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Original
Pigs' husbandry as case study to test effects of vegetable residues' hydrolysates on animals / Morlacchini, M.; Fusconi, G.; Montoneri, C.; Nisticò, R.; Montoneri, E.. - In: ARC JOURNAL OF ANIMAL AND VETERINARY SCIENCES. - ISSN 2455-2518. - ELETTRONICO. - 3:3(2017), pp. 11-23. [10.20431/2455-2518.0303003]

Availability:
This version is available at: 11583/2709208 since: 2018-06-01T14:25:09Z

Publisher:
ARC Publications

Published
DOI:10.20431/2455-2518.0303003

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12 October 2024

ARC Journal of Animal and Veterinary Sciences
Volume 3, Issue 3, 2017, PP 11-23
ISSN No. (Online) 2455-2518
DOI: http://dx.doi.org/10.20431/2455-2518.0303003
www.arcjournals.org

Pigs' Husbandry as Case Study to Test Effects of Vegetable Residues' Hydrolysates on Animals

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Abstract: *Soluble biopolymers (SBO) are research products obtained from alkaline hydrolysis of municipal and agriculture bio-residues. They contain a mix of soluble macromolecules with different C types and functional groups. These features make SBO multipurpose products for the chemical industry, agriculture and animal husbandry. As such, they may contribute to the development of bio-based circular economy by recycling renewable carbon over urban and agriculture environments. To foster production and commercialization, assessment of SBO's compliance with countries' regulations is mandatory. The authors performed the present in vivo pigs study to investigate quality, safety, and efficacy of SBO's as animal feed supplement. Total 106 male and female piglets were fed 42 days with protein-based diets containing 0.1 and 0.2 % SBO. Pigs grew from average 7.7 to 26 kg. Average mortality rate was 3.9 %. Relatively to control diets with no added SBO, the diets containing 0.1 and 0.2 % SBO evidenced no significant effects on pigs' growth, mortality, and anatomy and pathology of dead animals. A previous rabbit study reported similar results for SBO supplemented diets. The authors discuss the economic and environmental implications of the results in relation to SBO potential marketability.*

Keywords: *Bio wastes; bio macro molecules; pigs; rabbits; animal husbandry; regulatory requirements.*

Abbreviations: *Af, aliphatic carbon; CP, compost; COX, carboxyl carbon; HS, humic substances; IR, insoluble residue; MBW, municipal biowaste; NR, amine carbon; OCO, anomeric carbon; OME, methoxy carbon; Ph, aromatic carbon; PhOY, phenoxy carbon; SBO, soluble biopolymers*

1. Introduction

Soluble biopolymers (SBO) obtained from alkaline hydrolysis of bio-residues from municipal and agriculture source are multipurpose products for use in diversified fields (Montoneri, 2017). Examples are the fabrication and/or formulation of detergents, dyeing baths, flocculants, dispersants, binding agents for ceramics manufacture, emulsifiers, auxiliaries for soil/water remediation and enhanced oil recovery, nanostructured materials for chemical and biochemical catalysis, plastic materials, soil fertilizers and plant biostimulants for agriculture. The SBO are composed by a heterogeneous pool of macromolecules with 67–463 $kg \cdot mol^{-1}$ molecular weight and 6–53 polydispersity index. They contain aliphatic and aromatic C chains substituted by a wide variety of acid and basic functional groups of different strength (Tabasso et al., 2015). Their chemical nature bears chemical similarities with natural humic substances (HS).

The above findings prospect implementation of a bio-waste based circular economy, based on the following hypothetical scenario. The hydrolysis of municipal food wastes, private gardens and public parks trimmings, and post-harvest plant residues yields SBO for use in agriculture and for the manufacture of consumers' chemical product in everyday life.

Renewable C is recycled repeatedly over the agriculture and urban environment. Biowaste treatment plants (Montoneri et al., 2011) become biorefineries, producing bio-based fuel and chemicals. SBO replace products from fossil sources. This reduces the depletion of fossil sources and the environmental impact of not renewable fuel and chemicals. The economy of biowaste treatment plants improves. The bio-waste based economy produces socio-economic benefits; such has reduction of citizens' taxation for waste management and creation of new jobs (Montoneri, 2017).

The SBO are not commercial products yet. To contribute to the realization of the above perspectives, a hydrolysis prototype plant is under construction within the municipal biowaste (MBW) treatment plant of Acea Pinerolese in Pinerolo (TO), Italy. Acea collects MBW from 150,000 inhabitants of 47 municipalities (Montoneri et al. 2011). It process MBW through integrated anaerobic and aerobic fermentation. It produces biogas and compost, and thermal and electrical power from biogas for the nearby residential district. The prototype plant will be integrated into the existing biochemical facilities to hydrolyze the plant anaerobic fermentation digestate and/or the compost, produce 400-800 t/y SBO and validate the SBO performance and marketability in real operational conditions. In this scenario, it is essential assessing the compliance of SBO with countries' regulatory requirements to ensure the quality, safety, and efficacy of the product and obtain its marketing authorization (Handoo et al., 2012).

