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In vitro evaluation of fermentation characteristics of two types of insects as potential novel protein feeds for pigs[1]

C. Poelaert, Y. Beckers, X. Despret, D. Portetelle, F. Francis, and J. Bindelle[2]

Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium

ABSTRACT: Novel protein sources such as insects are suggested for pig nutrition. Protein availability might be impacted by the nature of the insect and by the thermal treatment applied to sanitize this ingredient. Their influence on protein availability and colonic fermentation is unknown. Plant proteins (beans, lentils, peas, and soybean, raw and vapor cooked) were compared to adult house crickets (*Acheta domesticus*) and mealworm larvae (*Tenebrio molitor*) that had been autoclaved, oven cooked (150 and 200°C), or used raw. Ingredients were run in an in vitro model of the pig gastrointestinal tract combining enzymes to simulate digestion in the stomach and the small intestine and subsequent fermentation by fecal microbes to simulate hindgut fermentation. In vitro crude protein disappearance (IVCPD) of insects decreased with oven cooking at 150°C or autoclaving ($P < 0.05$) while that of plants was unaffected ($P > 0.05$), except for soybean. IVCPD of raw mealworms (0.726) equaled that of the best plants (0.725 to 0.763) while crickets were less digestible ($P < 0.01$). Consequences on fermentation metabolites were lower propionate ($P < 0.01$) and branched-chain fatty acids (BCFA; $P < 0.05$) molar ratio in raw insects against oven-cooked or autoclaved insects. Both insect sources displayed greater BCFA ($P < 0.01$) and lower propionate ($P < 0.01$) than plants. Crickets produced 50% as much BCFA as mealworms ($P < 0.01$). In conclusion, feeding insect-sourced protein requires a careful choice of the species as well as the thermal treatment to avoid possible detrimental consequences on digestibility and intestinal health in pigs.

Key words: fermentation, insects, in vitro method, pig, short-chain fatty acid

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INTRODUCTION

Due to their low environmental impact in terms of greenhouse gas production, land requirement, and water consumption and their nutrient composition, insects are suggested as novel protein feeds (Schabel et al., 2010). Beside high protein content (50–82% DM) and well-balanced AA profiles, the quality of proteins from insects also depends on their digestibility (Sánchez-Muros et al., 2014). Edible insect species, such as the yellow mealworm (*Tenebrio molitor*) and the house cricket (*Acheta domesticus*), are now raised industrially in Western countries to feed domestic pets and zoo animals and are investigated as poultry feed (Makkar et al., 2014). However, little information is available on the influence of the nature of the insect and of the thermal treatment applied for sanitization on protein availability and on colonic fermentation in pigs. The aim of this study was to evaluate the influence of two insects (house crickets or mealworm larvae) and of the cooking procedure (raw, oven cooked, or autoclaved) on protein digestibility and on microbial fermentation characteristics. These insects were compared with grain legumes using an in vitro model of the pig gastrointestinal tract (GIT).

MATERIALS AND METHODS

Ingredients. Two insects and four grain legumes were used: adult house crickets (*Acheta domesticus*), mealworm larvae (*Tenebrio molitor*), beans, lentils, peas, and soybean. Insects were reared in the laboratory of Functional and Evolutionary Entomology of the University of Liege. Mealworm larvae were

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[2]Corresponding author: jérôme.bindelle@ulg.ac.be

Table 1. CP, crude fat, and NDF contents (g/kg of DM), in vitro DM (IVDMD), and CP (IVCPD) disappearance (%) during enzymatic hydrolysis (n = 15), final gas production (A, mL/g hydrolyzed ingredient), and half-fermentation time (B, h; n = 3) of insects and grain legumes

