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Novel biodegradable plastics in sheep nutrition 2. Effects of NaOH pretreatment of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) on *in vivo* digestibility and on *in vitro* disappearance (Rusitec)

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Introduction

The use of biodegradable polymers in animal nutrition can be considered not only as a possible way of waste recycling but could also be beneficial in the derivation of animal products from the conversion of these energy-rich substances within the nutritional chain. However, earlier studies with swine and sheep as well as *in vitro* studies have shown a poor digestibility of untreated biodegradable plastics (KUNKEL and SEO 1994; FORNI et al. 1999a,b). The low accessibility of the polymeric chains, particularly if only coarsely milled, to the gastric juices, enzymes and gastrointestinal micro-organisms of farm animals, and the relatively short incubation time during the passage through the digestive tract obviously limit the degradation of these biodegradable polymers. Therefore, the present study focused on the effects of pretreatment and of increasing the dietary proportion of the biodegradable plastic poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). PHBV is an aliphatic biodegradable polymer produced by bacteria (DE KONING et al. 1996). Pretreatment with NaOH was chosen to reduce the chain length and to form absorbable monomers such as β-hydroxybutyrate, a natural metabolite in the digestive turnover and metabolism of ruminants. NaOH is an inexpensive and effective substance for breaking down PHBV, which was found to increase the apparent PHBV digestibility by 37 % in swine (FORNI et al. 1999a). In addition, NaOH pretreatment is commonly used in ruminant nutrition to enhance the digestibility and intake of straw and poor quality hay which are both rich in biological polymers such as cellulose and lignin (FLACHOWSKY 1987).

Materials and methods

Pretreatment of PHBV

The pretreatment of PHBV consisted of a 24 h exposure to NaOH equivalent to an amount of 40 g/kg total feed dry matter (DM). Before mixing with the other feed ingredients, the pretreated PHBV was neutralized with HCl to a pH of 6 to 7.

Sheep trial

Six adult castrated male sheep of the Swiss Brown Hill breed were assigned to a 6 × 6 Latin square experiment. The animals were kept in metabolism crates and fed two equal meals in the morning and the afternoon. A coarsely chopped hay of medium quality (C) and a hay–

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Table 1. **Nutrient and gross energy composition of the ingredients and their dietary proportions**

	Hay n = 5	Corn meal n = 3	PHBV n = 1
Dry matter (DM; g/kg)	932	897	997
Organic matter (g/kg DM)	925	983	998
NDF (g/kg DM)	588	108	924
ADF (g/kg DM)	330	34	716
Crude fibre (g/kg DM)	291	20	0.5
Crude protein (g/kg DM)	114	89	8
Gross energy (MJ/kg DM)	18.2	18.8	22.5
Proportion in the diet (%)			
Diet C	100	–	–
Diet C1	50	50	–
Diet P10	50	40	10
Diet tP10	50	40	10[1]
Diet P20	50	30	20
Diet tP20	50	30	20[1]

[1] PHBV pretreated with NaOH for 24 h and neutralized with HCl

corn meal mixture (C1; 1 : 1 on dry matter basis) served as control diets (Table 1). This allowed the replacement of corn meal from the C1 diet by PHBV at a considerably higher proportion than was possible in the previous experiment (FORNI et al. 1999b) without suppressing the digestibility of the diet below that of the control diet with hay only (C). Either untreated or pretreated (t) PHBV substituted 10 % (P10, tP10, respectively) or 20 % (P20, tP20, respectively) corn on a dry matter basis. In contrast to the first experiment (FORNI et al. 1999b), for the present sheep trial the PHBV was delivered from the manufacturer as a powder without previous granulation, but nevertheless from the same batch as before. As a consequence, the average particle size of the nongranulated 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 %. The PHBV was thoroughly mixed with the corn meal to avoid feed selection.

Table 1 gives the composition of the ingredients as analysed. The diets were fed at maintenance level (SCHNEEBERGER and LANDIS 1984) considering body weight changes and assuming that the PHBV would have the same net energy lactation (NEL) content as straw. The diets were supplemented by 12 g/day of a commercial mineral–vitamin premix. Free access to sufficient amounts of water was ensured. Each experimental period lasted for 21 days including 17 days of adaptation in individual pens in a controlled environment before the period of total excreta collection. As only minor effects on energy metabolism had occurred in the previous experiment, no respiratory measurements were performed. The wethers were weighed weekly throughout the experiment. The average live weights of the sheep amounted to 76.7, 73.7, 74.4, 74.8, 74.9, and 75.1 kg (SEM 0.23) in treatments C, C1, P10, tP10, P20, and tP20, respectively. Further details of the experimental procedure are given by FORNI et al. (1999b).

