occurs according to DLG [39].

In freeze-dried silage material nutritional parameters were analysed according to the Weende feed analysis and alkaloids were analysis by GC-MS [37].

For statistical analysis, the computer software SPSS 14 for Windows (SPSS, Chicago, IL, USA) was used. Duncan test was applied to examine mean differences for significance. The level of significance was preset at p < 0.05.

#### 3. Results

#### 3.1. Nutrient contents of lupine seeds and silages after incubation of 90 days

The high energy and protein contents of moist harvested lupine grains from this study are in accordance with those reported by key feed tables (**Tables 1** and **2**). According to Jansen et al. [40], White et al. [41], and Wrigley [42], the crude starch in lupine corns analysed with enzymatical method contains approximately 2.4% in DM and is lower than contents after analysis with polarimetric method used for declarations in DLG. These authors supposed that crude starch contents analysed with polarimetric method could include sugar and non-starch polysaccharides.

	DLG (2014) <sup>1</sup>	Starting materi 'Borlu', 'Azuro'	al 'Bora', ; 2005/06, n = 6
Crude ash [% DM]	3.6	3.7	±0.2
Crude protein [% DM]	33.5	35.8	±0.9
UDP [% XP]	20	-	
nXP [%]	21.9	-	
RNB [g N/kg]	+19	-	
Crude fat [% DM]	5.5	6.2	±0.7
Crude fibre [% DM]	16.3	15.0	±1.8
Crude starch [% DM]	<sup>2</sup> 6.0	<sup>3</sup> 2.2	±0.2
Crude sugar [% DM]	5.6	4.0	±1.0
NEL [MJ/kg DM]	8.92	8.95	±0.09
ME <sub>cow</sub> [MJ/kg DM]	14.19	14.27	±0.13
ME <sub>pig</sub> [MJ/kg DM]	15.3	14.4	±0.19
AME <sub>Npoultry</sub> [MJ/kg DM]	8.8	8.02	±0.36

<sup>&</sup>lt;sup>1</sup>DLG 2014 [43] nutrient digestibility ruminants and UDP (undegrable protein): DLG [44].

**Table 1.** Contents of proximate nutrients and energy in grains of blue lupine from literature (mature seeds, in 100% DM) compared to experimental data with lupine seeds harvested at ~65% DM (blue lupine).

<sup>&</sup>lt;sup>2</sup>Polarimetric method.

 $<sup>^3</sup>$ Analysed by HPLC with enzymatic method; DM: dry matter; LAB: lactic acid bacteria [*Lactobacillus plantarum*  $3 \times 10^5$  cfu/g fresh matter (FM), DSM 8862, 8866]; ME<sub>pig</sub>: metabolizable energy for pigs; calculated according to the estimation equation from GfE [45] and digestibility from DLG [44].

 $<sup>^4</sup>$ AME<sub>N poultry</sub>: apparent metabolizable energy for poultry (N-corrected): calculated according WPSA [46]; NEL MJ/kg DM and MEcow MJ/kg DM: energy calculated according to DLG [44]; nXP: available crude protein calculated according to DLG [44]; RNB: ruminal N-bilance, RNB = (XP–nXP)/6.25; UDP: undegrable protein.

Blue lupine 'Bora' 2005, <i>n</i> = 3	Silage						
			Control		LAB		
DM [%]	67.2ª	±0.1	66.2 <sup>b</sup>	±0.6	65.9 <sup>b</sup>	±0.2	
Crude ash [% DM]	4.0ª	±0.1	3.9 <sup>b</sup>	±0.1	$3.8^{\circ}$	±0.1	
Crude protein [% DM]	35.8 <sup>b</sup>	±0.3	36.9ª	±0.6	36.6ab	±0.4	
Crude fat [% DM]	5.8	±0.0	6.1	±0.3	6.1	±0.2	
Crude fibre [% DM]	16.5ª	±0.1	15.8 <sup>b</sup>	±0.5	15.8 <sup>b</sup>	±0.2	
Crude starch [% DM]¹	2.4ª	±0.2	$0.7^{\rm c}$	±0.3	1.2 <sup>b</sup>	±0.6	
WSC [% DM]	4.9ª	±0.6	2.3 <sup>b</sup>	±0.7	2.5 <sup>b</sup>	±0.6	
NEL [MJ/kg DM]	8.89	±0.01	8.99	±0.02	8.99	±0.02	
ME <sub>cow</sub> [MJ/kg DM]	14.19	±0.01	14.32	±0.05	14.33	±0.04	
ME <sub>pig</sub> [MJ/kg DM]	14.24	±0.02	14.33	±0.08	14.37	±0.06	
AME <sub>Npoultry</sub> <sup>2</sup> [MJ/kg DM]	8.16	±0.03	8.08	±0.15	8.11	±0.09	

