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The possible application of fungal enriched substrates in ruminant nutrition. A review

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ABSTRACT. Microbial utilization of raw agro-substrates by solid-state fermentation (SSF) leads to an effective enrichment of prefermented cereal-derived substrates (PCS) with oleaginous fungi being a source of *γ*-linolenic acid (GLA, 18:3*n*-6). Such method could open up new possibilities in animal nutrition. In this review, the nutritional effects of various PCS used as components of basal diets are summarized through the integrating related studies. PCS with two oleaginous fungi (*Thamnidium elegans* and *Cunninghamella echinulata*) as GLA sources were described. Apart from fatty acids, other related fermentation parameters i.e. digestibility of dry matter, neutral detergent fibre, acid detergent fibre, methane and ammonia concentration, short-chain fatty acid profiles and protozoal counts were taken into account. The effectiveness of GLA sources in increasing ruminal GLA outputs varied, depending on the filamentous fungi used, in the order *C. echinulate* > *T. elegans*, but efficiency also depends on the cereal substrate type. However, *in vivo* studies are needed to determine the impact of using cereal substrates enriched with oleaginous fungi as a source of GLA on rumen metabolism as well as the quality of ruminant meat and dairy products.

Introduction

Concentrates used in ruminant nutrition are rich in proteins and carbohydrates, but many of them are deficient in several essential nutrients, such as lipids, in particular polyunsaturated fatty acids (PUFAs). Grains contain quite small amounts of fats (on average 3.6%), and while linoleic acid is the major *n*-6 fatty acid (FA) found in grains, *α*-linolenic acid (*n*-3 FA) is detected only in small quantities in cereals. Also, the calculated ratio of *n*-6 (18:2):*n*-3 (18:3) in cereals varies. Apart from this, cereals are also deficient in other essential PUFAs of both *n*-6 (γ-linolenic acid (GLA), dihomo-*γ*-linolenic acid (DGLA), arachidonic acid (AA)) and *n*-3 (eicosapentoenoic acid (EPA), docosahexaenoic acid (DHA)) families as was reported previously (Čertík et al., 2006, 2008). GLA, the key intermediate in the *n*-6 FA family, is involved in maintaining proper cell functions in mammals. The insufficient supply of GLA from agricultural and animal sources has led to the seeking for microorganisms capable of producing GLA in high yields.

Currently, the productivity of oleaginous microorganisms, as well as the biosynthesis of particular PUFAs that can be used as food additives (Bellou et al., 2016) are widely studied. Due to the extensive research on oleaginous lower filamentous fungi, the development of a process of solid state fermentation (SSF) in which microorganisms grow on a moist

solid substrate in the absence of free water is possible. During this process, PUFA-producing fungi utilize various agroindustrial materials (e.g., cereals and legumes) and convert them to prefermented products with the desired essential FA content. So, SSF is a good method of preparing innovative feedstuff additives rich in PUFA (Xie et al., 2016; Yang and Zhang, 2016).

To date, a limited number of studies on prefermented cereal-derived substrates (PCS) or microbial oils in ruminant nutrition field have been conducted. However, increasing interest in this technology (enriching the properties of feed and at the same time decreasing its antinutritional factors) is observed. PCS are mainly used for ruminant diet supplementing. In recent studies (Laho et al., 2011a; Wencelová et al., 2014; Čertík et al., 2017) it was shown that PCS enriched with GLA effectively enhanced the output of GLA in an artificial rumen (RUSITEC) without detectable differences in rumen fermentation patterns or microorganism populations.

Therefore, the aim of this review was to present an overview of recently published data on ruminal fermentation and lipid metabolism of PCS enriched with GLA by lower filamentous fungi in a SSF process. Also, the possibility of effective usage of these GLA-enriched substrates in ruminant diets was taken into account.

Solid state fermentation

Fermentation is one of the oldest known food processing methods. This technique allows to enrich different agro-materials with the desired PUFAs (Certik and Adamechova, 2009), carotenoids and pigments (Čertík et al., 2013a). An advantage of the SSF is that fermented materials can be directly used as food or feed additives without any downstream process. It makes this process attractive also from the financial point of view – such method is not very expensive.

