

## Digestive utilization of novel biodegradable plastic in growing pigs

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**Abstract** — Two experiments were carried out to determine the effect of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and polycaprolactone (PCL) inclusion (Exp. 1), and of PHBV pretreatment with sodium hydroxide (Exp. 2) on the digestibility of diets in growing pigs. In Exp. 1, eight male castrated Large White pigs (initial liveweight 24 kg; final liveweight 64 kg) received rationed amounts of a cereal-based diet containing 0 (C) or 8 % of either barley straw meal (S), PHBV (P1) or PCL (P2) in a replicated double 4 × 4 Latin square design. In Exp. 2, ten animals (23–84 kg liveweight) were fed the same standard diet including 0 (C), 10 or 20 % of either untreated (P10, P20) or pretreated PHBV (tP10 and tP20) in a replicated double 5 × 5 Latin square design. Inclusion of untreated biodegradable plastic and straw negatively influenced ( $P < 0.01$ ) organic matter and energy digestibility. Untreated PHBV was poorly digested. The analogy of data indicates that PCL was even less digested. Untreated plastic can be considered as undegradable material in the gastrointestinal tract of pigs. In contrast, pretreatment (Exp. 2) significantly increased ( $P < 0.01$ ) PHBV, organic matter and energy digestibility. Independently of the amount included, 37 % of pretreated PHBV was digested on average. (© Elsevier / Inra)

**pig / straw / biodegradable plastic / sodium hydroxide / digestibility**

**Résumé** — Utilisation digestive de nouveaux plastiques biodégradables chez les porcs en croissance. Deux expériences ont été réalisées chez des porcs en croissance pour déterminer, d'une part la digestibilité fécale de nouveaux plastiques biodégradables non traités tels le polycaprolactone (PCL) et le poly[3-hydroxybutyrate-co-3-hydroxyvalérate] (PHBV), d'autre part étudier l'effet d'un prétraitement chimique et d'une substitution échelonnée avec le PHBV. Dans l'expérience 1 (Exp. 1), huit mâles castrés Large White (poids initial : 24 kg – poids final : 63 kg) ont reçu une ration à base de céréales contenant 0 (C) ou 8 % de paille broyée (S), de PHBV (P1) ou de PCL (P2) selon un dispositif en carré latin 4 × 4 répété. Pour l'expérience 2 (Exp. 2) la même ration de base incluant 0 (C), 10 ou 20 % de PHBV prétraité (tP10, tP20) ou non (P10, P20) a été distribuée à

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10 mâles castrés selon un dispositif en carré latin  $5 \times 5$  répété. L'incorporation de plastiques non traités et de paille dans la ration a provoqué une diminution significative ( $p < 0,01$ ) de la digestibilité fécale de la matière organique et de l'énergie. La digestibilité du PHBV non traité a été très faible dans les deux expériences. L'analogie des données indique que la digestibilité du PCL a même été inférieure à celle du PHBV. Le plastique biodégradable peut être considéré comme une matière non dégradable dans les intestins du porc. En revanche, le prétraitement avec de la soude a amélioré de façon significative la digestibilité fécale du PHBV, de la matière organique et de l'énergie. L'augmentation a été suffisante pour obtenir des digestibilités fécales similaires au témoin dans le cas du régime *tP10*. En moyenne 37 % du PHBV prétraité a été digéré indépendamment de la quantité incluse dans l'aliment. En fait, la formation d'environ 40 % de monomères par le prétraitement avec de la soude semble avoir totalement contribué à l'augmentation de la digestibilité fécale du PHBV, de l'énergie et de la matière organique. En conclusion, les plastiques riches en énergie n'ont pas eu de graves effets négatifs sur la digestion, bien que les plastiques non traités n'aient pas été bien utilisés en tant qu'énergie digestible. (© Elsevier / Inra)

## porc / paille / plastique biodégradable / hydroxyde de sodium / digestibilité

### 1. INTRODUCTION

The use of renewable agricultural resources as raw materials for biodegradable polymers is presently being widely discussed [9]. Due to their alleged complete biodegradation and the high energy content, interest arose for using biodegradable plastics as a feed ingredient in diets for pigs.

Polycaprolactone (PCL) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) are aliphatic biodegradable polymers. PCL is chemosynthetically produced and PHBV is a copolymer by bacteria [10, 15]. Aliphatic polyesters are mainly considered carbon and energy storage polymers; however, PHBV was detected as a non-storage polymer in eukaryotic organisms [18].

