STUDY OF THE EFFECT OF EM SILAGE ON THE FERMENTATION CHARACTERISTICS, THE NUTRITIONAL CHARACTERISTICS AND THE AEROBIC STABILITY OF WHEAT BRAN AND SPELT BRAN

SUMMARY

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Title:	*Study of the effect of EM Silage on the fermentation characteristics, the nutritional characteristics and the aerobic stability of wheat bran and spelt bran – SUMMARY.*
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1. Aim

Aim of the trial was to test the effect of treatment of wheat bran and spelt bran with EM Silage product solution on fermentation characteristics, nutritional characteristics and aerobic stability.

2. Experimental Setup

The characteristics of the starting material are summarized in Table 1.

Table 1. Nutritional and microbial characteristics of starting material.

Wheat bran	
dry matter (g/kg FM)	610,9
ME dairy cattle (MJ/kg DM)	15,15
digestibility of organic matter (g/kg DM)	89,8
crude protein (g/kg DM)	177,0
crude fat (g/kg DM)	18,5
water-soluble carbohydrates (g/kg DM)	58,8
yeasts (cfu/g FM)	4,0E+02
moulds (cfu/g FM)	2,0E+02
lactic acid bacteria (cfu/g FM)	3,3E+02
Spelt bran	
dry matter (g/kg FM)	636,4
ME dairy cattle (MJ/kg DM)	14,87
digestibility of organic matter (g/kg DM)	87,5
crude protein (g/kg DM)	198
crude fat (g/kg DM)	41,00
water-soluble carbohydrates (g/kg DM)	50,70
yeasts (cfu/g FM)	<100
moulds (cfu/g FM)	4,0E+02
lactic acid bacteria (cfu/g FM)	1,4E+02
Counts on EM Silage	
yeasts (cfu/g)	4,4E+04
moulds (cfu/g)	<100
lactic acid bacteria (cfu/g)	1,8E+06

Wheat bran and spelt bran were treated with the appropriate solution:

control 5 liter tap water for 11,25 kg fresh matter

EM Silage 5 liter product solution for 11,25 kg fresh matter

Both solutions were sprayed onto the bran with hand-held sprayers.

For both treatments, 12 microsilos were filled:

- 5 microsilos at normal density, without aerobic stress
  - → "normal circumstances"
- 4 microsilos at normal density, with aerobic stress (24h) given after 10 days
  - → "aerobic stress given"
- 3 microsilos at low density, without aerobic stress
  - → "ensiled at low density"

Mean densities for both bran types were as follows:

- wheat bran
  - normal density: 311.63 ± 1.03 kg DM/m³
  - low density: 223.54 ± 1.49 kg DM/m³
- spelt bran
  - normal density: 323.26 ± 6.16 kg DM/m³
  - low density: 233.44 ± 3.28 kg DM/m³

All microsilos were opened after 28 days and samples were taken immediately after desiling.

The obtained data were statistically analyzed with SAS 5.1. Treatment with EM Silage was compared to the untreated control for the three regimes seperately: normal circumstances – aerobic stress applied – ensiled at low density.
Normality was tested by Kolmogorov-Smirnov and equality of variances by Levene's test. Normally distributed, homoscedastic data were subjected to two-sided one way ANOVA with Tukey as *post hoc* test. Otherwise, data were subjected to two-sided non-parametric one way ANOVA according to Wilcoxon. Significance was declared at $p < 0.05$.

3. Results

3.1. Wheat bran

- Fermentation losses during ensiled period

The evolution of the fermentation losses is visualized in Figure 1, while the detailed figures per object are presented in Table 2.