The main factor to obtain successful SSF is the selection of appropriate microorganisms able to grow on various substrates and simultaneously synthesize various compounds in large enough quantities. The screening of microorganisms has led to the selection of lower filamentous fungi, especially those belonging to the order *Mucorales*, as the best candidates for SSF. The species *Thamnidium elegans*, *Cunninghamella echinulata*, *Cunninghamella elegans* and in particular *Mortierella isabellina* were used to produce GLA, whereas *Mortierella alpina* to produce DGLA, AA and EPA (Certik and Adamechova, 2009; Certik et al., 2010). As a result of microbial growth and metabolism, various types of value-added substances of microbial origin with the desirable properties and containing PUFAs, pigments, sterols, organic acids, alcohols, esters, enzymes etc. are formed in the fermented food/feed.

The accumulation and amount of microbial PUFAs in prefermented cereals is also dependent on the used substrates and cultivation conditions. The process of regulation of fungal SSF is complicated, mainly at the semi-industrial or industrial levels (Certik et al., 2010), and comprises several technological steps, also pre-fermentation. SSF is often carried out with an internal solid matrix (e.g., spent malt grains) to improve the efficiency of respiration and aeration, to eliminate heat formed during fermentation and to reduce substrate particle agglomeration (Čertík et al., 2006). Adequate oxygen availability is necessary to maintain the high-activity of enzymes that transform carbon from the substrate into PUFA. The appropriate moistening of the substrate is another significant factor for optimal fungal growth and evaporative cooling of the fermentation mass. In addition, proper water activity of the cereal substrate prevents the growth of undesired microorganisms. To achieve the heterogeneity of cereal substrates and well-balanced utilization of nutrients the increasing availability of the carbon source is needed. This could be possible due to either partial hydrolysis (chemical, enzymatic) of the cereal substrates or gradual elevation of the carbon:nitrogen ratio made by supplementation of the substrate with an appropriate carbon source, PUFA precursors, and activators/inhibitors (e.g., isolated from plants) modifying the activities of the enzymes involved in the carbon flow to the targeted PUFAs (Certik et al., 2010; Certik et al., 2013b). Thus, the production of PUFA-rich cereals by SSF could offer a valuable opportunity to meet marketing demands in the food, feed, pharmaceutical, veterinary and environmental fields.

Overview and effectiveness

The data concerning the usage of fungal enriched substrates and microbial oil in ruminants is presented in Table 1. All rumen inocula (solid and liquid) in the experiments were collected from rumen-cannulated sheep, which were housed separately in pens and fed diet consisting mostly of meadow hay and barley grains in two equal meals per day (approximately 800:200 w/w), with free access to water. All procedures on animals were performed in accordance with guidelines and experimental protocol approved by the Ethical Committee of the Institute of Animal

Table 1. Selected studies concerning the usage of fungal enriched substrates and microbial oil in ruminant nutrition

Reference	*In vitro* method	Basal feed	GLA source	Sample collection
Čertík et al. (2017)	RUSITEC	Meadow hay and wheat bran (800:200 w/w)	*Cunninghamella echinulata*	on days 5–10
Wencelová et al. (2014)	Batch culture	Meadow hay and wheat bran (500:500 w/w)	*Cunninghamella echinulata*	after 24 h
Laho et al. (2011a)	RUSITEC	Lucerne hay and wheat bran + brewer's spent grains (800:200 w/w)	*Thamnidium elegans*	on days 6–11
Wencelová et al. (2014)	Batch culture	Meadow hay and wheat bran + brewer's spent grains (500:500 w/w)	*Cunninghamella echinulata*	after 24 h
Čertík et al. (2017)	RUSITEC	Meadow hay and wheat bran + brewer's spent grains (800:200 w/w)	*Cunninghamella echinulata*	on days 5–10
Laho et al. (2011a)	RUSITEC	Lucerne hay and maize meal (800:200 w/w)	*Thamnidium elegans*	on days 6–11
Wencelová et al. (2014)	Batch culture	Meadow hay and maize meal (500:500 w/w)	*Cunninghamella echinulata*	after 24 h
Čertík et al. (2017)	RUSITEC	Meadow hay and maize meal (800:200 w/w)	*Cunninghamella echinulata*	on days 5–10
Wencelová et al. (2014)	Batch culture	Meadow hay and maize meal + brewer's spent grains (500:500 w/w)	*Cunninghamella echinulata*	after 24 h
Čertík et al. (2017)	RUSITEC	Meadow hay and maize meal + brewer's spent grains (800:200 w/w)	*Cunninghamella echinulata*	on days 5–10
Wencelová et al. (2014)	Batch culture	Meadow hay and barley flakes (500:500 w/w)	*Cunninghamella echinulata*	after 24 h
Čertík et al. (2017)	RUSITEC	Meadow hay and barley flakes (800:200 w/w)	*Cunninghamella echinulata*	on days 5–10
Wencelová et al. (2014)	Batch culture	Meadow hay and barley flakes + brewer's spent grains (500:500 w/w)	*Cunninghamella echinulata*	after 24 h
Wencelová et al. (2014)	RUSITEC	Meadow hay and barley flakes + brewer's spent grains (800:200 w/w)	*Cunninghamella echinulata*	on days 5–10
Laho et al. (2011b)	RUSITEC	Meadow hay and barley ground (800:200 w/w)	*Thamnidium elegans*	on days 10–12
Laho et al. (2011b)	RUSITEC	Meadow hay and rye bran (800:200 w/w)	*Thamnidium elegans*	on days 10–12
Jalč and Čertík (2005)	RUSITEC	Lucerne and barley (400:600 w/w), monensin (66 ppm), fumarate (6.25 mmol)	*Thamnidium elegans*	on days 6–12
Jalc et al. (2005)	RUSITEC	Meadow hay and barley (800:200 w/w)	*Thamnidium elegans*	on days 6–12
Jalč et al. (2009)	RUSITEC	Meadow hay and barley (800:200 w/w)	*Thamnidium elegans*	on days 6–12
Kišidayová et al. (2006)	RUSITEC	Meadow hay and barley (800:200 w/w)	*Thamnidium elegans*	on days 6–12

