^{32}P uptake by erythrocytes in sheep supplemented with phosphorus at pasture

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Abstract

This study aimed to evaluate the of ^{32}P uptake by erythrocytes of young sheep, supplemented with phosphorus and kept at pasture. Twenty sheep, initially weighing 13.88 ± 2.51 kg, kept on *Andropogon gayanus* pasture, were divided in two treatments of 10 animals each. In the first group animals were supplemented with 3g phosphorus/animal/day and in the other no P supplementation was given. Five blood and faeces collections were taken on the 8th, 29th, 43rd, 57th and 71st days of the experiment to evaluate of ^{32}P uptake by erythrocytes, to determine the percentage of P in the faeces and concentrations of calcium, glucose and phosphorus in blood serum. Significant differences (P<0.05) for Ca concentrations were found on the 57th day of the experiment, but no significant differences were found for serum glucose and phosphorus in faeces (P>0.05) between treatments. The P concentration in plasma was significantly higher (P<0.05) in supplemented animals (5.37; 6.75; 6.97; 6.84 and 6.64 mg/100ml) compared to the non-supplemented animals (4.83; 3.91; 4.50; 4.35 and 4.40 mg/100ml) for the collection days respectively. With the exception of the first collection date, the ^{32}P uptake by the erythrocytes was significantly higher (P<0.05) in non-supplemented animals (12.2; 7.4; 8.4; 9.4 and 12.1%) compared to supplemented ones (9.8; 4.2; 4.3;

3.3 and 4.2%). The technique using of ^{32}P uptake is an important additional tool to evaluate P status with the identification of sub-clinical deficiency in young sheep.

Key words: deficiency, metabolism, Santa Inês, hair sheep.

1. Introduction

McDowell (1999) shows that the deficiency of phosphorus is the most common in animals kept at pasture in tropical and subtropical regions. Also, it is an economic limitation in animal production. Tropical pastures are, in general, deficient in P, and supplementation of this element is necessary to correct the disturbances (Salviano e Vitti, 1997).

The reduction in food consumption is one of the main symptoms of P deficiency, and physiological mechanisms have been studied in an attempt to explain the situation: P deficiency interferes in the intermediary metabolism and also in the reduction of microbial activity (Nicodemo *et al.*, 2000). The deficiency of P has been associated with a decrease in P levels in soft tissues. This decrease leads to a reduction in the metabolic rate, due to a lower availability of cyclic AMP or other nucleic acid molecules (Clark, 1990).

The use of inorganic P in plasma has been used as a criteria to determine the nutritional P status in domestic animals. Research has shown that this parameter, alone, is not adequate, because plasma P can suffer action of innumerous factors and show wide variation (Vitti, 2000).

Marginal deficiency is economically more serious as there is a lack of clinical signs, therefore no care is taken with the animals and productivity is affected. The development of methods for detection and diagnosis of sub-clinical deficiency is

valuable to correct these disturbances in the initial phases, which can be corrected with adequate diet (Vitti, 1987). This study was carried out to investigate the use of ^{32}P uptake by erythrocytes as a non-invasive technique to evaluate the sub-clinical deficiency of P in young sheep.

2. Material and Methods

Twenty male sheep, Santa Inês breed, with initial live weight of 13.8 ± 2.5 Kg, aged 3 months, were maintained in Andropogon *gaianus* grass and received concentrate (200 g/day of cassava flour and 10 g/day of urea) and 9.57 g/day of mineral mixture (0,009 KI; 0,0008 $CoSO_4$; 0,03 $CuSO_4$; 1,61 MgO; 3,0 NaCl; 0,32 $ZnSO_4$; 0,148 $MnSO_4$; 0,457 $FeSO_4$; 4,0 S g/dia). The lambs were randomly allocated to two dietary treatments (Group P: 16.66g of bicalcium phosphate per animal/day and Group N: not supplemented with P). Chemical analysis of the diet was carried out (Table 1) according to recommendations of the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (AOAC, 1995).