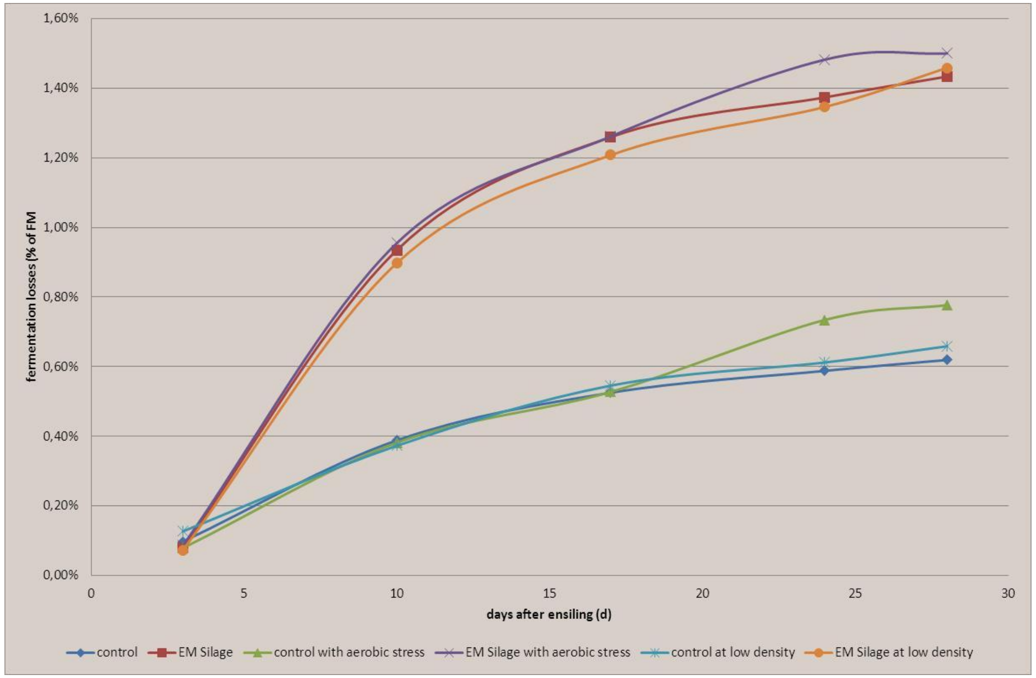

Figure 1. Evolution of fermentation losses (% of fresh matter) of wheat bran.

As can be observed in Figure 1, treatment with EM Silage clearly resulted in higher fermentation losses for all three regimes. In absolute value however, differences were limited.

Table 2. Fermentation losses (% of fresh matter) of wheat bran during ensiled period.

Fermentation losses (% of FM)	control (N=5)			control - aerobic stress (N=4)			control - low density (N=3)		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
3 days	0,10	0,01	a	0,08	0,01	a	0,13	0,01	a
10 days	0,39	0,01	a	0,38	0,01	a	0,37	0,01	a
17 days	0,53	0,01	a	0,53	0,02	a	0,55	0,03	a
24 days	0,59	0,02	a	0,73	0,03	a	0,61	0,06	a
28 days	0,62	0,04	a	0,78	0,03	a	0,66	0,06	a
	EM Silage (N=5)			EM Silage - aerobic stress (N=4)			EM Silage - low density (N=3)		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
3 days	0,08	0,01	a	0,09	0,01	a	0,07	0,01	b
10 days	0,93	0,02	b	0,95	0,01	b	0,90	0,01	b
17 days	1,26	0,02	b	1,26	0,01	b	1,21	0,02	b
24 days	1,37	0,02	b	1,48	0,01	b	1,35	0,02	b
28 days	1,43	0,02	b	1,50	0,01	b	1,46	0,02	b

These data confirm the pattern visible in Figure 1: from 10 days after ensiling on until desiling after 28 days, application of EM Silage resulted in significantly higher fermentation losses compared to the untreated control in all three regimes. In absolute value, differences were limited.

- Fermentation characteristics

Table 3 summarizes the results of the chemical analyses, the microbial analyses and the determination of the aerobic stability.

Many **chemical parameters** differ significantly between treatments, especially for the regime of normal circumstances and the regime with aerobic stress given: under normal circumstances, only dry matter content and butyric acid content did not differ significantly – when aerobic stress was given, only the butyric acid content did not differ significantly. Treatment with EM Silage resulted in a lower ammonia content and an altered fermentation fatty acid profile: lactic acid concentrations were lower, but acetic acid and propionic acid levels were increased, due to activity of heterofermentative lactic acid bacteria. These bacteria have however increased the ethanol content. A more intense fermentation was observed since only little water-soluble carbohydrates were detected at desiling.

