Replacement of dicalcium phosphate by alternative Cuban sources: study of the dry matter digestibility and intake of Pelibuey lambs

M. Valera, Natacha Dorta, J.G. Cairo and Odilia Gutiérrez
Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba
Email: mvalera@ica.co.cu

The dicalcium phosphate (DP) was replaced by a phosphorite and a phosphoric rock in a mineral premixture from a feedstuff for lambs, in order to validate them as substitutes of the imported product. The chemical composition of "Trinidad de Guedes" phosphorite (TGP) was evaluated regarding the amount of P and Ca as well as the solubility of theses elements in hydrochloric and citric acid. A 4x4 Latin square experimental design was used to analyze the influence of the replacement of DP by phosphorite and rock with Pelibuey male lambs. Two levels of addition of the premixture with TGP, and one with "Venegas" phosphoric rock. The TGP showed an 18 % of P and a 28 % of Ca. Its values of solubility were between 95.4 and 99.13 % for the P, and between 82.7 and 86 % for the Ca, in hydrochloric and citric acid, respectively. The animals showed, as average, consumptions of 1.09 kg of DM for the control, 0.96 and 0.98 kg for the treatments with TGP and 0.95 kg for the treatment with "Venegas" phosphoric rock. During the two first treatments the values of DM digestibility obtained were of 40.63 and 48.75 %. During the last treatment the values were of 49.60 %, equally superior. The value of DM digestibility for the control treatment was 45.61 %. The TGP has acceptable values of P and Ca, and it can replace the DP from the mineral premixture of the feedstuff for lambs, without affecting the consumption and digestibility of DM.

Key words: *Pelibuey, mineral premixture, feedstuff*

The replacement of mineral supplements like dicalcium phosphate (DP) is very important for achieving a proper animal diet. This product can have very high prices in the international market like, for instance, 1200.00 USD/t (UECAN 2008). The dicalcium phosphate was offered to animals as a P and Ca supplement, and it constitutes a basic component of the mineral premixture used in the production of feedstuff. Because of these conditions, it is necessary to find alternatives for replacing the imports of this type of products through profitable substitutes which allow reaching good productive indicators.

Studies carried out by Acosta *et al*. (2009) and Gutiérrez *et al*. (2009) demonstrated that it is possible to use the phosphorite from the Trinidad de Guedes deposit (TGP) (10-15 % P, 20-23 % Ca) as replacement of the DP in the diet of laying hens, dairy cows and fattening bulls.

With the objective of providing the bases to potentiate its use and contribute to the imports replacement, the chemical composition of the TGP and its possible effect on the consumption of DM and its digestibility was determined, when using it as a replacement of DP in the mineral premixture from the feedstuff for the diet of lambs. The influence of the phosphoric rock from the "Venegas" deposit, in Yaguajay, Sancti Spíritus province, included in the replacement, was also analyzed. The animals in this region consumed it freely. There was a particular emphasis on the TGP because its deposit was previously studied.

Materials and Methods

The study started with an amount of TGP, produced from the phosphoric rock of the deposit located in Matanzas province, Cuba. This deposit is georeferenced and its geological prospecting for the present extractions (10 000 t/year) suggests enough volumes for 50 years of exploitation. The cost of production is around 13.25 USD/t and nowadays it is only used as raw matter for the production of fertilizers. The "Venegas" phosphoric rock comes from a deposit from Sancti Spíritus province, which remains unstudied.

Determination of P and Ca. Thirty technological samples of TGP were taken at random. The Amaral (1972) technique was used to determine P. From a dissolution of 1g of phosphorite in 100 mL (1:100) of hydrochloric acid (1M), 1mL was taken, and deionised water was poured into a volumetric flask of 50 mL, until the half of its capacity. Two or three drops of the 2-4 dinitrophenol indicator were added. Later, a solution of NaOH (10 %) was added, drop by drop, until the dissolution turned yellow. Then, H_2SO_4 was added, drop by drop, until the discoloration is complete. Besides, 2mL of ammonium molybdate were added and deionised water was also used to reach the volume of 50 mL. Afterwards, 0.15 mL of $SnCl_2$. $2H_2O$ was added. It was shook until the mixture was homogeneous and the optical density at 660 nm against a target was determined.

