Assessment of *in vitro* ensilability of jack bean (*Canavalia ensiformis*) and cowpea (*Vigna unguiculata*) grains, sole or mixed with sorghum (*Sorghum bicolor*) grains

L.A. González[1], Sandra Hoedtke[2], A. Castro[1] and Annette Zeyner[2]

[1]*Research Centre of Agriculture and Animal Science, Central University of Las Villas, Carretera a Camajuaní km 5 ½, Villa Clara, Cuba*

[2]*Chair for Nutrition Physiology and Animal Nutrition, University of Rostock, Justus-von-Liebig-Weg 8, 18059 Rostock, Germany*

Email: luisgd@uclv.edu.cu

Jack bean (*Canavalia ensiformis*), cowpea (*Vigna unguiculata*) and sorghum (*Sorghum bicolor*) grains are alternative feedstuffs for pigs in the tropics. However, ensilage as a method for conservation has to be investigated. Ripe grains of cowpea (CWP) and jack bean (JBN) were chemically analyzed and ensilability was tested by the Rostock Fermentation Test (RFT). The buffering capacity (BC) was 8.9, 6.3 and 3.1 g lactic acid (LA)/100 g DM for JBN, CWP and sorghum (SOR), respectively. For RFT, 50 g milled grains were incubated with 200 mL of deionized water (30 ºC). Variants were performed in triplicate: control without additive, molasses (MOL, 4 %), *Lactobacillus plantarum* (LAB, $3x10^5$ cfu/g), MOL+LAB. As well, SOR was mixed with legume grains. At 0, 14, 18, 22, 26 and 38 h pH was measured and filtrates were analyzed for LA, volatile fatty acids, alcohol and NH_3 after 38 h. The lowest pH ($P < 0.05$) at 38 h was determined for JBN+LAB+MOL, JBN+LAB+SOR, CWP+LAB, CWP+LAB+MOL and CWP+LAB+SOR. For the variants CWP+LAB and CWP+LAB+MOL the highest LA production ($P < 0.05$) and the lowest levels of acetic and butyric acid as well as NH_3 were determined ($P < 0.05$). RFT revealed the necessity of LAB inoculation and addition of molasses for a sufficient acidification. Mixed silages are an option to be used when SOR grains have to be harvested at high moistures.

Key words: *Jack bean, cowpea, sorghum, in vitro ensilability, Rostock Fermentation Test*

Many of the underutilized legumes like jack bean (*Canavalia ensiformis*) and cowpea (*Vigna unguiculata*) contain adequate amounts of protein, essential amino acids, polyunsaturated fatty acids, dietary fiber, and essential minerals and vitamins comparable to other common legumes. They are adaptable to adverse environmental conditions and can thrive under extreme stress conditions (Amubode and Fetuga 1983, Sotelo *et al*. 1999 and Bhat *et al*. 2008). Furthermore, legumes are characterized by the ability of nitrogen fixation in the soil and in their tissues and are therefore seen as valuable plants.

Ensiling is reported to be a promising technique for the conservation and improvement of the feedstuff's nutritive value (Liener 1962, Gomez-Brenes *et al*. 1988, Belma *et al*. 1999). It is particularly useful for the processing of hard legume seeds, improving digestibility through reducing bean flatulence and the elimination of anti-nutritional factors, e.g. trypsin inhibitors (Deshpande and Salunkhe 2000). Fermentation of a cereal-legume mix is beneficial with respect to the complementation of the amino acid content. The sulfur-containing amino acids methionine and cystine are often limiting in legumes, while cereal proteins are generally deficient in lysine (Deshpande and Salunkhe 2000). Therefore, combined ensiling of cereal and legume grains can be seen as a possibility for harvesting and preserving high moisture mature grains, forming a complete diet at the same time.

To assess the ensilability of feedstuffs, the down-scale from a field level to an experimental unit by using model silages enables a comprehensive evaluation of the ensiling process. Thereby, it has been assumed that small-scale silos provide a reliable prediction of the farm-scale fermentation process (Wilson and Wilkins 1972, McDonald *et al*. 1991 and Cherney and Cherney 2003). Apart of being a time-consuming technique, model silages often require a lot of storage capacity and effort of the operator during the elaboration, depending on the number of experimental units under evaluation (Hoedtke and Zeyner 2010). In contrast, *in vitro* methods are generally characterized by a high throughput of samples and a minor expenditure of time. In the field of ensilage, an *in vitro* ensilability test was developed by Pieper *et al*. (1989) and advanced by Zierenberg (2000) to allow predictions about the necessity of silage additives with respect to the particular plant material to be ensiled. The principle of the method is the acidification of minced plant material in aqueous solutions. The Rostock fermentation test (RFT) is distinguished by a simple procedure, the possibility of testing a wide range of treatments contemporaneously and an availability of results within a short time. For this reason RFT was used in the present study to investigate the ensilability characteristics of jack bean and cowpea grains, sole or mixed with sorghum grains.