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In all presented studies experimental rumen fluid was mixed with artificial saliva (McDougall, 1948) at a ratio determined during the *in vitro* procedure. PCS as a source of GLA were: wheat bran, wheat bran with brewer's spent grains (BSG), maize meal, maize meal with BSG, barley flakes, barley flakes with BSG, barley ground and rye bran. PCS were mixed with meadow hay or lucerne hay in the ratios: 200:800 w/w for RUSITEC or 500:500 w/w for batch culture, respectively. The feed evaluation was conducted in *in vitro* experiments with the use of RUSITEC (Czerkawski and Breckenridge, 1977) or batch culture system of incubation (Váradyová et al., 2005). These *in vitro* methods are ethically superior, faster and less expensive than *in vivo* methods.

Impact on ruminal digestibility

Collated data pertaining to the digestibility of GLA-enriched PCS is presented in Table 2. The rumen eubacterial population was not pooled in the database due to limited studies reporting this respective parameter. It can be assumed that mean fat content of PCS was 70 g · kg^{-1} DM (*T. elegans*) and 50 g · kg^{-1} DM (*C. echinulata*). Jalč and Čertík (2005) showed in their study that purified microbial oil (30 g · kg^{-1} DM) originating from oleaginous fungi (GLA-enriched oil) did not directly affect the digestibility of the diet in the RUSITEC, which is consistent with data on PCS digestibility. This is in contrast to lipid supplementation of a diet with fish oil (60 g · kg^{-1} DM) and linseed or coconut oil (both up to 40 g · kg^{-1} DM), which can reduce sheep ruminal organic matter digestibility and neutral detergent fibre (NDF) digestibility (Sutton et al., 1983; Wachira et al., 2000). However, negative effect of oil supplements added to ruminants diets depends on various factors, like the type and form of diet, the amount of supplements or additives used etc. It was shown that the same amount of added oils do not disturb rumen fermentation and positively influence milk production and FA composition (Cieślak et al., 2015; El-Sherbiny et al., 2016). The values of NDF and acid detergent fibre (ADF) varied among GLA-enriched PCS (Table 2). Fungal mycelium covers and utilizes the cereal substrates. In some cases the PCS have higher contents of detergent fibre (ADF and NDF). These PCS are less digestible in comparison with PCS treated with BSG because

Table 2. Collated data pertaining to the digestibility of various prefermented cereal substrates with oleaginous fungi

