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Original article

Plasma phosphorus content and dietary phosphorus availability in adult sheep

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Abstract — This study was aimed at discriminating different quality phosphorus sources using plasma phosphorus kinetics in sheep. Four adult castrated Vendean sheep received successively two preliminary usual then low phosphorus diets (three weeks on 2.5 g P·kg^{-1} DM and three weeks on 1.5 g P·kg^{-1} DM), and then one of four compared diets (2.5 g P·kg^{-1} DM) in a Youden square design. The four compared diets included either dicalcium phosphate, calcium aluminium phosphate, standard rapeseed meal or formaldehyde-treated rapeseed meal as the main phosphorus source. Inorganic plasma phosphorus was determined in samples taken from the jugular vein from the last week of the first preliminary phase to the last week of the test phase. The first preliminary diet stabilised the plasma phosphorus content (51.2 mg·L^{-1}, no day effect; no animal effect). The low phosphorus diet induced an initial rapid decrease in plasma phosphorus. After four days, plasma phosphorus increased probably resulting from an adjustment in phosphorus homeostasis. During the test phase, dicalcium phosphate and both rapeseed meals were more efficient than calcium aluminium phosphate in restoring the initial plasma phosphorus levels. Both, rapeseed meal diets induced the highest plasma phosphorus concentration. The physiological significance of these results is discussed.

availability / phosphorus / plasma / sheep

Résumé — Phosphore plasmatique et disponibilité du phosphore alimentaire chez le mouton. L'objectif de cet essai était de caractériser le phosphore de différentes sources au travers des cinétiques de phosphatémie chez des moutons recevant préalablement un régime normal puis bas en phosphore puis alimentés avec les sources de phosphore étudiées. Quatre moutons vendéens adultes castrés ont reçu successivement deux régimes préliminaires (2,5 g P·kg^{-1} MS puis 1,5 g P·kg^{-1} MS), puis un des quatre régimes testés (2,5 g P·kg^{-1} MS) selon un carré de Youden. Les quatre régimes testés contenaient soit du phosphate bicalcique, du phosphate alumino-calcique, du tourteau de colza standard ou du tourteau de colza tanné au formol. A partir de la dernière semaine de la phase préliminaire jusqu'à la dernière semaine de la phase de test, la phosphatémie a été déterminée sur des échantillons de sang prélevés à la jugulaire. Le régime préliminaire normal en phosphore a stabilisé la phosphatémie (51,2 mg·L^{-1}, pas d'effet jour ; pas d'effet animal). Le régime bas en phosphore a

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rapidement diminué la phosphatémie qui a ré-augmenté après quatre jours, probablement suite à un ajustement homéostatique. Pendant la phase de test, le phosphate bicalcique et les tourteaux de colza ont été plus efficaces que le phosphate alumino-calcique à restaurer la phosphatémie initiale. Les régimes à base de tourteaux de colza ont induit des phosphatémies supérieures. La signification physiologique de ces résultats est discutée.

disponibilité / mouton / phosphore / plasma

1. INTRODUCTION

There is a current wave of interest in research on the ways to decrease livestock phosphorus rations in order to preserve the environment [22]. As in monogastrics, the measurement of phosphorus availability in ruminant diets is necessary to better adapt phosphorus intake to the requirements. During the 1980s and 1990s, several methods of assessing phosphorus availability were developed for monogastric animals using various global responses, bone ash phosphorus determinations, a slope ratio assay based on the bone-breaking moment [12], and plasma alkaline phosphatase activity [6]. Few such studies have been performed in ruminants and almost all of these have involved the determination of dietary phosphorus digestibility based upon faecal analyses in which endogenous phosphorus was quantified using ^{32}P radio-isotopic dilution [9].

The present study was aimed at characterising plasma phosphorus kinetics in sheep fed different diets following preliminary periods of usual then low phosphorus intake. The trial compared four tested diets supplying highly available or poorly available inorganic phosphates, or organic forms of phosphorus (standard or formaldehyde-treated rapeseed meal).

2. MATERIALS AND METHODS

2.1. Animals

The trial was carried out on four adult castrated Vendean sheep. Throughout the experiment, the body weight of the animals remained constant, averaging 65 kg. The sheep were kept in individual stalls filled with low-phosphorus wood shavings (< 0.1 g $P \cdot kg^{-1}$ of DM).