Materials and Methods

Plant material. Fully mature grains of jack bean

(*Canavalia ensiformis* L. DC, JBN), cowpea (*Vigna unguiculata* L. Walp., CWP) var. INIFAT-93 and sorghum (*Sorghum bicolor* L. Moench, SOR) var. CIAP-2E were hand harvested from small parcels planted on Brown Carbonated Soils in amounts corresponding to the necessities of the experiment at the Agricultural Experimental Station "Álvaro Barba" (Central University of Las Villas). No fertilizer and no irrigation were used. All the grains were sun dried and stored in nylon bags prior to analysis.

Chemical analysis. Dry matter (DM) was determined by oven drying at 105 ºC for 3 h. Ashing followed at 600 ºC for 5 h in a muffle furnace. Crude protein (N × 6.25) was analyzed with Kjeldatherm and Vapodest (Gerhardt, Königswinter, Germany; Kjeldahl 1883). Neutral detergent fiber (NDF, exclusive residual ash) and acid detergent fiber (ADF, exclusive residual ash) were determined by wet chemical analyses (Goering and van Soest 1970) and crude fiber (VDLUFA 1993) using a FOSS analyzer (Fibertec 2010, Rellingen, Germany). Water-soluble carbohydrates (WSC) were analyzed as monomeric and dimeric sugars in water extracts (1 h at 25 ºC) by high-performance liquid chromatography (HPLC, HPX-87C, Biorad, Hercules, CA, USA) according to Menge-Hartmann *et al*. (2009) with a flow rate of 0.65 mL/min at refractive index detector (column temperature 80 ºC). For determination of starch an enzymatic procedure using amylase (Thermamyl 120, Novo Nordisk A/S, Denmark) was chosen (Schmidt *et al*. 2005). Concentration of glucose was measured by HPLC with the conditions mentioned before and the starch content was calculated by considering the previously determined water soluble carbohydrate content (glucose). Buffering capacity (BC) was analyzed by titration with lactic acid (0.1 mol/L) to a pH of 4.0 (Weißbach 1967).

Rostock Fermentation Test (RFT). To conduct RFT (Pieper *et al*. 1989 and Zierenberg 2000) grains were milled to pass a 4 mm mesh size. From the coarsely ground material 50 g were mixed with 200 mL of deionized water in glass beakers of 600 mL capacity. If necessary, additives were applied and the following variants were performed (n=3): control without additive, molasses (MOL, 4 %), *Lactobacillus plantarum* (LAB, $3x10^5$ cfu/g, DSM 8862 and 8866), MOL+LAB. As well, sorghum was mixed with legume grains to reach 20 % of crude protein in the mix (table 1). The beakers were covered with aluminium foil and incubated in an oven at 30 °C. The pH value was measured after 0, 14, 18, 22, 26 and 38 h potentiometrically using a calibrated pH analyzer with glass and reference electrodes (precision 0.01, temperature compensation 0 - 70 ºC). Before every measurement, each sample was well stirred by hand using a glass stick. The pH electrode was disinfected (alcohol 70 %) between measurements to avoid microbial contamination of different variants.

Fermentation products were analyzed in the filtrated extracts after 38 h. Lactic acid was determined by HPLC (Aminex HPX-87H, Biorad) with a flow rate of 0.60 mL/min at the UV detector. Short-chain fatty acids and ethanol were quantitatively separated by gas chromatography (GC-14A, CLASS-VP, Shimadzu, Kyoto, Japan). Nitrogen was used as carrier gas at a pressure of 1 kg/cm². The temperature of the injector and flame ionization detector was kept constant at 190 ºC each; the temperature of the column oven was programmed at 110 ºC during the first 1.5 min, increasing to 170 ºC at a rate of 12 ºC/min thereafter. Ammonia was determined in the filtrates using a modified micro-diffusion technique (Voigt and Steger 1967).

Statistical analysis. Results were analyzed by SPSS 19.0 computer program (SPSS 19.0© for Windows; SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was performed to investigate the effect of treatments on pH kinetic and fermentation parameters with Duncan test for homogeneity of variance or Dunnett-T3 test without homogeneity of variance. Results are given as mean values (±SD) and the level of significance was preset at $P < 0.05$. A general linear mean model (univariat) was performed to find out interactions between treatments and fermentation time on pH during RFT.

Table 1. Variants used in the Rostock Fermentation Test.

