

Solid-state fermentation of fibrous residues*

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ABSTRACT

Three substrates, sago fibre, rice straw and sawdust supplemented with either palm kernel cake, rice bran, sodium nitrate or urea were fermented by the white-rot fungus, *Pleurotus sajor-caju*, for 0 (control), 10 or 25 days at 25°C in the dark. The rate of supplementation with palm kernel cake and rice bran was 200 g supplement/kg substrate and the rate with sodium nitrate and urea was 100 g supplement/kg substrate. After fermentation the spent waste was analysed for total ash (TA), neutral detergent fibre (NDF), crude protein (CP) and *in vitro* dry matter digestibility (IVD). All substrate-supplement combinations except with urea, promoted fungal growth. The characteristics of the spent waste of the three substrates after 25 days were different indicating fungus-substrate specificity during fermentation. With sago fibre, primary metabolism of the soluble carbohydrates was not followed by secondary metabolism of the structural carbohydrates. This was reflected in an increase in TA and NDF. With sago fibre, a depressed IVD was associated with an increase in CP reflecting antagonism between these two processes. With sawdust, primary and secondary metabolism of the carbohydrates was evident with the sodium nitrate supplement. With rice straw, although there was also a loss of organic matter, secondary metabolism of the structural carbohydrates occurred with the rice bran and sodium nitrate supplements. Of the three substrates, rice straw supplemented with either palm kernel cake or rice bran yielded a spent waste suitable for animal feeding, with an IVD of 63.3% and composed of 20.0% TA, 11.2% CP and 55.2% NDF.

KEY WORDS: fermentation, fibrous residues, spent waste

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INTRODUCTION

The major constraint to the use of by-products from agriculture and forestry as ruminant feed is its high content of lignocellulose. The degradation of lignocellulose by physical, chemical and biological means has been the subject of considerable research, the latter being a less expensive and an ecologically more benign procedure (Akin et al., 1996). One such method is solid-state fermentation, the cultivation of micro-organisms on moist solid raw materials.

The micro-organisms generally used in solid-state fermentation are fungi, because they are very efficient enzyme producers and can easily colonise and penetrate solid particles. White-rot fungi (basidiomycetes which degrade brown lignin and leave behind white cellulose) are the only known organisms that can degrade plant lignin but the delignification is selective, some species such as *Phanerochaete chrysosporium* degrading lignin, cellulose and hemicellulose (Jung et al., 1992; Akin et al., 1993), others such as *Cyathus stercoreus* degrading lignin and hemicellulose and leaving the cellulose intact (Karunanandaa et al., 1992). The degradation of lignin in these residues is mediated by the peroxidase, oxidase and laccase enzyme groups (Platt et al., 1984; Higuchi, 1990; Kerem et al., 1992). An additional benefit is the potential ability of these fungal species to decompose non-protein nitrogen from ligno-protein compounds in the substrate and to incorporate the released nitrogen into fungal protein such as chitin (Hadar et al., 1992). Chitinolytic bacteria have been isolated from rumen fluid (Kopečný et al., 1996) and chitinous compounds, such as shrimp shell waste, can be incorporated at levels of up to 15% in lamb diets (Cobos et al., 2002). Thus the spent waste which comprises the decayed substrate plus the fungal biomass, is anticipated to be higher in digestibility and crude protein than the original substrate and thus more valuable as an animal feed.

A variety of ligno-cellulosic residues are released from cropping and forestry activities in Malaysia. These include rice straw and rice husks from rice cultivation, palm press fibre, palm oil mill effluent and oil palm fronds from oil palm cultivation, sago fibre from the extraction of sago starch and sawdust from the processing of sawn timber. Present utilization of these residues as animal feed in Malaysia is poor. Pre-treatment strategies for improving their value as animal feed have been confined to the chemical treatment of rice straw (Tuen et al., 1991; Vadiveloo, 2000a) and the physical treatment of oil palm fronds (Zahari et al., 2002). Rice and oil palm cultivation also yield rice bran and palm kernel cake which are nitrogen sources in the diets of monogastrics and ruminants, respectively.

