

227 AGRO

227



THE BRITISH LIBRARY
Document Supply Centre

This document has been produced
and supplied by
The British Library Document
Supply Centre, Boston Spa, Wetherby,
West Yorkshire, UNITED KINGDOM,
LS23 7BQ

WARNING: Further copying of this document,
other than that allowed under the copyright law, is
not permitted without the permission of the
copyright owner or an authorised licensing body.

ren 4,9 mmol/l Pansensaft. Das könnte darauf hindeuten, daß bei Flavomycin-zulage weniger NH_3 für die Proteinsynthese verwendet worden ist.

Die Pansenprotozoenpopulation hat sich eindeutig auf die Ration eingestellt. Die beobachteten Vertreter der Gattung *Epidinium* verwerten Stärke und Zellulose, sind aber zur Nutzung löslicher Zucker, die in der angegebenen Ration kaum vorhanden, nicht befähigt (Kolb und Gürtler, 1971).

Literaturverzeichnis

- Autorenkollektiv: Biologielaborant. 2. Auflage, Deutscher Landwirtschaftsverlag, Berlin (1980)
- Bährecke, G., M. Baginski und G. Fischer: *Kraftfutter* 56, 328 (1973).
- Bährecke, G., M. Baginski und G. Fischer: *Kraftfutter* 57, 245 (1974)
- Bährecke, G., M. Baginski und B. Sebestyan: *Kraftfutter* 65 376 (1982)
- Burgstaller, G., K. Mader und a. Huber: *Bayer. Landwirtsch. Jb.* 58, 622 (1981)
- Dogiel, v. A.: *Monographie der Familie Ophryoscolecidae Arch. Protistenkd.* 59, 1 (1927)
- Ender, v. A.: *Monographie der Familie Ophryoscolecidae Arch. Protistenkd.* 59, 1 (1927)
- Ender, K. und M Hartung: In: Methoden zur Bestimmung des Schlachtkörperwertes (Ender). Tag. Ber., Akad. Landwirtsch.-Wiss. DDR 263, 67, ILID, Berlin (1987)
- Ferber, K.E. und T. Winogradowa-Federowa: *Biol. Zbl.* 49, 321 (1929)
- Flachowsky, G. und G. H. Richter: *Arch. Anim. Nutr., Berlin* 41, 303 (1991)
- Gürtler, H.: In: Ernährungsphysiologie der landwirtschaftlichen Nutztiere (E. Kolb and H. Gürtler), Gustav Fischer Verlag, Jena, 247 (1971)
- Hughes, J.: *Crichton Royal Farm Ann. Rep.* W 1/46 (1983)
- Hungate, R. E.: *The rumen and its microbes.* Academic Press, New York, London (1966)
- Kaminski, S., S. Wawrzynczak, Z. Wiczorek, a. Mandecki, J. Winnicka und T. Waszkiswicz: *Ruzniki Naukowe Zootechn.* 20, 157 (1982)
- Kay, M., N. Pejic und E.a. Hunter: *Anim. Prod.* 38, 528 (1983)
- v. Manteuffel, K.: *Rinderwelt* 6, 166 (1981)
- Pressmann, B. C.: *Ann. Rev. Biochem.* 45, 925
- Scheilling, G. T.: *J. of anim. sci.* 58, 1518 (1984)
- Schiemann, R.: *Arch. Anim. Nutr.* 31, 1 (1981)

ren 4,9 mmol/l Pansensaft. Das könnte darauf hindeuten, daß bei Flavomycin-zulage weniger NH_3 für die Proteinsynthese verwendet worden ist.

Die Pansenprotozoenpopulation hat sich eindeutig auf die Ration eingestellt. Die beobachteten Vertreter der Gattung *Epidinium* verwerten Stärke und Zellulose, sind aber zur Nutzung löslicher Zucker, die in der angegebenen Ration kaum vorhanden, nicht befähigt (Kolb und Gürtler, 1971).