 $<sup>^{1}</sup>$ Analysed by HPLC; with enzymatic method; control: without additive; DM: dry matter; LAB: lactic acid bacteria [*Lactobacillus plantarum*  $3 \times 10^{5}$  cfu/g fresh matter (FM), DSM 8862, 8866]; ME<sub>pig</sub>: metabolizable energy for pigs; calculated according to the estimation equation from GfE [45] and digestibility from DLG [44].

**Table 2.** Contents of proximate nutrients and energy in grains of blue lupine harvested at ~65% DM and therefrom produced silages after incubation of 90 days.

The content of nutritional feed value parameters is, apart from the fermentable carbohydrates (WSC), not affected by the silaging process, and the high feed and energy content of legumes are maintained in the grain silage.

#### 3.2. Quality of silages

To ensure optimal osmotic conditions for silaging, lupine grains can either be harvested as mature grains and remoistered immediately before being ensiled, or harvested immature and ensiled as harvested. Based on the fermentation quality of the sampled grain meal silage in the dry mass range of ~65% DM and further results, silaging is shown to be a suitable method of preservation (**Table 3**). Harvest-moist grains may be stored without problems, even without the addition of silage additives. The addition of high-performance lactobacilli ensures the fermentation through an earlier and more comprehensive production of lactic acid. The production of acetic acid and alcohol was reduced.

 $<sup>^2</sup>$ AME<sub>N poultry</sub>: apparent metabolizable energy for poultry (N-corrected): calculated according WPSA [46]; NEL MJ/kg DM and MEcow MJ/kg DM: energy calculated according to DLG [44]; WSC: water soluble carbohydrates: fructose, glucose, sucrose, galactose

<sup>&</sup>lt;sup>a,b</sup>Significant (p < 0.05) differences of means between variants starting material (lupine 'Bora') and silages.

	DM		pН		LA	LA AA		ΣΑL		NH <sub>3</sub> -N		
	[%]			[% DM]						[% N]	I	
SM	66.5	±0.6	5.9ª	±0.1	n. a.							
CON	66.3	±0.6	$4.8^{\circ}$	±0.3	2.5 <sup>b</sup>	±1.0	$0.5^{b}$	±0.2	$0.3^{a}$	±0.2	$1.0^{a}$	±0.1
LAB	66.1	±0.4	$4.2^{d}$	±0.1	5.3ª	±0.4	0.6a	±0.1	$0.1^{b}$	±0.0	$0.7^{b}$	±0.1

AA: acetic acid; CON: control (without additive); control without additive; DM: dry matter; LA: lactic acid; LAB: addition of lactic acid bacteria [Lactobacillus plantarum 3 × 10<sup>5</sup> cfu/g fresh matter (FM), DSM 8862, 8866]; n. a.: not analysed; NH<sub>3</sub>-N: ammonia nitrogen; SM: starting material; ΣAL: alcohol (ethanol, propanol, butanol, butandiol). a, b Significant (p < 0.05) differences of means between variants.

Table 3. Fermentation parameters of silages from lupine grains (harvested at ~65% DM) 90 days of ensiling and ensiled with or without lactic acid bacteria (LAB) as additive (own data, means of all samples from 2005 ('Bora', 'Borlu', 'Azuro'; n = 18).

