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Phosphorus kinetics in tissues of young Santa Ines sheep

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Abstract. Santa Ines is one of the most popular hair sheep breeds from Brazil used for meat production. This study was carried out to determinate the effect of phosphorus (P) intake on the P kinetics in tissues. Twelve Santa Ines sheep (20 weeks old and live weight 22.6 ± 2.21 kg), were housed in metabolism cages and fed 600 g/animal/day of Coast cross hay and concentrate mixture (200 g/animal/day of cassava flour and 15 g/animal/day of urea) with daily supplements of dicalcium phosphate (0, 1.5, 3.0 and 4.5 g P/animal). A group of 3 animals received each level of dicalcium phosphate and after 22 days they were given a 0.5 ml saline solution with an intravenous dose of 7.4 MBq ^{32}P to trace the P kinetics in tissues. Blood was collected for the subsequent seven days and radioactivity decay measured. Animals were slaughtered and samples from bone, liver, kidney, heart and muscle were taken. The concentration of P, biological half-life, retention, standardized activity [(activity in tissue/(mg P/g tissue))/(activity injected/live weight)] and relative activity (activity in tissue/activity in plasma) of ^{32}P were evaluated in relation to P intake and analysed by regression analysis. No significant relationship was observed on stable P concentration in tissues and P intake. Biological half-life, retention (liver, kidney and heart) and standardized activity (liver, kidney and heart) of ^{32}P were found to have a negative linear relationship with P intake. There was a positive linear relationship between relative activity (bone, kidney and heart) and P consumed. It was concluded that dietetic P influences the turnover of P in tissues of young Santa Ines sheep.

Keywords. Hair sheep – Metabolism – Mineral.

Cinétique du phosphore dans les tissus des agneaux de race Santa Ines

Résumé. *L'objectif de cette étude est d'évaluer la cinétique du phosphore dans les tissus des ovins. Douze agneaux de poids vif moyen 22,6 ± 2,21 kg ont été logés dans des cages à bilan. Ils ont été répartis en lots recevant des niveaux croissants de phosphate bicalcique dans la ration de base (0, 1,5, 3,0 et 4,5 g de phosphore/animal/jour). Vingt-deux jours après le démarrage de cet essai, 7,4 MBq de ^{32}P ont été injectés dans la veine jugulaire de chaque animal. Des prélèvements de sang ont été effectués pendant les 7 jours suivants. A la fin de cet essai, les animaux ont été abattus et des organes (os, foie, rein, coeur et muscle) ont été récupérés pour la détermination de la concentration du P stable et de la demi-vie biologique, de la rétention, de l'activité spécifique standardisée et relative du phosphore marqué (^{32}P). Le niveau de phosphore injecté n'a pas affecté la concentration du phosphore stable dans les tissus des organes prélevés (P > 0,05). Une régression linéaire négative pour la demi-vie biologique, la rétention (foie, rein et coeur) et l'activité spécifique standardisée (foi, rein et coeur) avec l'augmentation du P dans le régime a été mise en évidence. En revanche, l'augmentation du niveau de phosphore dans la ration s'est traduite par une activité relative et une régression linéaire positive entre l'os, le rein et le coeur. L'augmentation du P dans le régime a été associée à une augmentation du recyclage du P chez les agneaux. Il ressort de ce travail que le phosphore alimentaire affecte le passage de ce minéral dans les tissus des agneaux de race Santa Ines.*

Mots-clés. *Brebis de poil – Métabolisme – Minérale.*

I – Introduction

According to McDowell (1992), approximately 80% of phosphorus in sheep body is present in

bones and teeth. The other 20% which has no structural function is found in blood and soft tissues. Therefore, the biological half-life, retention, standardized specific activity and relative specific activity of the radiotracer in tissues are parameters used to determine the mineral kinetics in the organism (Bueno and Vitti, 1998). Each of these parameters is useful in determining specific properties of the mineral in the organism. While the biological half-life indicates recycling time of P, retention reflects the incorporation of the mineral by the tissue. Relative specific activity (RSA) refers to the metabolic activity of the element in the tissues in relation to the activity in the blood while the standardized specific activity uses the RSA divided by the live weight of the animal in relation to the injected dosage. The objective of this study was to evaluate the kinetics of P in tissues of young Santa Ines sheep.