Variants	Molasses (4%)	LAB ($3x10^5$ cfu/g)	SOR (20% CP in the DM)
LEG			
LEG+MOL	+		
LEG +SOR			+
LEG +LAB	+	+	
LEG +LAB+MOL	+	+	
LEG +LAB+SOR		+	+

cfu – colony forming units; CP - crude protein; DM - dry matter; LAB - lactic acid bacteria; LEG - legume grains (jack bean or cowpea); SOR- sorghum grains

Results and Discussion

The chemical composition of JBN, CWP and SOR grains is presented in table 2. All crude fractions lie within the range of tabulated values (Ensminger 1992, Cáceres *et al*. 1995, Ekanayake *et al*. 2000 and Sridhar and Seena 2006).

According to McDonald *et al*. (1991), legumes are regarded as rather unsuitable for ensiling due to three main factors: they are highly buffered, they tend to have low contents of water soluble carbohydrates (WSC), and they are low in dry matter (DM) content when used solely as pastures. In the case of CWP, low quantities of WSC were observed, whereas in JBN no WSC were determined (table 3). Especially high buffering capacities (BC) were determined in the legumes (9.0 and 6.3 g LA/100 g DM in JBN and CWP, resp.), as due to high contents of protein, legumes are more buffered than grasses (McDonald and Henderson 1962 and McDonald *et al*. 1991), which was the case for JBN and CWP compared to SOR (3.1 g LA/100 g DM). The ratio of WSC and BC (WSC/BC) showed that JBN (0.0) had more unfavorable ensilability characteristics compared to CWP with 0.4 (table 3). The ratios of all grains were found to be below 2, which is according to Weißbach (1967) the minimum value to expect a good fermentation quality. Nevertheless, a better ensilability was expected for CWP than for JBN.

Maize, barley, oats, wheat and millet have been used as additives in an attempt to improve both the fermentation quality and the nutritional value of silages (Murdoch *et al*. 1955, Stewart 1967, Ely 1978, Lindgren *et al*. 1983 and Jones 1988, cited by McDonald *et al*. 1991). Therefore, by the addition of SOR it was thought to reduce the BC in the mixture and as a consequence, to increase ensilability. Since WSC were low in JBN, CWP and SOR, molasses was considered to be added as an additional source of WSC. As well, lactic acid bacteria (LAB) as inoculants were considered to improve silage fermentation (Zimmer 1990, Muck and Bolsen 1991, Spoelstra 1991 and Muck 1993).

The use of LAB showed a clear impact on the rapidness of pH decrease and the final pH at 38 h in the RFT with CWP (table 4). However, no significant differences of pH at 38 h were detected among all the variants using LAB. Contrary to CWP, the decrease of pH in RFT of JBN was less influenced by the use of additives (LAB and MOL). However, in the variant JBN+LAB+SOR a faster decrease of pH was observed (between 0 and 22 h, table 5). The combined effect of the addition of LAB and the disolving effect of SOR on the buffering capacity could be associated with the faster drop of pH.

As fermentation in RFT takes place in an aqueous solution, the biochemical processes run faster in the *in vitro* method compared to silages. Therefore, it is expected that at the 38 h measurement time in RFT, corresponding to an anaerobically stable silage, the fermentation processes are completed (Pieper *et al*. 1989 and Zierenberg 2000), which is shown by the lowest pH at 38 h over all treatments (table 6). Beside the effects of fermentation time and treatment on the pH value, interactions of those factors were as well observed (table 6).

Although the content of WSC in CWP seemed to be too low (table 3), it has to be assumed that other sources of WSC in sufficient quantities were available to guarantee a good ensilability. The main sugars present in the WSC fraction of legumes are fructose, glucose and sucrose (Raguse and Smith 1966), although raffinose and stachyose have also been detected (Hirst *et al*. 1959). In a study with 9 varieties of cowpea, contents of raffinose and stachyose were found to be 0.7 - 6.9 % and 2.4 - 3.9 %, respectively (Nwinuka *et al*. 1997). Akpapunam

Table 2. Chemical composition of jack bean, cowpea and sorghum grains used in the Rostock Fermentation Test.

Grain	DM (%)	CP (%DM)	CF (%DM)	NDF (%DM)	ADF (%DM)	Starch (% DM)	Ash (%DM)
JBN	84.2	33.4	9.0	24.9	16.5	35.9	3.3
CWP	87.0	27.3	6.3	23.0	15.5	38.7	5.0
SOR	85.3	9.5	3.1	15.7	11.5	73.9	2.0

ADF - acid detergent fiber; CF - crude fiber; CP - crude protein; CWP - cowpea; DM - dry matter; JBN - jack bean; NDF - neutral detergent fiber; SOR - sorghum

Table 3. Content of water soluble carbohydrates (WSC), buffering capacity (BC), and WSC/BC ratio of the grains used in the Rostock Fermentation Test.