In the present investigation rice straw, sago fibre and sawdust were selected as examples of ligno-cellulosic residues for fermentation by the white-rot fungus *Pleurotus sajor-caju*. To optimize fungal growth, organic and inorganic nitrogen supplements were added to the residues. Palm kernel cake and rice bran were selected as organic supplements because of their ready availability; sodium nitrate

and urea were selected as inorganic supplements because they are used for this purpose in mushroom cultivation and ruminant feeding, respectively.

MATERIAL AND METHODS

Substrates and supplements

Three substrates, sago fibre, rice straw or sawdust were used as sources of carbon. Each of the substrates was supplemented with the following sources of nitrogen: palm kernel cake, rice bran, sodium nitrate or urea. For simplicity, supplements were applied on a dry matter basis, palm kernel cake and rice bran in a ratio of 1:5 (200 g supplement/kg substrate) whereas sodium nitrate or urea in a ratio of 1:10 (100 g supplement/kg substrate). On a N-basis, urea levels were therefore high. A mixed mineral solution of 0.2% KH_2PO_4 (w/v) and 0.05% MgSO_4 (w/v) was added to the sago, straw and sawdust treatments in a substrate/mineral solution ratio (w/v) of 1:4, 1:3 and 2:3, respectively. This ensured a moisture content of 70-80% and accommodated the differing moisture capacities of the substrates (Hadar et al., 1992). All except the urea treatments were then sterilized by autoclaving at 121°C for 20 min. For the urea treatments, the supplement was added after sterilization to prevent volatile loss as ammonia. Each treatment was prepared in seven replicates.

Pleurotus sajor-caju, grown on boiled wheat grains was inoculated into 6 treatment flasks and incubated at 25°C in the dark. After 10 and 25 days, respectively, the growth of mycelium in three flasks was observed. Mycelium growth was quantified as 100% if the entire surface area of the flask was covered with mycelium. The flasks were stored at -20°C until the spent waste was ready for chemical analysis. A seventh flask, not inoculated with *Pleurotus*, served as a control (0 days).

Chemical composition and digestibility

Substrates, supplements and the spent waste (as stored at -20°C) were weighed on a dry matter basis and analysed in triplicate for total ash (TA) and crude protein (CP) by standard methods (AOAC, 1984) and neutral detergent fibre (NDF) by the method of Van Soest et al. (1991). Dry matter digestibility was estimated *in vitro* (IVD) by the cellulase buffer-neutral detergent solution procedure (Bughara and Sleper, 1986) using the Onozuka 3S enzyme (Yakult Biochemicals).

Statistical analyses

For each supplement-substrate combination, the effect of duration of incubation was estimated by one-way analysis of variance and the combining ability of supplement with substrate by principal component analysis.

Principal component analysis is a multivariate statistical procedure in which several criterion variables (TA, CP, NDF and IVD) are evaluated simultaneously instead of singly as in univariate analysis. The procedure computes eigenvectors, which quantify the contribution of each criterion variable. In this instance, negative eigenvectors for TA and NDF were assumed to contribute negatively and positive eigenvectors for IVD and CP to contribute positively to the nutritive value of the spent waste. The relative contributions of the criterion variables can be quantified in a single value, the principal component score, which can be used to compare or rank the factors of interest. In this instance, the principal component score was used to rank substrates and supplements for their combining ability. Eigenvectors which made a net contribution to the principal component score were identified from their sign and magnitude, eigenvectors of similar magnitude but of opposite sign were deemed to make no net contribution to the principal component score. A ranking of 1 or 2 was described as good, 3 or 4 as poor.

Principal component analysis was preferred over a univariate procedure such as factorial analysis of variance because the latter assumes that criterion variables are independent and have equal discriminating value. As no single parameter can adequately describe nutritive value differences (Vadiveloo and Fadel, 1992), a multivariate procedure was employed.

All statistical analyses were run on a SAS Version 6 statistical package (Statistical Analysis Systems Institute Inc., 1987)

RESULTS

The composition and IVD of the substrates and organic supplements are shown in Table 1. The rice bran was finely ground and its solubility contributed to its high IVD.