Table 3. Chemical analyses, microbial analyses and aerobic stability of wheat bran.

Fermentation characteristics	control (N=5)			control - aerobic stress (N=4)			control - low density (N=3)		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
DM* at desiling (g/kg FM)	621,77	4,06	a	613,79	1,90	a	659,85	15,65	a
ammonia (g/kg DM)	0,945	0,023	a	0,950	0,030	a	0,865	0,035	a
ammonia-nitrogen/ total nitrogen	2,83	0,06	a	2,78	0,06	a	2,96	0,31	a
pH	4,08	0,07	a	4,28	0,01	a	4,26	0,07	a
lactic acid (g/kg DM)	53,59	5,82	a	44,56	0,57	a	40,81	4,61	a
acetic acid (g/kg DM)	7,61	0,51	a	7,51	0,21	a	7,70	1,03	a
butyric acid (g/kg DM)	0,00	0,00	a	0,00	0,00	a	0,00	0,00	a
propionic acid (g/kg DM)	0,00	0,00	a	0,00	0,00	a	0,04	0,06	a
ethanol (g/kg DM)	9,64	1,12	a	9,48	1,41	a	7,38	1,01	a
water-soluble carbohydrates (g/kg DM)	41,23	3,87	a	39,81	0,98	a	32,75	7,19	a
yeasts (log cfu/g FM)	3,89	0,74	a	5,15	0,73	a	5,29	0,80	a
moulds (log cfu/g FM)	2,68	1,39	a	2,98	2,01	a	4,35	2,13	a
lactic acid bacteria (log cfu/g FM)	7,16	0,15	a	7,19	0,10	a	7,27	0,23	a
aerobic stability (h)	117,17	45,85	a	45,31	10,64	a	61,91	25,57	a
max. temperature during Honig (°C)	36,03	4,33	a	64,66	2,89	a	40,28	6,56	a
	EM Silage (N=5)			EM Silage - aerobic stress (N=4)			EM Silage - low density (N=3)		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
DM* at desiling (g/kg FM)	635,34	20,52	a	622,91	3,90	b	645,79	37,48	a
ammonia (g/kg DM)	0,639	0,026	b	0,640	0,036	b	0,571	0,029	b
ammonia-nitrogen/ total nitrogen	1,94	0,05	b	1,91	0,13	b	1,78	0,07	b
pH	4,26	0,01	b	4,24	0,02	b	4,27	0,02	a
lactic acid (g/kg DM)	37,64	1,91	b	38,36	1,83	b	28,67	12,22	a
acetic acid (g/kg DM)	21,19	2,75	b	23,97	2,28	b	15,34	6,64	a
butyric acid (g/kg DM)	0,00	0,00	a	0,00	0,00	a	0,00	0,00	a
propionic acid (g/kg DM)	0,23	0,04	b	0,22	0,03	b	0,09	0,08	a
ethanol (g/kg DM)	15,68	2,30	b	16,51	0,44	b	20,01	1,47	b
water-soluble carbohydrates (g/kg DM)	0,38	0,86	b	0,00	0,00	b	0,00	0,00	b
yeasts (log cfu/g FM)	1,98 **	0,00	b	1,98 **	0,00	b	1,98 **	0,00	b
moulds (log cfu/g FM)	1,98 **	0,00	a	1,98 **	0,00	a	1,98 **	0,00	a
lactic acid bacteria (log cfu/g FM)	7,58	0,25	b	7,80	0,44	b	8,00	0,07	b
aerobic stability (h)	175,00 ***	0,00	b	175,00 ***	0,00	b	175,00 ***	0,00	b
max. temperature during Honig (°C)	20,56	0,32	b	20,44	0,42	b	20,21	0,35	b

* corrected for volatile compounds according to Dulphy & Demarquilly (1981)

** below detection limit (100 cfu/g FM)

*** no heating to 3°C above surrounding temperature within 170 hours

The results of the **microbial counts** and **aerobic stability** are similar for the three regimes: treatment with EM Silage significantly decreased yeast numbers and significantly increased lactic acid numbers. Aerobic stability of EM Silage treated wheat bran was significantly higher compared to the untreated control and the maximum temperature during the Honig protocol was significantly lower in case of EM Silage application.