In order to prepare the standard curve, 10mL of a standard solution of P (50 p.p.m.) were taken to a volumetric flask of 50 mL and deionised water was used to fulfill the rest of the flask content. Aliquots of 1, 2 and 3 mL were extracted from this solution that contains 10 p.p.m. of P and were taken to a volumetric flask of 50 mL. This procedure was repeated for determining P. These solutions had 0.2, 0.4 and 0.6 p.p.m. of P. A graph was built through the plotting of P concentrations in the X axis, and the optical density in the Y axis. The slope was calculated through the graph.

To calculate the results a formula was applied:

$$\%P = \frac{D.O}{m} \times \frac{1}{\frac{\text{Sample weight}}{\text{Taken volume}} \times \frac{\text{Aliquot taken}}{\text{Final volume}}}$$

Where:

OD: Optical Density

Sample weight: 1 g

Taken volume: 100 mL

Final volume: 50 ml

m: slope of the standard curve of P previously established

The procedure for determining the Ca was according to the AOAC (1965), from the formation of a chelate between the cation and the ethylenediaminetetraacetic acid (EDTA). An aliquot of 10 mL of the phosphorite sample dissolved in hydrochloric acid (1:100) was taken to a 100mL volume. Then, 25 mL of deionised water and 8 mL of a KOH (20 %) solution were added. Later, an amount of the calceine-KCl indicator, equivalent to the tip of a small spatula was added. Finally, the dissolution with a solution of disodium salt of EDTA was assessed. In order to prepare a standard solution of Ca, 0.625 g of $CaCO_3$ were weighed out, previously dried at 110 ºC during three hours, and they were dissolved in 6 mL of HCl (1:1). It was transferred to a volumetric flask of 1000 mL deionised water was used to fulfill the rest of the flask content. This solution contained 0.25 mg of Ca/mL.

The following formula was used to obtain data:

$$\% Ca = VCa \times FCa$$

$$F Ca = \frac{2.5}{V}$$

Where:

V Ca= volume of the EDTA solution used in the sample assessment

FCa= Factor of Ca

V= volume of the EDTA solution used in the assessment of the Ca standard solution.

Solubility of the P and Ca from the TGP in the hydrochloric and citric acids. In order to determine the solubility of mineral components in the hydrochloric and citric acids, 0.2 g of TGP were weigh and transferred to a volumetric flask of 250 mL, with dissolutions of citric (2 %) and hydrochloric (0.4 %) acid separately. The mixtures were homogenized and let to rest for 30 min. Later, they were filtered and the amount of P and Ca was determined, which were soluble in each of the supernatants.

Studies on comparison of DM consumption. Four lambs were used, with 30 kg of average live weight, with a 4x4 Latin square experimental design. Two levels of addition of the premixture formulated with TGP were evaluated, which replaces the DP (60 and 100 g), included in the 200 g of the feedstuff provided as protein supplement. During the fourth treatment, 60 g of the premixture formulated with phosphoric rock was included in the 200 g of feedstuff provided as supplement.

The apparent digestibility and consumption of DM were determined. A mineral premixture of feedstuff was used, which had a formula with imported DP. All the premixtures were manually prepared every day and were added to the feedstuff. Their composition is shown in table 1.

Table 1. Composition of the mineral premixtures used in the study

Premixtures	Composition
Premixture with DP (Control)	50 % DP 40 % NaCl 10 % Microelements
Premixture with TGP	60 % TGP 30 % NaCl 10 % Microelements
Premixture with "Venegas" phosphoric rock	60 % "Venegas" phosphoric rock 30 % NaCl 10 % Microelements

The animals received 2kg of low protein quality hay as food, with 89.67 % of DM, 6.96 % of ashes and 10 L of water. The intake of hay and water was determined, as well as the excretions of fresh and humid DM in each of them. The lambs were kept in the experimental stage for 36 d, with 5 days of adaptation for each diet and four days of samples collection for each treatment.