Variety						DM		Alkaloids		
					n	[%]		[% DM]		
Bitter lupine	Dry seed	'Azuro'	SM		1	65.0	±0.30	2.289	±1.09	
			Silage	Control	6	63.5	±0.72	2.246	±0.34	
				LAB	18	64.4	±0.85	2.615	±0.95	
	Moist grains		SM		3	66.1	±0.1	2.991	±1.42	
			Silage	Control	6	66.6	±0.2	2.765	±0.50	
				LAB	6	66.1	±0.7	2.743	±0.22	
Sweet lupine		'Borlu' + ''Bora'	SM	SM	6	67.2	±0.1	0.109ª	±0.02	
			Silage	Control	12	66.1	±0.7	0.079 <sup>b</sup>	±0.01	
				LAB	12	66.1	±0.2	$0.074^{b}$	±0.02	
		'Borlu'	SM		3	67.2	±0.0	0.116ª	±0.03	
			Silage	Control	6	66.1	±0.9	0.082 <sup>b</sup>	±0.01	
				LAB	6	66.2	±0.2	0.078 <sup>b</sup>	±0.01	
		'Bora''	SM		3	67.1	±0.1	0.103ª	±0.02	
			Silage	Control	6	66.2	±0.6	0.075 <sup>b</sup>	±0.01	
				LAB	6	65.9	±0.2	0.069 <sup>b</sup>	±0.02	

Control without additive; DM: dry matter; LAB: addition of lactic acid bacteria [Lactobacillus plantarum 3 × 10<sup>5</sup> cfu/g fresh matter (FM), DSM 8862, 8866]; SM: starting material; alkaloids: external standard at measurement with GC-MS: lupanin and spartein; a, b significant (p < 0.05) differences of means between variants.

Table 4. Alkaloids in dry corn from year 2002 ('Azuro') and in moist harvested grains (2005) from sweet lupines ('Borlu' and 'Bora') and bitter lupine ('Azuro') and their silages after 90 days incubation.

#### 3.3. Alkaloids

Table 4 shows the alkaloid content of lupine seeds (bitter 'Azuro' and sweet variants 'Bora') harvested at usual dry matter (2002) and approximate 65% DM (2005) and their silages after incubation of 90 days. The alkaloid content in analysed corns of bitter lupines ('Azuro') is higher than sweet lupines. Bitter lupines can contain high alkaloid contents of 4.5% DM [47, 48]. According to studies at the Julius Kühn Institute, the variety of the bitter lupine 'Azuro' contains 1.375% alkaloids in DM [29]. Sujak et al. [49] published a variety of alkaloids in sweet lupines between 0.05 and 0.24% in DM. Jansen et al. [16] published alkaloid contents in blue sweet lupines between 0006 and 0068% in DM. But under the environmental conditions of high temperatures, the accumulation of alkaloids may vary, and the alkaloid content in sweet lupines may be higher than the alkaloid limit for using lupines as feedstuff [5, 16, 17]. In blue sweet lupines 'Borlu' the alkaloid content reached a high level of 0.116% in DM and illustrated the variability between years. Otherwise, the grains were harvested earlier than normal, and the crop could contain more green corns with maybe high alkaloid content.

Against the statement of Camacho et al. [29], a reduction in alkaloid content in lupine silages through the fermentation process could not initially be shown because of the low differences and irregular dynamic in alkaloid contents. In all likelihood, the alkaloids are stabile in an acid environment [23]. A change in toxic potential of alkaloids in lupines after silaging should be reviewed in experimental feeding tests.

Pearson and Carr [50], Godfrey et al. [51], Bellof and Sieghart [52], and Allen [53] defined an experimental limit of tolerance of alkaloid contents in ration for feeding monogastric animals of 0.03%. But also contents of 200 mg alkaloid/kg could reduce feeding intake and growth of the animals [50, 51]. Petersen and Schulz [54] analysed an alkaloid content of 0.01% that effected the feeding intake.

#### 4. Conclusions

Making silages of moist legume grains could be a lower-cost and ecological conservation process for the production of protein-rich feed in conventional and organic farming.