Marketing approval of new products is quite complex process. It involves the assessment of critical parameters during product development. The regulatory requirements of various countries of the world vary from each other. Therefore, it is quite challenging to collect data, which is suitable to obtain approval in different countries. In the case of SBO, the authors have considered the Italian legislation on fertilizers, as starting point. The most recent Italian legislation on fertilizers (Presidente della Repubblica, 2010; Ministero delle Politiche Agricole e Forestali, 2017) allows marketing HS or HS-like products obtained by extraction of soil, peat or other fossil materials, or mixtures of vegetable materials after anaerobic and/or aerobic fermentation. Based on chemical composition, sourcing material and production process (see section 2.1), the SBO fall well into the above category of fertilizers.

The chemical similarity (Montoneri, 2017) of SBO with HS is a favorable feature, in relation to the perspective of successfully demonstrating the safety of SBO for commercial use. Humic substances isolated from soil or fossils are efficient for use as diet supplement in animal husbandry (Montoneri et al., 2013; Biagini et al., 2016). Use of SBO, in place of HS, appears highly desirable for reasons linked to the greater availability of biowastes and to the need to reduce landfill areas for biowaste disposal. Agriculture and animal husbandry constitute important markets for the potential allocation of large amounts of SBO. These facts offer worthwhile scope for investigating the full potential feasibility of SBO to enter the market.

Sortino et al. (2012), Montoneri et al. (2013) and Biagini et al. (2016) have dedicated studies to assessing the performance and safe use of SBO. Sortino et al. (2012) carried out an on field tomato cultivation study, adding SBO to the cultivation soil. They found that, compared to the no treatment and to the treatment using commercial fertilizers, the SBO enhance significantly plant growth and crop production, while not affecting chemical composition of soil and leaves. The authors concluded that the potential adverse environmental impact of periodically repeated applications of SBO to the same soil should raise no more concern than that contributed by conventional mineral and organic fertilizers.

Montoneri et al. (2013) performed an in vitro fermentation study to test SBO as diet supplements to modulate pig's cecal fermentation of a protein feed. They added SBO at 0.05-0.2 % level in the fermentation slurry. The results showed that, during fermentation, ammonia production in the fermentation slurry containing SBO decreased by 17 %, compared to the control fermentation with no added SBO. The authors ascribed the SBO effect to reduced proteolysis and N mineralization. They expected favourable economics and lower environmental impact for the animal production industry, stemming from the use SBO as animal feed supplements.

Biagini et al. (2016) carried out an in vivo animal study. They evaluated the effect of different doses of SBO as feed additives in diets for fattening rabbits. Rabbits are herbivorous non-ruminants. They are able to utilize a wide range of feed resources. The rich microbial intestinal population (particularly at the caecum level) influences the post-gastric digestion of

such animals. One of the leading causes of mortality is disease of the digestive apparatus, involving the microbiota. This feature makes the use of rabbits interesting to assess the influence of SBO on animal welfare and intestinal microflora. Rabbits serve to bridge the gap between the small animal models, which are perhaps more suitable for biomedical research, and larger animals used for animal production. The results of Biagini et al. (2016) study showed that diets containing 0.05–1 %w/w do not affect the live and slaughtering performances, except for the intestinal tract absorption area (higher in the intestines of rabbits fed with the high SBO content) or the apparent digestibility of the feed (lower in diets with low or no SBO content). The authors concluded that SBO might perform as feed additive for rabbits, without any adverse effects on animal productivity and health.

To find whether the encouraging results on the safe use of SBO in rabbits' diet were general for other animals, the present work reports the in vivo study on pigs husbandry performed with diets containing similar SBO amounts as in the rabbits study. Pigs are large animals. Pigs and humans have anatomical and physiological similarities (Kobayashi et al., 2012). Pigs are models in biomedical research and toxicology testing (Swindle et al., 2012).

2. Materials and Methods

2.1. SBO Preparation and Characterization

The SBO were obtained and characterized, as previously reported (Montoneri, 2017). Urban private gardening residues and public park trimmings collected in the province of Torino, Italy, were composted for 280 days. The compost was hydrolysed 4 h at 60 °C in aqueous KOH at pH 13 and 4 liquid/solid w/w ratio. Centrifugation of the recovered slurry separated the insoluble residue (IR) from the aqueous hydrolysate. Filtration of the recovered liquid phase through a 5 kDa cut off polysulphone membrane yielded the retentate and the permeate. Drying at 60 °C the retentate yielded the solid SBO in 30 % w/w amount, relative to the pristine compost (CP). The compost, and the IR and SBO products, were characterized by the data reported in Table 1 and 2.