Indices	DMD, g · kg^{-1}	NDF, g · kg^{-1}	ADF, g · kg^{-1}	IVDMD, g · kg^{-1}
Wheat bran + brewer's spent grains	940	404	208	610
Wheat bran	915	448	141	508
Wheat bran + brewer's spent grains	918	419	163	421
Wheat bran	904	260	382	530
Wheat bran + brewer's spent grains + *T. elegans*	935	419	243	500
Wheat bran + *C. echinulata*	956	614	239	401
Wheat bran + brewer's spent grains + *C. echinulata*	952	569	265	415
Wheat bran + *C. echinulata*	987	272	205	502
Maize meal	917	244	202	880
Maize meal	906	66	31	611
Maize meal + brewer's spent grains	906	92	66	556
Maize meal	909	239	236	625
Maize meal + *T. elegans*	921	185	206	806
Maize meal + *C. echinulata*	941	372	113	566
Maize meal + brewer's spent grains + *C. echinulata*	944	375	104	493
Maize meal + *C. echinulata*	978	294	241	584
Barley ground	910	162	82	896
Barley flakes	899	75	36	614
Barley flakes + brewer's spent grains	912	232	124	473
Barley flakes	900	165	130	632
Barley ground + *T. elegans*	993	216	115	670
Barley flakes + *C. echinulata*	934	245	117	561
Barley flakes + brewer's spent grains + *C. echinulata*	943	281	184	496
Barley flakes + *C. echinulata*	975	183	152	612
Rye bran	985	284	100	812
Rye bran + *T. elegans*	928	308	207	583

DMD – digestibility of dry matter, NDF – neutral-detergent fibre, ADF – acid-detergent fibre, IVDMD – *in vitro* dry matter digestibility, *C. echinulate* – *Cunninghamella echinulate*, *T. elegans* – *Thamnidium elegans*

dry BSG are high in digestible fibre and can successfully replace forage in ruminant rations (Younker et al., 1998; Firkins et al., 2002). In all presented studies, the effect of PCS on *in vitro* dry matter digestibility (IVDMD) was evident and ranged from 1 to 21%. The decrease in the IVDMD of PCS with BSG ranged from 1 to 3% (wheat bran + BSG) and from 5 to 7% (maize meal + BSG) with *C. echinulata* and by 18% (wheat bran) with *T. elegans*. Only IVDMD of barley flakes with BSG (*C. echinulata*) was not affected.

BSG is an abundant by-product mainly used in dairy cattle nutrition since it provides high contents of protein, fibre and energy (Preston et al., 1973; Mussatto et al., 2006). There are many options for its possible application (Mussatto, 2014). The presence of lignin in BSG decreases the efficiency of enzymatic hydrolysis, because its rigid structure makes the action of enzymes difficult (Mussatto et al., 2008). However, PCS with BSG in a ratio of 3:1 is optimal for the fungal biotransformation of cereals to GLA bioproducts with maximum GLA productivity.

It is well known that fungal growth and biosynthesis of GLA are rapidly bolstered when BSG is mixed with cereals as an internal support (Čertík et al., 2013a). On the other hand, cereals without BSG can negatively affect substrate utilization, since there is poorer availability of assimilable compounds from the substrates (Čertík et al., 2006). Finally, PCS has an adverse impact on the IVDMD of the diets, and the effect does not depend on the GLA source or the method of ruminal fermentation. Lower values of IVDMD are probably due to the higher detergent fibre content of less digestible fungal mycelium.

Impact on ruminal fermentation parameters

The effectiveness of different PCS added to the ruminal fermentation feed ratios depends on several factors. The types of PCS affecting carbohydrate metabolism can partly explain the variation among treatments in the individual SCFA. When diets with direct supplementation of microbial oil (i.e., GLA-enriched oil; 30 g · kg^{-1} DM) or microbial oil blends are incubated, the molar proportion of acetate is reduced whereas the molar proportion of propionate increases (Jalc et al., 2005; Jalč and Čertík, 2005; Jalč et al., 2009). However, diet supplementation with PCS reduced especially the molar proportion of *n*-butyrate. The decreased proportions of *n*-butyrate in the majority of PCS are associated with a decrease in the protozoal counts in the rumen fluid. The ruminal protozoal population produces *n*-butyrate as an end product of carbohydrate fermentation, and rumen defaunation is often associated with a decreased rumen *n*-butyrate concentration (Ikwuegbu and Sutton, 1982; Williams and Coleman, 1992; Ueda et al., 2003), though this was not fully confirmed in an experiment with oils rich in linoleic acid in sheep rumen fluid incubated *in vitro* (Szumacher-Strabel et al., 2009). It seems from the research data collated in this study that the protozoal population is unable to grow with PCS-rich in starch. In addition, the direct supplementation of purified microbial oil (GLA-enriched oil; 30 g · kg^{-1} DM) into diets decreased the protozoal population in RUSITEC effluent (Kišidayová et al., 2006). This observation suggests that the specific antiprotozoal fungal effects of PCS or GLA-enriched microbial oil do not interfere with hydrogenesis, since methane production in experiments was not reduced (Laho et al., 2011a,b; Wencelová et al., 2014). However, it is known that PUFA sources distinctly inhibit the ruminal protozoal population, but they do not suppress bacterial activity (Hristov et al., 2004), although the reduction in the protozoal population may also lead to a decrease in the methanogen population (Toprak, 2015; Szczechowiak et al., 2016). Alternatively, the metabolic responses of rumen ciliates and bacteria to different forms and concentrations of PUFA sources vary (Cieślak et al., 2009a,b), and the absence of protozoa from the rumen microbiota did not systematically reduce methane production in the rumen environment (Morgavi et al., 2012). Reducing the ruminal protozoal population can also be associated with lower concentrations of ammonia (Hristov et al., 2005).