From the results of the experiments with fermented grain meal with high moisture content, it is possible to draw the following conclusions:

- 1. Harvest-moist grains at 65% DM are possible.
- Lupine grains are a valuable feed due to their high energy and protein content and their composition. There are no degradations of nutritional parameters when harvested at dough-ripe stage, because dimerization is finished.
- **3.** Despite unfavourable chemical silage properties, the preservation of legume grains is possible even with a high amount of dry matter.

- **4.** The preservative effect of acid formation is primarily dependent on the moisture content. In respect of the process safety in industrial conditions, moisture content of 35% for lactic acid silage inputs is to be recommended.
- 5. Lactic acid bacteria stabilize the ensiling process and the silage quality.
- 6. The content of nutritional feed value parameters is, apart from the fermentable carbohydrates, not affected by the silaging process, and the high feed and energy content of legumes are maintained in the grain meal silage.
- 7. A reduction in the alkaloid content during the silaging cannot be assumed (statistical) due to the irregular dynamic of the observed content. The number of samples should be considered for future investigations. Maybe other bacillus could reduce alkaloid contents.
  - In own studies, the alkaloid contents could decrease tendencially by additional different lactobaccilus. A possible change in toxicity of the alkaloids during fermentation should be investigated by feeding studies.
- 8. The fermentation of early-harvested legume grains with high moisture content can therefore be recommended as a suitable method of conservation. With respect to the economic and organisational benefits when compared to the discussed methods of chemical conservation, the lactic acid fermentation of grains under anaerobic conditions conforms to the requirements of organic agriculture and is therefore also interesting for this branch. Silaging in plastic tubes is recommended (Figure 5).



Figure 5. Silaging in plastic tubes is recommended (picture: A. Priepke, LFA MV).

#### Acknowledgements

The study was financed by Bundesministerium für Ernährung und Landwirtschaft. We are grateful to the H. Wilhelm Schaumann Stiftung for funding a scholarship.

#### **Author details**

Annett Gefrom<sup>1\*</sup> and Annette Zeyner<sup>2</sup>

\*Address all correspondence to: annett.gefrom@gmx.de

1 State Research Centre of Agriculture and Fishery Mecklenburg-Western Pomerania, Germany

2 Group Animal Nutrition, Faculty of Natural Sciences III, Institute of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

#### References

- [1] Waldroup PW, Smith KJ. Animal feed uses of legumes. In: Matthews RH, editor. Legumes. Chemistry, Technology and Human Nutrition. Marcel Dekker Inc., New York und Basel, 1989. pp. 245-327. doi:19911433828
- [2] Birk Y. Antinutritional factors (ANFs) in lupins and in other legume seeds. In: Proceedings of the 7th International Lupin Conference; 18-23 April 1993; Évora. Portugal. pp. 424-429.
- [3] Garcia AG. Cultivos herbaceous extensivos. Mundi-Prensa, Madrid, 1984.
- [4] Jeroch H. Körner und Samen. In: Jeroch H, Flachowsky G, Weissbach F, editors. Futtermittelkunde. 1st ed. Gustav Fischer Verlag Jena, Stuttgart, 1993. pp. 281-293. ISBN:9783334003848
- [5] Jansen G, Jürgens HU, Ordon F. Effects of temperature on the alkaloid content of seeds of *Lupinus angustifolius* Cultivars. Journal of Agronomy and Crop Science. 2009;195:172-177. doi:10.1111/j.1439-037X.2008.00356.x
- [6] Petterson M. Composition and food uses of lupins. In: Gladstones JS, Atkins CA, Hamblin J, editors. Lupins as crop plants: biology, production and utilization. Wallingford: CAB International, Cambridge; 1998. pp. 353-384. ISBN:0851992242
- [7] ANZFA (Australia New Zealand Food Authority) (2001). Lupin alkaloids in food—a toxicological review and risk assessment. Technical Report, Series 3, Canberra; Available from: http://www.foodstandards.gov.au/publications/documents/TR3.pdf [Accessed: 2016-09-27].
- [8] Wink M. Evolution of secondary metabolites from an ecological and molecular phylogenic perspective. Phytochemistry. 2003;64(1):3-19. doi:10.1016/S0031-9422(03) 00300-5
- [9] Wink M. Methoden zum Nachweis von Lupinen-Alkaloiden. In: Wink M, editors. Lupinen 1991 Forschung, Anbau und Verwertung. Universität Heidelberg; 1992. 78-90.

