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THE IMPACT OF THE APPLICATION OF BREWER'S BY-PRODUCT ROASTED BARLEY HUSKS ON SELECTED PARAMETERS OF RUMEN FERMENTATION OF DAIRY COWS. *IN VITRO* STUDY

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Abstract. The aim of the study was to evaluate the effect of brewer's by-product roasted barley husks additive (RBH) on the cow's rumen fermentation process, in *in vitro* conditions. Ruminal fluid for the research was collected from 9 dairy cows selected on the basis of analogues. Animals were fed total mixed ratio (TMR). The substrates for *in vitro* experiment were composed on the basis of TMR. Control substrate contained only TMR. Substrates D1, D2, D3 contained TMR with the addition of respectively: 5, 10 and 15% of dry matter of RBH. Substrate D4 contained only RBH. Samples of ruminal fluid were incubated for 8 hours, in anaerobic conditions, at 39°C. A desirable effect of applying RBH was observed in groups D3 and D4 – the production of volatile fatty acids (VFA) increased. Furthermore, positive effects were observed in groups D2 and D4 – acetic acid production decreased (by up to 7%). Reduced methane production (up to 10%) was observed in experimental groups which indicates that animals would have smaller energy losses. Most beneficial changes in the VFA profile and the highest growth of non-glucogenic and glucogenic acids ratio (NGR) were recorded in D3 group samples. Positive results in this matter are promising as the roasted barley husks are a by-product of beer production and its usage has both economic and ecologic benefits. Obtained results in the *in vitro* study are the prerequisites to carry out an *in vivo* experiment.

Key words: barley husks, microbial fermentation, rumen, methane, volatile fatty acids.

INTRODUCTION

Beer produced on the basis of barley grain was already known in Egypt 5000 years before common era (Cabras and Higgins 2016). Currently the roasted barley is used in the manufacture of very dark beers, such as Porter, Stout or Dark Beer. About 10% of grains used in the beer production are barley. Grain for beer manufacturing is processed by roasting the crude grains at a temperature of approx. 200°C. Roasted barley grain can also be used as an addition to coffee (Dłużewski 2001). The roasted barley husks are a by-product of the roasting process of barley (Urbaniak and Błędzki 2015).

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Production of beer in Europe and Central Asia amounts 72 million tons per year, and in Poland alone, 3.8 million tons (FAO 2014). By-products constitute approx. 20% of the beer production. Spent grain, mainly consisting of husks, makes 85% of those by-products (Gupta et al. 2010). Barley husks based brewing by-products can be used, *inter alia*, as an additive in animal and human nutrition (Mussatto et al. 2006; McCarthy et al. 2012), for the production of biogas as a source of energy (Zanker and Kepplinger 2002), for the manufacture of carbon (Sato et al. 2001; Okamoto et al. 2002), for the manufacture of paper (Ishiwaki et al. 2005) and in the biotechnology (Mussatto et al. 2006).

The high content of crude fibre and crude protein make the spent grain (which consists mostly of barley husks) an excellent addition to the diet of dairy cows (Mussatto et al. 2006). Other authors studied the nutritional value of brewing by-products consisting mainly of barley husks (Davis et al. 1983; Mussatto et al. 2006; McCarthy et al. 2012). Results of their studies are the reason to research the effect of adding RBH to TMR on cow's rumen fermentation profile. In the available literature no research describing the influence of roasted barley husks on rumen fermentation has been found. Such research is important because rumen fermentation processes influence the health of animals and the quality of animal products.

The aim of this study was to evaluate the effect of roasted barley husks additive (brewing by-product) on the cow's rumen fermentation process, in *in vitro* conditions.

MATERIAL AND METHODS

Animals and ruminal fluid

The ruminal fluid used in the research was collected using a probe (Basko) from nine cows of the Polish Holstein-Friesian breed. Animals for the experiment were selected by analogy for age (second lactation), stage of lactation (peak lactation, 40th–50th day) and condition (3.5 points in Body Condition Score – BCS) (Edmonson et al. 1989). All of the animals were kept in the tie stall barn and fed TMR composed of: corn silage 56%, spent grain 21%, grass silage 13%, straw 4%, dried beet pulp 2% and 4% balanced concentrate.