Degradation of aliphatic polyesters in a living environment can result from enzymatic action or simple hydrolysis of ester bonds or both [12]. In the case of PHBV, biodegradation occurs in both aerobic and anaerobic microbial active environments [2]. Biodegradation appears to proceed by colonization of the polymer surface by bacteria or fungi, which secrete an extra cellular depolymerase capable of degrading the polymer near the cell. The soluble degrada-

tion products are then absorbed through the cell wall and metabolized [5].

The aim of the first experiment (Exp. 1) was to evaluate the digestibility and the effects of straw inclusion and of the biodegradable plastic PHBV and PCL in diets for growing pigs. In a second experiment (Exp. 2), the effect of chemical pretreatment of PHBV by means of sodium hydroxide and the addition of 10 or 20 % PHBV on the digestibility of plastic in growing pigs were studied.

### 2. MATERIALS AND METHODS

#### 2.1. Animals and experimental procedures

Two digestibility trials were conducted using 18 castrated male Large White pigs weighing  $23.5 \pm 1.4$  kg. In Exp. 1, eight and in Exp. 2 ten pigs were assigned to four and five diets, respectively. In both experiments the animals were housed in individual pens in a controlled environment and had free access to water. The animals were fed once a day according to a feeding scale adapted weekly to the liveweight (LW) ( $\text{kg feed} = 0.1 \times \text{LW}^{3/4}$ ). The pigs were weighed once a week. The experiments were carried out according to a double  $4 \times 4$  (Exp. 1) and  $5 \times 5$

Latin square design (Exp. 2). Each experimental period lasted three weeks and consisted of 17 days of adaptation period followed by faeces collection on four consecutive days. Faeces samples were stored in plastic bags at  $-20^{\circ}\text{C}$ . Prior to analysis, the samples were thawed overnight and homogenized at room temperature. An aliquot was taken for nitrogen determination and the remaining material was dried (48 h,  $60^{\circ}\text{C}$ ) and ground (0.5 mm). The faeces samples from each collection period were analyzed separately. Organic matter (OM), nitrogen, energy, PCL and PHBV digestibility were estimated by means of the indicator method, using 4 N-HCl insoluble ash as indicator [17]. Celite 545 (acid-washed diatomaceous earth) was added to the diets as an additional acid insoluble ash (AIA) source.

## 2.2. Diets

In Exp. 1, the animals received a standard diet based on barley and wheat containing 0 (C) or 8 % of either barley straw meal (S), PHBV (P1) or PCL (P2), respectively (table 1). PCL and PHBV were delivered by the manufacturer as granules. To avoid feed selection by the animals during the feeding trial, the granules were milled to powder. The particle size distribution within the PCL powder was: < 0.063 mm, 2.6 %; 0.063–0.125 mm, 9.2 %; 0.125–0.25 mm, 20.1 %; 0.25–0.5 mm, 40.7 %; 0.5–1.0 mm, 26.5 %; 1.0–1.4 mm, 0.5 % and > 1.4 mm, 0.4 %. The corresponding values for PHBV were: < 0.063 mm, 5.1 %; 0.063–0.125 mm, 18.5 %; 0.125–0.25 mm, 24.2 %; 0.25–0.5 mm, 32.5 %; 0.5–1.0 mm, 5.1 %, 1.0–1.4 mm, 7.0 % and > 1.4 mm, 7.6 %. Diet S served as a negative control since straw is poorly digested by pigs and is rich in plant fibre.

For Exp. 2, the same standard diet as in Exp. 1 was fed to the pigs, including 0 (C), 10 or 20 % of either untreated (P10, P20) or pretreated PHBV (tP10, tP20) (table 1). Pretreatment of PHBV consisted of a 24-h exposure to NaOH equivalent to an amount of  $40\text{ g}\cdot\text{kg}^{-1}$  total feed. Before mixing the other feed ingredients, the pretreated plastic was neutralized with HCl to a pH of 7. In contrast to Exp. 1, for Exp. 2, the PHBV was delivered from the manufacturer as a powder without previous granulation, but still from the same batch as before. As a consequence, the average particle size of the non-granulated PHBV was considerably smaller, with the following distribution determined: < 0.063 mm,

19.7 %; 0.063–0.125 mm, 66.6 %; 0.125–0.25 mm, 4.9 %; 0.25–0.5 mm, 2.9 %; 0.5–1.0 mm, 2.5 %, 1.0–1.4 mm, 1.8 % and > 1.4 mm, 1.6 %.