Rusitec trial

Three of the diets used in the *in vivo* experiment (C1, P10, tP10) were also evaluated *in vitro* in order to measure the effect of the NaOH pretreatment on PHBV degradation by rumen fermentation. The equipment used was a six vessels Rusitec apparatus (Rumen Simulation Technique) described by MACHMÜLLER et al. (1998). The Rusitec system has been repeatedly approved to be suitable for simulating ruminal degradation (e.g., ABEL et al. 1990). For the Rusitec trial the same milled granulated PHBV, which had been used in the previous experiment (FORNI et al. 1999b), was employed but only using the fraction with a particle size between 0.125 mm and 0.25 mm, which accounted for 24.2 % of total PHBV.

Each diet was tested in duplicate for a total period of 10 days. The feed supply per fermenter amounted to 14 g DM/day, supplemented by 0.2 g of the mineral–vitamin premix. Each daily feed portion remained for 48 h within the system. The experimental procedures used were as described by MACHMÜLLER et al. (1998), but in the present study the nylon bags had greater dimensions (80 mm × 160 mm). Quantitative data on PHBV degradation were obtained by recording the PHBV recovery in the nylon bag residues as well as in the outflowing quantity of rumen fluid. Fermentation gases were quantitatively collected in gas-proof bags.

Analyses

To determine the effects of pretreatment with NaOH and subsequent neutralization with HCl on the PHBV itself, the soluble part of the pretreated sample was analysed to determine the structure of the compounds by ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR). The still insoluble polymeric fragments were analysed with gel permeation chromatography (GPC) for chain length. These analyses were performed by Dr V. TONCHEVA (Vakgroep voor Organische Chemie, University of Gent, Belgium).

The contents of DM and nutrients in the feed and faeces as well as the PHBV contents in refusals, faeces, *in vitro* fermentation residues and rumen fluid samples (Rusitec) were analysed as previously described by FORNI et al. (1999a,b). The PHBV contents of feed refusals were used to estimate the proportions of the ingredients in the refusals by the PHBV : corn ratio of the respective diets. In rumen fluid samples that were directly drawn from the fermenters and in the fermentation gas several fermentation parameters were analysed according to standard procedures that were mainly based on gas chromatographic techniques (MACHMÜLLER et al. 1998). The urinary allantoin content was determined by reversed phase high performance liquid chromatography (HPLC) (HAN 1991).

Statistical analysis

Data were analysed by a multifactorial ANOVA with Statgraphics Plus for Windows (Version 2.1, Statistical Graphics Co., Manugistics Inc., Rockville, MD, USA). In the *in vivo* trial, treatments, experimental periods and animals were taken into account according to the Latin square design. Multiple comparisons among means were performed using the Bonferroni multiple range test. In the Rusitec trial, data were analysed as a split plot over time using the General Linear Model procedure of Systat (Version 6.0.1. for Windows, Systat Inc., Evanston, IL, USA) for repeated measurement analysis. Effects included in this model were treatment, fermenter within treatment, day and day × treatment.

Results

Effect of pretreatment on PHBV

Table 2 gives the proportion and variety of soluble monomers formed with the equivalent of 40 g NaOH per kg feed DM. Furthermore, the proportions and average molecular weights of the four polymeric fractions (F_1 to F_4) found in the insoluble part are presented.