		Composition of undigested ingredients			Enzymatic hydrolysis		Fermentation parameters	
Ingredient	Treatment	CP	Fat	NDF	IVDMD	IVCPD	A	B
House crickets	Raw	746	151	227	56.1^{f}	65.5^{e,f}	47.4^{e,f}	22.5^{a}
	Oven at 150°C	745	147	309	46.1^{i}	59.3^{g}	45.5^{e,f}	10.3^{c,d,e,f}
	Oven at 200°C	745	147	281	47.3^{g,h,i}	61.1^{f,g}	51.0^{e}	13.8^{b,c,d}
	Autoclaved	746	152	332	48.7^{g,h}	59.5^{g}	47.7^{e,f}	12.4^{c,d,e}
Mealworm larvae	Raw	597	240	174	76.2^{b}	72.5^{b,c,d}	43.0^{e,f}	20.4^{a,b}
	Oven at 150°C	597	253	236	69.8^{c}	64.1^{e,f,g}	38.2^{e,f}	6.52^{e,f}
	Oven at 200°C	596	251	253	69.4^{c}	63.9^{e,f,g}	32.3^{f}	5.24^{f}
	Autoclaved	596	259	288	63.8^{d}	59.5^{g}	34.5^{e,f}	5.26^{f}
Beans	Raw	231	17	200	27.2^{l}	68.5^{d,e}	259^{a}	16.4^{a,b,c}
	Vapor cooked	235	21	202	46.9^{h,i}	68.8^{c,d,e}	236^{b}	7.57^{d,e,f}
Lentils	Raw	254	7	157	32.6^{k}	68.8^{c,d,e}	252^{a,b}	12.3^{c,d,e}
	Vapor cooked	261	15	178	60.2^{e}	72.5^{b,c,d}	207^{c}	8.94^{d,e,f}
Peas	Raw	238	12	119	37.1^{j}	74.1^{a,b,c}	251^{a,b}	12.2^{c,d,e}
	Vapor cooked	244	16	88.1	63.4^{d}	76.0^{a,b}	209^{c}	7.45^{d,e,f}
Soybean	Raw	450	239	65.9	49.6^{g}	60.8^{f,g}	134^{d}	8.74^{d,e,f}
	Vapor cooked	495	258	83.8	46.2^{i}	76.3^{a,b}	127^{d}	7.50^{d,e,f}
Casein		922	1	5.59	92.5^{a}	78.5^{a}	—	—
SEM					1.03	0.494	10.0	0.705
P-value					<0.01	<0.01	<0.01	<0.01

$^{a–l}$Within a column, means without a common superscript differ ($P < 0.05$).

maintained in the dark at 25°C and were fed a mixture of wheat flour (50%), wheat bran (30%), and beer yeast (20%). The house crickets were fed the same diet and were reared at 25°C in ventilated containers with light 10 h/d. After freeze killing, insects were used under several forms: raw, autoclaved (25 min), and oven cooked (150°C for 30 min and 200°C for 10 min). Grain legumes were used raw and vapor cooked (30 to 60 min). Casein was used as control.

In Vitro Enzymatic Digestion and Fermentation. The in vitro model of the pig's GIT described by Bindelle et al. (2007a) was used. Briefly, the ingredients were incubated with porcine pepsin (pH 2, 2 h, 39°C) and pancreatin (pH 6.8, 4 h, 39°C). The residues were centrifuged (2,000 × g, 15 min, 4°C) and freeze-dried to calculate in vitro DM (IVDMD) and CP disappearance (IVCPD) during enzymatic hydrolysis. Subsequently, the residues were incubated for 72 h with a bacterial inoculum prepared with fresh feces from 3 sows and mixed to a buffer solution with 2 modifications: 1) the reducing agent Na_2S was omitted and 2) the buffer was N-free. The pressure inside 3 glass bottles per substrate was regularly recorded, and after 72 h, fermentation broth was centrifuged (13,000 × g, 10 min) and the supernatant sampled for SCFA analysis. Hydrolyses were replicated 15 times, and fermentation was done in triplicates.

Chemical Analyses. All ingredients were analyzed for DM, CP, crude fat, and NDF as described in Bindelle et al. (2007b). Fermentation supernatants were analyzed for SCFA contents with a Waters 2690 HPLC system (Waters, Milford, MA) fitted with a HPX 87 H column (Bio-Rad, Hercules, CA) and an UV detector (210 nm; Bindelle et al., 2007b).

Calculations and Statistical Analyses. Gas production curves were modeled according to Groot et al. (1996): $G = \frac{A}{1+\frac{B^C}{t^C}}$ if t > 0, where G (mL/g of fresh weight) denotes cumulative gas production; A (mL/g of hydrolyzed residue), maximal gas volume for t = ∞; B (h), time to reach 50% of A; and C, the stiffness constant of the curve.

IVDMD and IVCPD during enzymatic hydrolysis, fermentation parameters (A and B), total SCFA production, and molar ratio after 72 h of fermentation were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) and LSMEANS with a general linear model according to the ingredient × treatment (one criteria of classification). The hydrolysis or fermentation flask was used as experimental unit.

RESULTS AND DISCUSSION

In Vitro Enzymatic Digestion. IVDMD of insects decreased with thermal treatments ($P < 0.01$) while that of grains, except for soybean, increased ($P < 0.05$). For a similar treatment, mealworm larvae were more digestible than crickets ($P < 0.01$). This difference is

Table 2. Total short-chain fatty acid production (SCFA; mg/g of hydrolyzed residue) and molar ratio (%) of insects and grain legumes after in vitro fermentation by pig fecal bacteria for 72 h ($n = 3$)