- Nutritional characteristics

The nutritional characteristics are given in Table 4.

Table 4. Nutritional characteristics of wheat bran.

Nutritional characteristics	control			control - aerobic stress			control - low density		
(N=3, except crude protein: N=5)	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
digestibility of organic matter (%)	86,30	1,37	a	86,89	0,56	a	87,88	0,25	a
crude ash (g/kg DM)	67,79	0,86	a	68,43	1,64	a	66,89	1,53	a
crude protein (g/kg DM)	170,01	1,93	a	174,37	2,14	a	149,60	15,52	a
crude fat (g/kg DM)	23,25	0,74	a	21,87	0,17	a	20,55	0,12	a
crude fiber (g/kg DM)	138,51	4,06	a	134,01	2,64	a	124,79	1,52	a
starch (g/kg DM)	173,90	17,11	a	169,90	4,66	a	179,63	7,65	a
	EM Silage			EM Silage - aerobic stress			EM Silage - low density		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
digestibility of organic matter (%)	86,50	0,46	a	85,92	0,78	a	85,24	2,41	a
crude ash (g/kg DM)	68,36	0,23	a	67,94	0,19	a	68,31	0,62	a
crude protein (g/kg DM)	168,31	6,27	a	171,99	2,68	a	164,49	7,79	a
crude fat (g/kg DM)	22,32	0,67	a	22,53	0,24	b	19,99	0,52	a
crude fiber (g/kg DM)	132,46	6,87	a	122,15	4,22	b	126,15	1,60	a
starch (g/kg DM)	179,40	5,40	a	196,73	33,92	a	182,46	1,81	a

Very few significant differences in nutritional characteristics were observed. Only in the regime where aerobic stress was given, a significantly higher crude fat content and a significantly lower crude fiber content was observed in EM Silage treated wheat bran compared to the untreated control. The other two regimes revealed no significant differences in nutritional parameters.

Table 5 summarizes the energy content for dairy cattle, horse and pig.

Table 5. Energy content (MJ/kg DM) of wheat bran for dairy cattle, horse and pig.

Energy content (MJ/kg DM) (N=3)	control			control - aerobic stress			control - low density		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
ME for dairy cattle	14,50	0,29	a	14,54	0,07	a	14,72	0,05	a
ME for horse	8,82	0,04	a	8,84	0,03	a	8,86	0,03	a
NE for pig	7,20	0,21	a	7,04	0,06	a	6,91	0,19	a
	EM Silage			EM Silage - aerobic stress			EM Silage - low density		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
ME for dairy cattle	14,48	0,10	a	14,43	0,18	a	14,17	0,44	a
ME for horse	8,85	0,05	a	8,92	0,02	b	8,86	0,02	a
NE for pig	6,97	0,06	a	7,23	0,36	a	6,92	0,17	a

No significant differences between treatments were observed, except for the regime were aerobic stress was given: the metabolizable energy for horse was significantly higher in case of treatment with EM Silage compared to the untreated control.

3.2. Spelt bran

- Fermentation losses during ensiled period

The evolution of the fermentation losses is visualized in Figure 2, while the detailed figures per object are presented in Table 5.