## 2.3. Chemical analyses

Dry matter (DM), ash, crude protein (CP), soxhlet fat (SF) and gross energy (GE) were determined for all diets. The faeces were submitted to the same analyses except SF. The analyses of DM, ash and SF were determined according to the AOAC [1] procedures. Kjeldahl nitrogen determination was performed with an automated Büchi 323 distillation unit (Büchi Laboratory-Techniques Ltd., Flawil, Switzerland), 665 Dosimat and 678 EP/KF Processor (Metrohm Ltd., Herisau, Switzerland). The CP content was calculated as  $6.25 \times$  nitrogen content. The energy content was assessed through an anisothermic bomb calorimeter (System C 700T, IKA Analysetechnik GmbH, Heiterstheim, Germany). The AIA content of the diets and faeces was determined according to the method of Prabucki et al. [17].

The method of measurement of PHBV and PCL in the pig faeces was described by Forni [6]. The calibration of the high performance liquid chromatography (HPLC) unit was done prior to analysis with a dilution series of pure PHBV and provided close linear relationships (mean  $R^2 = 0.99$ ). To determine the effects of pretreatment, the soluble part of the pretreated sample was analyzed by  $^1\text{H}$  nuclear magnetic resonance spectroscopy ( $^1\text{H}$  NMR) and the insoluble polymeric part was submitted to a gel permeation chromatography (GPC) analysis (by Dr. V. Toncheva, Vakgroep voor Organische Chemie, University of Gent, Belgium).

## 2.4. Statistical analyses

The statistical analysis was performed with Statgraphics Plus for Windows (Version 2.1, Statistical Graphics Corp., Manugistics Inc., Rockville, MD, USA). Exps. 1 and 2 were analyzed separately. The data were subjected to multifactorial analyses of variance (ANOVA). Treatments, experimental periods and the individual pig were taken into account according to the Latin square design. Multiple comparison of means was performed using the Bonferroni multiple range test. The means were considered significantly different at  $P < 0.01$ .

Table I. Composition and chemical analysis of the diets in Exps. 1 and 2.

	Exp. 1					Exp. 2		
	C	S	PI	P2	C	PI0/P10	P20/P20	P20/P20
<i>Composition of the diets (in %)</i>								
Barley	48	44.2	44.2	44.2	48	43.2	38.4	38.4
Wheat	35.5	32.6	32.6	32.6	35.5	31.95	28.4	28.4
Potato proteins	4	3.7	3.7	3.7	4	3.6	3.2	3.2
Soya cake (40 %)	5	4.6	4.6	4.6	5	4.5	4	4
Meat-and-bone meal	2.2	2.02	2.02	2.02	2.2	1.98	1.76	1.76
L-Lysine	0.54	0.50	0.50	0.50	0.54	0.49	0.43	0.43
Calcium carbonate	0.86	0.79	0.79	0.79	0.86	0.77	0.69	0.69
Sodium chloride	0.6	0.55	0.55	0.55	0.6	0.54	0.48	0.48
Premix <sup>1</sup>	0.8	0.74	0.74	0.74	0.8	0.72	0.64	0.64
Celite <sup>2</sup>	2.5	2.3	2.3	2.3	2.5	2.25	2	2
Barley straw meal	-	8	-	-	-	-	-	-
PHBV	-	-	8	-	-	10	20	20
PCL	-	-	-	8	-	-	-	-
<i>Chemical analysis (dry matter)</i>								
Ash (g·kg <sup>-1</sup> )	84	85	75	78	87	76	67	67
Crude protein (g·kg <sup>-1</sup> )	182	171	165	165	189	170	149	149
Soxhlet fat (g·kg <sup>-1</sup> )	10	10	9	9	16	15	14	14
Crude fibre (g·kg <sup>-1</sup> )	38	64	nd	nd	44	42	39	39
Nitrogen-free extract (g·kg <sup>-1</sup> )	686	670	-	-	664	697	731	731
Gross energy (MJ·kg <sup>-1</sup> )	17.47	17.70	17.89	18.42	17.70	18.29	18.91	18.91

<sup>1</sup> The content of 1 kg premix: 10.0 g Ca; 55.0 g Mg; 5.0 g S; 2.0 g Cu; 6.0 g Fe; 20.0 g Zn; 8.0 g Mn; 0.2 g I; 0.06 g Se; 2 × 10<sup>6</sup> IU vit. A; 2 × 10<sup>5</sup> IU vit. D3; 8 000 IU vit. E; 300 mg vit. K3; 800 mg vit. B2; 800 mg vit. B6; 3 mg vit. B12; 20 mg biotin; 3.0 g Ca-pantothenate; 4.0 g niacin; 40 mg folic acid. <sup>2</sup> Acid-washed diatomaceous earth added as indicator. nd: Interference of plastics to crude fibre analysis did not allow accurate results for P1 and P2.