Grain	WSC (g/100 g DM)	BC (g LA/100 g DM)	WSC/BC ratio
JBN	0.0	9.0	0.0
CWP	2.3	6.3	0.4
SOR	0.2	3.1	0.1

BC - buffering capacity; CWP - cowpea; DM - dry matter; JBN - jack bean; LA - lactic acid; SOR - sorghum; WSC - water soluble carbohydrates

Table 4. The pH kinetic during incubation of cowpea grains sole or mixed with sorghum grains in the Rostock Fermentation Test.

	0 h	14 h	18 h	22 h	26 h	38 h
CWP	6.37^{a} ±0.00	5.77^{c} ± 0.06	5.80^{d} ± 0.07	5.57d ± 0.09	5.31^{c} ± 0.13	5.50^{c} ± 0.06
CWP+MOL	6.40^{b} ±0.04	5.84^{c} ± 0.05	5.77^{d} ± 0.05	5.22^{c} ± 0.02	5.00^{b} ± 0.06	4.99^{b} ± 0.10
CWP+SOR	6.38^{ab} ±0.00	5.79^{c} ± 0.04	5.59^{c} ± 0.04	5.04^{b} ± 0.06	4.92^{b} ± 0.17	4.95^{b} ± 0.25
CWP +LAB	6.36^{a} ±0.00	5.18^{a} ± 0.06	4.45^{a} ± 0.06	4.29^{a} ± 0.03	4.18^{a} ± 0.02	4.02^{a} ± 0.02
CWP +LAB+MOL	6.37^{a} ±0.00	5.37^{b} ± 0.07	4.58^{b} ± 0.04	4.24^{a} ± 0.02	4.19^{a} ± 0.02	4.04^{a} ± 0.01
CWP+LAB+SOR	6.35^{a} ±0.00	5.18^{a} ± 0.04	4.46^{a} ± 0.04	4.24^{a} ± 0.01	4.10^{a} ± 0.01	3.99^{a} ± 0.01

CWP – cowpea; LAB – lactic acid bacteria; MOL – molasses; SOR – sorghum

^{abc} Mean values with different superscripts in the same column differ significantly (P < 0.05).

Table 5. The pH kinetic during incubation of jack bean grains sole or mixed with sorghum grains with the Rostock Fermentation Test.

	0 h	14 h	18 h	22 h	26 h	38 h
JBN	6.10^{c} ± 0.01	5.99^{c} ± 0.02	5.73^{c} ± 0.04	5.15^{c} ± 0.30	4.89^{b} ± 0.29	4.62^{b} ± 0.20
JBN+MOL	6.14^{e} ± 0.00	6.02^{c} ± 0.01	5.73^{c} ± 0.22	5.10^{bc} ± 0.49	4.77^{b} ± 0.31	4.51^{ab} ± 0.25
JBN+SOR	6.13^{d} ± 0.00	5.91^{b} ± 0.04	5.55^{bc} ± 0.04	4.99^{bc} ± 0.07	4.58^{ab} ± 0.04	4.50^{ab} ± 0.04
JBN+LAB	6.07^{a} ± 0.01	5.93^{b} ± 0.01	5.51^{b} ± 0.02	4.89^{abc} ± 0.05	4.63^{ab} ± 0.02	4.51^{ab} ± 0.02
JBN+LAB+MOL	6.09^{b} ± 0.00	5.92^{b} ± 0.03	5.43^{b} ± 0.08	4.65^{ab} ± 0.06	4.43^{a} ± 0.02	4.35^{a} ± 0.00
JBN+LAB+SOR	6.11^{c} ± 0.01	5.74^{a} ± 0.03	4.91^{a} ± 0.02	4.48^{a} ± 0.02	4.43^{a} ± 0.01	4.33^{a} ± 0.01

JBN – jack bean; LAB – lactic acid bacteria; MOL – molasses; SOR – sorghum

^{abc} Mean values with different superscripts in the same column differ significantly (P < 0.05).