- Available from: http://www.uni-heidelberg.de/institute/fak14/ipmb/phazb/pubwink/ 1992/27.1992.pdf [Accessed: 2016-09-27].
- [10] Wink M. Biological activities and potential application of lupin alkaloids. In: Proceedings of the 7th International Lupin Conference; 18-23 April 1993; Évora, Portugal, pp. 161-178.
- [11] Wink M, Witte L. Turnover and transport of quinolizidine alkaloids: diurnal variation of lupanine in the phloem sap, leaves and fruits of Lupinus albus L. Planta. 1984;161:519-524.
- [12] Lee MJ, Pate JS, Harris DJ, Atkins CA. Synthesis, transport and accumulation of quinolizidine alkaloids in Lupinus albus L. and L. angustifolius L. Journal of Experimental Botany. 2007;58(5):935-946. doi:10.1093/jxb/erl254
- [13] Wink M. Stoffwechsel und Funktion von Chinolizidinalkaloiden in Pflanzen und pflanzlichen Zellkulturen [thesis]. University Braunschweig; 1984.
- [14] Wink M. Chemische Verteidigung der Lupinen: Zur biologischen Bedeutung der Chinolizidinalkaloide. Plant Systematics and Evolution. 1985;150:65-81. doi:10.1007/ BF00985568
- [15] Wink M, Witte L: Quinolizidine alkaloids as nitrogen source for lupin seedlings and cell cultures. Zeitschrift für Naturforschung. 1985;40c:767-775.
- [16] Jansen G, Jürgens HU, Beyer H, Seddig S. Alkaloidgehalt in Blauen, Gelben und Weißen Lupinen [Internet]. Available from: http://lupinenverein.de/wp-content/uploads/ 2014/04/Alkaloidgehalt-in-Lupinen.pdf [Accessed: 2016-09-02].
- [17] Jansen G, Jürgens HU, Flamme W. Breeding of sweet lupines for the organic farming —quality investigations according to feed Suitability [Internet]. Available from: http:// forschung.oekolandbau.de [Accessed: 2016-09-02].
- [18] Christiansen JL, Jornsgard B, Buskov S, Olsen CE. Effect of drought stress on content and composition of seed alkaloids in narrow-leafed lupin, Lupinus angustifolius L. European Journal of Agronomy. 1997;7:307-314. ISSN:1161-0301
- [19] McDonald P, Henderson AR, Heron SJE. The biochemistry of silage. 2nd ed. Cambrian Printers Ltd: Aberystwyth; 1991. 340 p. doi:19811424455
- [20] Santana FMC, Fialho AM, Sa-Correia I, und Empis JMA. Isolation of bacterial strains capable of using lupanine, the predominant quinolizidine alkaloid in white lupin, as sole carbon and energy source. Journal of Industrial Microbiology. 1996;17:110-115. doi: 10.1007/BF01570053
- [21] Weissbach F. Beziehungen zwischen Ausgangsmaterial und Gärungsverlauf bei der Grünfuttersilierung [thesis]. Rostock: Universität Rostock; 1968.
- [22] Münte H. Einsäuerungsversuche mit grünen Lupinen. Dissertation [thesis]. Kiel: Universität Kiel; 1931.