### 3. RESULTS

#### 3.1. Chemical composition of biodegradable plastic

Biodegradable plastics have an almost negligible content of water and crude ash (*table II*). In contrast, they showed a higher content of neutral detergent fibre (NDF) than barley straw, a highly fibrous material. The CP content was minimal and the GE exceeded the value for straw since biodegradable plastic is mostly an energy and carbon storage compound.

*Table III* gives the relative amount and variety of soluble monomers formed with the equivalent of 40 g NaOH per kg feed DM. Pretreatment led to a substantial formation of soluble monomers, namely hydroxybutyric, hydroxyvaleric and crotonic acid and of insoluble polymeric fractions ( $F_{1-4}$ ) with different mean molecular weight. The higher NaOH amount per unit of PHBV applied in the *tP10* treatment degraded 52 % and the lower amount in *tP20* about 35 % of PHBV to monomers. Neutralization with HCl reduced the soluble polymer part in treatment *tP10* by 11 % units and caused a further decrease in treatment *tP20*. GPC analysis of the polymeric part showed that pretreatment generally

**Table II.** Chemical composition (g·kg<sup>-1</sup> dry matter [DM]) and gross energy content (MJ·kg<sup>-1</sup> DM) of the supplemented ingredients.

	Straw	PHBV	PCL
<i>Chemical composition</i>			
Organic matter	929	998	1 000
Neutral detergent fibre	756	982	923
Acid detergent fibre	468	826	843
Crude fibre	644	13	886
Crude protein	69	12	3
Gross energy	18.25	23.30	28.84

decreased the initial numeric PHBV molecular weight of 174 000. Four fractions of average numeric molecular weights (representing the residual PHBV polymer chain length) of about 100 000, 20 000, 5 000 and 2 000 were found, each of them considerably shorter than the original polymer.

#### 3.2. Digestibility of diets, straw and biodegradable plastics

Inclusion of straw and untreated biodegradable plastic in the diets decreased the OM and energy digestibility ( $P < 0.01$ ) (*table IV*). Energy digestibility of the diets

**Table III.** Formation of monomer and polymer fractions by NaOH pretreatment of PHBV for the *tP10* (PHBV<sub>10</sub>) and *tP20* (PHBV<sub>20</sub>) diets.

	Monomeric part				Polymeric part				
	%	Composition (%)			%	Polymer fractions (M <sub>n</sub> )			
		Hydroxy-butyric acid	Hydroxy-valeric acid	Crotonic acid		F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>
PHBV <sub>10</sub> <sup>1</sup>	51.6	81.0	5.7	13.3	48.4	103 500	19 000	5 140	1 970
PHBV <sub>10</sub> <sup>2</sup>	40.2	91.5	6.4	2.1	59.8	89 600	18 900	5 320	2 130
PHBV <sub>20</sub> <sup>1</sup>	35.3	83.8	7.1	9.1	64.7	—*	18 920	5 510	1 980
PHBV <sub>20</sub> <sup>2</sup>	21.6	90.3	7.6	2.1	78.4	99 700	19 900	5 370	2 050

<sup>1</sup> Exposure to NaOH for 24 h; <sup>2</sup> after 24 h exposure to NaOH neutralized with HCl to a pH of 7; \* not detected.

**Table IV.** Effects of straw (S), PCL (P2) and PHBV (P1, P10 and P20) supplementation as well as PHBV pretreatment with NaOH (tP10 and tP20) on dry matter (DM) content of faeces, digestible energy (DE) content, nutrient and energy digestibility of the diets, and their digestibility calculated by difference.