Table 6. Effect of fermentation time and treatments on pH of jack bean and cowpea grains in the Rostock Fermentation Test.

	pH	
	CWP	JBN
Fermentation time (h)		
0	6.37^{a}	6.11^{a}
14	5.52^{b}	5.92^{b}
18	5.11^{c}	5.48c
22	4.77^{d}	4.88^{d}
26	4.62^{e}	4.62^{e}
38	4.58^{e}	4.47^{f}
Treatment		
LEG	5.72^{a}	5.41^{a}
LEG+MOL	5.54^{b}	5.38^{a}
LEG+SOR	5.44^{c}	5.28^{b}
LEG+LAB	4.75^{e}	5.26^{b}
LEG+LAB+MOL	4.80^{d}	5.15^{c}
LEG+LAB+SOR	4.72^{e}	5.00^{d}
Pooled SD	0.0707	0.138
P-values		
Fermentation time	<0.001	<0.001
Treatment	<0.001	<0.001
Fermentation time*treatment	<0.001	0.002

^{abc} Means with different superscript in the same column refer to the general linear means models (univariat)

CWP, cowpea; JBN, jack bean; LEG, legume; LAB, lactic acid bacteria; MOL, molasses; SOR, sorghum

and Markakis (1979) evaluated 13 varieties of cowpea (*Vigna sinensis*) with average contents of 2.2 % sucrose, 1.2 % raffinose, 3.4 % stachyose and 0.9 % verbascose (all sugars on a DM basis). Therefore, it is possible that CWP used in this study contained reasonable contents of oligosaccharides, which should be proven in further studies.

The variants of RFT with addition of LAB showed significant higher contents of lactate and lower contents of volatile fatty acids compared to those treatments without inoculant. Nevertheless, in CWP+LAB+SOR the production of lactate was lower (4.34 % DM) compared to CWP+LAB and CWP+LAB+MOL (6.83 and 6.85 % DM, respectively; table 7).

were marginal in all variants of RFT, CWP+LAB and CWP+LAB+MOL showed the lowest contents compared to the other treatments. When only SOR was added to CWP, less alcohol and NH_3 was detected, but only in the variants without LAB. When LAB was used, this effect was not observed. Presumably, the action of tannins in SOR grains could be a reason. Salawu *et al*. (1999) reported a similar finding in small-scale laboratory silages when tannins from different sources were used for ensiling perennial ryegrass. They reported an effect of the supplementation of the tannins, which led to a rapid acidification and the protection of protein by binding to tannins. As well, Grabber *et al*. (2002) referred to the effect of tannins on binding proteins in

Table 7. Contents of lactate and volatile fatty acids (% DM) in filtrates of the Rostock Fermentation Test with cowpea grains after 38 h of incubation.

	Lactate	Acetate	Propionate	Butyrate
CWP	$0.00^{a} \pm 0.00$	$1.76^{d} \pm 0.00$	ND	$1.64^{a} \pm 0.16$
CWP+MOL	$2.40^{b} \pm 0.41$	$1.41^{c} \pm 0.11$	ND	$1.20^{b} \pm 0.30$
CWP+SOR	$0.22^{a} \pm 0.12$	$1.04^{b} \pm 0.01$	ND	$1.85^{a} \pm 0.16$
CWP+LAB	$6.83^{d} \pm 0.04$	$0.67^{a} \pm 0.00$	ND	ND
CWP+LAB+MOL	$6.85^{d} \pm 0.14$	$0.62^{a} \pm 0.05$	ND	ND
CWP+LAB+SOR	$4.34^{c} \pm 0.04$	$0.62^{a} \pm 0.01$	ND	ND

CWP - cowpea; LAB - lactic acid bacteria; MOL - molasses; ND, not detected; SOR - sorghum

^{abc} Mean values with different superscripts in the same column differ significantly ($P < 0.05$).

When ground maize was added (54.5 kg/t) to a mixture of lucerne and bromegrass, increases of the silage DM as well as of total acid content and lactic acid content were found (Allen and Porter 1954, cited by McDonald *et al*. 1991). As the supplementation of SOR did not show a comparable effect, it can be assumed that the content of condensed tannins in the SOR variant used (CIAP-2E) might have restricted the development of LAB. Furthermore, it is known that the main carbohydrate in cereals is starch (73.9 % in the SOR grains used in present study), a polysaccharide not available for the majority of LAB (McDonald and Whittenbury, 1973). However, Gefrom *et al*. (2009) found reduced starch contents in silages of high moisture lupine grains. Therefore, there are assumptions that starch could possibly be used by other starch degrading microbes interacting with the applied LAB after ensiling. As well, an increased activity of endogenous enzymes of the grains might be possible (Pieper *et al*. 2010). As a consequence, it can be suggested that SOR should not be used as the only source of WSC in those silages.

Although levels of alcohols and NH_3 (table 8) silages when two varieties of alfalfa (with or without tannins) where used, reducing the formation of NH_3 as a consequence.

The addition of LAB alone or in combination with MOL led to the highest lactate production in JBN (table 9). The butyric acid production was generally marginal for all variants. Acetic acid fermentation occurred as well during RFT, and the lowest value was detected for JBN+LAB+MOL (0.45 % DM), which was the variant with the generally lowest content of volatile fatty acids and the highest content of lactate (4.63 % DM), suggesting good ensilability characteristics of this treatment.