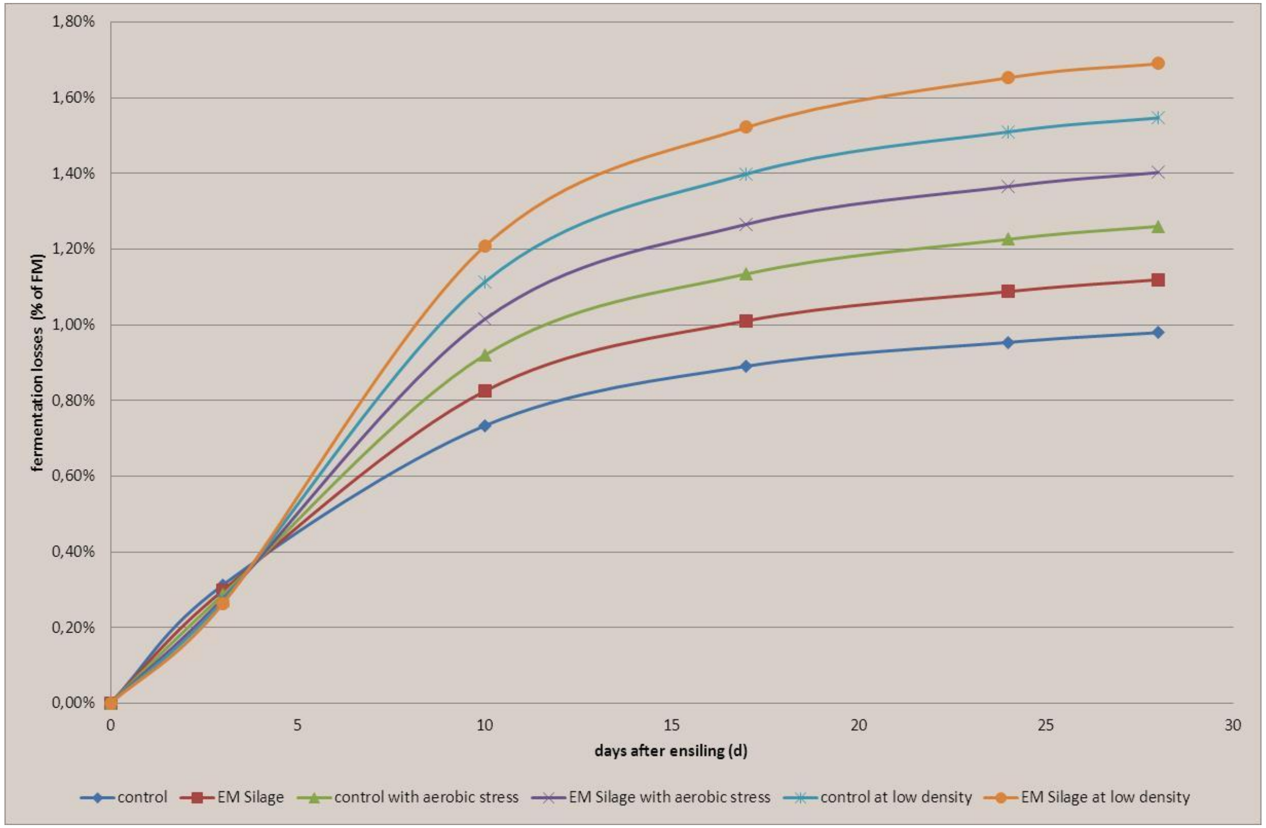

Figure 2. Evolution of fermentation losses (% of fresh matter) of spelt bran.

As visualized in Figure 1, the fermentation losses are higher after application of EM Silage for all three regimes. This is confirmed by the data in Table 5. However, differences are limited in absolute value.

Table 5. Fermentation losses (% of fresh matter) of spelt bran during ensiled period.

Fermentation losses	**control** (N=5)			**control - aerobic stress** (N=4)			**control - low density** (N=3)		
(% of FM)	**mean**	**st.dev.**	**≠**	**mean**	**st.dev.**	**≠**	**mean**	**st.dev.**	**≠**
3 days	0,31	0,01	a	0,29	0,01	a	0,29	0,01	a
10 days	0,73	0,02	a	0,72	0,01	a	0,72	0,01	a
17 days	0,89	0,02	a	0,89	0,03	a	0,88	0,01	a
24 days	0,95	0,02	a	1,05	0,03	a	0,95	0,03	a
28 days	0,97	0,02	a	1,06	0,01	a	0,97	0,03	a
	EM Silage (N=5)			**EM Silage - aerobic stress** (N=4)			**EM Silage - low density** (N=3)		
	mean	**st.dev.**	**≠**	**mean**	**st.dev.**	**≠**	**mean**	**st.dev.**	**≠**
3 days	0,26	0,01	b	0,25	0,01	b	0,24	0,03	a
10 days	1,21	0,01	b	1,20	0,02	b	1,12	0,02	b
17 days	1,52	0,01	b	1,52	0,01	b	1,46	0,02	b
24 days	1,65	0,01	b	1,74	0,01	b	1,59	0,02	b
28 days	1,69	0,01	b	1,75	0,01	b	1,64	0,03	b

- Fermentation characteristics

Table 6 summarizes the results of the chemical analyses, the microbial analyses and the determination of the aerobic stability.