Table1. *Analytical data for pristine compost (CP), SBO and IR*

	pH		Volatile Solids, w/w %[a]		C, w/w %[a]		N, w/w %[a]		C/N				
CP	7.3		42.9		22.4±0.4		1.91±0.03		11.7				
SBO	8.2		72.1		38.2 ± 0.1		4.01 ± 0.03		9.54				
IR	8.7		28.7		18.4±0.7		1.15±0.09		16.0				
	Mineral elements: Si, Fe, Al, Mg, Ca, K, Na as % w/w;[a] Cu, Ni, Zn, Cr, Pb, Hg as ppm[a]												
	Si	Fe	Al	Mg	Ca	K	Na	Cu	Ni	Zn	Cr	Pb	Hg
CP	12.1	1.03	0.59	1.67	4.86	1.18	0.06	73	100	157	49	43	0.31
SBO	2.55	0.77	0.49	1.13	6.07	3.59	0.16	202	92	256	19	85	0.15
IR	15.0	1.10	0.67	1.45	4.19	1.49	0.06	51	102	123	34	39	0.07
	C types and functional groups[b] concentration as % of total organic C												
	Af	OME + NR	OR	OCO	Ph	PhOY	COX	C=O	Af/Ar				
CP	32.9	8.33	23.8	6.34	12.3	6.73	8.21	1.37	1.7				
SBO	36.9	7.24	13.2	4.18	13.4	6.84	13.5	4.69	1.8				
IR	31.7	8.43	24.6	6.14	11.5	7.23	7.74	2.68	1.7				

[a]*Concentration values referred to dry matter: averages and standard deviation calculated over triplicates.*

[b]*Aliphatic (Af), aromatic (Ph), methoxy (OMe), carboxyl (COX, X = OH or NR), ammine (NR), alkoxy (RO), phenoxy (PhOY, Y = OH or R) and anomeric (OCO) C atoms, R = H, alkyl or phenyl C.*

Table2. *Proximates' composition (% w/w) for SBO*

H_2O	2.34
Crude protein	18.0
Crude fat	0.07
ADF	52.4
ADL	33.8
P	0.27
Gross energy, MJ/kg	13.3

The data in Table 1 were obtained by 13 C NMR spectroscopy, potentiometric titration, elemental C and N, and mineral elements, according to a protocol specifically developed for biowaste derived materials (Tabasso et al., 2015; Montoneri et al., 2013; Sortino et al.,

2012). The data in Table 2 were obtained according to AOAC (2006).

2.2. Pigs Feeding Trials

The animal study was performed in 2011, in compliance with the Italian national legislation on the use of animals for research (Presidente della repubblica, 1992). The experimental work was performed according to the commonly accepted '3Rs' EU guidelines at the Cerzoo facilities (Università Cattolica del Sacro Cuore, 2017) in Piacenza, Italy. Before starting the work, the authors submitted the ethics committee of the local Italian authority (A.USL di Piacenza, 2017) the 2011 document (see supplementary material file), which contained all experimental details of the planned work. According to the above regulation, this document accomplished the legal requirements to start the work. The test animals were 54 castrated male and 54 female weaned piglets with average 7.74±0.46 kg weight, genetic type commercial hybrids. Agricola Serena di Lingiardi Sandro Fabrizio, Cascina Serena, Via Strada Provinciale, 104 – 24051, Antegnate (BG), Italy, AUSL code 007 LO 011 supplied the animals. These were housed in pens within a sun-lighted building, where temperature and humidity were controlled through water heaters and a computerized automatic ventilation system. The pens were equipped with mangers and automatic water nipple drinkers to dispense potable water analyzed for Na, K, Ca, Cl and E. coli.

Before starting the trials, pens were disinfected with non-toxic substances. Animals were subjected to veterinary examination health conditions and then placed in the pens, each containing three males or three females. The experimental plan comprised testing each feeding diet (Table 3) over 36 animals, corresponding to twelve replicates for each diet. Each treatment was equivalent for animal weight and sex. The diets did not contain zootechnical additives according to EU Reg.1831/03 (European Commission, 2017). They were iso-nutritive in each feeding period, according to the feed requirements as a function of age and weight (Fanimo et al., 2003). Mangimificio Rossana, Revere (Mantova), Italy prepared the two basal diets for the two different feeding periods. Cerzoo Piacenza, Italy, prepared the treatment diets by adding the required SBO amount to the basal diet and homogenizing through a horizontal mixer. Before the addition of SBO, examination of the basal diets guaranteed no cross contamination. Except for the added SBO, no other products were added to the basal diets. During the feeding periods, the trials were monitored twice a day, in the morning and in the afternoon, by examining the animals' health conditions, and checking the availability of feed and water, and the correct functioning of the temperature and humidity control system. Animal growth was monitored by measuring the animal body weight at day zero, 14 and 42, and the feed consumption at day 14 and 42. Diets' analyses were carried out on 250 g samples. Diets were analysed according to ISO methods, for the content of water (ISO, 2016a), ashes (ISO, 2013a), starch (ISO, 2013b), crude protein (ISO, 2014), ether extracts (ISO, 2016b), crude fibre (ISO, 2017), digestible (Morgan et al., 1987) and net (Noblet et al., 1994) energy. Values for these analytes, and for NDF, ADF, ADL and amino acids, were also calculated using Brill formulation feed management systems version 2.03, 2011, Brilliant Alternatives Inc., USA.