In contrast, supplementation of PCS to the diet increased ammonia concentration in both batch culture and RUSITEC, by 20–30% and 10%, respectively. Importantly, the PCS effects probably do not interfere with methanogenesis, because the increase in hydrogen from bacteria was consistent with the increase in ammonia from bacteria. The positive effect of PCS on ammonia-producing bacteria leads to a significant increase in the concentration of ammonia. Hyperammonia-producing bacteria (HAP, e.g., *Peptostreptococcus anaerobius*, *Clostridium sticklandii* and *Clostridium aminophilum*) are in a relatively small number in the rumen, though they are important due to their ammonia-production rate (Russell et al., 1988,1991; Chen and Russell, 1989). HAP bacteria are asaccharolytic and can generate NH_3 at a rate far greater than the most numerous ruminal species; they can also contribute to overall NH_3 production in the rumen of cattle and sheep (Russell et al., 1991; Wallace, 1996). This is in contrast to the results of Richardson et al. (2013) who

reported that HAP bacteria were detected during human faecal bacteria fermentation without the presence of sugars and in spite of the protein metabolism kinetics, which are similar to that of the rumen. It seems that HAP bacteria have access to a readily available energy source, increasing microbial protein synthesis or reduction by using amino acids as a microbial energy source (Nocek and Russell, 1988). In addition, it was shown that BSG have a more favourably balanced amino acid profile in rumen-undegraded protein than soyabean meal (Cozzi and Polan, 1994). Many HAP bacteria are clostridia, and they can recover the reducing equivalent *via* hydrogenases or utilized pairs of amino acids during deamination of amino acids, producing acetate, *n*-butyrate and ammonia through the Stickland reaction (Gano, 2013). However, HAP bacteria are capable of utilizing certain substrates and thereby producing ammonia on various substrates (Eschenlauer et al., 2002). Based on the 16S-PCR-DGGE (denaturing gradient gel electrophoresis) method, the species *C. echinulata* is uncapable to produce sufficient concentrations of bioactive compounds that will have an impact on the eubacterial community (Wencelová et al., 2014). However, the DGGE method indicates species richness, but not individual population size, and this limitation of the 16S-PCR-DGGE method may point to the changes at least in the population of ammonia-producing bacteria (Wencelová et al., 2014). When diets are supplemented with lipids, no effect (Jalc et al., 2005) or mostly a reducing effect (Doreau et al., 1991; Machmüller et al., 1998; Messana et al., 2013) of supplemented fats on ammonia concentrations are found. Interestingly, it seems that PCS with BSG produces higher concentrations of ammonia when compared with PCS without BSG (Wencelová et al., 2014). Studies have shown that BSG, as a partial replacement of concentrates in the diet of cows, increase the concentration of ammonia 2 h after feeding (Cozzi and Polan, 1994; Miyazawa et al., 2007). In Table 3 there is presented a summary of the effects of various PCS on ruminal fermentation parameters.