Many **chemical parameters** differ significantly between treatments. Generally, treatment with EM Silage resulted in a lower pH and an altered fermentation fatty acid profile: lactic acid concentrations were higher, as well as acetic acid levels due to activity of heterofermentative lactic acid bacteria. These bacteria have however increased the ethanol content. A more intense fermentation was observed since only low water-soluble carbohydrate contents were detected at desiling.

Table 6. Chemical analyses, microbial analyses and aerobic stability of spelt bran.

Fermentation characteristics	control (N=5)			control - aerobic stress (N=4)			control - low density (N=3)		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
DM* at desiling (g/kg FM)	635,60	1,53	a	633,01	0,89	a	639,15	1,02	a
ammonia (g/kg DM)	0,508	0,107	a	0,398	0,032	a	0,627	0,083	a
ammonia-nitrogen/ total nitrogen	1,39	0,30	a	1,09	0,08	a	1,71	0,24	a
pH	4,71	0,18	a	4,94	0,10	a	4,70	0,02	a
lactic acid (g/kg DM)	8,80	2,31	a	10,66	1,84	a	29,82	1,50	a
acetic acid (g/kg DM)	1,36	0,28	a	2,16	0,40	a	4,87	0,13	a
butyric acid (g/kg DM)	0,00	0,00	a	0,00	0,00	a	0,00	0,00	a
propionic acid (g/kg DM)	0,00	0,00	a	0,00	0,00	a	0,00	0,00	a
ethanol (g/kg DM)	14,77	0,31	a	14,02	0,80	a	15,02	0,35	a
water-soluble carbohydrates (g/kg DM)	43,14	4,09	a	40,95	4,11	a	46,14	5,32	a
yeasts (log cfu/g FM)	4,12	0,80	a	5,35	0,26	a	3,90	0,54	a
moulds (log cfu/g FM)	1,98 **	0,00	a	2,59	1,23	a	1,98 **	0,00	a
lactic acid bacteria (log cfu/g FM)	6,61	0,15	a	5,66	0,10	a	6,36	0,11	a
aerobic stability (h)	80,55	17,33	a	40,11	2,70	a	108,06	27,13	a
max. temperature during Honig (°C)	33,73	8,52	a	35,74	3,25	a	28,47	4,93	a
	EM Silage (N=5)			EM Silage - aerobic stress (N=4)			EM Silage - low density (N=3)		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
DM* at desiling (g/kg FM)	641,52	4,54	b	642,65	1,41	b	642,71	5,09	a
ammonia (g/kg DM)	0,511	0,084	a	0,420	0,033	a	0,428	0,036	b
ammonia-nitrogen/ total nitrogen	1,39	0,23	a	1,15	0,08	a	1,25	0,29	a
pH	4,45	0,03	b	4,44	0,01	b	4,45	0,01	b
lactic acid (g/kg DM)	14,96	4,86	b	21,28	12,04	a	30,83	4,23	a
acetic acid (g/kg DM)	4,65	1,46	b	6,97	3,96	b	9,64	1,33	b
butyric acid (g/kg DM)	0,00	0,00	a	0,00	0,00	a	0,00	0,00	a
propionic acid (g/kg DM)	0,00	0,00	a	0,04	0,07	a	0,11	0,11	a
ethanol (g/kg DM)	20,40	3,29	b	20,11	3,41	b	19,63	1,69	b
water-soluble carbohydrates (g/kg DM)	6,56	2,91	b	0,00	0,00	b	0,00	0,00	b
yeasts (log cfu/g FM)	1,98 **	0,00	b	1,98 **	0,00	b	1,98 **	0,00	b
moulds (log cfu/g FM)	2,16	0,41	a	1,98 **	0,00	a	2,54	0,97	a
lactic acid bacteria (log cfu/g FM)	7,43	0,41	b	7,66	0,43	b	7,71	0,34	b
aerobic stability (h)	175,00 ***	0,00	b	175,00 ***	0,00	b	175,00 ***	0,00	b
max. temperature during Honig (°C)	20,41	0,40	b	20,38	0,38	b	20,54	0,46	a