2.3. Statistical Analyses

Data were analysed by Anova using SAS, 2002-2008, version9.2 software. Averages over replicates were compared by Student t-test. The body weight data were analysed also for outliers. None was evident on day 14 and 42.

3. Results

3.1. Chemical Composition of SBO and sourcing compost

The SBO used in the pigs' husbandry study is the same as the one used in the pigs in vitro study (Montoneri et al., 2013) and in the rabbits in vivo study (Biagini et al., 2016). They were obtained by hydrolysis in alkaline water of composted urban private gardening residues and public park trimmings collected in the province of Torino, Italy (see Section 2). The process yields SBO and an insoluble residue (IR). Table 1 and 2 report the collected chemical data for the pristine compost (CP) and the SBO and IR hydrolysates. Determination of the chemical structure of these materials is not as easy as for synthetic molecules. As anticipated in section 1, they contain molecules of different molecular weights and chemical composition. These materials are characterized at best for the content of the analytes reported in Table 1. It is

not possible to identify a specific molecular structure. Nevertheless, the analytical data give some important information. The three materials contain organic and mineral matter. Compared to CP and IR, SBO contain more organic matter and less mineral matter, except for Ca, K, Cu, Zn and Pb.

The higher content of K, Ca, Cu, Zn and Pb in SBO stems from the different C types and functional groups distribution in the three materials (Table 1). 13 C NMR spectroscopy measurements yielded the functional groups data in Table 1. This analytical technique allows identifying the reported C types, but not obtaining their breakdown into more specific components. For example, the carboxyl (COX), phenoxy (PhOY), methoxy and amine (OMe + NR) groups cannot be broken down into free carboxylic acid, amide, ester, phenol, phenyl ethers, methoxy, and amine groups. Nevertheless, Table 1 shows the following significant differences among the three products. Compared to CP and IR, SBO contain about half alkoxy (RO) groups and twice more carboxyl (COX) groups. This is likely due to the hydrolysis of ester bonds in CP and formation of SBO carboxylate groups in the alkaline reaction medium. Potentiometric titration measurements yielded further details on the chemical nature of SBO. The authors did not carry the same measurements for CP and IR, due to their poor solubility properties.

Potentiometric titration allowed obtaining the breakdown of the SBO COX, PhOY and OMe and NR groups into carboxylates (COO^-), amide (CON), phenol (PhOH), phenoxy (PhOR), and amine (NR) groups. The results of these measurements indicated that, in SBO, 92 % of the COX groups are in COOH form and 56 % of PHOY are in PhOH form. They also suggested the higher content of elemental N in SBO, compared to CP and IR, to arise from the presence of NR groups. These functional groups are the likely cause of the stronger capacity of SBO to complex and transport the K, Ca, Cu, Zn and Pb elements into the reaction soluble hydrolysate.

The data in Table 1 is not normally provided in as much detail for materials of biological sources, as those investigated in the present work. Montoneri et al. (2011) developed a specific analytical protocol to investigate the nature of the molecular pool representing these materials. For the specific use of SBO as diet supplement, Table 2 reports the conventional data on the product proximates' composition. The data in Table 2 is consistent with the data in Table 1. The functional groups given in Table 1 are associated to the proximates listed in Table 2. For example, the OR and OCO groups may be associated to the polysaccharide fraction. The Ph and PhOY groups may be associated to the lignin fraction. The COX and NR groups may be associated to the protein fraction of the material.

3.2. Animal Diets and Live Performance

The animals were fed with the control diet (T1) and with the treatment diets containing SBO at 0.1 % (T2) and 0.2 % (T3) concentration, over two fattening periods, from 0 to 14 days and from 14 to 42 days. For the two periods, Table 3 reports the diets' formulations and chemical compositions. The T1, T2 and T3 diets in each period have close nitrogen and energy content (see also Table 1S in supplementary material file).