The production of alcohols in JBN was lower in most of the variants compared to CWP (table 10). Nevertheless, JBN and JBN+MOL showed higher levels compared to the other treatments. Like in CWP, the inclusion of sorghum was associated with the lowest levels of NH_3, where JBN+LAB+SOR showed the lowest quantities (0.06 % DM), representing a reduction of 55 % in respect to the control variant. Tabacco *et al*. (2006) achieved a 15 % reduction compared to the control when 4 % of chestnut tannins (hydrolysable) were added to alfalfa lab-scale silos. The different experimental conditions

Table 8. Contents of alcohol (% DM) and NH_3 (% DM) in filtrates of the Rostock Fermentation Test with cowpea grains after 38 h of incubation.

	Ethanol	Propanol	2,3 Butanediol	NH_3
CWP	$1.53^{e} \pm 0.19$	ND	$2.08^{d} \pm 0.59$	$0.30^{e} \pm 0.03$
CWP+MOL	$0.99^{d} \pm 0.00$	ND	$1.68^{c} \pm 0.16$	$0.24^{d} \pm 0.01$
CWP+SOR	$0.52^{c} \pm 0.06$	ND	$0.83^{b} \pm 0.12$	$0.14^{c} \pm 0.01$
CWP+LAB	$0.16^{a} \pm 0.00$	ND	$0.05^{a} \pm 0.00$	$0.07^{a} \pm 0.00$
CWP+LAB+MOL	$0.15^{a} \pm 0.00$	ND	$0.07^{a} \pm 0.02$	$0.08^{a} \pm 0.01$
CWP+LAB+SOR	$0.30^{b} \pm 0.01$	ND	$0.05^{a} \pm 0.02$	$0.10^{b} \pm 0.00$

CWP - cowpea; LAB - lactic acid bacteria; MOL - molasses; ND, not detected; SOR - sorghum

^{abc} Mean values with different superscripts in the same column differ significantly ($P < 0.05$).

Table 9. Contents of lactate and volatile fatty acids (% DM) in filtrates of the Rostock Fermentation Test with jack bean grains after 38 h of incubation.

	Lactate	Acetate	Propionate	Butyrate
JBN	$2.26^{a} \pm 0.24$	$1.14^{bc} \pm 0.26$	ND	0.05 ± 0.04
JBN+MOL	$2.80^{c} \pm 0.00$	$1.28^{c} \pm 0.06$	ND	ND
JBN+SOR	$2.23^{a} \pm 0.03$	$0.83^{b} \pm 0.03$	ND	0.05 ± 0.01
JBN+LAB	$3.71^{d} \pm 0.06$	$0.93^{b} \pm 0.09$	ND	ND
JBN+LAB+MOL	$4.63^{e} \pm 0.07$	$0.45^{a} \pm 0.36$	ND	ND
JBN+LAB+SOR	$2.51^{b} \pm 0.01$	$0.84^{b} \pm 0.00$	ND	ND

JBN - jack bean; LAB - lactic acid bacteria; MOL - molasses; ND, not detected; SOR - sorghum

^{abc} Mean values with different superscripts in the same column differ significantly ($P < 0.05$).

Table 10. Contents of alcohol (% DM) and NH_3 (% DM) in filtrates of the Rostock Fermentation Test with jack bean grains after 38 h of incubation.

	Ethanol	Propanol	2,3 Butandiol	NH_3
JBN	$0.34^{b} \pm 0.04$	ND	$0.11^{ab} \pm 0.03$	$0.11^{e} \pm 0.01$
JBN+MOL	$0.36^{b} \pm 0.04$	ND	$0.23^{b} \pm 0.12$	$0.12^{f} \pm 0.01$
JBN+SOR	$0.19^{a} \pm 0.03$	ND	$0.14^{ab} \pm 0.05$	$0.07^{b} \pm 0.01$
JBN+LAB	$0.18^{a} \pm 0.07$	ND	ND	$0.09^{d} \pm 0.00$
JBN+LAB+MOL	$0.15^{a} \pm 0.00$	ND	$0.03^{a} \pm 0.00$	$0.09^{d} \pm 0.00$
JBN+LAB+SOR	$0.19^{a} \pm 0.02$	ND	$0.03^{a} \pm 0.00$	$0.06^{a} \pm 0.00$

JBN - jack bean; LAB - lactic acid bacteria; MOL - molasses, ND, not detected; SOR - sorghum

^{abc} Mean values with different superscripts in the same column differ significantly ($P < 0.05$).

(different sources of tannins) and plant materials could be the reason for such a difference.