Evaluating the available data, it can be concluded that it is difficult to draw clear conclusions regarding the impact of GLA-enriched PCS on rumen fermentation characteristics. It seems that the amount of fat supplement primarily affected obtained results from fermentations. However, ruminal fermentation parameters may be modified (Jenkins, 1993) or not (Beauchemin et al., 2007) by the addition of lipid

Table 3. Collated data pertaining to the fermentation parameters of prefermented cereal substrates with oleaginous fungi

Indices	Acetate, mol%	Propionate, mol%	*n*-Butyrate, mol%	SCFA, mmol · l^{-1}	Methane, %	NH_3-N, mg · l^{-1}	Protozoa, 10^3 · ml^{-1}
Wheat bran + brewer's spent grains	62	19	12	53	6	396	30.0
Wheat bran	65	17	13	50	7	203	34.2
Wheat bran + brewer's spent grains	64	17	12	49	7	207	34.4
Wheat bran	66	18	13	49	4	140	37.6
Wheat bran + brewer's spent grains + *T. elegans*	68	19	12	46	5	444	<10.0
Wheat bran + *C. echinulata*	66	17	12	47	6	218	23.2
Wheat bran + brewer's spent grains + *C. echinulata*	66	17	11	48	6	223	27.3
Wheat bran + *C. echinulata*	65	18	11	50	3	141	22.1
Maize meal	67	18	12	48	4	343	35.3
Maize meal	65	18	14	54	7	101	32.9
Maize meal + brewer's spent grains	65	18	13	52	8	169	40.4
Maize meal	67	19	12	56	3	96	33.6
Maize meal + *T. elegans*	69	19	13	42	4	363	<10.0
Maize meal + *C. echinulata*	64	18	13	51	8	152	30.2
Maize meal + brewer's spent grains + *C. echinulata*	66	18	12	50	7	229	37.6
Maize meal + *C. echinulata*	67	17	11	56	3	98	30.0
Barley ground	69	17	14	51	5	273	18.0
Barley flakes	66	17	13	55	7	183	43
Barley flakes + brewer's spent grains	66	17	12	53	7	217	41
Barley flakes	68	18	14	50	6	80	45
Barley ground + *T. elegans*	68	17	13	50	5	270	<5.0
Barley flakes + *C. echinulata*	67	17	12	54	7	219	27
Barley flakes + brewer's spent grains + *C. echinulata*	66	17	12	52	7	223	31
Barley flakes + *C. echinulata*	61	18	14	53	8	86	26
Rye bran	64	15	12	52	5	351	11.3
Rye bran + *T. elegans*	60	16	11	48	6	304	<5.0

SCFA – short-chain fatty acids, *C. echinulate – Cunninghamella echinulate*, *T. elegans – Thamnidium elegans*

sources to the ruminant diet; however, the extent of the changes also depends on the composition of the basal diet (Toral et al., 2009). In general, PCS reduce *n*-butyrate and protozoal population in all cases in RUSITEC or 24-h batch culture, respectively; however, they do not have an impact on methane production. It can be suggested the PCS appear to be more effective in influencing fermentation parameters in continuous culture in RUSITEC than in 24-h batch culture, because more time for microbial adaptation to the substrates is important. However, the effectiveness differs depending on factors such as the type of PCS, the method and duration of the experiment, the forage:concentrate ratio as well as the source and amount of GLA.

Impact on lipid metabolism

Nowadays, the demand for feeds containing health beneficial PUFAs that are not produced by the body and must be obtained through diet or diet supplementation is increasing (Szumacher-Strabel et al., 2015). An effective way to enhance the concentration of PUFAs in ruminant-derived food products are diets supplemented with PCS as a significant source of GLA. To date, only limited research is available on the effect of PCS with oleaginous fungi on lipid metabolism in ruminants, but the data clearly indicate that PCS might positively enhance daily outputs of GLA from RUSITEC effluent by approximately 40–90%. The effectiveness of GLA sources in increasing ruminal GLA outputs varied by the filamentous fungi used in the order *C. echinulate* > *T. elegans*; however, efficiency also depends on the cereal substrate type used (Table 4). The concentrations of GLA found in *in vitro* experiments ranked by prefermented substrates are: barley flakes BSG + *C. echinulata* > wheat bran BSG + *T. elegans* > wheat bran BSG + *C. echinulate* > maize meal BSG + *C. echinulate* > maize meal + *T. elegans* > rye bran + *T. elegans* > barley ground + *T. elegans*. Probably due to physical structure, barley flakes seem to be better source of GLA than ground barley. It is clear that dietary lipids are subjects of hydrolysis and biohydrogenation by the rumen microbial population and that such factor as an increased dietary level of fat can reduce the extent of transformations of dietary lipids (Beam et al., 2000).