Literaturverzeichnis

- Autorenkollektiv: Biologielaborant. 2. Auflage, Deutscher Landwirtschaftsverlag, Berlin (1980)
- Bährecke, G., M. Baginski und G. Fischer: *Kraftfutter* 56, 328 (1973).
- Bährecke, G., M. Baginski und G. Fischer: *Kraftfutter* 57, 245 (1974)
- Bährecke, G., M. Baginski und B. Sebestyan: *Kraftfutter* 65 376 (1982)
- Burgstaller, G., K. Mader und a. Huber: *Bayer. Landwirtsch. Jb.* 58, 622 (1981)
- Dogiel, v. A.: *Monographie der Familie Ophryoscolecidae Arch. Protistenkd.* 59, 1 (1927)
- Ender, v. A.: *Monographie der Familie Ophryoscolecidae Arch. Protistenkd.* 59, 1 (1927)
- Ender, K. und M Hartung: In: Methoden zur Bestimmung des Schlachtkörperwertes (Ender). Tag. Ber., Akad. Landwirtsch.-Wiss. DDR 263, 67, ILID, Berlin (1987)
- Ferber, K.E. und T. Winogradowa-Federowa: *Biol. Zbl.* 49, 321 (1929)
- Flachowsky, G. und G. H. Richter: *Arch. Anim. Nutr., Berlin* 41, 303 (1991)
- Gürtler, H.: In: Ernährungphysiologie der landwirtschaftlichen Nutztiere (E. Kolb and H. Gürtler), Gustav Fischer Verlag, Jena, 247 (1971)
- Hughes, J.: *Crichton Royal Farm Ann. Rep.* W 1/46 (1983)
- Hungate, R. E.: *The rumen and its microbes.* Academic Press, New York, London (1966)
- Kaminski, S., S. Wawrzynczak, Z. Wieczorek, a. Mandecki, J. Winnicka und T. Waszkiswicz: *Rozniki Naukowe Zootechn.* 20, 157 (1982)
- Kay, M., N. Pejic und E.a. Hunter: *Anim. Prod.* 38, 528 (1983)
- v. Manteuffel, K.: *Rinderwelt* 6, 166 (1981)
- Pressmann, B. C.: *Ann. Rev. Biochem.* 45, 925
- Scheilling, G. T.: *J. of anim. sci.* 58, 1518 (1984)
- Schiemann, R.: *Arch. Anim. Nutr.* 31, 1 (1981)

each case. Indeed, fungi may preferentially digest the more easily degradable fibre fraction, leaving a less digestible residue (Agosin et al. 1985; Zadrazil 1984).

White rot fungi are able to increase the digestibility of plant residues without chemical and physical pretreatment through selective lignin degradation (Zadrazil, 1984). They produce polyphenyloxidases (ligninase; Ishakawa et al., 1963) which destroy lignin or split up linkages between lignin and polysaccharides. The microbial lignin degradation follows pathways similar to those of lignin autoxidation. Hydrogen peroxide plays a key role in the mechanism of fungal lignin degradation (Forney et al. 1982; Greene and Gould, 1984). H_2O_2 has been produced by mycelia of the lignin-degrading fungi. Lignin-C-C bonds have been ruptured in presence of hydrogen peroxide into nonphenolic units (Glenn et al., 1983; Tien and Kirk, 1983).

The selection of suitable microorganism is of decisive importance for the bioconversion of lignocelluloses. Particularly suitable are fast growing cellulolytic fungi which allow fermentation times from 50 to 120 hours connected with specific substrate consumption coefficients of about 2.3 g substrate per g microbial biomass and crude protein yields of more than 12 %. Strains of the genera *Aspergillus*, *Chaetomium*, *Gliocladium*, *Penicillium* and *Trichoderma* proved to be the most appropriate ones. When we use active and adapted mycelia as inoculum instead of spores it is possible to shorten the fermentation time.

Much research work has been done concerning the bioconversion of lignocelluloses by solid-state-fermentation as an alternative to submerged fermentation. Favourable conditions of solid-state-fermentation are moisture content in the substrate of 65–80% and mineral salt concentrations lower than 40 g/l. This implies a compromise with respect to the degree of conversion and the protein yield (Klappach, 1991). Solid-state fermentation will be applicable to bioconversion of lignocelluloses with good success when it is focussed on the enrichment of lignocellulosic substrates with protein, essential amino acids, vitamins etc. by just sufficient fungal growth rather than on the total conversion into fungal biomass.

The objective of the experiment was to investigate the influence of cellulolytic enzymes in by solid state fermented fungi substrates on the in sacco dry matter degradability of wheat straw.

2. MATERIAL AND METHODS

2.1. Fermented substrates

A substrate consisting of 20 % ground wheat straw (mash size of sieve: 2 mm), 40% wheat bran and 40 % dried sugar beet pulp was homogeneously mixed and inoculated with four different fungi (Table 1). The solid state fermentation was carried out over 120 hours.

After this time the fermented substrates had enzymatic activities as shown in Table 1. Dry matter losses of solid state fermentation were not measured.

