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Original article

Utilisation of phytate phosphorus by rumen bacteria in a semi-continuous culture system (Rusitec) in lactating goats fed on different forage to concentrate ratios

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Abstract — Experimental data on phytate phosphorus utilisation by ruminants are scarce. The aim of this study was to estimate the phytase activity of rumen micro-organisms when phytate phosphorus supply is high. A semi-continuous culture system fermentor (RUSITEC) was used. The inoculum was obtained from eight goats fed on either high or low forage level diets. Experimental buffers only differed by the nature of phosphorus monosodium phosphate vs. corn sodium phytate. The nylon bags containing 15 g DM of substrate were removed after a 48-hour incubation period. The system was maintained for 15 days: 5 days for adaptation, in order to obtain a steady state, and 10 days for sampling and recording. No significant differences were observed for DM digestibility, gas production, pH, N-NH_3, and SCFA for the different treatments. Bacterial efficiency of phytate phosphorus utilisation was significantly higher ($p < 0.001$) with organic P, but remained lower than the data usually reported in the literature. These results may be explained by the relative saturation of bacterial phytase activity when the buffer contains a high level of phytate phosphorus.

phosphorus / phytate / ruminant / bacteria / in vitro

Résumé — Mesure en fermenteur semi-continu (Rusitec) de l'utilisation du phosphore phytique par les bactéries du rumen chez la chèvre laitière recevant des régimes de différentes proportions fourrage-concentré. Les informations sur l'utilisation du phosphore phytique par les bactéries du rumen sont peu nombreuses. Le but de cet essai était d'évaluer in vitro (RUSITEC) l'efficacité de l'activité phytasique des micro-organismes recevant une proportion élevée de phosphore phytique. L'inoculum a été prélevé sur des chèvres recevant des régimes haut ou bas en aliment concentré. Dans les solutions nutritives, seule la nature du phosphore (phosphate monosodique vs. phytate de sodium de maïs) était différente. Les sachets nylon contenant 15 g de MS de substrat étaient maintenus 48 h

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dans le système de fermentation. Après une période d'adaptation de 5 jours, les mesures ont été réalisées pendant 10 jours consécutifs. Aucune différence significative n'a été observée pour les paramètres classiques de l'activité bactérienne. L'efficacité de l'utilisation du phosphore phytique a été significativement supérieure ($p < 0.001$) avec l'apport de P organique tout en restant plus faible que les données habituellement rapportées par la littérature. Ces résultats pourraient être expliqués par une relative saturation de l'activité phytasique avec des quantités élevées de phosphore phytique présentes dans la solution nutritive.

phosphore / phytate / ruminant / bactérie / in vitro

1. INTRODUCTION

Experimental data on phytate phosphorus utilisation by ruminants are scarce. Most studies have been performed with low levels of phytate phosphorus. At such levels, phytate phosphorus may be almost completely hydrolysed by the enzymes of rumen micro-organisms. When the proportion of phytate phosphorus is less than 50% of total P supply, about 90% of phytate phosphorus is hydrolysed by rumen bacteria [3, 17, 18, 21, 24].

At the present time in Europe, especially in France, economic considerations are leading towards an increasing utilisation of cereal grains (up to 50% of DMI) in ruminant diets, especially for high-yielding cows. In view of the high phytate phosphorus content (60–80%) of these foodstuffs, are the rumen microbes able to completely hydrolyse phytate phosphorus? P release in the rumen represents the first and essential step of P utilisation by the animals. Under these conditions, a limitation of phytate hydrolysis can occur as reported by Ellis and Tillman [7] with 91% of P as phytate in the diet. Moreover, animal nutrition now faces universal new goals especially in pollution control, and a better knowledge of dietary P availability is needed to more precisely assess the amount of P in ruminant waste.

The aim of this study was to estimate the in vitro utilisation of phytate phosphorus vs. inorganic phosphorus by rumen micro-organisms with inocula from animals adapted or not to a high level of concentrate in their diet.