Ingredient	Treatment	SCFA	Acetate	Propionate	Butyrate	BCFA[1]
House crickets	Raw	250^{f,g}	50.3^{f,g}	9.64^{k}	13.7^{b}	26.3^{b}
	Oven at 150°C	256^{f,g}	49.2^{g}	12.9^{i}	11.5^{d,e}	26.5^{b}
	Oven at 200°C	271^{e,f,g}	47.3^{h}	12.4^{i,j}	11.7^{c,d}	28.6^{a}
	Autoclaved	293^{e,f}	46.3^{h}	11.9^{j}	11.3^{d,e}	30.4^{a}
Mealworm larvae	Raw	282^{e,f}	65.6^{a}	10.5^{k}	16.6^{a}	7.22^{g}
	Oven at 150°C	204^{g,h}	55.7^{c}	15.9^{g}	13.3^{b,c}	15.2^{d}
	Oven at 200°C	253^{f,g}	58.1^{b}	14.4^{h}	14.6^{b}	12.9^{e}
	Autoclaved	181^{h}	51.8^{e,f}	18.0^{f}	11.7^{c,d}	18.3^{c}
Beans	Raw	455^{a,b}	53.3^{d,e}	33.6^{b,c}	9.94^{e,f}	3.20^{h}
	Vapor cooked	398^{b,c}	54.7^{c,d}	34.4^{b}	7.47^{g,h}	3.39^{h}
Lentils	Raw	418^{b,c}	54.4^{c,d}	32.9^{c}	10.1^{d,e,f}	2.66^{h}
	Vapor cooked	365^{c,d}	55.9^{c}	35.5^{a}	6.10^{h}	2.50^{h}
Peas	Raw	493^{a}	53.4^{d,e}	34.3^{b}	9.55^{f}	2.75^{h}
	Vapor cooked	380^{c,d}	55.3^{c}	35.8^{a}	6.03^{h}	2.90^{h}
Soybean	Raw	357^{c,d}	51.9^{e,f}	26.8^{e}	11.5^{d,e}	9.75^{f}
	Vapor cooked	325^{d,e}	54.5^{c,d}	28.6^{d}	9.13^{f,g}	7.74^{g}
SEM		14.3	0.689	1.40	0.418	1.40
P-value		<0.01	<0.01	<0.01	<0.01	<0.01

$^{a–k}$Within a column, means without a common superscript differ ($P < 0.05$).

[1]BCFA = branched-chain fatty acids (valerate, isovalerate, and isobutyrate).

explained by their respective contents in chitin. This polysaccharide, found in the cuticle, is not digestible and is present in higher amounts in adult crickets (67.6 mg/kg of DM) than in mealworm larvae (55.7 mg/kg of DM; Finke, 2007). For both insects, oven cooking at 150°C or autoclaving reduced IVCPD more markedly for mealworm larvae ($P \leq 0.0015$) than for crickets ($P \leq 0.028$). As for meat, cooking insects could lead to physical-chemical modifications of proteins, such as oxidation, aggregation, and Schiff bases formation, resulting in the reduction of protein accessibility and susceptibility to digestive enzymes (Bax et al., 2012). In terms of IVCPD, raw mealworms (0.726) equaled that of the best plants (0.725 to 0.763) while crickets were less digestible ($P < 0.01$).

Fermentation Characteristics. Fermentation of grain legumes generated greater ($P < 0.01$) gas production than insects (Table 1). Except for soybean, cooking of tested grain legumes decreased ($P < 0.01$) the gas production. Final gas production was not affected by the thermal treatment in both insects but hydrolyzed residues fermented faster when insects were previously oven cooked or autoclaved ($P < 0.01$). Fermentation end products were influenced by both the ingredient and the thermal treatment. Except for soybean, total SCFA production was higher for grain legumes than for insects ($P < 0.05$; Table 2). Both insect sources displayed greater BCFA (including valerate; $P < 0.01$) and lower propionate ($P < 0.01$) than grain legumes, except for soybean. Moreover, crickets produced approximately 50% as much BCFA as mealworms (0.263 to 0.304 vs. 0.072 to 0.183, respectively, $P < 0.01$). Consequences of the thermal treatment were reduced propionate ($P < 0.01$) and BCFA ($P < 0.05$) molar ratio in raw insects against their oven cooked at 200°C or autoclaved counterparts and, as compensation, greater acetate and butyrate molar ratio ($P < 0.05$). When a thermal treatment is applied to mealworms, the molar ratio of BCFA is approx. two times higher ($P < 0.01$) than for raw insects. This could be explained by reduced IVCPD ($P < 0.01$) for cooked mealworms leading to a higher proportion of undigested proteins available for microbial fermentation by comparison with raw ones.

In conclusion, the application of a thermal treatment seems to negatively affect the digestibility of insects and to modify the fermentation metabolites, with notably an increase of the proportion of BCFA. Mealworm larvae display better digestibility and produce less BCFA than crickets. Hence, feeding insect-sourced protein requires a careful choice of the species as well as the thermal treatment to avoid possible detrimental consequences on intestinal health in pigs.

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