- [23] Holzschuh W, Schmidt W. Silage. Herstellung, Fütterung. VEB Deutscher Landwirtschaftsverlag. 1963.
- [24] Hopper DJ, Kaderbhai MA, Marriott SA, Young M, Rogozinski J. Cloning, sequencing and heterologous expression of the gene for lupanine hydroxylase, a quonocytochrome c from a Pseudomonas sp. Biochemical Journal. 2002;367:483-489. doi:10.1042/ BJ20020729
- [25] Hopper DJ, Rogozinski J, Toczko M. Lupanine hydroxylase, a quinocytochrome c from an alkaloid degrading Pseudomonas sp. Biochemical Journal. 1991;279:105-109. doi: 10.1042/bj2790105
- [26] Toczko M. Induced lupanine hydroxylase from Pseudomonas lupanini. Biochimica et Biophysica Acta (BBA) — Enzymology and Biological Oxidation. 1966;128:570-573. doi: 10.1016/0926-6593(66)90018-X
- [27] Toczko M, Brzeski W, Kakolewska-Baniuk A. Microbial degradation of lupanine. V. Identification of 17-hydroxylupanine. Bulletin of the Polish Academy of Sciences. 1963;Cl. II, 11: 161-164.
- [28] Santana FC, Empis J. Bacterial removal of quinolizidine alkaloids from Lupinus albus flours. European Food Research and Technology. 2001;212:217-224. doi:10.1007/ s002170000221
- [29] Camacho L, Sierra C, Marcus D, Guzman E, Campos R, von Bäer D, Trugo L. Nutritional quality of lupine (Lupinus albus cv. Multolupa) as affected by lactic acid fermentation. International Journal of Food Microbiology. 1991;14:277-286. doi:10.1016/0168-1605(91) 90119-A
- [30] Gefrom A, Ott EM, Hoedtke S, Zeyner A. Ensiling legume seeds and the influence of conservation on contents of alkaloids, oligosaccharides, phytate-phosphorus and tannins. Züchtungskunde. 2013;85(2):154-168. ISSN:0044-5401
- [31] Gefrom A, Ott EM, Hoedtke S, Zeyner A. Ensiling legume grains to reduce antinutritional factors; Effect of ensiling moist field bean (Vicia faba), pea (Pisum sativum) and lupine (Lupinus spp.) grains on the contents of alkaloids, oligosaccharides and tannins. Journal of Animal Physiology and Animal Nutrition. 2012. doi:10.1111/jpn. 12024
- [32] Martinez-Villaluenga C, Gulewicz P, Frias J, Vidal-Valverde C. Amino acid profile in fermented Lupinus angustifolius cv. Zapaton. Integrating legume biology for sustainable agriculture. In: Proceedings of the 6th European Conference on Grain legumes. 12-16 November 2007; Lisboa. Portugal. p. 181.
- [33] Hackl W, Pieper B, Pieper R, Korn U, Zeyner A. Effects of ensiling cereal grains (barley, wheat, triticale and rye) on total and pre-caecal digestibility of proximate nutrients and amino acids in pigs. Journal of Animal Physiology and Animal Nutrition. 2010;94(6): 729-735. doi:10.1111/j.1439-0396.2010.01032.x