2.2. Experimental diets

The animals were fed 1200 g per day of pelleted feeds, 600 g at 8:30 am and 600 g at 4:30 pm. The four sheep were all preliminary fed three weeks on a diet supplying 2.5 g of phosphorus per kg DM, in order to homogenise phosphorus plasma content, and then three weeks on a diet, supplying 1.5 g of phosphorus per kg DM, in order to restrict dietary phosphorus supply to the plasma. In the following test phase, the tested diet supplied 2.5 g phosphorus per kg DM. Each of the four tested diets incorporated one of the four compared sources of phosphorus in such a level to provide 70% or more of total dietary phosphorus.

Each period was the succession of three phases of three weeks each. From week 1 to week 3, the sheep were fed the usual phosphorus diet (first preliminary phase), then from week 4 to week 6, the sheep were fed the low phosphorus diet (second preliminary phase) and then from week 7 to week 9, the sheep were fed the tested diets (test phase).

The tested diets were optimised for maintenance in energy, nitrogen and mineral supplies [11] and were balanced to present a chemical and nutritional composition as close as possible (Tab. I). The variable mineral mix was adjusted so that each diet provided 0.6% Ca on a DM basis, and

Table I. The composition of the preliminary diets (usual and low phosphorus contents) and of the test diets supplying either standard or formaldehyde-treated rapeseed meal (S-RM and FT-RM, respectively) or dicalcium phosphate (DCP) or calcium-aluminium phosphate (CAP).

Composition (g·100 g^{-1} DM)	Preliminary diet		Test diet			
	Usual P	Low P	S-RM	FT-RM	DCP	CAP
Wheat straw[1]	47.00	47.95	47.00	47.00	47.00	46.80
Cassava[1]	17.10	17.10	15.93	8.36	17.10	17.10
Wheat gluten[1]	8.700	8.700	6.30	3.78	8.70	8.70
Corn starch[1]	15.00	15.00	5.67	15.29	15.00	15.00
Citrus pulp[1]	3.0	3.0	3.00	3.00	3.00	3.00
Beet molasses[1]	3.80	3.80	3.00	3.00	3.80	3.80
Mineral mix[1]	3.0	3.0	3.00	3.00	3.00	3.00
Urea[1]	1.45	1.45	0.32	1.21	1.45	1.45
Dicalcium phosphate[1]	0.95				0.95	
Ca-Al phosphate[1]						1.15
S rapeseed meal[1]			15.78			
FT rapeseed meal[1]				15.36		
Balanced nutritional values[2]						
Net energy (UFV·kg^{-1} DM)	63.1	63.1	62.4	62.7	63.1	63.1
PDIN[3] (g·kg^{-1} DM)	99	99	100	99	99	99
PDIE[3] (g·kg^{-1} DM)	99	99	99	99	99	99
Analytical composition						
Dry matter	91.6	90.7	91.6	91.8	91.6	91.1
Crude protein[1]	13.7	12.8	16.3	12.7	13.7	14.5
Crude fibre[1]	25.7	23.8	23.0	22.5	25.7	23.2
Starch[1]	25.6	27.2	23.9	24.7	25.6	28.3
Calcium[1]	0.58	0.31	0.58	0.55	0.58	0.65
Phosphorus[1]	0.30	0.14	0.28	0.23	0.30	0.29

[1] In % of dry matter.
[2] According to Jarrige [11].
[3] Protein digestible in the intestines from microbial origin and dietary origin according to nitrogen (PDIN) and energy (PDIE) supplies.

6% Na, 3.3% Mg, 1000 ppm Zn, 1000 ppm Mn, 170 ppm Cu, 7 ppm Co, 13 ppm I, 5 ppm Se, 85000 IU vit A, 17000 IU vit D3 and 500 IU vit E on a feed basis. The tested phosphorus sources were standard (S) rapeseed meal (diet S-RM), formaldehyde-treated (FT) rapeseed meal (diet FT-RM), dicalcium phosphate (diet DCP), and calcium aluminium (Ca-Al) phosphate (diet CAP). The FT rapeseed meal was industrially processed with 3000 ppm formaldehyde (i.e., 1% of a commercial 30% formaldehyde

solution). Low phosphorus feedstuffs (wheat straw, wheat gluten, corn starch, cassava, beet molasses, citrus pulp, urea, and vegetable oil) completed the four compared phosphorus sources (Tab. II) to obtain almost similar diets without an interaction on dietary phosphorus availability.

2.3. Blood measurements

Immediately prior to the morning feeding, from Monday to Friday from the last week of the first preliminary phase until the last week of the test phase, a blood sample was taken from the jugular vein in a heparinised collection tube and the plasma was retained for inorganic phosphorus analysis.