Analysis of feeding components

A comprehensive analysis of the composition was made (apparatus NIRS DS 2500, FOSS) for roasted barley husks, TMR and TMR components: grass silage and corn silage. Following feeding components of substrates were analyzed: dry matter (method 934.01 of AOAC 2005), crude ash (method 942.05 of AOAC 2005), crude protein (Kjeldahl method, method 984.13 of AOAC 2005 using a conversion rate N x 6.25), crude fat (method 920.39 of AOAC 2005), crude fibre (method 978.10 of AOAC 2005), neutral-detergetnt fibre – NDF (method of Holst, 1973), acid-detergent fibre – ADF (method 973.18 of AOAC 2005) and starch (method 996.11 of AOAC 2005). Feed unit for lactation (UFL) was determined. Results are presented in Table 1. The diet of cows was arranged according to the French standard INRA (IZ-INRA 2009).

Table 1. The chemical composition of fresh forage [%] used to make substrates for *in vitro* experiment

Item	TMR	Roasted barley husks	Grass silage	Corn silage
Dry matter	35.14	90.67	35.40	40.18
Crude ash	2.80	10.56	9.35	6.05
Crude protein	5.29	25.96	15.88	6.03
Crude fat	1.14	1.72	3.60	3.55
Crude fibre	6.62	14.11	–	–
NDF	14.56	74.82	51.98	38.71
ADF	8.34	37.49	32.07	23.65
Starch	9.63	6.93	0.00	32.42

The substrates for *in vitro* experiment were composed on the basis of dried TMR ingredients. Control substrate (C) contained only TMR. Substrates D1, D2, D3 contained the same components as the control substrate, but with the addition of 5, 10 and 15% of dry matter of roasted barley husks. The last substrate D4 contained only roasted barley husks. The composition of substrates used in experiment is presented in Table 2.

Table 2. The chemical composition of substrates (in % of dry matter) for *in vitro* experiment

Item	C (only TMR)	D1 (TMR + 5% roasted barley husks)	D2 (TMR + 10% roasted barley husks)	D3 (TMR + 15% roasted barley husks)	D4 (only roasted barley husks)
Dry matter [%]	92.21	90.80	90.88	90.59	90.67
Crude ash	9.48	10.35	10.50	10.39	10.56
Crude protein	11.16	16.95	15.66	16.72	25.96
Crude fat	0.72	0.78	0.82	0.91	1.72
Crude fibre	22.95	14.71	16.99	15.83	14.11
Starch	9.80	9.67	9.44	9.29	6.93

Fermentation *in vitro.* Samples of ruminal fluid were collected two hours after the morning feeding of the animals and transported to the laboratory at the temperature of 36–39°C. The obtained samples of ruminal fluid were filtered through surgical gauze, partitioned into 20 ml portions and poured to serum bottles with a capacity of 125 ml (Sigma-Aldrich). To each bottle the appropriate buffer was added in an amount of 60 ml to dilute the content (McDougall 1948). The samples were divided into five groups and incubated with 1g of the prepared substrate. Serum bottles were thoroughly flushed with carbon dioxide to obtain anaerobic conditions and hermetically sealed with a manual crimper. Samples were incubated for 8 hours of *in vitro* fermentation in a shaking water bath at 39°C.

Analysis of selected products of fermentation

After incubation, the headspace pressure was measured in each bottle. Methane content in the gas was measured through gas chromatography (Agilent Technologies 7890 GC System, Santa Clara, USA). The chromatograph was equipped with a flame ionisation

detector (FID), thermal conductivity detector (TCD), two Supelco columns HayeSep Q and Porapak Q (Supelco, Bellefonte, USA), as well as a 5A Molecular Sieve. Helium was used as the carrier gas with flow rate: 0.42 ml/s.