- [34] Frank A, Hackl W, Ott EM, Gefrom A, Zeyner A. Untersuchungen zum Einsatz von Lupinenkornsilage in der Ferkelaufzucht. In: Proceedings of the DLG Forum angewandte Forschung in der Rinder- und Schweinefütterung; 01-02 April 2009; Fulda. Germany. pp. 168-171.
- [35] VDLUFA—Association of German Agricultural Analytic and Research Institutes e. V.: Handbook, Vol. III: Feedstuff Analysis, 3th ed. Darmstadt; 1997. 2190 p. ISBN: 978-3-941273-14-6
- [36] Schmidt T, Krawielitzki K, Voihgt J, Gabel M. Methodical aspect of the in situ technique for estimating ruminal nutrient degradation: microbial contamination and lag time. Übersichten zur Tierernährung. 2005;33(2):87-100. ISSN:0303-6340
- [37] Wink M, Meissner C, Witte L. Patterns of quinolizidine alkaloids in 56 species of the genus Lupinus. Phytochemistry. 1995;38:139-153. doi:10.1016/0031-9422(95)91890-D
- [38] Hoedtke S, Zeyner A. Comparative evaluation of laboratory-scale silages using standard glass jar silages or vacuum-packed model silages. Journal of the Science of Food and Agriculture. 2011;91:841-849. doi:10.1002/jsfa.4255
- [39] DLG (2006). Grobfutterbewertung, Teil B, DLG-Schlüssel zur Beurteilung der Gärqualität von Grünfuttersilage auf Basis der chemischen Untersuchung. Informationen 2/2006, DLG-Verlag, Frankfurt am Main. Available from: http://www.dlg.org/ fileadmin/downloads/fachinfos/futtermittel/grobfutterbewertung\_B.pdf [Accessed: 2016-09-27].
- [40] Jansen G, Seddig S, Jürgens HU. Untersuchungen zum "Stärkegehalt" in Blauen Süßlupinen. 8. Gesellschaft für Pflanzenzüchtung-Tagung, Freising-Weihenstephan, 2006, 14.03.-16.03.2006, Vortrag Pflanzenzüchtung, 68, 73.
- [41] White CL, Hanbury CD, Young P, Phillips N, Wiese SC, Milton JB, Davidson RH, Siddique KHM, Harris D. The nutritional value of Lathyrus cicera and Lupinus angustifolius grain for sheep. Animal Feed Science and Technology. 2002;99:45-64. doi:10.1016/ S0377-8401(02)00035-4
- [42] Wrigley C. The lupin the grain with no starch. Cereal Foods World. 2003;48:30-31.
- [43] DLG (2014). Deutsche Landwirtschafts-Gesellschaft, DLG-Futterwerttabellen Schweine. DLG-Verlag, Frankfurt am Main.
- [44] DLG (1997). Deutsche Landwirtschafts-Gesellschaft, DLG-Futterwerttabellen Schweine. DLG-Verlag, Frankfurt am Main.
- [45] GfE (Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie) Flachowsky G: Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen. 1st ed. Band 10 von Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere. DLG Verlag, Frankfurt am Main; 2006. 247 p. doi:9783769006834
- [46] WPSA (World's Poultry Science Association) Subcommittee Energie of the Working Group nr. 2 Nutrition of the Federation of Branches of the World's Poultry Science

- Association, European Table of Energie Values for Poultry Feedstuffs, 3rd Edition. 1989, 104.
- [47] Marquard R. III. Nutritive und antinutritive Inhaltsstoffe der Leguminosen. In: Schuster WH, Alkämper J, Marquard R, Stählin A, editors. Leguminosen zur Kornnutzung [Internet]. 2002. Available from: http://geb.uni-giessen.de/geb/volltexte/2000/320/ [Accessed: 2016-09-02].
- [48] Römer P. Lupinen—Verwertung und Anbau [Internet]. 2007. Available from: http://lelf.brandenburg.de/sixcms/media.php/4055/lupine07.15564210.pdf [Accessed: 2016-09-02].
- [49] Sujak A, Kotlarz A, Strobel W. Compositional and nutritional evaluation of several lupin seeds. Food Chemistry. 2006;98:711-719. doi:10.1016/j.foodchem.2005.06.036
- [50] Pearson G, Carr JR. A comparison between meals prepared from the seeds of different varieties of lupin as protein supplements to barley-based diets for growing pigs. Animal Feed Science and Technology. 1977;2:49-58. doi:10.1016/0377-8401(77)90040-2
- [51] Godfrey NW, Mercy AR, Emms Y, Payne HG. Tolerance of growing pigs to lupine alkaloids. Australian Journal of Experimental Agriculture and Animal Husbandry. 1985;25:791-795. doi:10.1071/EA9850791
- [52] Bellof G, Sieghart S: Süßlupinen und Rapskuchen. Eiweißalternativen in der Schweinemast. Deutsche Geflügelwirtschaft und Schweineproduktion. 1996;5:45-49.
- [53] Allen JG. Toxins and lupinosis. In: Gladstones JS, Atkins CA, Hamblin J, editors. Lupins as crop plants: biology, production and utilization. CAB International, Cambridge; 1998. pp. 411-435. doi:10.1023/A:1017590829523
- [54] Petersen U, Schulz E. Examination about the suitability of faba beans (Vicia faba L. minor), sweet lupins (Lupinus luteus L.) and rapeseed meal (Brassica napus L. var napus) as protein source in pig fattening. 2. Landwirtsch. Forsch. 1978;31:269-280.

## IntechOpen

# IntechOpen