II – Materials and methods

Twelve Santa Ines lambs, 20 weeks old, entire, with live weight 22.6 ± 2.21 kg were allocated into four treatments with 3 animals each. The animals were kept in individual cages, and fed a basal diet of roughage (*ad libitum*), concentrate mixture (200 g/day of cassava flour and 15 g/day of urea) and 10 g/day of mineral mixture. The treatments consisted of adding, to the basal diet, different amounts of dicalcium phosphate corresponding to 0, 1.5, 3.0 and 4.5 g P/animal/day. Chemical analysis of the feed was carried out according to recommendations of the AOAC (1995) (Table 1).

Table 1. Chemical composition of diets fed sheep receiving different levels of phosphorus

Chemical composition (g/kg DM)	Hay	Treatments (P g/day)			
		0	1.5	3.0	4.5
Dry matter (DM)	899.6	880.1	887.1	888.5	894.0
Crude protein	61.7	232.0	237.2	238.0	231.6
Ether extract	28.0	11.1	10.9	14.8	21.4
Neutral detergent fibre	733.6	142.7	147.6	143.5	142.4
Acid detergent fibre	380.8	32.6	32.9	31.5	32.0
Ash	63.4	28.6	66.6	84.1	122.3
Phosphorus	1.9	0.9	8.1	16.4	25.8

On the 21st day, a single dose of 7.4 MBq ^{32}P was injected intravenously in each lamb. Blood samples with heparine were collected over the following 7 days. On 8th day, the sheep were slaughtered and samples from bone, liver, kidney, heart and muscle were taken. The inorganic P level in plasma was determined by colorimetric analysis. Radioisotope activity was estimated by placing 1 ml of plasma in 19 ml distilled water into counting vials. Radioactivity of ^{32}P was measured in a Liquid Scintillation Spectrometer using Cerenkov radiation.

Tissues samples were milled, dried overnight (105℃), and burned (500℃ for 8 h). Ten ml of sulphuric acid 18 N (1:1) were added to the ash on a hot plate. After digestion, this material was transferred to counting vials for radioactivity measurements. The inorganic P level in tissue was determined by colorimetric analysis following digestion by concentrated hydrochloric acid.

The biological half-life of the ^{32}P (T1/2) was calculated using the decay curve for specific activity in plasma with time. The retention was measured using the equation: (activity in tissue/g DM)/[injected dose/(mg P/g DM)]. In tissues, the standardized activity was calculated by: [activity in tissue/(mg P/g tissue)]/(activity injected/live weight) and relative activity (activity in tissue/activity in plasma). The animals were assigned to four treatments (four phosphorus levels) in a fully randomized design. Variance and polynomial regression analysis were carried out (SAS, 1999).

III – Results and discussion

Live weight and stable phosphorus in blood and tissues were not affected (P > 0.05) by increasing P in the diet (data not presented). Variables related to ^{32}P are shown in Table 2. A negative linear regression was found between consumed P and biological half-life, retention (liver, kidney and heart) and standardized activity (liver, kidney and heart). For relative activity in bone, kidney and heart, there was a positive linear relationship with ingested P.

Table 2. ^{32}P kinetics in young Santa Ines sheep fed increasing levels of P in the diet