The liquid samples had pH measured (CP-401 pH-meter Elmetron with an EPP-3 electrode and temperature sensor), then the samples were centrifuged and formic acid was added (0.1 ml of formic acid per 2 ml of sample) to stop fermentation. Gas chromatograph (Agilent Technologies 7890 GC System) with FID detector and column (DB-23, Agilent J&W) with helium as the carrier gas (flow rate 25 ml/min) was used to indicate total concentration of VFA and the percentage of individual acids: acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic. Peak identification of volatile fatty acids was carried out by comparing the retention times to standards supplied by Sigma (Free Acid Mix Supelco).

Calculations and statistical analysis

Mutual relations were calculated between acids: acetic acid and propionic acid (A : P), propionic acid and butyric acid (P : B). Utilization rate of VFA was calculated by expressing the ratio of non-glucogenic acids to glucogenic acids (NGR) according to the following formula:

NGR = (A + B 2 + Pc) / (P + Pc)

A, P, B, Pc is respectively: acetic acid, propionic acid, butyric acid, and valeric acid in mol% in the total VFA volume (Abrahamse et al. 2008). Nine samples were analyzed in each experimental group with the specific substrate (C, D1, D2, D3, D4). Therefore a total of 45 samples of rumen gastric juices were analyzed.

The test results were statistically compiled through a one-way analysis of variance (ANOVA) using a computer program Statistica 10.0. Differences were considered at the significance level of $P < 0.185$ using Duncan's new multiple range test for five groups. In the range of $0.05 < P < 0.1$ statistical trend was additionally estimated and analyzed.

RESULTS

Profile of VFA in fresh ruminal fluid (Table 3) was characterized by the highest share of acetic acid, lower of propionic acid and the lowest of the butyric acid when compared to the values presented in Table 4. In the general pool of these three major VFA, observed molar proportions were 58 : 28 : 14 (acetic : propionic : butyric). Measured proportions indicate normal physiological processes in samples collected from all cows. After 8 hours of fermentation with the addition of substrates (Table 4), increase in acetic acid and propionic acid and a slight decrease in the share of butyric acid were observed. Production of isobutyric acid in groups D2 and D4 decreased.

In analyzed samples (Table 4) from the rumen, after 8 hours *in vitro* fermentation, the pH level stayed between 6.09 and 6.14 in four research groups (D1, D2, D3 and D4) and only in the control group (C) fell below 6 and averaged at 5.97. The metabolic energy for TMR was 11.5 MJ EM, while for the roasted barely husks 9.5 MJ EM.

Fermentation indicator calculated on the basis of the level of VFA (P : B) implies a reduction of butyric acid, especially in D3 group (Tables 3 and 4). Improvement in results was observed with increased amount of roasted barley husks added to TMR.

Table 3. Fermentation profile of fresh ruminal fluid after adding the buffer

Fermentation parameters	Fresh ruminal fluid	SEM
pH	7.91	0.168
SCFA [a]	89.78	8.669
Acetate [b]	54.49	2.923
Propionate [b]	26.39	1.871
Isobutyrate [b]	2.01	1.045
Butyrate [b]	13.64	1.493
Isovaleriate [b]	2.30	0.397
Valeriate [b]	1.09	0.589
Caproate [b]	0.08	0.036
A : P [b]	2.20	0.246
P : B [b]	2.09	0.227
NGR	2.91	0.293

SCFA – short chain fatty acids.
[a] mmol/kg of undiluted ruminal content.
[b] percentage in the total molar concentration of SCFA [mol %].

Table 4. Changes in ruminal parameters after 8 hours *in vitro* fermentation

Fermentation parameters	C	D1	D2	D3	D4	SEM	P-value
pH	5.97	6.14	6.13	6.09	6.11	0.051	0.849
SCFA [a]	187.86	148.56	184.68	208.83	211.19	12.156	0.516
Acetate [b]	42.46	49.34	41.09	52.63	39.49	2.159	0.120
Propionate [b]	42.83	38.02	46.61	37.81	46.45	1.852	0.293
Isobutyrate [b]	0.41	0.48	0.20	0.00	0.29	0.079	0.376
Butyrate [b]	11.86	9.38	10.21	7.12	10.16	0.694	0.300
Isovaleriate [b]	1.51	1.75	0.76	1.83	2.04	0.233	0.484
Valeriate [b]	0.76	0.42	1.02	0.43	1.48	0.137	0.062
Caproate [b]	0.18	0.02	0.10	0.18	0.09	0.035	0.540
A : P [b]	1.13	1.43	0.94	2.34	0.95	0.238	0.332
P : B [b]	4.05	1.43	4.38	6.15	5.09	0.319	0.176
NGR	1.65	1.76	1.33	2.67	1.35	0.229	0.369
Gas production [a]	3.24	3.27	3.35	3.22	3.25	0.167	1.000
Methane [a]	1.14	1.03	1.09	1.07	1.05	0.054	0.339