Blood and faeces were collected and animals weighed 8, 29, 43, 57 and 71 days after the start of supplementation with P. Blood samples were collected with EDTA and used to determine 32P uptake of ^{32}P, and blood without EDTA for serum determination of P, Ca and glucose, using commercial Labtest ® kits.

Faeces samples (1g) were milled, dried overnight (105^{o} C) and ashed (500^{o} C for 8 h). Concentrated hydrochloric acid (HCl) was added to the ash and P determined by colorimetric method (Sarruge & Haag, 1974).

The rate of ^{32}P uptake by the erythrocytes was carried out by preparing a radioactive solution, by adding 1ml NaCl at 0.85% and 3.710^{6} Bq ^{32}P as Na_2HPO_4, so

that each 10µl solution had an activity of 1.8510^3Bq. After this, 3 eppendorf tubes, each containing 1 ml of blood and 10 µl glucose, were used. In two eppendorfs, 10 µl of a saline solution we added, and in the third "blank", 10 µl of KCN at 10% was added, which cancelled the uptake of the ^{32}P by the red cells in this tube. Thereafter, 10 µl of radioactive ^{32}P were added to each eppendorf and these were incubated for three hours at 38 °C with 95% O_2 and 5% CO_2. The eppendorfs were then taken to a cold room where they remained for 15 minutes at approximately 5˚C, to interrupt haemocyte metabolism. After this, 0.5 ml of the solution was transferred from each eppendorf to their respective porcelain crucibe, representing ^{32}P in total blood.

To the remainder of the solution in the eppendorfs, 1 ml of NaCl at 0.85% was added and centrifuged at 14.000 rpm for six seconds with the removal of supernatant, receiving the name "cellular wash". This was repeated twice more, thereby obtaining erythrocytes with ^{32}P uptake which were transferred to another 3 porcelain crucible and together with the total blood crucible were dried at 120 ˚C for three hours and afterwards incinerated at 550 ˚C for eight hours in an furnace.

The remaining ash in the crucibles were heated on a hot plate to 200 ˚C and digested with H_2SO_4-6N. The resultant liquid was transferred to special cyntilography flasks for detection of the activity by the Cerenkov effect (International Atomic Energy Agency, 1979). The percentage of ^{32}P uptake was calculated using the formula by Burk Junior et al. (1967):

$$^{32}P\,uptake = \frac{activity\ in\ erythrocytes \times 100 \times 40^{*}}{activity\ in\ total\ blood \times cellular\ volume}\%$$

*Where the factor 40 was used to standardized the haematocrit value

2.1 Statistical analysis

The data were analyzed as a fully random design with two treatments and 10 replicates, using GLM (General Linear Model) procedure of SAS (Statistical Analysis System ®, Cary, Indiana, 1991). When measures were collected over time, these were analyzed using the REPEATED option to test the hypothesis of parallelism and decomposition of effects over time and correlation between variable. Significant differences were considered at $P<0.05$.

3. Results

Animal live weight, serum glucose and calcium are shown in Table 3, there being no significant differences between the two treatments during the experimental period ($P<0.05$).

Glucose levels are within the normal values, with no significant differences between the two treatments and collection dates. Only on the 57^{th} day was Ca level higher in the group unsupplemeted with P ($P<0.05$) with a tendency for a negative correlation between P and Ca in the plasma ($r= -0.18$; $P<0.0910$)

Figure 1 shows data of P in feaces, serum and ^{32}P uptake by eritrocytes. The concentration of P in the faeces did not show significant differences between treatments. The group receiving P supplementation showed higher plasma P values in the second collection ($P<0.05$).

The ^{32}P uptake by erytrocites was lower for the supplemented animals, showing a negative correlation between plasma P and the uptake of ^{32}P ($r=-0,47$; $P<0.0001$). The unsupplemented group also showed an increase in uptake over time ($P<0.05$).