2. MATERIALS AND METHODS

Two units with four semi-continuous culture system fermentors (RUSITEC) adapted from Czerkawski and Breckenridge [4] were used. The liquid inoculum was obtained from two groups of four female Alpine or Saanen goats adapted to either high forage levels (diet F, 80% of DM) or low forage levels (diet C, 60% of DM). These diets were fed to the animals for three weeks prior to sampling for rumen inoculum. The composition of the pre-experimental diets is given in Table I. At the beginning of the experiment, each one-litre-capacity fermentor was filled with 500 mL of liquid inoculum previously filtered through gauze and 100 mL of distilled water, then topped up to one litre with an artificial buffer. Experimental buffers only differed by the nature of the phosphorus; the total P content was the same: 130 $mg \cdot L^{-1}$. Buffer A had 75% inorganic phosphorus, with P_i as monosodium phosphate, and buffer B had 75% organic phosphorus, with P_o as corn sodium phytate (Sigma P8810). The buffer composition is given in Table II. The treatments represented a 2×2 factorial arrangement (diets and buffers). On the first day of the experiment, two nylon bags were placed in each fermentor. The first bag with 80 g rumen solid content was removed 24 hours

later. The second bag contained 15 g of substrate DM. The composition of the substrate (as fed) was as follows: cellulose (Filtralfa, Eurofiltec) 69%, corn starch (Cérestar, Beghin-Say) 30%, mineral and amino-acid premix 1% (Tab. III). The substrate was granulated into 5-mm pellets (Unité de Préparation des Aliments Expérimentaux, INRA, Jouy-en-Josas, France). The nylon bags containing the substrate were removed after a 48-hour incubation time. The artificial buffers were infused at a continuous rate of 850 mL·d^{-1}. The system was maintained for 15 days: 5 days for adaptation, in order to obtain a steady state, and 10 days for sampling and recording.

Gas production (GP) and pH in the fermentors were recorded daily and DM disappearance (DMd) was calculated. N-NH_3, from Berthelot adapted for Technicon Autoanalyzer (TM), short chain fatty acid (SCFA) [12] productions and P_i [8] were determined in the effluents.

An estimation of phosphorus uptake by rumen micro-organisms was performed from the De Meyer and Van Nevel [5] equation for hexose fermented in the rumen and with an assessment of 5 g of P per kg of fermented hexose, calculated according to [23].

We assessed the phosphorus balance in the fermentor as follows:

$$P_{t_{buf}} = P_{i_{efl}} + P_{o_{efl}} + P_{mic},$$

where $P_{t_{buf}}$ is the total amount of P in the buffer ($P_i + P_o$), $P_{i_{efl}}$ is the amount of inorganic P recovered in the effluents (measured), ($P_{o_{efl}}$) is the amount of inorganic P

Table I. Diet composition (% DM basis).

	Forage (F)	Concentrate (C)
Alfalfa hay	34.0	25.5
Grass hay	16.0	12.0
Beet pulp silage	26.0	19.5
Barley straw	4.0	3.0
Corn	6.4	12.8
Barley	6.4	12.8
Dehydrated beet pulp	2.0	4.0
Soybean meal	4.0	8.0
Sodium bicarbonate	0.5	0.5
Mineral mix	0.7	0.7
CP (%)	13.3	14.2
UFL*	0.92	0.95
Phosphorus	0.40	0.44
Phytate phosphorus**	0.05	0.10

* Unité fourragère lait = 1730 kcal net energy.
** Calculated from io7 Tables [11].

Table II. Buffer composition (g·L^{-1}).

	Buffer A	Buffer B
$NaHCO_3$	10.26	10.26
NaCl	0.52	0.52
KCl	0.75	0.75
$MgCl_2, 6H_2O$	0.17	0.17
$CaCl_2$	0.06	0.06
Na_2SO_4	0.16	0.16
$NaH_2PO_4, 2H_2O$	0.503	0.1684
Sodium phytate*	0.155	0.465
Urea	0.85	0.85

***** Sigma P8810.

Table III. Substrate premix composition (g·kg^{-1}).