Statistical treatment. The statistical analysis of the results was performed through the Infostat system (version 5.1) (Balzarini *et al*. 2001).

Results and Discussion

The contents of P and Ca of the TGP were 18 and 28%, respectively. These results corresponded to the ones reported by Uzcátegui and Rodríguez (2007), who carried out studies in Venezuelan phosphoric rocks, and also coincided with the informed by Acosta (2009). However, these results are superior when comparing them to the ones of NRC (2005) for DP (P: 18.5% and Ca: 20-24 %), which has always been a source of reference for the supplementation of the macroelements in the animal diets.

As an indicator of P bioavailability, its solubility in citric acid (2 %) (Sindirações 2005 and Sakomura and Rostagno 2007) and in hydrochloric acid (0.4 %) was determined. The solubility of Ca was also determined. The values are represented in table 2.

The figures of solubility in acids were higher than the

Table 2. Solubility of the Ca and P from the TGP in the hydrochloric and citric acids.

Indicators	Solubility (%)	
	Ca	P
Citric acid	86.00	99.13
Hydrochloric acid	82.70	95.40

ones informed by Martínez de Acurero *et al*. (1993), who determined that the solubility measuring of an inorganic phosphate in several solvents can be considered also as a methodology for determining the biological availability of the elements of this phosphate.

According to Casanova (2007), the average relative bioavailability of P in the animal diet presents the following values: monocalcium phosphate (93-98 %), hydrated dicalcium phosphate (92-101 %), anhydrous tricalcium phosphate (86 %), deflourinated tricalcium phosphate (95-96 %), triple phosphate of sodium, calcium and magnesium (96 %), urea phosphate (90-96 %), bone meal (90 %), phosphoric rock (20-50 %), calcium, iron and aluminum phosphates (15 %), regarding the monosodium phosphate (100 %). This bioavailability varies with the specie and, at the same time, represents a potential use of the phosphoric rocks in the food portions, that can totally or partially substitute the traditional source of imported DP.

Cornejo *et al*. (1998) stated that the quality controls performed to the phosphates used in the animal diet include, among other requirements, the solubility of P in citric acid (2 %) to be superior of 95 %. The solubility of P in this study fulfills this regulation.

Acosta *et al*. (2009), in studies carried out with TGP, reported 98.83 % of solubility values of P in citric acid. In this study, the results of the test were higher than the ones referred by Acosta (2009). Likewise, the values of solubility of P in citric acid (2%) also surpassed the values of DP.

Table 3 shows the results for the consumption and digestibility of DM, regarding the applied treatments. According to Covacevich (2001), the consumption of DM in a lamb is between 3 and 3.5 % of its LW. There has to be taken into account that the level of voluntary intake of forage depends on the dynamic interactions that involve the animal, the microbial population and the provided forage (Orskov 1991). The values obtained did not differ from the control.

DP was replaced by the phosphoric rock, a consumption of 3.1 % regarding the average LW was achieved. Results are located in the interval referred by Covacevich (2001). Therefore, the replacement of the DP from the feedstuff by the TGP did not affect the consumption or the apparent digestibility of DM in these animals.

The values of apparent digestibility of DM had interest results. No significant differences were found with regard of the control during the treatment with supplementation of 60 g of mineral premixture with TGP.

There were no significant differences during the treatment with 100g of premixture either and the same happened when including the phosphoric rock.

When comparing the two treatments where the DP was replaced by TGP, significant differences ($P < 0.05$) were found according to the apparent digestibility of DM. When supplementing with 100 g of premixture, higher percents of digestibility were obtained. In this case, animals received 10.8 g of P through the premixture. However, they only received 6.48 g of P during the supplementation with 60 g.