2.4. Physicochemical analyses

DM content was determined in feed after drying at 80 °C for 48 h. Phosphorus and starch were determined using, respectively, the spectrometric method [16] and the polarimetric method [7]. Plasma phosphorus was obtained by the molybdovanadate colorimetric procedure. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest et al. [23]. Crude protein (CP, N × 6.25) was obtained after Kjeldahl mineralisation. Calcium was measured by atomic absorption spectrophotometry [17].

2.5. Experimental design and statistical analysis

A Youden square design was used as a balanced incomplete block with 4 animals as experimental blocks, 4 diets as treatments and 3 periods as the Youden factor [1]. Each period was the succession of three phases of three weeks each: the two preliminary phases and then the test phase.

Statistical analyses were performed using the GLM procedure of SAS [21]:

Considering both preliminary phases results together: $Y = \mu + A + B + C + E + \varepsilon$

Considering the test phase results:
$Y = \mu + A + B + C + D + \varepsilon$

where Y = plasma phosphorus content, μ = overall mean, A = period, B = day (considered as replication), C = animal, D = test diet (DCP, CAP, S-RM or FT-RM), E = phase (preliminary phase 1 or 2), ε = error term. These models were also used for each animal results. The means were compared using the Bonferronni test.

3. RESULTS (Tab. III)

During the first preliminary phase (usual levels of dietary phosphorus), there was neither animal ($P = 0.17$) nor day ($P = 0.13$) effects and there was no interaction between the animal and the day ($P = 0.799$). There was a slight period effect ($P = 0.09$) but the Bonferronni test did not discriminate the difference between period 3 (49.9 mg·L^{-1}) and the two other periods (period 1: 49.9 mg·L^{-1} and period 2: 53.5 mg·L^{-1}, $P > 0.1$). The plasma phosphorus value during this first preliminary phase averaged 51.2 mg·L^{-1}.

During the low-phosphorus preliminary phase, in spite of the lower dietary phosphorus supply, no feed refusal was observed and phosphorus intake was 1.44 g·day^{-1}. During this phase, a day effect ($P < 0.01$) corresponded to a significantly lower plasma phosphorus on the third (44.9 mg·L^{-1}) and fourth (44.2 mg·L^{-1}) days after the beginning of the period. There was also an animal effect ($P < 0.01$) since two animals presented higher plasma phosphorus content than the two others (51.1 mg·L^{-1} and 49.0 mg·L^{-1} vs. 47.4 mg·L^{-1} and 46.5 mg·L^{-1}). When comparing usual and low phosphorus preliminary phases, we observed a slight but significant decrease of mean plasma phosphorus content: 48.5 mg·L^{-1} vs. 51.2 mg·L^{-1} ($P < 0.01$).

During the test phase, no day effect was observed ($P = 0.34$), but the experimental

Table II. Physicochemical analyses of the feedstuffs used in the experimental diets.

	Rapeseed meals		Inorganic phosphates		Other ingredients					
	Standard	Formaldehyde treated	Dicalcium	Calcium aluminium	Wheat straw	Wheat gluten	Corn starch	Fat	Citrus pulp	Cassava
Moisture[1]	12.4	12.9	0	7.6	7.3	6.0	12.3	3.7	14.2	12.46
Ash[2]	7.7	7.7	nd	89.2	7.16	0.82	0.07	21.1	6.76	8.20
Crude protein[2]	39.0	38.2	nd	nd	1.94	82.02	0.28	nd	6.47	6.51
Crude fibre [2]	14.7	14.6	nd	nd	47.3	0.15	0.11	nd	nd	5.93
Fat[2]	2.3	2.7	nd	nd	nd	2.61	0.23	6.0	2.1	0.71
Starch[2]	4.9	4.0	nd	nd	nd	10.5	99.3	nd	8.3	66.1
Calcium[2]	0.9	0.8	22.5	3.6	0.43	0.11	0.02	nd	1.9	0.30
Phosphorus[2]	1.3	1.3	17.8	12.2	0.06	0.21	0.01	0.01	0.09	0.11

[1] Expressed in %.
[2] Expressed in % DM.
nd: not determined.

Table III. Mean plasma phosphorus concentration expressed in $mg \cdot L^{-1}$ during the preliminary phases and the test phase. The results are presented by animal (sheep 1 to 4) and by diet. The test diets incorporated different phosphorus sources: dicalcium phosphate (DCP), calcium aluminium phosphate (CAP), standard rapeseed meal (S-RM) and formaldehyde-treated meal (FT-RM). The figures between brackets are the standard error of the mean.