4. Discussion

The level of P in the forage was 0.23%, not as deficient as some tropical grasses which have levels less than 0.15%, which have a more severe deficiency (McDowell, 1999). Live weight as a single trait, especially in marginal deficiencies of P, appears not to be an adequate parameter to evaluate this situation. Exposition time to lack of mineral assumes an important role on animal performance, not apparent over the 75 days of this experiment.

Villaroel et al. (1991), working with lambs on 3 treatments at pasture (supplementation with 1: commercial mineral mixture - 7.83% P and 9.50% Ca; common salt - 35% Na and 25% Cl; and no mineral supplement), showed that those receiving a commercial mixture or common salt did not differ in body development. Vitti et al. (1988) did not find significant differences in weight gain between a group of calves supplemented for 9 weeks with P and another not supplemented.

Louvandini et al. (2004), in a study that continued from this evaluated performance and carcass traits in these animals and showed that the weight of the 12^{th} rib, bone tissue in the 12^{th} rib and abdominal viscerae in the P group were heavier than the N group ($P < 0.05$), with a tendency for the former to have more muscle in the 12^{th} rib ($P=0.0954$). It is therefore necessary to evaluate other traits, not only liveweight, as evaluation parameters for P supplementation in sheep.

The Ca values in the two groups were close to normal (9 - 12 mg/100 ml - McDowell, 1992). The fact that no significant differences were found on days 8; 29; 43 and 71, are probably due to the homeostasis mechanism which maintains serum levels of Ca (Fisher et al., 1972; Salviano, 1996; Bueno et al., 1999). Higher levels of Ca were observed in the plasma of the N group compared to the CP group, which may be due to greater bone absorption due to the deficiency of P in the diet, in agreement with 12^{th} rib data (Louvandini et al., 2004). This stimulates the parathormone (PTH) and dihydroxycolecalciferol (DCC), which together stimulate reabsorption of bone, resulting in liberation of P and Ca. DCC by itself increases reabsorption of Ca by the gastrointestinal tract (Vitti, 2000).

It was expected that supplemented animals would show higher glucose values compared to non-supplemented, but no significant differences were found. The non-supplemented group had marginal P deficiency and the time exposed to this deficiency may not have been sufficient to affect glucose metabolism.

Silva Filho et al. (1997) working with cattle to evaluate the effect of P supplementation on the of ^{32}P uptake by erythrocytes in animals kept at pasture for six weeks (Phase 1) and later the same animals kept six weeks at pasture with a commercial mineral supplement containing 6% P (Phase 2), verified mean glucose values in the plasma of 40.69 ± 3.43 and 44.09 ± 1.98 mg/100ml respectively for phases 1 and 2 ($P < 0.01$). The authors suggest that the greater values in phase 2 were influenced by greater concentration of inorganic phosphorus in the plasma, tereby influencing the energy metabolism in these animals.

Plasma P values were within normal range (Thompson, 1978), varying between 4 and 9 mg/100 ml. Read et al. (1986) suggest that the deficiency limit for P is 2.0 mg/100 ml. Nevertheless, it can be seen in animals without P supplementation in the diet are close to the lower limits suggesting marginal deficiency of the mineral in question Braithwaite (1985) and Ternouth & Servilha (1990) showed a positive linear relationship between P consumed and P in the plasma.

Dayrel et al. (1973) described that plasma P levels in ruminants are subject to changes and may suffer variations due to prolonged fasting, stress and agitation at blood collection as well as due to sample storage temperature, among others. Vitti (2000) states that plasma P values may be considered parameters of P status of the animal, but care should be taken in the interpretation of P data in plasma, as plasma P levels do not always reflect the nutritional state of the animal with respect to this element, as there is a complex homeostatic system involved and the bone is a reservoir of this mineral.

The P levels in the faeces were not significantly different between the two groups, although P loss in the faeces are not directly related to that ingested by the animal (Barrow & Lambourne, 1962). Silva Filho (1995) observed that P levels in the faeces increased in a linear fashion with the level ingested by the animal.