Table3. *Formulas and chemical composition (g/kg as-fed basis, unless otherwise indicated) for experimental diets used in the first (0-14 days) and second (14-45) fattening periods.*

	First period (0-14 days)			Second period (14-45 days)		
	T1 (control)	T2 (0.1 % SBO)	T3 (0.2 % SBO)	T1 (control)	T2 (0.1 % SBO)	T3 (0.2 % SBO)
Ingredients						
Corn flour	450	440	430	460	460	460
Barley flour	109	119.5	130	191.5	189.5	188.5
Hamlet protein (HP200)	142	140	138	120	120	120
Extract of soy flour 44%	100	100	100	90.0	90.0	90.0
Sweet whey powder	70.0	70.0	70.0	50.0	50.0	50.0
Soft wheat bran	50.0	50.0	50.0			
Vegetable oil	21.5	23.0	23.5	22.0	23.0	23.0
Potato protein	10.0	10.0	10.0	15.0	15.0	15.0
Calcium carbonate	18.0	17.0	17.0	18.0	18.0	18.0
Monocalcium phosphate	20.0	20.0	20.0	23.0	23.0	23.0

Sodium chloride	3.0	3.0	3.0	3.0	3.0	3.0
Lysine hydrochloride	3.0	3.0	3.0	4.0	4.0	4.0
DL methionine	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin mineral premix	2.5	2.5	2.5	2.5	2.5	2.5
SBO	0.0	1.0	2.0	0.0	1.0	2.0
Chemical Composition[a]						
Dry matter	896	894	895	893	891	892
Crude protein	197	193	196	184	181	185
Crude fat	54.4	54.4	52.1	44.2	43.7	47.3
Crude fiber	38.7	36.3	38.2	39.9	39.1	40.4
Ash	63.5	67.6	65.9	70.7	69.8	70.2
Starch	390	387	390	435	435	434
Digestible energy, MJ/kg	14.4	14.4	14.4	14.3	14.3	14.3
Net energy, MJ/kg	10.7	10.7	10.7	10.7	10.7	10.7

[a]*Analytical data determined as reported in section 2. Calculated values for these analytes and for NDF, ADF, ADL and amino acids are reported in the Supplementary material file.*

In the present work testing a research product not yet known in the market, a first very important result was the animals' mortality. This was generally very low. The animals' death rate was zero for all groups in the first fattening period. In the second fattening period, the dead animals were two for the T1, one for the T2 and one for the T3 groups. Apparently, the mortality rate in each treated T2 and T3 groups was half that of the control T1 group. The dead animals were too few to run a statistical analysis and make a reasonable, reliable assessment of the significance level of this apparent result. It is a fact that, over the control and treated groups, the average death rate was 3.9 %. This value is in the normal range for pigs of the same age and weight, as those fed with the diets in Table 3. The anatomical and pathological examination of the dead animals did not evidence abnormalities possibly caused by the treatment diets.

Table 4 reports the feed average daily intake for the 54 males' group and 54 females' group, separately, in each fattening period, and the average intake calculated over the entire duration of the test. In each period, there are no significant differences in the intake of the control (T1) and of the treated diets (T2 and T3). Females tend to intake less feed than males, but this difference is not proven by the statistical analysis of the males' and females' pooled intake values.

Table4. *Animals' feed average daily intake (g) in each fattening period,[a] and average intake calculated over the entire duration of test, for males' groups and females' groups treated with control and treated diets in Table 3*

	Feed Intake (g)			Statistical parameters[b]	
	T1 (control)	T2 (0.1 % SBO)	T3 (0.2 % SBO)	P	SEM
Males					
D0-D14	518	481	525	0.35	22
D14-D42	932	915	907	0.92	42
D0-D42	785	764	774	0.81	23
Females					
D0-D14	478	520	444	0.17	27
D14-D42	881	874	881	0.74	8
D0-D42	747	756	736	0.52	12

[a]*First period from day zero to 14 (D0-D14), second period from day 14 to day 42 (D14-D42), entire test duration from day zero to day 42 (D0-D42).*

[b]*Significance level of effect of treatment (P) and standard error of means (SEM) from statistical analysis of feed intake data in each row.*

Table 5 reports the animals' growth performance. The data are averages of the data collected for male and female groups, in each control and treated group. For males, there are no significant effects of the diets with added SBO compared to the control diet. On the contrary, for females, the average growth of the group fed with the T2 diet in the first fattening 0-14 days period was significantly about 13 % lower than that of the T0 and T1 groups. This is true only for the first fattening period. At day 42, the performances of the females' T0, T1 and T2 groups are not significant anymore.

Table5. *Animals'growth performance. Data as averages calculated over males' groups only and females' groups only, in each control and treated group*

	Weight (W) and daily growth rate (DG)[a]			Statistical parameters[b]	
	T1 (control)	T2 (0.1 % SBO)	T3 (0.2 % SBO)	P	SEM
Males					
W D0	7.86	7.78	8.14	0.22	0.15
W D14	12.3	11.8	12.4	0.47	0.4
W D42	27.6	24.9	27.6	0.14	1.0
DG D0-D14	319	284	307	0.64	26
DG D14-D42	506	464	531	0.38	38
DG D0-D42	444	402	454	0.23	22
Females					
W D0	7.72	7.71	7.24	0.14	0.18
W D14	11.9a	12.1a	10.4b	0.02	0.4
W D42	25.7	26.6	23.9	0.21	1.0
DG D0-D14	302	315	229	0.14	27
DG D14-D42	490	516	480	0.63	27
DG D0-D42	427	449	396	0.32	24