Table 2. **Formation of monomer and polymer fractions by NaOH pretreatment of PHBV intended for the tP10 and tP20 diets**

	Monomeric part (soluble)				Polymeric part (insoluble)				
	%	Composition (%)			%	Average molecular weight of fractions (GPC analysis)			
		hydroxy-butyric acid	hydroxy-valeric acid	crotonic acid		F_1	F_2	F_3	F_4
tP10	88.7	71.3	5.3	3.3	11.3	89 300	–**	5 160	1 950
tP10*	60.7	87.2	6.0	6.7	39.3	101 000	20 300	5 100	2 070
tP20	41.8	84.3	5.9	3.8	58.2	91 020	18 950	5 060	2 060
tP20*	50.7	84.2	5.5	9.7	49.3	101 600	19 900	5 300	2 100

* subsequently neutralized with HCl
** not detected

Table 3. **Daily intake of dry matter, nutrients and energy as well as water consumption and urinary excretion of the sheep**

	C	C1	P10	tP10	P20	tP20	SEM
Dry matter (g/day)	1075[a]	854[b]	931[c]	929[c]	1008[d]	982[cd]	12.1
NDF (g/day)	632[a]	296[b]	398[c]	397[c]	512[d]	501[d]	5.7
ADF (g/day)	356[a]	155[b]	232[c]	231[c]	320[d]	312[d]	3.6
Crude protein (g/day)	122[a]	86[b]	88[b]	88[b]	89[b]	87[b]	1.1
Water (kg/day)	4.4[a]	4.8[a]	4.9[a]	6.8[b]	5.4[ab]	6.6[b]	0.32
Urine (kg/day)	3.1[a]	3.5[a]	3.7[a]	5.1[b]	4.1[ab]	5.0[b]	0.28

[abcd], values in the same row with different superscripts are significantly different ($p > 0.05$)

Pretreatment led to substantial formation of monomers, namely hydroxybutyric, hydroxyvaleric and crotonic acid. The higher NaOH amount per unit of PHBV applied in the tP10 treatment degraded 89 % and the lower amount in tP20 degraded about 42 % of PHBV to monomers. Neutralization with HCl reduced the soluble polymer part in treatment tP10 by 28 % units and caused a further increase in treatment tP20. The GPC analysis of the polymeric part showed that pretreatment generally decreased the initial numeric PHBV molecular weight of 174 000. With the exception of tP10, four fractions of average numeric molecular weights (representing the residual PHBV polymer chain length) of about 100 000, 20 000, 5000 and 2000 were found, each of them considerably shorter than the original polymer.

Sheep trial

The DM allowances were highest for the hay treatment C since the other treatments contained corn, which reduced the amount necessary to cover requirements. Furthermore, in the PHBV treatments the sheep received more feed than the C1 sheep due to the assumed low NEL content of PHBV. Since no greater treatment differences in feed refusal occurred, the differences in allowance were also found in the intake (Table 3). The relatively high

Table 4. **Effect of untreated and pretreated PHBV on apparent nutrient digestibilities of the diets and on urinary allantoin excretion of the sheep**

	C	C1	P10	tP10	P20	tP20	SEM
Digestibility coefficient							
Organic matter (OM)	0.629[a]	0.759[b]	0.762[b]	0.780[b]	0.777[b]	0.782[b]	0.0147
Gross energy (GE)	0.587[a]	0.728[b]	0.733[b]	0.756[b]	0.752[b]	0.762[b]	0.0167
NDF	0.598[ab]	0.576[a]	0.647[abc]	0.685[bc]	0.727[c]	0.727[c]	0.0198
ADF	0.569[ab]	0.528[a]	0.597[ab]	0.667[bc]	0.676[bc]	0.732[c]	0.0263
Crude protein	0.678	0.715	0.605	0.646	0.643	0.610	0.0319
PHBV	–	–	0.566[a]	0.868[b]	0.655[a]	0.938[b]	0.0434
Allantoin (g/day)	0.45	0.26	0.32	0.35	0.29	0.33	0.068

[abc], values in the same row without or with different superscripts are significantly different ($p > 0.05$)

neutral detergent fibre (NDF) and acid detergent fibre (ADF) intakes reflected the fibrous character of PHBV. Crude protein intake was lower for the supplemented treatments than for C because of the lower DM intake and the lower protein content of the corn and PHBV relative to hay. Both NaOH pretreatments caused significantly greater water consumption and, consequently, higher urine volumes (Table 3). The higher level of untreated PHBV also tended to enhance water consumption and urine output compared with the 10 % level.