TABLE 1

Chemical composition (% in DM) and IVD (%) of the substrates and supplements

By-product	Ash	CP	NDF	IVD
Sago fibre	10.2	1.7	42.3	67.0
Rice straw	19.8	4.2	65.1	57.2
Sawdust	3.5	1.7	90.2	12.5
PKC	4.8	14.4	71.1	60.7
Rice bran	5.6	10.4	36.5	93.8

IVD - *in vitro* dry matter digestibility

The sago and sawdust substrates supplemented with palm kernel cake, rice bran or sodium nitrate resulted in mycelium growth which covered the entire surface area of the flask (100%) within 10 days but did not promote fruiting bodies even after 25 days of incubation. The rice straw substrate supplemented with palm kernel

TABLE 2

Variable		Effect of supplement and substrate on the chemical composition (% in DM) and IVD (%) of the spent waste											
		Supplement			Days fermentation on sago fibre			Days fermentation on rice straw			Days fermentation on sawdust		
		0	10	25	SE diff	0	10	25	SE diff	0	10	25	SE diff
Ash	PKC	7.2	7.8	9.2	0.29***	16.9	19.2	20.5	1.22 ns	2.5	3.2	2.7	0.41 ns
	RB	7.8	8.7	9.9	0.18***	15.1	18.7	21.5	0.53***	3.0	3.3	3.9	0.29 ns
	SN	9.4	9.5	10.5	0.51 ns	21.5	23.0	22.8	0.69 ns	5.2	5.8	6.4	0.20**
	UR	6.9	7.2	8.8	0.31***	17.8	19.1	19.5	0.40**	1.7	2.0	2.4	0.16**
CP	PKC	3.2	5.3	7.8	0.57***	7.1	7.9	10.1	0.82*	5.1	5.6	4.5	0.64 ns
	RB	2.8	5.1	7.3	1.19*	6.0	7.9	12.2	0.77***	4.0	4.3	6.4	0.65*
	SN	3.1	5.9	6.6	0.76**	7.1	7.4	9.5	0.89 ns	4.8	5.5	6.5	0.52*
	UR	33.1	42.0	38.6	1.28***	25.8	23.6	21.6	1.97 ns	35.8	22.4	16.1	1.99***
NDF	PKC	59.4	46.5	83.9	3.24***	55.7	59.1	60.6	3.03 ns	89.6	84.2	90.0	1.77*
	RB	49.5	43.5	69.6	4.45**	54.2	56.6	49.7	1.89*	83.7	72.3	83.5	3.70*
	SN	47.9	36.5	54.3	3.47**	65.6	57.7	57.6	2.71*	86.5	81.5	82.2	4.65 ns
	UR	56.1	51.6	45.6	4.15 ns	66.0	60.7	60.6	2.20 ns	84.4	81.3	84.7	2.24 ns
IVD	PKC	72.6	72.3	48.5	1.15***	52.4	47.6	61.6	1.34***	23.2	15.1	30.1	1.44***
	RB	82.1	79.2	57.8	2.76***	62.5	53.1	64.9	2.21**	27.8	41.9	35.4	2.45**
	SN	80.9	80.1	68.8	2.64**	55.4	48.7	57.7	2.35*	17.6	16.2	33.1	1.25***
	UR	81.0	74.9	68.0	3.47*	58.4	55.0	61.1	2.17 ns	22.1	14.5	21.2	2.78 ns

ns - P<0.05 * P<0.05 ** P<0.01 *** P<0.001

IVD - *in vitro* dry matter digestibility

PKC - palm kernel cake, RB - rice bran, SN - sodium nitrate, UR - urea

cake, rice bran or sodium nitrate promoted 100% mycelium growth within 10 days and promoted fruiting bodies after 25 days with palm kernel cake and rice bran. All substrates supplemented with urea promoted only about 10% growth of mycelium even after 25 days of incubation.

The composition and IVD of the spent waste is shown in Table 2. Comparing the palm kernel cake, rice bran and sodium nitrate supplements with sago fibre, the results show that fermentation after 10 or 25 days significantly increased TA above control levels (0 days) for palm kernel cake and rice bran only. For all the three supplements, CP content significantly increased, NDF content decreased after 10 days and then increased after 25 days and IVD significantly declined below control levels after 25 days.

For rice straw, fermentation increased TA and significantly for rice bran ($P<0.001$) whereas NDF significantly decreased with rice bran and SN ($P<0.05$). Relative to 0 days and 10 days, respectively, IVD declined after 10 days and increased after 25 days ($P<0.05$).