[a]*Average weight (W, kg) at day zero (D0), 14 (D14) and 42 (D42), and daily growth (DG, g) over the first (D0-14) and second (D14-42) fattening periods, and over the entire duration of the experiments (D0-D429; values followed by different letters are significantly different at P <0.05.*

[b]*Significance level of effect of treatment (P) and standard error (SEM) from statistical analysis of W and DG data in each row.*

Table 6 reports the feed conversion rate, calculated from the average daily feed intake in Table 4 divided by the average daily growth rate in Table 5. The data shows no significant differences between the control and the treated groups. Over the entire duration of the fattening trials (D0-D42), the females treated with the SBO T2 diet show apparently better-feed conversion rate than the female T1 and T3 groups. This results from the apparently higher daily growth of the T2, compared to T1 and T3 groups.

Table6. *Feed conversion index (FCI, w/w) in each fattening period and over the entire duration of test.[a]Data as averages calculated over males' groups only and females' groups only, in each control and treated group.*

	Feed Conversion Index (FCI)			Statistical Parameters[a]	
	T1 (control)	T2 (0.1 % SBO)	T3 (0.2 % SBO)	P_T	SEM
Males					
FCI D0-D14	1.70	1.71	1.76	0.93	0.13
FCI D14-D42	1.95	2.03	1.72	0.53	0.20
FCI D0-D42	1.80	1.93	1.71	0.41	0.11
Females					
FCI D0-D14	1.70	1.67	2.22	0.31	0.22
FCI D14-D42	1.83	1.70	1.88	0.45	0.09
FCI D0-D42	1.78	1.69	1.90	0.29	0.09

[a]*First period from day zero to 14 (D0-D14), second period from day 14 to day 42 (D14-D42), entire test duration from day zero to day 42 (D0-D42).*

[b]*Significance level of effect of treatment (P) and standard error of means (SEM) from statistical analysis of FCI data in each row.*

4. Discussion

The lack of effects on pigs' growth and mortality in the present work indirectly indicates that, at the tested concentration, SBO are not toxic. The animals exhibited no signs of toxicity, and no pathologies related to the SBO-supplemented diets. Biagini et al. (2016) reported similar conclusions in the study performed on rabbits fed with the same SBO and at the same concentrations in the feed. The agreement between the two studies performed on different animal constitutes an encouraging data's base line for focusing further studies to meet regulatory requirements and to market SBO as multipurpose bio-based chemicals. One important aspect for regulatory compliance is the presence of heavy metals in SBO (Table 1). The present study did not comprise any specific experiment to address this issue. The reason is that the experiments by Biagini et al (2016) and

Sortino et al.(2012) performed with the same SBO have excluded potential toxicity by SBO' s heavy metal accumulation in rabbits meat, soil and plants. On this basis, no risk for human health can reasonably be expected, even at the highest SBO 0.2 % concentration in feed and at 140 kg ha^{-1} dose applied in cultivation soil.

The data obtained in the present pig study and in the previous rabbit study (Biagini et al., 2016) do not confirm the economic expectations proposed in the vitro pigs' cecal fermentation study by Montoneri et al. (2013). The reduced protein N mineralization during fermentation reported by these authors allowed hypothesizing enhanced meat production upon using protein diets added with SBO. This SBO effect on animal growth has not been evidenced in both the pigs' and rabbits' husbandry studies. However, the reduced protein mineralization is likely to have a positive environmental effect in reducing the ammonia content in manure and emission of GHG from the animal production industry. Ji et al. (2006) have reported that humic substances (HS) mined from different geological sites, and used as dietary supplements for weaning pigs, reduce ammonia emission from manure by 16 – 18 %. Montoneri et al. (2013) have measured similar ammonia reduction in the in-vitro pigs' cecal fermentation in the presence of 0.05-0.2 % SBO. This shows that SBO are similar to HS not only for chemical nature (Tabasso et al., 2015), but also for properties. It is also a further argument supporting the compliance of SBO with the Italian regulation on fertilizers (Presidente della repubblica, 2010).

Two other studies have reported reduction of ammonia in SBO assisted anaerobic fermentation of municipal biowastes (Francavilla et al., 2016) and cow manure Riggio et al., 2017) carried out in bioreactor dedicated to biogas production. These findings have contributed in starting the construction of the hydrolysis prototype at the Acea Pinerolese waste treatment plant (see Section 1) under the EU cofunded LIFE16 ENV/IT/000179 LIFECAB project within the LIFE2016 project proposals. The project specific aim is to produce SBO in sufficient quantities, use it as additive in the Acea plant biowaste fermentation reactors, and validate in real operational environment the SBO assisted anaerobic fermentation process yielding biogas and digestate with reduced ammonia content.

Under the above circumstances and perspectives, it is worthwhile to build on the established research base line and to carry on further studies dedicated to assessing the SBO compliance with specific countries' regulatory requirements, and the positive economic and/or environmental effects in the chemical industry, agriculture and animal production activities.