	Exp. 1				Exp. 2						
	C (n=8)	S (n=8)	P1 (n=8)	P2 (n=8)	SEM	C (n=10)	P10 (n=10)	tP10 (n=10)	P20 (n=10)	tP20 (n=10)	SEM
Intake (kg·d <sup>-1</sup> )	1.384	1.419	1.413	1.383	0.020	1.684 <sup>a</sup>	1.776 <sup>b</sup>	1.731 <sup>ab</sup>	1.705 <sup>ab</sup>	1.687 <sup>a</sup>	0.019
DM content of faeces (%)	37.5 <sup>a</sup>	32.7 <sup>a</sup>	44.8 <sup>b</sup>	44.2 <sup>b</sup>	0.8	29.6 <sup>a</sup>	38.1 <sup>b</sup>	35.8 <sup>b</sup>	45.0 <sup>c</sup>	41.7 <sup>b</sup>	0.7
<i>Digestibility</i>											
Organic matter	0.864 <sup>a</sup>	0.808 <sup>b</sup>	0.768 <sup>c</sup>	0.755 <sup>c</sup>	0.004	0.819 <sup>a</sup>	0.751 <sup>b</sup>	0.793 <sup>a</sup>	0.668 <sup>c</sup>	0.734 <sup>b</sup>	0.008
Energy	0.841 <sup>a</sup>	0.786 <sup>b</sup>	0.727 <sup>c</sup>	0.693 <sup>d</sup>	0.005	0.801 <sup>a</sup>	0.710 <sup>b</sup>	0.765 <sup>a</sup>	0.611 <sup>c</sup>	0.697 <sup>b</sup>	0.006
Nitrogen	0.868 <sup>a</sup>	0.826 <sup>b</sup>	0.832 <sup>b</sup>	0.835 <sup>b</sup>	0.004	0.830	0.836	0.850	0.818	0.835	0.009
PHBV			0.16	nd		–	–0.118 <sup>a</sup>	0.386 <sup>b</sup>	0.024 <sup>c</sup>	0.369 <sup>b</sup>	0.025
Energy digestibility calculated by difference		0.271	–0.274	–0.326			0.097	0.529	0.045	0.394	
DE (MJ·kg <sup>-1</sup> DM)	14.692	13.912	13.006	12.765		14.178	12.986	13.992	11.554	13.180	

a, b, c, d Means followed by different letters within rows differ significantly at  $P < 0.01$ ; nd: digestibility of PCL could not be determined by the HPLC method.

containing untreated PHBV and PCL was reduced by 13.6 and 17.6 % for *P1* and *P2* in Exp. 1 and by 11.3 and 23.7 % for *P10* and *P20* in Exp. 2 compared to treatment *C*. Reduction of digestibility of energy was more pronounced ( $P < 0.01$ ) for the diet supplemented with PCL than the diet supplemented with untreated PHBV. Pretreatment of PHBV increased mean OM and energy digestibility ( $P < 0.01$ ) in comparison to the analogous untreated diets. The OM and energy digestibility of the *tP10* treatment was similar to that of treatment *C*, whereas treatment *tP20* was lower ( $P < 0.01$ ). Nitrogen digestibility was decreased by straw and biodegradable plastic inclusion in Exp. 1, but not in Exp. 2 when compared to treatment *C*.

The highest DM content of faeces was recorded for the treatments supplemented with plastic ( $P < 0.01$ ) (table IV). In addition, an increase of faecal DM content ( $P < 0.01$ ) was observed with the higher inclusion of untreated biodegradable plastic in the diet (*P20*, Exp. 2). Pretreatment reduced the faecal DM content for *tP20* in comparison to *P20* ( $P < 0.01$ ).

The digestible energy (DE) content of the diets (table IV), which were calculated from the current data, reflect the effects on energy digestibility induced by untreated plastic inclusion, pretreatment and an increased dietary level of PHBV.

According to recovery by HPLC, 38.6 (*tP10*) and 36.9 % (*tP20*) of the pretreated PHBV were digested, whereas low or even negative digestibility values were detected for the untreated PHBV. A partial volatilization of PCL during the drying process of the faeces before analysis occurred, since faeces were dried at 60 °C for 48 h, whereas the melting point of PCL is between 60 and 65 °C. Therefore, no PCL digestibility assessed by the HPLC method was estimated.

## 4. DISCUSSION AND CONCLUSION

### 4.1. Characterization of biodegradable plastic as a feedstuff

The biodegradable polymers were analytically recovered in the fibre fraction. The analytical values were particularly high for NDF. However, in the case of biodegradable plastic, fibre analysis may act as a test of resistance against the chemicals used in the different analyses rather than as a reference value for their nutritional characteristics. Although biodegradable polymers were recovered in the fibre fraction, they may not necessarily have acted like structural carbohydrates in the intestinal digesta of the animals. In agreement with the results of Hadorn [7], greater excretion of undigested feed led to increased faecal DM content in all treatments supplemented with biodegradable plastic.