For sawdust, TA increased significantly with sodium nitrate ($P<0.01$), CP increased significantly with rice bran and sodium nitrate ($P<0.01$). However, NDF after 25 days was not significantly different from control levels although IVD significantly increased after 25 days for all supplements ($P<0.01$).

The urea treatment increased TA above control levels for all substrates. With sago, CP was increased but with rice straw and sawdust, CP was decreased. The NDF content decreased with sago and rice straw but was unchanged with sawdust. Compared to control levels, IVD decreased with sago after both 10 and 25 days, but with rice straw and sawdust, IVD decreased after 10 days but increased after 25 days (Table 2).

Table 2 also shows that the NDF of the palm kernel cake/rice straw treatment at 0 days was lower (55.7%) than either palm kernel cake (71.1%) or rice straw (65.1%; Table 1). Unusually, the IVD at 0 days was also lower (52.4%) than the individual constituents (60.7 and 57.2%). In the case of palm kernel cake/sago treatment, the IVD was higher (72.6%) than the IVD of its constituents (60.7 and 67.0%; Table 1) at 0 days.

The results of the principal component analysis are shown in Tables 3 and 4. Although the urea supplement did not promote mycelium growth, it was included in the analysis to allow for a comparison between chemical and biological effects. Only statistics on the first principal component, which accounted for at least 50% of the total variance, are reported. The eigenvectors which made a net contribution to the principal component score are identified (*).

Comparing supplements, sago combined poorly with palm kernel cake and rice bran at 25 days, whereas rice straw combined well with rice bran (Table 3). However, sago and rice straw combined best with urea and sawdust combined best with sodium nitrate. Comparing substrates, palm kernel cake, rice bran and sodium nitrate combined best with rice straw at 25 days (Table 4).

TABLE 3
First principal component statistics of the supplements

Statistic	Fermentation days on sago fibre				Fermentation days on rice straw				Fermentation days on sawdust						
	0	10	25	53.1	0	10	25	56.8	0	10	25	49.8	0	10	25
Variance, %	60.8	79.9	61.8	53.1	61.9	61.9	56.8	56.8	49.8	54.9	59.3	49.8	54.9	59.3	59.3
Eigenvectors															
Ash	0.57	0.56	-0.17	0.60*	-0.38	-0.38	-0.65	-0.65	0.61*	-0.17	0.55	0.61*	-0.17	0.55	0.55
CP	-0.30	-0.43	0.54*	0.34	0.61*	0.61*	0.56*	0.56*	-0.50	0.45*	-0.50	-0.50	0.45*	-0.50	-0.50
NDF	-0.63	-0.55	-0.61	0.65*	0.48	0.48	0.50	0.50	0.42	0.60	-0.27	0.42	0.60	-0.27	-0.27
IVD	0.42*	0.45*	0.55*	-0.31	0.51*	0.51*	0.11*	0.11*	-0.44	-0.64	0.61*	-0.44	-0.64	0.61*	0.61*
Scores															
PKC	-0.98	-0.43	-1.02	-0.35	-0.31	-0.31	0.23	0.23	0.35	0.45	-0.23	0.35	0.45	-0.23	-0.23
RB	0.58	0.48	-0.47	-1.25	-0.17	-0.17	-0.53	-0.53	-0.49	-1.40	0.56	-0.49	-1.40	0.56	0.56
SN	1.12	1.11	0.18	0.91	-0.93	-0.93	-1.00	-1.00	1.21	0.02	0.97	1.21	0.02	0.97	0.97
UR	-0.70	-1.16	1.31	0.69	1.42	1.42	1.29	1.29	-1.08	0.92	-1.30	-1.08	0.92	-1.30	-1.30
Rank															
PKC	4	3	4	2	3	3	2	2	3	2	3	3	2	3	3
RB	2	2	3	1	2	2	3	3	2	4	2	2	4	2	2
SN	1	1	2	4	4	4	4	4	4	3	1	4	3	1	1
UR	3	4	1	3	1	1	1	1	1	1	4	1	1	4	4

* net contribution to the principal component score
IVD - *in vitro* dry matter digestibility

PKC - palm kernel cake, RB - rice bran, SN - sodium nitrate, UR - urea

DISCUSSION

The objectives of cultivating fungi on crop residues are to increase the digestibility and protein content of the spent waste. In this study, different sources of carbon (sago fibre, rice straw and sawdust) and nitrogen (palm kernel cake, rice bran, sodium nitrate and urea) were combined in ratios to optimize conditions for fungal growth.