5. Conclusion

The results of this in vivo pigs' study and the previous in vivo rabbits' study confirm that SBO supplemented diets do not affect negatively the growth and health status of animals. The similarity of chemical nature and properties of SBO and natural humic substances support the compliance of SBO with the Italian regulation on fertilizers. These facts encourage further studies dedicated to assessing SBO compliance with specific countries' regulatory requirements, in order to scale up SBO production to commercial level and market the products for use in the chemical industry, agriculture, and animal husbandry.

Acknowledgments

This work was carried partly with Regione Piemonte funds within the Biochemenergy project (www.biochemenergy.it) and with Ministero delle Politiche Agricole funds within the Agrienergia project. In addition, the authors acknowledge Polytechnic of Torino for contributing through the funding project Starting Grant RTD (project number: 54_RSG17NIR01).

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SUPPLEMENTARY MATERIAL

Calculated Chemical composition of experimental diets (Table 1S)

Document submitted to the ethics committee of the local Italian authority (A.USL di Piacenza, 2017), which contained all experimental details of the planned work

Table1S. *Chemical composition (g/kg as-fed basis, unless otherwise indicated) of experimental diets used in the first (0-14 days) and second (14-45) fattening periods.*[a]

	First period (0-14 days)			Second period (14-45 days)		
	T1 (control)	T2 (0.1 % SBO)	T3 (0.2 % SBO)	T1 (control)	T2 (0.1 % SBO)	T3 (0.2 % SBO)
Dry matter	904	904	903	903	903	903
Crude protein	196	196	195	184	184	184
Fat	50.5	51.7	52.0	50.3	51.2	51.1
Crude fiber	34.2	34.4	34.6	32.0	31.9	31.9
Ashes	75.7	75.1	75.4	74.4	74.7	75.1
NDF	93.6	94.3	94.9	82.4	82.1	82.0
ADF	36.2	37.0	37.8	33.1	33.5	33.9
ADL	9.4	9.8	10.1	8.2	8.5	8.9
Starch	380	378	376	415	414	414
Lysine	12.8	12.8	12.7	12.7	12.7	12.7
Methionine	4.1	4.1	4.0	4.0	3.9	3.9
Cystine	3.3	3.3	3.3	3.2	3.2	3.2
Triptophan	2.3	2.3	2.3	2.1	2.1	2.1
Threonine	7.6	7.6	7.5	7.1	7.1	7.1
Glycine + Serine	16.7	16.6	16.5	15.0	15.0	15.0
Leucine	16.8	16.7	16.6	15.9	15.9	15.9
Isoleucine	8.7	8.6	8.6	8.1	8.1	8.1
Valine	9.7	9.6	9.6	9.1	9.1	9.1
Histidine	4.9	4.9	4.9	4.6	4.6	4.6
Arginine	12.1	12.0	12.0	11.0	11.0	11.0
Phenylalanine + Tyrosine	17.1	17.0	16.9	16.1	16.1	16.1
Ca	11.4	11.0	11.0	11.6	11.7	11.7
Total P	8.8	8.8	8.8	8.9	8.9	8.9
Disodium phosphate	5.5	5.6	5.6	5.1	6.1	6.1
Na	1.9	1.9	1.9	1.8	1.8	1.8
Linoleic acid	10.2	10.1	10.0	11.1	11.1	11.1
CA/P	1.3	1.2	1.2	1.3	1.3	1.3
Digestible Energy	14.1	14.2	14.1	14.2	14.2	14.2
Metabolized	13.7	13.7	13.7	13.8	13.8	13.8

Energy						
Net Energy	10.1	10.1	10.1	10.0	10.0	10.0
Gross Energy	13.8	13.8	13.8	14.1	14.1	14.1

[a]*Data calculated based on diets formulas in Table 3 main manuscript, using Brill formulation feed management systems version 2.03, 2011, Brilliant Alternatives Inc., USA.*

Document submitted to the ethics committee of the local Italian authority (A.USL di Piacenza, 2017), which contained all experimental details of the planned work

SCHEMAPERLAPRESENTAZIONEDIUNPROGETTODIRICERCAAISENSIDELDECRETOLEGISLATIVO116/921

RESPONSABILE DEL PROGETTO DI RICERCA

COGNOME MORLACCHINI NOME MAURO

CODICE FISCALE MRLMRA58E06G535J RESIDENZA VIAMORSELLI16-PIACENZA

DIPARTIMENTO O ISTITUTO DI AFFERENZA: CERZOO (CentrodiRicercheperla Zootecniael' Ambiente)-LOC.POSSESSIONEDIFONDO-S.BONICO-PIACENZA

RESPONSABILE DELL'ESECUZIONE DEGLI ESPERIMENTI COGNOME FUSCONI NOME GIORGIO

CODICE FISCALE FSCGRG59D04D357W RESIDENZA VIAROSSI,19-PIACENZA

DIPARTIMENTO O ISTITUTO DI AFFERENZA: CERZOO (Centrodi Ricercheperla Zootecniael' Ambiente)-LOC. POSSESSIONEDIFONDO-S.BONICO-PIACENZA