Portilho (2003) as well as verifying a positive linear relationship between P consumed and excreted by Santa Inês sheep of similar age and weights to those in the present study, also observed that those that did not receive P supplement in the diet had

a negative balance for this element, with retention of –42.26 mg/kg livweight day, therefore P metabolism was maintained by mobilizing body reserves of this element, whose excretion is by faeces. This explains the lack of a significant difference between the two treatments in this experimet in terms of faeces P.

Here, the higher uptake rate in non-supplemented animals reflect the nutritional system the animals were exposed to. Since ^{32}P uptake increased with time shows this system to be more sensitive than plasma P which remained approximately 4 mg/100ml, with no significant differences from the 2^{nd} to 5^{th} collection. These are in agreement with Silva Filho et al. (1997) who found mean values for ^{32}P uptake of 7.04 ± 1.20% in crossbred calves with no mineral supplementation for 6 weeks and 5.91 ± 1.24% in the same calves posteriorly fed mineral mixture with a 6% P content for 6 weeks.

An inverse relationship was found between of ^{32}P uptake and plasma P levels (r = -0,80). Silva Filho et al. (1997) also founded an inverse relationship between these parameters (r = -0,33). They thought that this is probably due to higher circulating P levels. The erythrocyes therefore are less avid for this element, incorporating therefore less radioisotope. Vitti, et al. (1988) found higher, but not significant, ^{32}P uptake values for the group kept on a P deficient diet, suggesting that the ^{32}P uptake could be used as a means of detecting P deficiency.

5. Conclusions

The uptake of ^{32}P seems to be an important additional tool to evaluate sub-clinical P deficiency in sheep at pasture.

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Table 1. Nutrient composition of diets

Chemical composition (mg/kg DM)	Grass	Dietary Treatment	
		Supplement P	Non supplement P
Dry Matter	322.6	888.5	880.1
Crude Protein	61.7	238.0	232.0
Ether Extract	28.0	14.8	11.1
NDF [a]	733.6	143.5	142.7
ADF [b]	380.8	31.5	32.6
Ash	63.4	84.1	28.6
Phosphorus	2.1	16.4	0.9