Fermentation conditions were the same for all substrates but the characteristics of their spent waste were different. The factors contributing to fungus-substrate specificity during fermentation include nitrogen supplementation, concentration of oxygen, moisture content and the presence of regulatory co-factors such as manganese (Villas-Boas et al., 2002). With sago fibre and rice straw supplemented with palm kernel cake or rice bran, the increase in TA indicated loss of organic matter arising from metabolism of the soluble constituents of the substrates. Similar findings were reported with cotton straw fermented by *Pleurotus ostreatus*, where some 20-35% of the organic matter, depending on treatment, was lost (Hadar et al., 1992). With the sago substrate, primary metabolism of the soluble carbohydrates was not followed by secondary metabolism of the structural carbohydrates, hence NDF increased and IVD was depressed. With sawdust, primary and secondary metabolic activities were evident with sodium nitrate.

With rice straw supplemented with rice bran and sodium nitrate, primary metabolism of the soluble carbohydrates was followed by secondary metabolism of the structural polysaccharides as NDF declined significantly between 0 and 25 days ($P < 0.05$) and IVD increased significantly between 10 and 25 days ($P < 0.05$; Table 2). With rice straw, there is evidence that *Pleurotus sajor-caju* selectively degrades hemicellulose from the sclerenchyma and vascular bundles to facilitate degradation of mesophyll tissue by rumen microorganisms (Karunanandaa et al., 1995). This is mediated by the manganese peroxidase, lignin peroxidase and laccase enzyme systems (Cohen et al., 2002). With the palm kernel cake treatment however, there was a non-significant increase in NDF but a significant increase in IVD between 0 and 25 days. The reasons for this are not clear, but may be related to the fact that mannans, the principal polysaccharide in palm kernel cake, do not inhibit digestion (Vadiveloo and Fadel, 1992).

It is postulated that improvement in digestibility and increase in protein content, the twin aims of solid-state fermentation, are antagonistic processes (Kamra and Zadrazil, 1988). This was the case with the sago substrate which recorded a depressed IVD but an increase in CP. Zadrazil and Brunnert (1980, 1982) noted that the digestibility of wheat straw fermented by *Pleurotus eryngii*, *Lentinus edoides* or *Stropharia rugosoannulata* decreased only when supplemented with ammonium nitrate. The increase in CP recorded with all substrates may be partially attributed to fungal protein, specifically chitin, a major component of the fungal cell wall (Hadar et al., 1992).

TABLE 4

Statistic	Fermentation days on PKC			Fermentation days on RB			Fermentation days on SN			Fermentation days on UR		
	0	10	25	0	10	25	0	10	25	0	10	25
Variance, %	61.4	60.7	96.8	63.6	59.5	94.3	55.7	61.2	73.4	71.5	71.2	76.4
Eigenvectors	0.58	0.54	0.51	0.55	0.54	0.51	-0.19	0.50	0.53	0.51	0.27	0.35
Ash	0.18*	0.37*	0.50*	0.18*	0.49*	0.50*	0.26*	0.50*	0.47*	-0.49	0.48*	0.48*
CP	-0.63	-0.55	-0.49	-0.60	-0.54	-0.51	0.67	-0.50	-0.54	-0.51*	-0.59	-0.57
NDF	0.49*	0.51*	0.50*	0.55*	0.42*	0.48*	-0.67	0.50*	0.45*	0.49	0.59*	0.57
IVD	0.28	0.36	-0.08	0.42	0.46	-0.11	-0.98	0.40	0.15	0.38	0.89	0.80
Sago fibre	0.83	0.77	1.04	0.72	0.69	1.05	-0.04	0.74	0.91	0.76	0.20	0.32
Rice straw	-1.11	-1.13	-0.96	-1.14	-1.15	-0.94	1.02	-1.14	-1.07	-1.13	-1.08	-1.12
Sawdust	2	2	2	2	2	2	3	2	2	2	1	1
Rank	1	1	1	1	1	1	2	1	1	1	2	2
	3	3	3	3	3	3	1	3	3	3	3	3