$FeCl_2, 4H_2O$	66.7
$MnCl_2, 4H_2O$	146.7
$ZnSO_4, 7H_20$	29.3
$CoCl_2, 6H_2O$	8
$CuSO_4, 5H_2O$	6.7
Ammonium molybdate	1.3
Valine	73.3
Leucine	86.7
Isoleucine	86.7
Methionine	25
Lysine	15
Choline chlorhydrate	50
Vit B_{12}	0.33
Biotin	0.66
Para amino benzoic acid	1.33
Thiamine	3.35
Vit B_6	0.66
Panthotenic acid	1.33
Folic acid	0.33

recovered in the effluents (calculated by difference) and P_{mic} is the estimation of P uptake by micro-organisms.

We assume the sodium phytate availability (%) as:

$$((P_{o_{buf}} - P_{o_{efl}})/P_{o_{buf}}) \times 100.$$

Statistical treatment was performed according to the GLM procedure followed by one-way analysis of variance [15]. The model included the effect of buffer, pre-experimental diet and buffer–diet interaction.

3. RESULTS

The experimental conditions were: AF (buffer A, Forage diet), AC (buffer A, Concentrate diet), BF (buffer B, Forage diet) and BC (buffer B, Concentrate diet). We did not observe any significant difference for the different treatments neither in DMd, pH and N-NH3 (Tab. IV) nor in SCFA production (Tab. V). The gas production was higher for AC treatment.

The results of P measurements are summarised in Table VI. The daily amount of P_i ($P_{i_{buf}}$) and P_o ($P_{t_{buf}}$) infused logically differed for each buffer, according to the experimental design, the total P supply ($P_{t_{buf}}$) did not differ. P_i in the effluents ($P_{i_{efl}}$) was significantly higher ($p < 0.001$) with buffer A, while no diet effect was observed. Better phytate phosphorus availability was observed with buffer B.

4. DISCUSSION

The results obtained for usual rumen inoculum activity parameters such as DMd,

Table IV. DM disappearance, pH, gas production (GP) and ammoniac concentration.

	Treatments							
	AF	AC	BF	BC	SEM	Buffer	Diet	Interaction
	$n = 2$							
DMS (%)	61.48	66.22	63.29	62.62	3.06	NS	NS	NS
$N\text{-}NH_3$ ($mg \cdot L^{-1}$)	273.6	238.9	238.2	263.7	20.5	NS	NS	NS
pH	6.76	6.76	6.77	6.76	0.02	NS	NS	NS
GP ($mL \cdot day^{-1}$)	2882[b]	3103[a]	2945[b]	2584[b]	121	*	NS	NS

[a, b] Within a row, means with different superscripts differ (* $p < 0.01$).
NS: not significant.

Table V. Production of short chain fatty acids (SCFA).

	Treatments							
	AF	AC	BF	BC	SEM	Buffer	Diet	Interaction
	$n = 2$							
Total SCFA ($mmol \cdot L^{-1}$)	51.08	51.83	51.90	52.36	1.69	NS	NS	NS
C2 (%)	56.10	57.21	55.12	55.61	0.88	NS	NS	NS
C3 (%)	31.87	30.51	31.50	32.25	0.79	NS	NS	NS
C4 (%)	8.93	9.27	10.07	8.90	0.34	NS	NS	NS

Table VI. Phosphorus measurements.