Both the use of corn and of PHBV in the diet significantly improved the digestibility of DM, organic matter (OM) and gross energy (GE) in comparison with hay alone (diet C; Table 4). An increased proportion and the pretreatment of PHBV tended to further enhance DM, OM and GE digestibility in comparison with the control diet C1. The effects on fibre digestibility were less uniform. However, the PHBV-containing treatments showed a higher fibre fraction digestibility than the only corn-supplemented diet C1. Pretreatment seemed to increase NDF digestibility for the 10 % PHBV-containing diets, but there was no difference between pretreated and untreated diets for the 20 % substitution of corn by biodegradable plastic. Significant differences were recorded for NDF digestibility either with 20 % untreated or pretreated PHBV in comparison with both control diets. In the 10 % diet, pretreatment of PHBV also led to a significantly higher NDF digestibility than was found in diet C1. Pretreatment and an increased proportion of PHBV significantly increased the ADF digestibility relative to the controls. The pretreated diet containing 20 % PHBV showed significantly greater ADF digestibility than the untreated P10 diet. The crude protein digestibility was numerically lower in the treatments containing biodegradable plastic with the lowest values for P10 and P20.

Apparent PHBV digestibility was 0.57 to 0.66 for untreated PHBV. Pretreatment of PHBV led to a significantly higher digestibility of PHBV by about 30 % independently of the amount of biodegradable plastic added. The values of the treatments with 20 % PHBV tended to be higher than the corresponding values with 10 % but the difference did not reach the level of significance.

The diets showed no significant influence on the urinary excretion of allantoin N (Table 4). The numerically highest values were found in treatment C, and pretreatment as well as increased dietary proportion slightly increased allantoin N in comparison to C1.

Rusitec trial

Pretreatment numerically increased the rate of DM disappearance from the nylon bags (Table 5). The disappearance of untreated PHBV was significantly lower than that of pretreated PHBV ($p < 0.05$), and the level of disappearance was higher than the *in vivo*

Table 5. **Effect of untreated and pretreated PHBV[1] on the fermentation pattern in the continuous culture system Rusitec (Averages of days 5 to 10)**

	C1	P10	tP10	SEM	Level of probability of error (p) for Treatment	Day	Day × treatment
In vitro DM disappearance (g/g supply)	0.763	0.754	0.817	0.0117	0.184	0.491	0.696
In vitro PHBV disappearance (g/g)	–	0.840	0.946	0.0212	0.004	0.017	0.330
Rumen fluid properties							
pH	6.82	6.94	6.96	0.030	0.075	0.001	0.320
Ammonia (mmol/L)	1.7	1.9	1.8	0.21	0.373	0.000	0.361
Volatile fatty acids							
Acetate (molar %)	55.2	58.2	55.5	0.66	0.510	0.180	0.052
Propionate (molar %)	29.7	27.9	29.7	0.36	0.428	0.157	0.913
Butyrate (molar %)	10.3	9.6	10.4	0.41	0.624	0.007	0.510
Valerate (molar %)	3.7	3.1	3.3	0.22	0.456	0.511	0.018
Acetate : Proprionate	1.87	2.09	1.88	0.040	0.453	0.238	0.409
Gaseous release							
Methane (mmol/day)	6.59	5.03	5.77	0.660	0.282	0.624	0.302
Hydrogen (mmol/day)	0.96	0.25	0.03	0.264	0.097	0.022	0.030

[1] PHBV powder with a particle size greater than 0.125 and smaller than 0.250 mm

total tract digestibility (Table 4). However, part of the PHBV that had disappeared from the nylon bags was recovered in the rumen fluid (7 % with P10 and 21 % with tP10). Combining the *in vitro* fermentation residues and the amounts of PHBV in rumen fluid shows that no difference in fermentation rate between untreated and pretreated PHBV occurred.

The relatively higher amount of corn in the control group (C1) reduced the average pH (Table 5). As the NaOH was neutralized by HCl before application no further increase in pH occurred in the tP10 treatment. Rumen fluid ammonia concentrations and volatile fatty acid proportions were not significantly affected by the use of untreated and pretreated PHBV. The inoculum contained on average 185 mmol/l of volatile fatty acids (VFA). The dietary inclusion of either untreated or pretreated PHBV showed no significant effect on the release of methane and hydrogen. With hydrogen, significant changes with time suggest that a different time-dependent evolution of the treatment effects had taken place. In the control cultures an increase of hydrogen release was observed, whereas with PHBV the hydrogen release remained low.