The results revealed RFT as an appropriate tool to predict ensilability within a short time, whereas only few facilities are needed in the lab. In the present study it was concluded, that at the end of RFT (38 h) good fermentation characteristics and low pH values of CWP and JBN grains sole or mixed with SOR grains were obtained. However, the use of inoculants alone or in combination with molasses should be considered. Further studies with laboratory scale model silages are recommended to investigate the effect of SOR on NH_3 reduction, as it was shown in RFT of CWP and JBN.

Acknowledgements

The authors are thankful for the support of the technical staff of the chair of nutrition physiology and animal nutrition of the University of Rostock for the chemical analysis and for the help of the workers of the Experimental Station "Álvaro Barba" University of "Las Villas" establishing and harvesting the experimental parcels.

References

Akpapunam, M.A. & Markakis, P. 1979. Oligosaccharides of 13 American cultivars of cowpeas (*Vigna sinensis*). J. Food Sci. 44:1317

Amubode, F.O. & Fetuga, B.L. 1983. Proximate composition and chemical assay of the methionine, lysine and tryptophan concentration of some forest tree seeds. Food Chem. 12:67

Belmar, R., Nava-Montero, R., Sandoval-Castro, C. & McNab, J.M. 1999. Jack bean (*Canavalia ensiformis* L. DC) in poultry diets: antinutritional factors and detoxification studies – a review. World´s Poultry Science Journal, Vol. 55

Bhat, R., Sridhar, K.R., Young, C.C., Arun, A.B. & Ganesh, S. 2008. Composition and functional properties of raw and electron beam-irradiated *Mucuna pruriens* seeds. Int. J. Food Sci. Technol. 43:1338

Cáceres, O., González, E. & Delgado, R. 1995. *Canavalia ensiformis*: leguminosa forrajera promisoria para la agricultura tropical. Pastos y Forrajes, 18:2

Cherney, J.H. & Cherney, D.J.R. 2003. Assessing silage quality, In: Silage Science and Technology, ed. by Buxton DR, Muck RE and Harrison JH. American Society of Agronomy, Madison, WI

Deshpande, S.S. & Salunkhe, D.K. 2000. Grain legumes, seeds and nuts: rationale for fermentation. Fermented Grain Legumes, Seeds and Nuts: A Global Perspective. FAO Agricultural Services Bulletin, FAO, Rome, 142:1-32

Ekanayake, S., Jansz, E.R. & Nair, B.M. 2000. Literature review of an underutilized legume: *Canavalia gladiata* l. Plant foods for human nutrition 55:305

Ensminger, M.E. 1992. The Stockman´s Handbook. Seventh edition by Interstate Publishers. U.S.A.

Gefrom, A., Ott, E.M. & Zeyner, A. (2009). Ensiling moistly harvested lupine seeds and the influence of conservation on oligosaccharides. Proc. Soc. Nutr. Physiol. 18:115

Goering, H.K. & van Soest, P.J. 1970. Forage fibre analysis. ARS, USDA, Washington, DC

Gomez-Brenes, R., Bendana, G., Gonzalez, J.M., Jarquin, R., Braham, J.E. & Bressani, R. 1988. Effects of the treatment of coffee pulp, fresh or ensilaged, with calcium hydroxide, on its nutritive value. Arch. Latinoam. Nutr. 38:173

Grabber, J., Rotz, C. & Muck, R. 2002. Tannin-containing alfalfa: a way to improve nitrogen-use and profitability of dairy farms? Abstract. American Grassland Council Conference Proceedings. 17 July 2002. Available: http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=134825

Hirst, E.L., Mackenzie, D.J. & Wylam, C.B. 1959. Analytical studies on the carbohydrates of grasses and clovers. J. Sci. Food Agric. 10:19

Hoedtke, S. & Zeyner, A. 2011. Comparative evaluation of laboratory-scale silages using standard glass jar silages or vacuum-packed model silages. J. Sci. Food Agric. 91:841

Kjeldahl, J. 1883. Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. Fresenius J. Anal. Chem. 22:366

Liener, I.E. 1962. Toxic factors in edible legumes and their elimination. American Journal of Clinical Nutrition, Vol 11

McDonald, P. & Henderson, A.R. 1962. Buffering capacity of herbage samples as a factor in ensilage. J. Sci. Food Agric. 13:395

McDonald, P., Henderson, N. & Heron, S. 1991. The biochemistry of silage. Chalcombe Publications,Marlow, UK

McDonald, P. & Whittenbury, R. 1973. Chemistry and biochemistry of herbages, (Butler, G.W. and Bailey, R.W., Eds.) Academic Press, New York. 3:33