RESPONSABILE DELLO STABULARIO

COGNOME MORLACCHINI NOMEMAURO

CODICE FISCALE MRLMRA58E06G535J RESIDENZA VIAMORSELLI16-PIACENZA

DIPARTIMENTO O ISTITUTO DI AFFERENZA: CERZOO (CentrodiRicercheperla Zootecniael' Ambiente)-LOC.POSSESSIONEDIFONDO-S.BONICO-PIACENZA

Medico VeterinarioResponsabiledeicontrolli di cui all'articolo 6 delDecretoLegislativo 116/92

COGNOME BONVICINI NOME FLAVIO

CODICE FISCALE BNVFLV63T31B910I RESIDENZA VIALECADORNA3, CASALPUSTERLENGO(LO)

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SI PREVEDE DI UTILIZZARE TIPO DI ANIMALE

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GLI ANIMALI SARANNO UTILIZZATI NEI LOCALI FORNITORI

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CERZOO

Az.AgricolaSerenaCascinaVincereCamairago(LO)

CodiceAll.:007LO011

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DURATA DELLA SPERIMENTAZIONE (MASSIMO TRENTASEI MESI) 4mesi

PERSONALECHEATTENDEALLAESECUZIONEDEGLIESPERIMENTIE/OALCONTROLLO DEGLI ANIMALI (COMPRESO IL RESPONSABILE DEGLIESPERIMENTI)

COGNOME E NOME	TITOLO DI STUDIO	QUALIFICA	DIPARTIMENTI/ ISTITUTO AFFERENZA
Mauro Morlacchini	LaureaScienzeAgrarie	Direttore	CERZOO
Giorgio Fusconi	LaureaScienzeAgrarie	Ricercatore	CERZOO
TizianoGeroldi	Licenza media	Operaio	CERZOO
NicoliNazareno	Diploma	Operaio	CERZOO
Gianfranco Piva	LaureaScienzeAgrarie	Resp. Scientifico	CERZOO

DESCRIZIONE DEI FINI DELL'ESPERIMENTO (ARTICOLO 3 D.LVO 116/92)

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METODOLOGIAETECNICADELL'ESPERIMENTO

1) CHE TIPO DI SOFFERENZA SI RITIENE DI INFLIGGERE AGLI ANIMALI? xPOCA O NESSUNA SOFFERENZA

o ALCUNE SOFFERENZE (DI BREVE DURATA)

o ESTREME E INTOLLERABILI SOFFERENZE IN ANIMALI COSCIENTI o FORTI PRIVAZIONI, MUTILAZIONI O TRAUMI

2) ANESTESIA SI [ ] NO [X]

Noncompatibileconilfinedell'esperimentoinquantolapresenzadisostanzeanestetizzantinell'organismoanimaleinterferisconoconlavalutazionedell'efficaciadellasostanzaoggettodellostudio

3) TIPO DI ANESTESIA

4) GLI ANIMALI SONO SOPPRESSI TRAMITE

Nessunsoggettosaràabbattutoperfinisperimentali.

AL TERMINE DELLE PROCEDURE SPERIMENTALI GLI ANIMALI SONO RIUTILIZZATI SI [ ] NO [X]

Alterminedellasperimentazioneglianimalientrerannonelnormaleciclodi allevamentoinquantolasostanzaoggettodellostudiosiconfiguracomeunmangimecomplementare.

DICHIARAZIONE RIFERITA ALLE PRESCRIZIONI DI CUI ALL'ARTICOLO 4 (INEVITABILITA' DEL RICORSO AD ESPERIMENTI SU ANIMALI E NECESSITA' DEL

RICORSO AD UNA SPECIE DETERMINATA ED AL TIPO DI ESPERIMENTO) E DI CUI ALL'ARTICOLO 5(RISPETTO DELLE CONDIZIONI)

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DOCUMENTAZIONE A CORREDO (BIBLIOGRAFIA, PUBBLICAZIONI, ECC.):

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RESPONSABILE DELL'ESECUZIONE DELL'ESPERIMENTO DR.FUSCONIGIORGIO

PIACENZA,14NOVEMBRE 2011

IL RESPONSABILE SCIENTIFICO

(Prof.GianfrancoPiva)

IL DIRETTORE DEL CENTRO DI SAGGIO

(Dr.MauroMorlacchini)

Citation: *M. Morlacchinia, G. Fusconia, C. Montonerib, R. Nisticòc, E. Montonerid. Pigs' Husbandry as Case Study to Test Effects of Vegetable Residues' Hydrolysates on Animals. ARC Journal of Animal and Veterinary Sciences. 2017; 3(3):11-23. doi: dx.doi.org/ 10.20431/2455-2518.0303003.*

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