* net contribution to the principal component score

IVD - *in vitro* dry matter digestibility

PKC - palm kernel cake, RB - rice bran, SN - sodium nitrate, UR - urea

All substrate-supplement combinations (except the urea treatments) were sterilized at a moisture content of 70-80% at 121°C for 20 min. This is effectively steam pre-treatment which can cause chemical (hemicellulose hydrolysis, lignin de-polymerization) and physical (particle size and pore distribution) changes (Castro et. al, 1993; Liu et. al., 1999). However, the efficiency of steam treatment depends on the type of lignocellulosic material (Grethlein et al., 1984), which may explain why the IVD at 0 days for the sago/palm kernel cake treatment was higher, whereas the IVD at 0 days for the straw/palm kernel cake treatment was lower, than the calculated value of 58.8 and 57.8% respectively based on the IVD and ratio of the constituents (Tables 1 and 2).

Although the urea supplement did not promote fungal growth, the results permit a comparison of chemical (urea) and biological (fungal fermentation) treatment methods to upgrade lignocellulosic residues. With all substrates, the urea treatment significantly increased ash content ($P < 0.01$), but the effect on NDF content and IVD after 25 days was not significant ($P > 0.05$) for rice straw and sawdust, but IVD was reduced significantly for sago ($P < 0.05$). Studies on the urea treatment of whole straw of six rice varieties corroborate the present findings with respect to ash and NDF content (Vadiveloo, 2000a) but differ in that urea treatment improved IVD through improvements in the leaf fraction (Vadiveloo, 2000b).

Urea treatment of rice straw after 25 days was ranked higher than fermentation (Table 3) due to the higher CP content (Table 2). As only 7-11 g urea/kg feed is required for maximum intake and feed utilization (Mehrez and Ørskov, 1978) and rumen microbial nitrogen synthesis does not exceed 32 g microbial N/kg digestible organic matter (Armstrong, 1993), much of the nitrogen in the urea treatment would not be incorporated into bacterial protein but would be lost as ammonia.

Of the three substrates, rice straw combined best with palm kernel cake and rice bran (Table 4). Consequently, these treatments make potential ruminant feeds, composed of (%): TA 20.0, CP 11.2, NDF 55.2 and of IVD 63.3.

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STRESZCZENIE

Fermentacja w stanie stałym włóknistych odpadów roślinnych

Trzy substraty, włókno sago, słoma ryżowa i trociny, z dodatkiem makuchu palmowego, otrąb żytnich, azotanu sodowego lub mocznika poddano fermentacji przy użyciu grzyba *Pleurotus sajor-caju*, w ciągu 0 (kontrola) 10 lub 25 dni w temperaturze 25°C, w ciemności. Dodatek makuchu palmowego i otrąb ryżowych wynosił 200 g/kg substratu, dodatek azotanu sodowego i mocznika 100 g/kg substratu. Po fermentacji w uzyskanym materiale oznaczano zawartość popiołu całkowitego (TA), NDF, białko ogólne (CP) oraz strawność s.m. *in vitro* (IVD). Wzrost grzyba był, stymulowany przez wszystkie kombinacje substrat+dodatek, z wyjątkiem mocznika. Charakterystyka pozostałości trzech badanych substratów po 25 dniowej fermentacji była zróżnicowana, co wskazuje na specyficzność układów grzyb-substrat. W przypadku włókna sago, po pierwotnym metabolizmie węglowodanów rozpuszczalnych nie nastąpiła wtórna fermentacja węglowodanów strukturalnych, co znalazło odbicie w zwiększeniu TA i NDF. W przypadku włókna sago obniżyła się również IVD, czemu towarzyszyło zwiększenie zawartości CP, co wskazuje na antagonizm między tymi dwoma procesami. Wstępny i wtórny metabolizm węglowodanów trocin był wyraźnie zwiększony przy dodatku azotanu sodowego. W przypadku słomy ryżowej, pomimo straty masy organicznej, dodatek otrąb ryżowych i azotanu sodowego wpływał na wtórną fermentację węglowodanów strukturalnych.

Z trzech badanych substratów, słoma ryżowa uzupełniona makuchem palmowym oraz otrębami ryżowymi nadaje się jako pasza dla zwierząt; zawiera (%): całkowitego popiołu 20,0; białka ogólnego 11,2 oraz NDF 55,2, o IVD 63,3%.