### 4.2. Digestibility of the diets

In Exp. 1, the OM, nitrogen and energy digestibility of diet *C* were higher than in Exp. 2. Even though the ingredient composition of the diet was the same in both experiments (table I), the ingredients were not from the same batches and this might be responsible for the differences in the energy and nutrient digestibility between Exp. 1 and 2.

### 4.3. Effects of straw inclusion

The present study confirms the poor digestibility of straw in swine [16]. Barley straw had a similar effect on energy digestibility as reported by other workers [8, 11, 14]. Energy and nitrogen digestibilities declined by 2.1 and 1.6 units, respectively, per unit of additional dietary crude fibre in the feed. However, Pfirter and Halter [16] found a smaller decrease in nitrogen digestibility than the present value.

#### 4.4. Effects of untreated plastic inclusion

The dietary supplementation of untreated bioplastic caused in both trials a significant reduction in the OM and energy digestibility. The results suggest that untreated bioplastic is undegradable in the gastrointestinal tract of pigs. Furthermore, the decrease in the energy digestibility as compared to diet C was higher than the level of plastic supplementation. This could be an indication for a negative effect on the digestibility of other nutrients as observed for nitrogen (Exp. 1). In Exp. 2, dietary supplementation of untreated PHBV (*P10*) did not result in decreased digestibility coefficients like in Exp. 1 (*P1*) when a still lower percentage of untreated PHBV (8 %) had been used. The greater energy digestibility of PHBV (*P10*) in Exp. 2 in comparison to Exp. 1 might be predominantly due to the smaller average particle size of the PHBV used in Exp. 2. Although generally coming from the same production batch, the first delivery was PHBV granules, which were subsequently milled, whereas the second delivery (the one used in Exp. 2) consisted of PHBV powder without being further processed to granules. Since in the trials employing small particle size PHBV digestibility, as calculated by the difference method, was higher, although a higher amount was supplemented, the smaller particles obviously favoured to a certain extent the degradation and prevented negative effects on nitrogen digestibility.

The absence of specific gastrointestinal microorganisms or enzymes that are capable of degrading PHBV or PCL may be one of the reasons for the poor digestibility of untreated plastic. Mergaert (Laboratorium voor Microbiologie, Vakgroep Biochemie, Fysiologie en Microbiologie, Universiteit Gent, Belgium, personal communication) could not isolate PHBV degrading bacteria from the faeces of pigs fed PHBV or PCL. Seppälä and Malin [19] reported that PHBV seems to be insensitive to hydrolytic degradation. Another explanation for the poor

digestibility might be the faster passage rate of biodegradable plastic through the gastrointestinal tract compared to degradation in other environments, where degradation may last months [13]. In contrast to our results, Brune and Niemann [3] found higher digestibility values for PHB. The amorphous state of the PHB which leads to a faster degradation [2] could be one of the reasons for the better results.

A discrepancy exists between the digestibility values for energy and for PHBV. The relative decrease in the energy digestibility between the *P1*, *P10* and *P20* diets and the diet C, as well as between diets *P10* and *P20* did not reflect the determined PHBV digestibility values. In Exp. 1, the PHBV digestibility determined from the HPLC analysis amounted to 0.16, whereas in Exp. 2 the values were negative (-0.118 for *P10*) or almost zero (0.024 for *P20*). The results reveal that the pigs did not digest untreated PHBV and therefore small differences in PHBV concentration in the faeces assessed by HPLC affected the digestibility values calculated by the indicator method.

#### 4.5. Effects of pretreatment

Digestibility of PHBV was improved, independently of the amount included in the diet, by almost 40 %. It was of interest that the formation of polymer fractions had the same average molecular weights ( $M_n$ ) for all pretreatments. Pretreatment apparently contributed directly to supply soluble and degradable products that are absorbed through the cell wall and metabolized [5] since the percentage of the formed soluble polymeric part corresponded to the coefficient of digested PHBV. In disagreement with Cox [4], the insoluble part was apparently not degraded despite the presence of low molecular weight fractions.

In conclusion, growing pigs fed diets with up to 20 % PHBV over a long period could withstand without severe repercussions to their health. In addition, no severe negative



effects due to plastic inclusion were observed for nitrogen digestibility. In growing pigs, untreated plastic was less digested than straw. Pretreatment improved OM, energy and PHBV digestibility, showing similar coefficients to the control for the treatment containing 10 % pretreated PHBV. Although no significant difference was observed between treatments *iP10* and *C*, fattening trials are needed to confirm the potential of pretreated PHBV as an ingredient of diets for growing-finishing pigs.

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