	Treatments							
	AF	AC	BF	BC	SEM	Buffer	Diet	Interaction
	$n = 2$							
$P_{i_{buf}}$ (mg·d^{-1})	84.8^a	85.0^a	27.9^b	28.1^b	0.59	***	NS	NS
$P_{o_{buf}}$ (mg·d^{-1})	28.5^a	28.6^a	83.4^b	83.8^b	0.66	***	NS	NS
$P_{i_{efl}}$ (mg·d^{-1})	89.7^a	89.4^a	72.5^b	76.6^b	1.5	***	NS	NS
P_{mic} (mg·d^{-1})	9.6	9.6	9.5	9.7	0.2	NS	NS	NS
$P_{o_{efl}}$ (mg·d^{-1})	14.0^a	14.7^a	29.3^b	25.6^b	1.4	***	NS	NS
Phytate availability (%)	51.0^a	48.3^a	65.0^b	69.5^b	2.3	***	NS	NS

$^{a, b}$ Within a row, means with different superscripts differ (*** $p < 0.001$).

pH, N-NH_3 and total SCFA could indicate that the microbial fermentation activity was not affected by our experimental conditions. The P supply in an inorganic as well as in an organic form, was adequate to meet bacterial requirements [2, 6]. We cannot clearly explain the higher gas production for the AC treatment but it must be noted that the gas production per g DM fermented ratio did not show any statistical differences between treatments: 327, 324, 320 and 293 (SEM = 19) for AF, AC, BF and BC, respectively. Higher AC gas production could be explained by a tendency towards higher fermentation degradability of dry matter for this treatment.

The data obtained for phytate phosphorus utilisation in this experiment, with 75% of P supply as sodium phytate, are slightly lower than those reported in the literature. It must be underlined that almost all of these data [14, 16, 17, 22] were obtained by using phytate phosphorus total collection balance trials. In this case, phytate hydrolysis may occur beyond the rumen which leads to an overestimation of phytate phosphorus absorption because the hydrolysed phytate phosphorus is measured as inorganic phosphorus in the faeces. This could also explain the low absorption value of total phosphorus reported in some experiments where the level of phytate phosphorus was high [7] and the post-rumen phytate hydrolysis may have been incomplete.

By contrast, our data are similar to those obtained recently with the nylon bag technique in the rumen [13, 18] which is more relevant to use as a comparison. In these experiments, phytate phosphorus degradation in sacco varied between 68 and 80%, which is consistent with our results. This incomplete hydrolysis of phytate phosphorus in rumen fluid may be explained by a saturation of enzymatic activity (buffer B), as reported by Raun et al. [20] in vitro, and by the decrease of phytase activity that could be explained by a high level of inorganic phosphate in the medium (buffer A) [10, 25]. The phytate availability calculated with buffer B treatments was lower than expected or reported in the literature with a low level of phytate [3, 16]. In our experimental conditions, the level of free inorganic phosphate in the medium was low. Saturation of enzymatic activity relating to the high level of substrate in the medium may have occurred which would explain these results.

The variations observed in phytate phosphorus release in the rumen among feedstuffs [1, 18] probably indicate that different phytic acid salts are not hydrolysed with the

same efficiency by rumen micro-organisms. In the present study, we used sodium phytate which is quite abundant in cereal grains (corn and rice) but may not reflect the phytate phosphorus availability of all the phytate-rich feedstuffs (cotton meal for example is rich in calcium phytate) [9]. Further investigation is needed to better understand this aspect.

The phytate phosphorus availability data obtained with phytate-rich buffer (B) are slightly lower than those reported by Park et al. [19]: a mean value of 67.2 vs. 78.0. Park et al. [19] used the dietary phytate phosphorus recovered in the duodenum. This discrepancy may be explained by the level of phytic phosphorus used (which was much higher in our trial). We must also consider that an additional hydrolysis of phytate phosphorus may have occurred in the strong acid conditions in the abomasum.

5. CONCLUSION

In this study, we did not observe any diet effect as expected as reported by Yanke et al. [25] and according to our own observations of an increase of rumen phytasic activity in goats fed high-concentrate diets (Godoy and Meschy, unpublished data). This was probably due to a shift in the microbial population species when fed a high cellulose substrate in RUSITEC.

The results of this study highlight the need for more research on phytate phosphorus utilisation by ruminants, especially when there are high levels of cereal grains in the diet.

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