[a]NDF: Neutral Detergent Fiber
[b]ADF: Acid Detergent Fiber

278 Table 3. Mean with standard error, live weight, serum glycose and calcium in sheep
279 supplemented and non supplemented with P.
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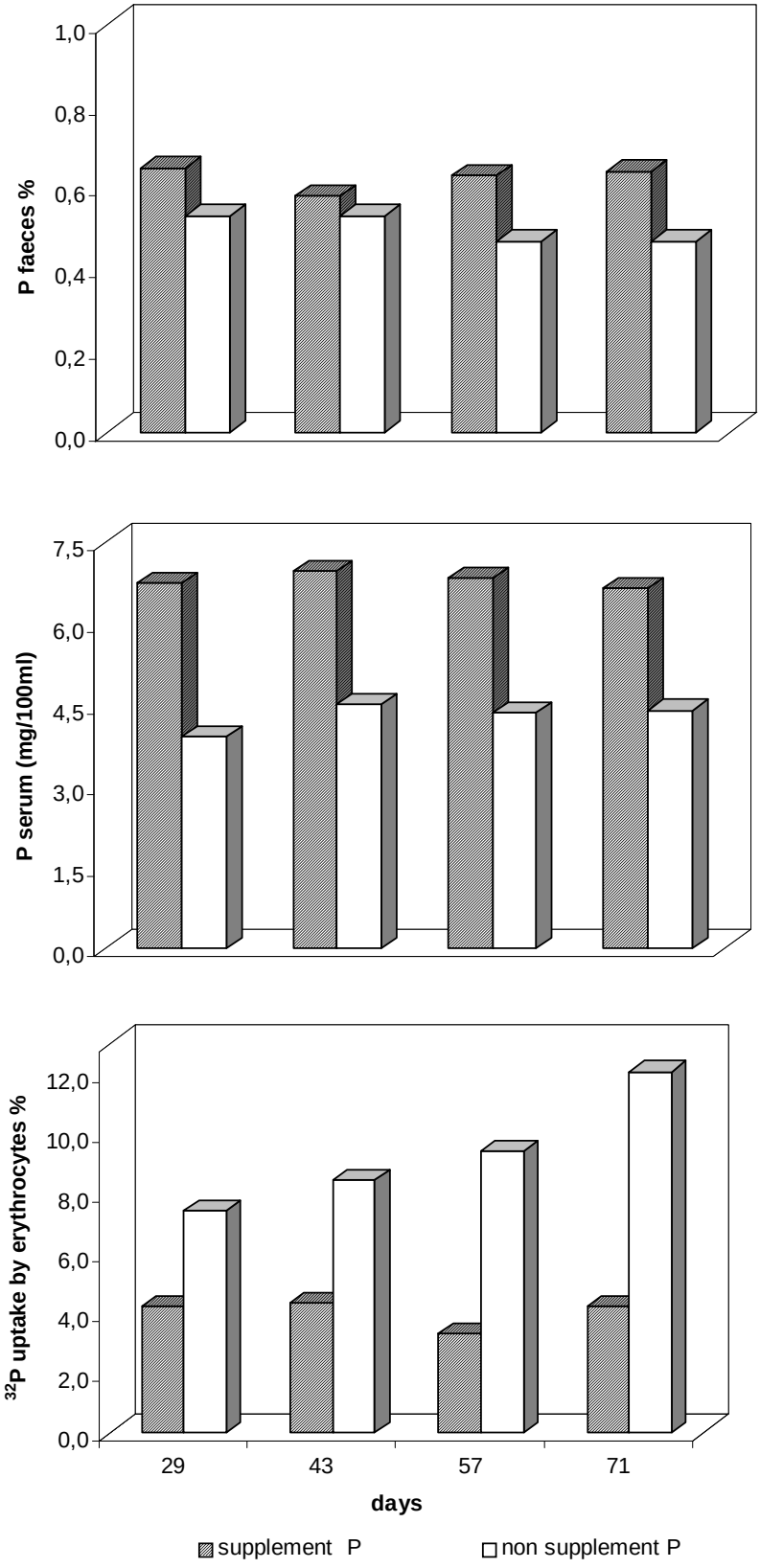
Days	Live weight (kg)		Glycose (mg /100 ml)		Calcium (mg /100 ml)	
	P	N	P	N	P	N
1	13.9 ± 2.7	13.8 ± 2.5	70.0 ± 14.6	75.1 ± 26.7	11.2 ± 2.2	10.1 ± 6.4
8	15.4 ± 2.8	15.2 ± 2.3	69.9 ± 14.6	75.1 ± 26.7	11.4 ± 2.2	10.08 ± 6.4
29	16.5 ± 3.5	16.8 ± 3.0	84.1 ± 19.8	90.1 ± 20.2	9.6 ± 2.8	11.43 ± 2.8
43	18.6 ± 3.0	17.8 ± 3.7	49.6 ± 30.2	68.5 ± 27.2	13.5 ± 2.1	13.57 ± 2.4
57	18.6 ± 3.2	19.7 ± 3.9	45.5 ± 18.2	51.3 ± 12.6	11.5^{a} ± 1.8	14.12^{b} ± 1.9
71	19.5 ± 3.5	19.6 ± 4.3	51.8 ± 21.1	40.8 ± 20.3	14.3 ± 1.7	14.52 ± 2.2

281 [a] and [b] Means with different letters are significantly different ($P<0.05$).



304 Figure 1. Serum, faeces phosphorus and ^{32}P uptake by red blood cells of sheep offered

305 diets supplement and non-supplement P.