Discussion

Characterization of untreated PHBV as a feedstuff

According to the chemical analyses of the diets, PHBV behaves like a highly fibrous material. However, as previously discussed (Forni et al. 1999b), fibre analysis is a chemical procedure and might give little indication on digestibility in the case of biodegradable plastics. There was no negative relationship between the fibre content of the diet and nutrient digestibility and even higher digestibilities of ADF and NDF were recorded when higher contents of fibre fractions were present in the diet. Nevertheless, PHBV may serve as the structural compound in cells since Seebach et al. (1994) found PHBV accumulated, but not as storage material, in prokaryotic and eukaryotic organisms. The crude protein content of PHBV is low since the PHBV used for experimental work was produced by bacteria under nitrogen-limiting conditions (BRANDL et al. 1990).

Dietary incorporation of PHBV did not result in decreased digestibility coefficients (Table 4) as occurred in the previous experiment with sheep when an even lower percentage of untreated PHBV (8 %) had been used (FORNI et al. 1999b). An increased dietary proportion even led to a further augmentation of the *in vivo* digestibility of OM, fibre fractions and GE. The differences found mainly resulted from the varying apparent digestibility of PHBV. In the previous study, an average digestibility of untreated PHBV of only 4 % was found whereas digestibility was more than tenfold greater in the present sheep experiment when PHBV had been provided at a similar (10 %) or even higher (20 %) dietary percentage. Also the *in vitro* degradation rate in the rumen fluid was astonishingly high.

Several observations suggest that the greater digestibility of PHBV in comparison with the previous study might be predominantly due to the smaller average particle size of PHBV used in the present trials. Although generally coming from the same production batch, the first delivery were PHBV granules, which were subsequently milled, whereas the second delivery (the one used in the present sheep trial) consisted of PHBV powder without being further processed to films or granules. The powder used in the Rusitec trial also originated from the first delivery, but only particles greater than 0.125 mm and smaller than 0.25 mm were used. This was not too different from the average particle size of the nongranulated powder used in the present sheep trial. Since in all trials employing small particle size the PHBV degradation was high, smaller particles obviously favoured degradation and/or fermentation, possibly due to the easier access of microbes and enzymes to the polymers.

Another explanation for the variability in digestibility of PHBV could be random differences in the affinity of adequate enzymes or the capacity of bacteria colonizing the gastrointestinal tract to degrade PHBV. Hydrolysis of aliphatic polyesters is known to be slow, but recent data show that biodegradation proceeds rapidly in the presence of suitable micro-organisms (HUANG and EDELMAN 1995). However, since the differences were systematic among animals of the same treatment group, individual differences in colonizing of the digestive tract seem to be unlikely as the major explanation for the differences observed in PHBV digestibility between the trials.

The digestibility of PHBV may reflect the sole effect of hydrolysis, however the pattern of digestibility of PHBV obtained in the present experiment suggests the contribution of microbes or agents able to degrade PHBV. A certain further evidence for a significant fermentation of PHBV is given by the slight increase in urinary allantoin excretion of the sheep as compared with the C1 control diet. Allantoin is correlated with bacterial protein synthesis in the digestive tract in ruminants (SMITH 1969). ROTH and KIRCHGESSNER (1978) estimated in retrospect that 1 g bacterial protein is metabolized to 38 mg allantoin N. Thereafter, the average daily incorporation of nitrogen by bacteria amounted to 6.9, 8.3 and 7.7 g for C1, P10, and P20, respectively, and was highest with 11.7 g with hay alone (treatment C). The daily dietary N supply was about 14 g, and was the same for all treatments except for C which excludes the possibility that the variation in allantoin came from a different protein supply (KREUZER et al. 1986).

Effect of pretreatment of PHBV

Pretreatment resulted in the formation of soluble acids and polymeric fractions with lower average numeric molecular weights than the untreated polymer (Table 2). Neutralization with HCl reduced the percentage of the soluble part for tP10 and increased this part for tP20. Previously, FORNI et al. (1999a) observed a decrease of monomers independently of the level of NaOH applied before neutralization. The total quantity of alkali and acid used were similar in both investigations, however, in the present study 4-molar NaOH and HCl were applied instead of 2-molar concentrations as in the previous study. The intensity of degradation to monomers was lower in the treatment containing 20 % PHBV since the NaOH concentration was based on the amount of DM offered. The NaOH showed a similar kind of cleavage of the polymeric chain independently from the amount of alkali applied since polymeric fractions of similar average molecular weights were formed. The presence of fractions with relatively low molecular weights should favour biodegradation (COX 1992). In any case, pretreatment directly contributed to the supply of soluble degradation products that might be absorbed through the cell wall and presumably are also metabolized (DOI et al. 1994).