Variables	Treatments (P g/day)				Regression	R^2	P†
	0	1.5	3.0	4.5			
P intake	0.9	2.5	4.4	6.6			
Biological half-life (hour)	86.67	54.55	58.58	54.84	y = –4.65x + 81.15	0.39	0.0293
Retention (mg/g DM)							
Bone	1.86	1.51	1.21	1.67			ns
Liver	0.61	0.43	0.34	0.33	y = –0.049x + 0.60	0.60	0.001
Kidney	0.35	0.23	0.20	0.19	y = –0.027x + 0.34	0.71	0.0006
Heart	0.30	0.20	0.17	0.18	y = –0.020x + 0.28	0.52	0.008
Muscle	0.15	0.11	0.08	0.10			ns
Standardized activity							
Bone	0.06	0.06	0.05	0.07			ns
Liver	0.34	0.27	0.22	0.22	y = –0.020x + 0.38	0.57	0.005
Kidney	0.30	0.24	0.19	0.22	y = –0.016x + 0.29	0.32	0.05
Heart	0.27	0.22	0.16	0.20	y = –0.012x + 0.26	0.39	0.03
Muscle	0.16	0.14	0.10	0.12			ns
Relative activity							
Bone	0.10	0.17	0.17	0.27	y = 0.028x + 0.077	0.35	0.04
Liver	0.52	0.74	0.80	0.85			ns
Kidney	0.47	0.66	0.65	0.78	y = 0.047x+ 0.47	0.42	0.02
Heart	0.40	0.59	0.58	0.79	y = 0.060x + 0.37	0.39	0.03
Muscle	0.26	0.38	0.36	0.47			ns

†ns = not significant.

As the level of phosphorus increased in the diet, there was a reduction in biological half-life (T1/2 = –4.65 P intake + 81.15; R^2 = 0.39; P = 0.0293), indicating that increasing intake leads to shorter time in the animal, or faster metabolism. This was also found by Louvandini (1995) in adult wool sheep (40 kg LW), and a biological half-life varying from 119.41 to 86.34 h, for lowest (0 g) to highest (3 g) P supplementation in the diet, respectively. Nevertheless, the ^{32}P retention time found by this author was higher than that found here, as animal here were younger, indicating faster metabolism for this mineral and higher nutritional requirements for P for this animal category.

Metabolic activity depends on intra-cellular activity, which is adjusted for phosphorus present in the diet. When the intake of this mineral increases the cellular activity also increases (Ternouth and Sevilha, 1990). The faster metabolism for higher levels of P in the diet leads to higher carcass production in animals, as there is a better mineral balance as well as an increase in feed intake.

P profiles in different tissues may be evaluated by retention, standardized activity and relative activity. Bone was shown to be the principal reserve of this mineral, with a higher retention level and lower metabolic activity when compared with other tissues. This is due to the physiological characterization of this tissue which is for structural support, and results here indicate that the processes of synthesis and re-absorption occur slower than in other tissues. This was confirmed when the exchange of P between blood and bone was observed, comparing angular coefficients for the regressions relative to bone (0.028), kidney (0.047) and heart (0.060) tissues. Nevertheless, its

retention and standardized activity were constant from the lower to higher level of P in the diet, showing no mineral saturation for this tissue, as young fast growing sheep were used in this study.

Liver, kidney and heart showed similar P kinetic profiles, with a fall in retention and standardized activity for higher P intake. The soft tissues analyzed had high metabolic rates, and the need for P decreased as P offer in the diet increased. This was in agreement with biological half-life data. Nevertheless, for kidney and heart the relative activity has a positive regression with P in the diet, in disagreement with liver data. Although, Vitti *et al.* (1992) used older animals and different sources of P, they found that P kinetics in soft tissues were similar to that one in this study. Bueno and Vitti (1998), using increasing levels of P in the diet of goats, found similar profiles in tissues for retention, standardized activity and relative activity.

Lopes *et al.* (1999), studying P kinetics in young swine, found lower retention in bone compared with soft tissues and a lack of connection between this variable and P in the diet. According to Underwood and Suttle (1999), P metabolism in monogastrics is different from ruminants, especially swine, due to the low use of P linked to phytic acid in these animals, which are more dependent on inorganic sources of P in the diet (Teixeira *et al.*, 2004). Vital activities should be maintained in detriment to bone tissue in this case. Comparisons between ruminants and monogastrics should be made with caution due to differences between these animal groups.

IV – Conclusions

Increase of phosphorus in the diet increases P kinetics in young sheep, with greater retention by bone tissue followed, in decreasing order, by liver, kidney, heart and muscle. The translocation of P in sheep occurs faster in liver, kidney and heart followed by muscle and slowest in bone.

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