SCFA – short chain fatty acids.
[a] mmol/kg of undiluted ruminal content.
[b] percentage in the total molar concentration of SCFA [mol %].

DISCUSSION

According to research done by Deepak et. al. (2013), barley husks consist of 39% cellulose, 12% hemicellulose, 22% lignin, 11% starch, 4% protein and 4% fat. Roasted barley husks used in this study had different parameters. The biggest difference was observed in protein content – almost 26% in our husks (Table 1). The research shows that the irrigation level of barley cultivations does not affect the composition of the barley husks but early planting date may result in a higher content of lignin (Grove et al. 2003). More important, however, is the absorption of water by the whole seeds, grain endosperm and the husk of barley. The research on this matter helps to facilitate and reduce the cost of analysis of barley grains intended for brewing while improving the production (Cozzolino et al. 2015). Ferulic and p-coumaric acids are primary coreless phenolic lignins (Moore and Hatfield 1994). The level of lignin and acid content of p-coumaric in husks of barley are rising along with ripening of plants while the level of ferulic acid remains constant (Grove et al. 2003).

Rumen fermentation requires adequate and stable physicochemical conditions. One of them is the pH of which optimal value is from 6.2 to 6.7 (Abdel Hameed et al. 2013). The pH of the ruminal fluid depends largely on the time of feeding and the type of forage given to cows (Křižova et al. 2011). Decrease of pH level below 6 results in reduced VFA production (Bhatta et al. 2006). In our study, the pH level after fermentation stayed at a desirable level (above 6) for all research groups containing roasted barley husks (D1, D2, D3, D4).

VFA produced in the process of microbial fermentation in the rumen are a source of energy. Their levels and composition depend primarily on the diet (Morvay et al. 2011). In addition to the content of individual fatty acids, their relative proportions are also important. It is assumed that the ratio of acetic to propionic to butyric acids should be around 77 : 15 : 8 (Zhou et al. 2012) before rumen fermentation *in vivo* begins. The proportions of VFA most similar to the literature data (Zhou et al. 2012) were achieved in our research in samples with the addition of 15% of roasted barley husks (D3) – 54 : 39 : 7 (acetic : propionic : butyric).

VFA covers approx. 80% of the energy needs of the animals (Heinhrisch and Varga 1996). Along with the increase of VFA production, milk yield increases (Piva et al. 1993). Therefore, an increase of VFA production in groups D3 and D4 from our study is beneficial. Levels of NDF and ADF reported for barley husks were consistent with Grove's studies (Grove et al. 2003), but higher than earlier research results (Bell and Keith 1988). Increasing the proportion of structural carbohydrates (NDF and ADF) in the feed leads to an increase of acetic production and reduction of the butyric acid production. Reduced level of non--structural carbohydrates results in lower production of VFA (Grochowska et al. 2012).

Iso-acids are produced as a result of catabolism of amino acids created by rumen microflora, their presence indicates the activity of proteolytic microorganisms (Del Bianco Benedeti et al. 2015). The highest and most favorable level of iso-acids in our results was observed in D4 group. The high amount of methane emitted causes energy loss for the animal which results in lower milk production. Observed in the D4 group reduced level of acetic acid to propionic acid reduces methane production (Stewart et al. 1997). Decreased methanogenesis in the rumen is a wanted phenomenon. Currently, there is a trend in agriculture for the introduction of feed additives and forage that reduce emission of methane (Yan et al. 2007). In our study, a slight decrease in the production of methane after the addition of roasted barley husks was observed.