This could explain the positive influence of pretreatment of the PHBV digestibility and, consequently, of OM, GE, NDF and ADF. Pretreatment increased PHBV digestibility from 0.56 to 0.87 and from 0.66 to 0.94 with 10 % and 20 % of PHBV in the diet, respectively. This seems to have been true absorption since all of the insoluble polymeric parts would have been analysed and the monomers should be widely absorbable. However, the site of degradation remains uncertain. The rumen seems not to be exclusively responsible for this additional degradation to absorbable monomers as can be concluded from the corresponding improvement in digestibility in swine (FORNI et al. 1999a) and from the results of the Rusitec trial. The amount of PHBV additionally disappearing from the nylon bags with pretreated compared to untreated PHBV was completely recovered in rumen fluid. Maybe the varying pH conditions in the stomach and small intestine alleviate microbial degradation and monomer absorption in the hindgut. The urinary allantoin excretion further indicates that, compared with untreated PHBV, with pretreated PHBV a certain increase in microbial activity had taken place. Applying the equation of ROTH and KIRCHGESSNER (1978), the daily microbial protein synthesis was nonsignificantly higher by 11 to 13 % (9.2 g and 8.7 g microbial protein/day with 10 and 20 % pretreated PHBV) when the PHBV was pretreated with NaOH. The slightly higher methane release with pretreated instead of untreated PHBV suggests that methanogenic microbes could be involved in PHBV degradation to some extent.

In the present study a significant increase of water consumption and urine excretion occurred with the use of the NaOH/HCl pretreated PHBV, as has been observed by FAHMY and ØRSKOV (1984) when NaOH-treated straw (60 g NaOH/kg) was fed to sheep. Sodium is a strong electrolyte and the major function of electrolytes is to assist in the maintenance of water balance within the body (PATIENCE 1993). In particular, sodium plays a primary role in the regulation of extracellular fluid volume and a critical role in cellular transport systems (Na-K-ATPase, PATIENCE 1993). A change in osmolality of only 1 % is sufficient to cause a measurable change in antidiuretic hormone and subsequent adjustment in urinary excretion (ZERBE and ROBERTSON 1983). The pretreatment applied in the present experiment corresponded to a dose of 23 g Na/kg feed DM. One further gram Na/kg DM was supplied by the feedstuffs. Although FLACHOWSKY (1987) recommends Na doses lower than 10 g/kg DM for long-term nutrition of sheep and goats with NaOH-treated straw, treatments using 40 g NaOH/kg and more were subject of various studies (FAHMY and ØRSKOV 1984). The free availability of water is the most essential prerequisite to alleviate Na surcharge. Another disadvantage of NaOH treatment is the excess of Na ion excretion in the urine. This might adversely modify the soil structure if applied as slurry (FAHMY and ØRSKOV 1984).

Conclusions

In the present investigation the influence of pretreatment and dietary level of PHBV on degradation and digestibility in ruminants was studied. In contrast to a previous study (FORNI et al. 1999b) PHBV was digestible to a considerable amount even if not pretreated. Further studies have to be conducted to investigate the variability in digestibility of processed PHBV due to particle size, which seems to be the key issue for the digestibility of

PHBV. Pretreatment with NaOH enhanced the degradation and digestion also to a certain degree thus suggesting that pretreatment might be beneficial for practical application. The NaOH effect may well have mostly resulted from the reduction in polymer chain length. Once more the feeding of the bioplastic in considerable dietary proportions turned out to be without adverse effects on animal health. There was not even an effect on feed intake which, however, had been restricted to a comparatively low level to meet the calculated requirements of adult sheep. It remains still open as to how far PHBV may be considered as an energy-providing feed ingredient.