Table 1 Enzymatic activities of fermented substrates

<i>Fungi varieties</i>	<i>Xylanase</i> (Units g ⁻¹ DM)	<i>Cellulase</i> (Units g ⁻¹ DM)
Wild variety	36.4	14.0
<i>Chaetomium cellulolyticum</i>	32.1	0.47
<i>Trichoderma harzianum</i>	22.1	2.3
<i>Penicillium</i> colony 10	78.6	4.9

2.2. Straw treatment

Ground wheat straw (mash size of sieve: 2 mm) was homogeneously mixed with the cellulolytic enzymes containing fermented substrate. Fermented substrate and straw were mixed in relations of 1 : 2, 1 : 5 and 1 : 10 and moistened to 30 or 50 dry matter (DM). All mixtures were stored for 24 h at 50°C in airtight glasses and then deep frozen.

2.3. In sacco degradability

The rumen dry matter degradability (DMD) of all samples was measured, using the nylon bag technique as described by Ørskov et al. (1980) and adapted to our institute (Flachowsky et al., 1988). Four rumen fistulated male sheep (inner diameter of fistula: 40 mm) of the Merino breed (body weight: 50 ± 3 kg) were fed with 1 kg artificially dried ryegrass. Minerals and vitamins were added to meet the requirements of sheep. All samples (2.0 ± 0.1 g DM) were incubated in nylon bags (70 × 100 mm, pore size: 36 µm) into the rumen for 48 h. After removal from the rumen the bags were washed by washing machine within cold water until the wash water was clear. After washing all samples were dried at 105°C for 24 h. The in sacco DMD (%) was calculated as difference before and after incubation of samples divided by the sample weight before incubation.

2.4. Chemical and statistical methods

Enzyme activities (Table 1) were determined as described by Ghose (1987) and Ghose and Bisaria (1987). Ligninase activity of fermented substrates was not determined.

Statistical analyses of data included three factorial analysis of variance (fungi substrate, DM content, ratio between fermented substrate and straw).

3. RESULTS AND DISCUSSION

The in sacco DMD of fermented substrate (without straw, 70 %) was higher than that of wheat straw (44 % after 48 h incubation time). Therefore higher portions of straw and lower portions of fermented substrate within the mixture decreased significantly ($P < 0.05$) in sacco DMD from 52.0 to 44.2 % on the average (Table 2). In earlier experiments (Flachowsky and Klappach, 1991) we found a higher

Table 2 In sacco DMD (% , 48 h) of wheat straw mixed with various fermented substrates

DM content of mixture (%)	Ratio between fungi substrate and straw	Control (Substrate without fungi)	Added fungi substrate				Overall
			Wild variety	Chaetomium cellulolyticum	Trichoderma harzianum	Penicillium colony 10	
							In sacco dry matter degradability (%)
30	1 : 2	55.0 ^a ± 2.1	52.4 ^{ab} ± 1.0	53.7 ^a ± 3.7	49.5 ^b ± 0.8	47.9 ^b ± 1.4	51.7
	1 : 5	46.8 ^{ab} ± 1.0	46.3 ^a ± 2.0	49.9 ^b ± 2.2	46.7 ^a ± 0.7	47.2 ^{ab} ± 0.8	47.2
	1 : 10	46.2 ^a ± 2.5	45.5 ^a ± 1.9	44.0 ^{ab} ± 2.5	42.0 ^b ± 1.1	42.0 ± 1.6	43.9
	1 : 2	53.3 ^a ± 0.3	52.6 ^{ab} ± 1.5	53.4 ^a ± 2.5	51.8 ^{ab} ± 2.7	50.7 ^b ± 1.8	52.4
50	1 : 5	47.5 ^{ab} ± 1.2	46.1 ^b ± 3.2	50.4 ^a ± 1.8	46.4 ^b ± 1.5	41.2 ^c ± 2.5	46.3
	1 : 10	44.1 ^b ± 1.0	45.6 ^{ab} ± 2.3	42.4 ^{bc} ± 2.6	49.8 ^a ± 2.3	40.7 ^c ± 2.6	44.5
	Overall	48.8	48.1	49.0	47.7	45.0	

a,b,c Means with different superscripts in the same line are significantly different ($P < 0.05$)

DMD when cellulase treated straw contained 30% DM compared with 50 % DM.

Cellulolytic enzymes from fermented substrate did not improve the DMD of the straw-fermented substrate-mixtures (Table 2). On the contrary added fungi substrates effected a decrease of DMD in some cases. *Trichoderma harzianum* and *Penicillium colony 10* decreased DMD in comparison to the control group when DM content was 30 %. *Penicillium colony 10* decreased DMD also in the case of 50 % DM (Table 2).