The ratio of non-glucogenic acids to glucogenic acids (NGR) influences milk production, energy balance and methane production (Morvay et al. 2011). In our research a clear increase in NGR was observed in a sample with the largest addition of roasted barley husks (15%). Increase in the production of methane associated with the increase of NGR was not observed, however, it was reported in other studies (Dos Santos Pedreira et al. 2013). Glucogenic propionic acid is responsible for the deposition of fat in body tissues and together with non-glucogenic acids: acetic and butyric is the source for the synthesis of long chain fatty acids (LCFA) (Abrahamse 2009).

The authors of previous studies indicate that a greater share of the dry matter in the feed results in reduction of gases production, in particular methane. They conclude existence of a negative correlation between the protein content of feed and gas production in rumen in *in vitro* conditions (Getachew et al. 2004). In our study, we measured that the substrate of the control group was characterized by the highest level of dry matter and the lowest level of protein compared to the experimental groups (Table 2). In opposition to the results of other studies, our experiment did not result in an increase of methane production in experimental groups (D1, D2, D3, D4).

CONCLUSIONS

Maintaining the production of VFA on a physiologically correct level is a desirable effect of applying roasted barley husks. The activity of the symbiotic microorganisms involved in the fermentation has been impaired by the use of roasted barley husks additive. Most beneficial changes in the profile of VFA were observed in D3 and D4 groups. Using substrate with 15% RBH addition resulted with the highest growth of the P : B indicator.

The obtained results indicate lack of negative impact of roasted barley husks on profile of *in vitro* cow's rumen fermentation. Stated changes in VFA profile, such as reduction of acetic acid level participation in groups D2 and D3 are beneficial. Results in this matter are promising as the roasted barley husks are a by-product of beer production and its usage has both economic and ecologic benefits. Obtained results in the *in vitro* study are the prerequisites to carry out *in vivo* experiments.

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WPŁYW ZASTOSOWANIA PRAŻONYCH ŁUSEK JĘCZMIENIA, BĘDĄCYCH PRODUKTEM UBOCZNYM PRZEMYSŁU BROWARNIANEGO, NA PROCESY FERMENTACJI ŻWACZOWEJ KRÓW. BADANIA *IN VITRO*

Streszczenie. Celem pracy była ocena wpływu zastosowania prażonych łusek jęczmienia (RBH), będących produktem ubocznym przemysłu browarnianego, na procesy fermentacji żwaczowej, w warunkach *in vitro*. W badaniach użyto płyn żwaczowy pobrany od 9 krów mlecznych wybranych na podstawie analogów. Zwierzęta karmiono w systemie dawki wymieszanej całkowicie (TMR). Substraty do eksperymentu *in vitro* ustalono na podstawie dawki TMR. Substrat kontrolny zawierał tylko TMR. Substraty D1, D2, D3 zawierały TMR z dodatkiem odpowiednio: 5, 10 i 15% suchej masy RBH. Substrat D4 zawierał tylko RBH. Próbki płynu żwacza inkubowano przez 8 godzin, w warunkach beztlenowych, w 39°C. Pożądany efekt stosowania prażonych łusek jęczmienia zaobserwowano w grupach D3 i D4 – zwiększyła się produkcja lotnych kwasów tłuszczowych (LKT). Kolejne pozytywne efekty odnotowano w grupach D2 i D4 – produkcja kwasu octowego zmniejszyła się do 7%. W grupach eksperymentalnych zaobserwowano zmniejszone wytwarzanie metanu (do 10%), co wskazuje na obniżenie strat energii przez zwierzęta. Najkorzystniejsze zmiany w profilu LKT i znaczny wzrost NGR odnotowano w próbkach z grupy D3. Ponieważ prażone łuski jęczmienia są produktem ubocznym warzenia piwa, ich stosowanie przynosi korzyści zarówno ekonomiczne, jak i ekologiczne. Pozytywne wyniki badania *in vitro* stanowią warunki wstępne do przeprowadzenia eksperymentu *in vivo*.

Słowa kluczowe: łuski jęczmienne, fermentacja mikrobiologiczna, żwacz, metan, lotne kwasy tłuszczowe.