Summary

The effects of pretreatment of the novel biodegradable polymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with 40 g NaOH per kg feed dry matter (DM) for 24 h and subsequent neutralization by HCl on its degradability and digestibility in ruminants were evaluated. Six wethers were fed hay (C) or hay and corn meal (C1, 1 : 1 on DM basis) at approximately maintenance level. Corn was substituted by 10 or 20 % PHBV either untreated (P10, P20, respectively) or pretreated (tP10, tP20, respectively). In a trial with the Rumen simulation technique (Rusitec) only the diets C1, P10 and tP10 were applied. Pretreatment degraded part of the PHBV to monomers and reduced average chain length of the residual polymers. The digestibility trial showed that the energy-rich PHBV was quite well digested by the wethers even when supplied untreated (57 % of intake). Both high dietary inclusion and pretreatment increased the apparent digestibilities of PHBV, organic matter, acid detergent fibre, neutral detergent fibre and gross energy. Apparent PHBV digestibility was higher by about 50 % with pretreatment, independent of the proportion of PHBV included in the diet. Compared with the control diet C1, the urinary allantoin excretion was slightly elevated by PHBV. Also the Rusitec *in vitro* disappearance of PHBV was relatively high thus confirming the effects on total tract digestibility measured *in vivo.* The average disappearance from the nylon bags was significantly increased by pretreatment, however, a correspondingly higher amount of PHBV was recovered in the rumen fluid. The fermentation pattern was not significantly altered by PHBV of any kind. In comparison with a previous study, the digestibility of PHBV was unexpectedly high. The smaller particle size of the PHBV used in the present study may have favoured microbial or hydrolytic degradation of PHBV in the present study. This requires further investigation.

Zusammenfassung

Neuartige bioabbaubare Kunststoffe in der Schafernährung. 2 Einfluß der Vorbehandlung von Poly (3-hydroxybutyrat-co-3-hydroxyvaleriat) mit NaOH auf die in vivo *Verdaulichkeit und die* in vitro *Abbaurate (Rusitec)*

Es wurden die Effekte einer Vorbehandlung des neuartigen bioabbaubaren Polymers Poly(3-hydroxybutyrat-co-3-hydroxy-valeriat) (PHBV) mit 40 g NaOH/kg Futter-T und anschließender Neutralisation mit HCl auf die Abbaurate und Verdaulichkeit beim Wiederkäuer untersucht. Sechs Hammel wurden mit Heu (C) oder Heu und Körnermais (C1, 1 : 1 bezogen auf T) nach Erhaltungsbedarf gefüttert. Mais wurde zu 10 oder 20 % durch unbehandeltes (P10 bzw. P20) oder vorbehandeltes PHBV (tP10 bzw. tP20) ersetzt. Mit dem Pansensimulationssystem Rusitec wurden nur die Rationen C1, P10 und tP10 geprüft. Die NaOH-Vorbehandlung resultierte in einem teilweisen Abbau von PHBV zu Monomeren und verringerte die mittlere Kettenlänge der verbleibenden Polymere. Der Verdauungsversuch ergab, daß das energiereiche PHBV vergleichsweise gut verdaut wurde, selbst wenn keine Vorbehandlung erfolgte (57 % der Aufnahme). Sowohl ein erhöhter Anteil in der Ration als auch die Vorbehandlung von PHBV verbesserten die scheinbare Verdaulichkeit von PHBV, organischer Masse, Faserfraktionen und Bruttoenergie. Die PHBV-Verdaulichkeit wurde dabei durch Vorbehandlung um ca 50 % gesteigert und zwar unabhängig von dem PHBV-Anteil in der Ration. Im Vergleich zur Kontrollration C1 wurde die renale Allantoinausscheidung durch PHBV leicht erhöht. Die Verschwindensrate von PHBV im Rusitec-Sytstem war relativ hoch und bestätigte somit die Ergebnisse der *in vivo* gemessenen Verdaulichkeit im gesamten Verdauungstrakt. Die Vorbehandlung führte zu einer Erhöhung der PHBV-Verschwindensrate, die aber durch eine entsprechend höhere Wiederfindungsrate an PHBV im Pansensaft kompensiert wurde. Die Fermentationsvorgänge wurden durch PHBV gleich welcher Art nicht signifikant beeinflußt. Im Vergleich zu einer vorangegangenen Untersuchung war die PHBV-Verdaulichkeit unerwartet hoch. Dies beruht wahrscheinlich auf der geringeren Partikelgrösse des Pulvers, welches in den vorliegenden Untersuchungen zur Anwendung kam, was den mikrobiellen oder hydrolytischen Abbau gefördert haben könnte. Dazu bedarf es jedoch weiterer Untersuchungen.

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