In this review, in the majority of PCS the concentration of conjugated linoleic acid (CLA) was relatively similar, however, vaccenic acid (TVA) varied among the tested PCS (Table 4). The accumulation of TVA is probably due to a overabundance of free fatty acids, which inhibits the final hydrogenation of TVA to stearic acid during ruminal bacterial biohydrogenation. Biohydrogenation involved the hydrogenation of CLA to produce TVA and the hydrogenation of TVA to produce stearic acid (Bauman et al., 1999). It is well known that the majority of CLA isomers in ruminant-derived food products originate from the isomerization of 18:2*n*-6 and 18:3*n*-3 in the rumen (reactions proceed *via* different mechanisms catalysed by bacterial enzymes); however, diet is the principal determinant of the amount and distribution of CLA isomers formed in the rumen (Shingfield and Wallace, 2014). Diets supplemented directly with PUFA-enriched oils increased the production of TVA and CLA, resulting in incomplete FA biohydrogenation (Jalc et al., 2005; Jalč et al., 2009; Szumacher-Strabel et al., 2009). Butyrate-forming populations in the rumen are highly active in the biohydrogenation of PUFAs (Newbold et al., 2001),

Table 4. Collated data pertaining to the C18 fatty acid outputs of various prefermented cereal substrates, mg · day^{-1} per vessel

Indices	18:0	18:1	18:2	18:3	TVA	CLA	GLA
Wheat bran + brewer's spent grains	91	2.71	31	0.6	9.43	0.56	0.007
Wheat bran + brewer's spent grains + *T. elegans*	75	2.38	13.6	0.53	9.28	0.72	0.340
Wheat bran	75	3.02	3.88	1.52	1.75	0.17	0.022
Wheat bran + brewer's spent grains + *C. echinulata*	40	3.18	2.61	1.15	1.55	0.35	0.207
Maize meal	111	13	8.94	1.72	8.46	0.81	0.003
Maize meal + *T. elegans*	113	14	4.21	1.31	11.7	0.71	0.180
Maize meal	62	2.46	2.46	1.33	7.09	1.49	0.123
Maize meal + brewer's spent grains + *C. echinulata*	88	5.62	3.98	1.65	10.7	0.63	0.205
Barley ground	99	8.30	4.05	0.78	11.4	0.52	0.010
Barley ground + *T. elegans*	101	3.03	2.36	0.49	10.3	0.16	0.020
Barley flakes + brewer's spent grains	290	12	11.3	9.35	40	0.23	0.607
Barley flakes + brewer's spent grains + *C. echinulata*	188	9.53	8.97	6.81	26.2	0.26	1.377
Rye bran	121	5.70	3.63	1.27	16.0	0.59	0.007
Rye bran + *T. elegans*	116	10	3.44	0.97	14.2	0.23	0.040

TVA – vaccenic acid, CLA – conjugated linoleic acid, GLA – gamma-linolenic acid, *C. echinulate* – *Cunninghamella echinulate*, *T. elegans* – *Thamnidium elegans*

but in experiments presented in the review a clear decrease in *n*-butyrate values was shown. Previously, nutritional manipulation associated with dietary addition of microbial oil (GLA-enriched oil) from oleaginous fungi resulted in higher production of PUFAs and incomplete FA biohydrogenation (Jalc et al., 2005; Jalč et al., 2009). This has been fully confirmed in PCS with oleaginous fungi, however the effect on ruminal biohydrogenation varied according to the type of fermented substrates added to the ruminal diet (Laho et al., 2011a,b; Wencelová et al., 2014). Furthermore, the FA composition of various PCS is not constant and can, in many cases, be enhanced by diet ratio.

Conclusions

There are very few direct comparisons enabling the effects of prefermented cereal-derived substrates (PCS) enriched with *γ*-linolenic acid (GLA) by oleaginous fungi (*Thamnidium elegans* and *Cunninghamella echinulata*) on ruminal fermentation and lipid metabolism to be evaluated precisely. However, indirect comparisons suggest that PCS are less digestible and have an adverse effect on the rumen *n*-butyrate concentration and ciliate protozoal population and exert no effect on methane production. On the other hand, PCS positively enhanced the output of GLA in the effluent from ruminal fermentation; however, they are not effective for increasing polyunsaturated fatty acids concentration. This review presents the most recent prospects for application of PCS enriched with GLA by oleaginous fungi in the field of ruminant nutrition; however, *in vivo* studies are needed to support the *in vitro* results. Research is required to fully characterize the benefits associated with using these substrates in ruminant diets and to understand how the levels of these substrates in diets can be enhanced.

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