It seems that some fungi use available nutrients (hemicelluloses, cellulose) from straw for their own growth and residual substrates were enriched with less degradable fractions. A higher moisture content of straw may be favourable for straw degradation by some fungi (e.g. *Penicillium colony 10*) and effected a decrease of DMD (Table 2). Similar results were reported by Flachowsky and Klappach (1991).

The fungi used in the present experiment (Tables 1 and 2) probably did not produce ligninase for lignin destruction.

In conclusion, a supply of cellulolytic enzymes containing fungi treated substrate (22.1–78.6 Units xylanase; 0.47–14.0 Units cellulase g^{-1} DM) to wheat straw did not increase the in sacco DMD of straw. The cellulolytic activities of rumen microbes seem high enough for degradation of available carbohydrates.

On the contrary some living fungi degraded straw, used it for their own metabolism, enriched it with less degradable fractions and decreased in sacco DMD.

References

- Agosin, E., Monties, B. and Odier, E.: *J. Sci. Food Agric.* **36**, 925 (1985)
- Bakshi, M.P.S. Gupta, V.K. and Langar, P.N.: *Agricult. Wastes* **13**, 51 (1985)
- Basten, R. and Schöner, F.J.: *Das wirtschaftseigene Futter* **30**, 71 (1984)
- Chanal, D.S. and J.M. Hachey, J.M.: 197th. Nat. Meeting of American Chem. Soc., ACS Symp. Series **433**, 304 (1990)
- Flachowsky, G. Klappach, G.: Int. Sci. Conf., "Industrial Enzymes, Probiotics and Biological Additives", Kaunas, Lithuania, 14.-16.5. 1991, 45
- Flachowsky, G. and Ørskov, E.R.: *Tierernährung und Fütterung* **15**, 244 (1986/87)
- Flachowsky, G., Schneider, M., Ochrimenko, W.I., Richter, G.H. and Löhnert, H.J.: *Schriftenreihe der Lehrgangseinrichtung für Fütterungsberatung*, Remderoda **11**, 20 (1988)
- Forney, K.J., Reddy, C.A., Tein, M. and Aust, S.J.: *J. Biol. Chem.* **257**, 1455 (1982)
- Henicz, Z.: 18. Jahrestagung, Sektion Tierprod. und Vet. Med. der Univ. Leipzig, Leipzig 3./4. 2. 1987, 57
- Ghose, T.K.: *Pure and Appl. Chem.* **59**, 257 (1987)
- Ghose, T.K. and V.S. Bisaria: *Pure and Appl. Chem.* **59**, 1739 (1987)
- Glenn, J.K., Morgan, M.A., Mayfield, M.B., Kuwahara, M. and Gold, M.H.: *Biochem. and Biophys. Res. Commun.* **114**, 1077 (1983)
- Greene, R.V. and Gould, J.M.: *Biochem. and Biophys. Res. Commun.* **118**, 437 (1984)
- Ishakawa, H., Schubert, W.J. and Nord, F.F.: *Arch. Biochem. Biophys.* **100**, 131 (1963)
- Jaster, E.H. and Moore, K.J.: *J. Dairy Sci.* **71**, 705 (1988)
- Kirk, T.K. and Moore, W.E.: *Wood Fiber* **4**, 72 (1972)
- Klappach, G.: Int. Sci. Conf. "Industrial Enzymes, Probiotics and Biological Additives", Kaunas, Lithuania, 14.-16.5. 1991
- Lancaster, C.M. and Patterson, J.A.: *J. Anim. Sci.* **66**, 1800 (1988)
- Morrison, I.M.: *J. Sci. Food Agric.* **42**, 295 (1988)
- Ørskov, E.R., Deß Hovell, F.D. and Mould, F.: *Trop. Anim. Prod.* **5**, 195 (1980)
- Rai, S.N. and Mudgal, V.D.: *Biol. Wastes* **24**, 175 (1988)
- Tien, M. and Kirk, T.K.: *Science* **221**, 661 (1983)
- Zadrazil, F.: *Europ. J. Appl. Microbiol.* **4**, 273 (1977)
- Zadrazil, F.: *Kali-Briefe* **14**, 759 (1979)
- Zadrazil, F.: Microbial conversion of lignocellulose into feed. in: Sundstøl, F. and Owen, E.: Straw and other fibrous by-products as feed. *Elsevier, Dev. in Anim. and Vet. Sci.* **14** 276-292 (1984)

ACKNOWLEDGEMENTS

The authors acknowledge the technical help of Gisela Sallen, Carmen Galambos and K. Tiroke.