Phase	Preliminary phases			Test phase				
Diet	Usual P	Low P	*P* value	DCP	CAP	S-RM	FT-RM	*P* value
Sheep 1	53.9 (1.60)	51.1 (0.85)	0.112	49.4 (1.5) G	40.7 (1.7) H	62.9 (1.9) F	–	0.0001[4]
Sheep 2	50.4 (1.36) α	47.4 (0.82) β	0.070[1]	48.5 (1.1) G	39.6 (1.2) H	–	66.5 (2.0) F	0.0004[4]
Sheep 3	49.6 (1.73) α	46.5 (0.82) β	0.090[1]	52.4 (1.1) F	–	49.2 (1.2) G	44.2 (1.3) H	0.001[4]
Sheep 4	51.1 (1.5)	49.0 (1.01)	0.231	–	53.4 (4.2) F	46.0 (1.6) G	44.5 (1.8) G	0.001[4]
Mean	51.2 (0.79) D	48.5 (0.45) E	0.001[3]	50.1 (0.7) B	44.5 (1.8) C	52.7 (1.4) A	51.7 (1.8) B	0.0001[2]

[1] α, β: means followed by different letters differ ($P < 0.1$);
[2] A, B, C: means followed by different letters differ ($P < 0.01$);
[3] D, E: means followed by different letters differ ($P < 0.01$);
[4] F, G, H: means followed by different letters differ ($P < 0.01$).

period significantly influenced plasma phosphorus concentration ($P < 0.01$) since the plasma phosphorus concentration was higher ($P < 0.01$) during the test phase of period 3 (58.8 $mmol \cdot L^{-1}$) compared to the other two periods (period 1: 45.6 $mmol \cdot L^{-1}$ and period 2: 44.8 $mmol \cdot L^{-1}$). Comparing the animals, the mean plasma phosphorus content varied from 47.9 $mg \cdot L^{-1}$ to 51.0 $mg \cdot L^{-1}$.

The diet effect was significant ($P < 0.01$). Mean plasma phosphorus contents were higher with the dicalcium phosphate diet than with the calcium aluminium phosphate diet (44.5 $mg \cdot L^{-1}$ vs. 50.1 $mg \cdot L^{-1}$, respectively, $P < 0.01$) and with the standard rapeseed meal diet than with the formaldehyde treated rapeseed meal (52.7 $mg \cdot L^{-1}$ vs. 51.7 $mg \cdot L^{-1}$, $P < 0.01$). Finally, the standard rapeseed meal phosphorus elicited higher mean plasma phosphorus levels than the formaldehyde treated rapeseed meal and both inorganic forms of phosphorus. Only rapeseed meals elicited higher plasma phosphorus content than the low phosphorus preliminary phase (respectively, 52.7 $mg \cdot L^{-1}$ and 51.7 $mg \cdot L^{-1}$ vs. 48.5 $mg \cdot L^{-1}$, $P < 0.1$). The calcium aluminium phosphate diet induced a plasma phosphorus content even lower but not significantly than the second preliminary diet (44.5 $mg \cdot L^{-1}$ vs. 48.5 $mg \cdot L^{-1}$, $P > 0.1$).

4. DISCUSSION

4.1. Low dietary phosphorus and phosphorus depletion

In the present trial, in spite of a low dietary phosphorus supply during the low phosphorus preliminary phase, plasma

phosphorus began to rise on the fourth day. This increase in plasma phosphorus content should illustrate the efficiency of phosphorus homeostasis in the maintenance sheep [10] when the sheep were switched to the low phosphorus preliminary diet. We expected a decrease and a stabilisation of plasma phosphorus content as observed in dairy goats with 9 days of low phosphorus supply (1.0 g·kg^{-1} of DM) [19]. This result confirms that milk phosphorus excretion is an obligatory phosphorus loss much more efficient in terms of body phosphorus drain than faecal or urinary excretion, which represents the only pathways of phosphorus loss in mature sheep [19]. Moreover, we presume that in the present experiment, bone phosphorus was also mobilised to readjust the plasma phosphorus content since the maintenance sheep presents large phosphorus storage in the bone [20]. For these reasons, in spite of a lower dietary phosphorus supply, the plasma phosphorus content was not maintained at a low level. At lower phosphorus supply, a low plasma phosphorus level was reported to persist in 45-kg sheep fed a diet supplying 0.96 g of phosphorus per day [5] and in 65-kg sheep fed a diet providing 1.0 g·kg^{-1} of DM [8]. Three weeks of a diet supplying 1.44 g·kg^{-1} DM appear not severe enough in extent and duration to induce a phosphorus depletion with plasma phosphorus responses. However, the lower mean plasma phosphorus content during the low phosphorus preliminary diet compared with the first one would suggest that the homeostasis did not completely restore the level of plasma phosphorus.