Menge-Hartmann, U., Soufan, W. & Greef, J.M. 2009. The influence of plant development stage and N fertilization on the content of watersoluble carbohydrates and fructans in different varieties of *Lolium perenne*. J. Kulturpflanzen 61:365

Muck, R.E. 1993. The role of silage additives in making high quality silages. Proceedings of the National Silage Producers Conference. NREAS-67, Ithaca, NY, 106

Muck, R.E. & Bolsen, K.K. 1991. Silage preservation and silage additive. In: Hay and silage management in North America (Bolsen, K.K., Baylor, J.E., McCullough, eds). National Feeds Ingredients Association, West Des Moines, Iowa, 105

Nwinuka, N.M., Abbey, B.W. & Ayalogu, E.O. 1997. Effect of processing on flatus producing oligosaccharides in cowpea (*Vigna unguiculata*) and the tropical African yam bean (*Sphenostylis stenocarpa*). Plant Foods Hum. Nutr. 51:209

Pieper, R., Hackl, W., Korn, U., Zeyner, A., Souffrant, W.B. & Pieper, B. 2010. Effect of ensiling triticale, barley and wheat grains at different moisture content and addition of *Lactobacillus plantarum* (DSMZ 8866 and 8862) on fermentation characteristics and nutrient digestibility in pigs. Anim. Feed Sci. Technol. 164:96

Pieper, B., Kleemann, J., Poppe, S., Allert, H., Losch, K., Wittchen, H., Mielitz, E. & Schulz, A. 1989: Verfahren zur Bestimmung der Vergärbarkeit von Futterpflanzen. Patent specification DD 281 255 A5. Patent office of the GDR

Raguse, C.A. & Smith, D. 1966. Some nonstructural carbohydrates in forage legume herbage. J. Agric. Food Chem. 14:423

Salawu, M.B, Acamovic, T., Stewart, C.S., Hvelplund, T. & Weisbjerg, M.R 1999.The use of tannins as silage additives: effects on silage composition and mobile bag disappearance of dry matter and protein. Anim. Feed Sci. Technol. 82:243

Schmidt, T., Krawielitzki, K., Voigt, J. & Gabel, M. 2005. Methodical aspects of the *in situ* technique for estimating ruminal nutrient degradation: microbial contamination and lag time. Übers. Tierernährg. 33:87

Sotelo A, Migliaro P, Toledo A & Contreras J. 1999. Chemical composition, digestibility and antinutritional factors content of two wild legumes: *Styphonolobium burseroides* and *Acacia bilimekii*. Plant Foods Hum. Nutr. 54:59

Spoelstra, S.F. 1991. Chemical and biological additives in forage conservation. In: Forage Conservation Towards 2000 (Pahlow, G., Honig, H., eds). Institute of Grassland Forage Research, Braunschweig, Germany, 40

SPSS, 2010. Software for Windows, version 19, Inc., Chicago, IL, USA

Sridhar, K.R. & Seena, S. 2006 Nutritional and anti-nutritional significance of four unconventional legumes of the genus Canavalia – A comparative study. Food Chem. 99:267

Tabacco, E., Borreani, G., Crovetto, G.M., Galassi, G., Colombo D. & Cavallarin L. 2006. Effect of chestnut tannin on fermentation quality, proteolysis, and protein rumen degradability of alfalfa silage. J. Dairy Sci. 89:4736

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA, Ed.) 1993. Die chemische Untersuchung von Futtermitteln. In:

Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Vol. III. VDLUFA-Verlag, Darmstadt

Voigt, J. & Steger, H. 1967. Zur quantitativen Bestimmung von Ammoniak, Harnstoff und Ketokörpern in biologischem Material mit Hilfe eines modifizierten Mikrodiffusionsgefäßes. Archiv Tierernähr. 17:289

Weißbach, F. 1967. Die Bestimmung der Pufferkapazität der Futterpflanzen und ihre Bedeutung für die Beurteilung der Vergärbarkeit. Akademie der Landwirtschaftswissenschaften der DDR 92:211

Wilson, R.F. & Wilkins, R.J. 1972. An evaluation of laboratory ensiling techniques. J. Sci. Food Agric. 23:377

Zierenberg, B. 2000. *In vitro* Methode zur Beurteilung der Fermentationsleistung von Milchsäurebakterien und deren Einfluss auf die Stoffwechselaktivität weiterer für die Silierung relevanter Mikroorganismen bei unterschiedlichen Fermentationsbedingungen. PhD thesis, University of Rostock

Zimmer, E. 1990. Evaluation of fermentation parameters from the silages experiments. In: Proceedings of he EUROBAC Conference (S. Lindgren and K.L. Pettersson, eds). Swedish University of Agricultural Sciences, Uppsala, 19

Received: May 26, 2011