* corrected for volatile compounds according to Dulphy & Demarquilly (1981)

** below detection limit (100 cfu/g FM)

*** no heating to 3°C above surrounding temperature within 170 hours

The results of the **microbial counts** and **aerobic stability** are similar for the three regimes: treatment with EM Silage significantly decreased yeast numbers and significantly increased lactic acid bacteria numbers. The aerobic stability of EM Silage treated wheat bran was significantly higher compared to the untreated control. The maximum temperature during the Honig protocol was significantly lower in case of EM Silage application (except for the low density regime).

- Nutritional characteristics

The nutritional characteristics are given in Table 7.

Table 7. Nutritional characteristics of spelt bran.

Nutritional characteristics	control			control - aerobic stress			control - low density		
(N=3)	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
digestibility of organic matter (%)	85,62	1,05	a	86,57	0,42	a	84,95	0,59	a
crude ash (g/kg DM)	83,43	0,45	a	88,79	3,31	a	83,95	2,55	a
crude protein (g/kg DM)	194,90	5,44	a	197,77	1,44	a	197,64	2,00	a
crude fat (g/kg DM)	36,84	0,69	a	36,62	0,96	a	36,93	1,12	a
crude fiber (g/kg DM)	111,70	1,55	a	110,02	1,06	a	119,50	7,65	a
starch (g/kg DM)	179,86	3,56	a	183,43	3,33	a	179,04	3,50	a
	EM Silage			EM Silage - aerobic stress			EM Silage - low density		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
digestibility of organic matter (%)	85,28	0,37	a	83,65	1,24	b	85,08	1,41	a
crude ash (g/kg DM)	99,73	2,59	b	89,68	3,69	a	86,25	1,99	a
crude protein (g/kg DM)	198,07	1,60	a	193,46	2,08	b	197,52	1,11	a
crude fat (g/kg DM)	36,52	0,69	a	38,30	1,28	a	37,80	1,14	a
crude fiber (g/kg DM)	112,86	2,16	a	120,46	6,62	a	119,41	2,73	a
starch (g/kg DM)	184,61	6,24	a	181,90	0,85	a	184,83	3,96	a

Only few significant differences in nutritional characteristics were observed. Under the normal regime, the crude ash content was significantly higher in the EM Silage treated spelt bran. In the regime where aerobic stress was given, digestibility of organic matter and crude protein content were decreased significantly by application of EM Silage.

Table 8 summarizes the energy content for dairy cattle, horse and pig.

Table 8. Energy content (MJ/kg DM) of spelt bran for dairy cattle, horse and pig.

Energy content (MJ/kg DM) (N=3)	control			control - aerobic stress			control - low density		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
ME for dairy cattle	14,46	0,21	a	14,51	0,11	a	14,33	0,14	a
ME for horse	9,04	0,02	a	9,00	0,04	a	8,98	0,04	a
NE for pig	7,27	0,11	a	7,29	0,03	a	7,49	0,08	a
	EM Silage			EM Silage - aerobic stress			EM Silage - low density		
	mean	st.dev.	≠	mean	st.dev.	≠	mean	st.dev.	≠
ME for dairy cattle	13,98	0,14	b	13,99	0,36	a	14,35	0,37	a
ME for horse	8,88	0,02	b	8,93	0,00	b	8,97	0,04	a
NE for pig	7,07	0,10	a	7,22	0,09	a	7,30	0,05	b

In the normal circumstances regime, metabolizable energy contents for dairy cattle and for horse were decreased significantly by EM Silage application, while only the metabolizable energy content for horse was decreased significantly in the regime where aerobic stress was given. In the low density regime, application of EM Silage resulted in a significantly lower net energy content for pig compared to the untreated control.

4. Conclusion

Generally, application of EM Silage on bran clearly altered the fermentation characteristics: a more intense hetero-fermentative WSC-fermentation was observed, with a higher acetic acid content and higher ethanol content. A higher aerobic stability was observed as a result, as well as a lower maximum temperature during the Honig protocol for determination of the aerobic stability. Influence of EM Silage treatment on the nutritional characteristics was only minor.