Another consequence of phosphorus deficiency is the decrease of DM intake, that we did not observe on our feed restricted sheep. This decrease of DM intake is commonly related to the high phosphorus requirement of rumen microbes [18]; phosphorus deficiency may induce a reduction in dietary fibre degradation in vitro [14] and decrease microbial protein synthesis in vivo [5]. However, rumen microbe populations have been reported to survive severe phosphorus depletion [13]. In in vivo studies involving sheep, a severe phosphorus-depleted diet (0.45g·kg^{-1} DM) was not found to affect neither the ability of the rumen to digest DM nor cell walls [3]. After 2.5 months at a low level of phosphorus intake (0.83g·kg^{-1} DM), the level of soluble phosphorus in the rumen of sheep was still sufficient to satisfy microbial requirements [20]. For this reason, with much less severe phosphorus deficiency we did not observe a DM intake decrease in our trial.

4.2. Plasma phosphorus content: a way to test dietary phosphorus quality?

In our study, plasma phosphorus variation in response to dietary phosphorus supply was highly dependent on the animal. This confirmed a study conducted in sheep showing that the plasma phosphorus concentration varies from 50 mg·L^{-1} to 150 mg·L^{-1} after one year of low phosphorus supply [2]. Since we did not observe the expected plasma phosphorus decrease, the test phase could not be considered as a true repletion phase.

However, some differences appear: for instance the lowest plasma phosphorus content in the test phase was obtained when the calcium aluminium phosphate diet was fed and it was different from the value obtained with the dicalcium phosphate diet According to the differences in phosphorus availability of both phosphorus sources, an average of 70% for the dicalcium phosphate vs. less than 13% for the calcium aluminium phosphate were expected [15]. Moreover, the plasma phosphorus content elicited by the calcium aluminium phosphate diet was lower than when the low phosphorus diet was fed during the second preliminary phase. This would illustrate the difficulty for homeostasis to maintain constant plasma phosphorus levels since the sheep fed the calcium aluminium

phosphate were kept on a diet either low in phosphorus quantity or poor in phosphorus quality for 6 weeks.

Rapeseed meal diets induced different but close plasma phosphorus concentrations. The same diets were tested in another study of phosphorus digestibility where the phosphorus digestibility of formaldehyde rapeseed meal was higher than that of the standard rapeseed meal (respectively, 0.67 vs. 0.60, $P < 0.01$; Bravo et al., unpublished). The plasma phosphorus content determination was probably not a sensitive enough approach to show these differences. It also appears that the phosphorus content of the standard rapeseed meal diet was also higher (0.28% vs. 0.23% for the FT-RM diet).

The standard rapeseed meal induced higher plasma phosphorus content than the dicalcium phosphate diet despite close phosphorus availability. The rumen is the place for standard rapeseed meal phosphorus solubilisation [4] and the abomasum is the place for dicalcium phosphate solubilisation [15]. When solubilised in the rumen, standard rapeseed meal phosphorus would be integrated into the microbial phosphorus content. The results of this study suggest that microbial phosphorus is of high quality for the host animal and probably of a better quality than inorganic dietary phosphorus.

5. CONCLUSIONS

The iterative measurements of plasma phosphorus concentration after common preliminary phases of usual and then low phosphorus intake did not permit the reliable discrimination among diets in terms of dietary phosphorus availability in adult sheep on maintenance diets. In dairy animals, homeostasis is probably less efficient in maintaining the level of plasma phosphorus when low quality phosphorus is fed because of the drain upon body phosphorus stores by the phosphorus lost in the milk.

In our trial, homeostasis appeared to override other factors and partially restore plasma phosphorus concentration when the sheep received low quality phosphorus diets (aluminium phosphate). The efficacy of this putative homeostatic control may have been enhanced by the large bone phosphorus stores of the experimental animals used (adult sheep in maintenance). However, a slight difference exists in the plasma phosphorus levels when the sheep were fed the calcium aluminium phosphate and dicalcium phosphate diets as expected given the large difference in phosphorus digestibility of these two phosphate sources. The two organic sources of phosphorus used in this study (standard and formaldehyde treated rapeseed meal) induced plasma phosphorus concentrations close to the concentration induced by the dicalcium phosphate diet.

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