It is possible that the concentration of P in the 60 g of premixture did not cover completely the needs of ruminal microorganisms. That is why the microbial activity could be increased with respect of the ruminal microorganisms from the animals that consumed 100g of premixture. During this treatment with 100g of premixture the microorganisms covered mainly the requirements of P, and could develop a superior cellulolytic activity. This brings as result a higher degradation of the cell walls.

In a study of Ramírez-Pérez and Meschy (2005) it is stated the importance of P for the activity of the ruminal microorganisms. These authors refer that these ruminal microorganisms need this macroelement. Besides, they give examples of the increase and decrease of the cellulolytic activity before the differences in the concentration of P. The treatment with phosphoric rock had higher percentages of apparent digestibility of DM when comparing them to the control. The concentration of P and Ca was not determined for this rock. These

Table 3. Behavior of the apparent digestibility and intake of the DM during the different treatments.

Indicator	Treatments (g premixture/200 g concentrated)				SE ± Sign
	DP (60)	TGP (60)	TGP (100)	Venegas (60)	
DM intake (kg/animal/d)	1.09	0.96	0.98	0.95	1.07
DM digestibility (%)	45.61^{ab}	40.63^{a}	48.75^{b}	49.60^{b}	1.81*

^{ab}Values with different letters within the same line differ significantly at $P < 0.05$ (Duncan 1955) *$P < 0.05$

The consumption of DM for the control treatment represented 3.6 % of the average LW. The true consumption was of 3.2 % for the first treatment with TGP.

The treatment, which included 100 g of premixture and 200 g of feedstuff, provided as a protein supplement, had results of 3.3 % of the DM consumption. When the results suggest the need of further studies with this source.

Regarding the results of this study, and considering the values of solubility in the acids and the apparent digestibility of DM, it can be concluded that the biological availability of the P from TGP is acceptable for its use in the diet of ruminants.

It is possible to replace DP by TGP in the formulation of mineral premixture in feedstuff for the diet of lambs. The source presented high amounts of P and Ca and high values of solubility in the analyzed acids. The consumption of DM and its apparent digestibility were not affected by the replacement. The availability of P is acceptable for its use in the animal diet. Among the analyzed treatments the highest value of digestibility was achieved with 100 g of the premixture formulated with TGP, except the phosphoric rock which presented higher values.

Acknowledgements

The authors thank the work of George E. Vasallo Jamet, DMVZ, for the support provided for these studies.

References

AOAC 1965. Official Methods of Analysis. Ass. Off. Agric. Chem. Washington, DC.

Acosta, A. 2009. Evaluation of the phosphorite from the Trinidad de Guedes (TG) deposit as potential source of phosphorus in laying hens. Cuban J. Agric. Sci. 43:161

Acosta, A., Lon-Wo, E., Cárdenas, M., Febles, M., Dieppa, O., & Almeida, M. 2009. Determination of relative phosphorus bioavailability in phosphorite from the Trinidad de Guedes deposit through tests of growth and bone mineralization in chickens and laying hens. Cuban J. Agric. Sci. 43:53

Amaral, A. 1972. Técnicas analíticas para evaluar macronutrientes en cenizas de caña de azúcar. Laboratorio de Nutrición de la Caña. Escuela de Química. Universidad de La Habana. Cuba

Balzarini, M., Casanoves, F., Di Rienzo, J. A., González, L. A. & Robledo, C. W. 2001. Software estadístico: InfoStat, versión 5.1. Manual de usuario. Universidad Nacional de Córdoba, Argentina.

Casanova, O. 2007. Efecto de las rocas fosfóricas naturales y modificadas sobre la cantidad y calidad de pastos introducidos en Venezuela. Agronomía Trop. 57:271

Cornejo, S., González, N., González, J., Camus, J., & Pokniak, J. 1998. Efectos del empleo de tres diferentes fuentes de fósforo en las dietas, sobre los rendimientos productivos de pollos broiler. Arch. Med. Vet. 30:2

Covacevich, N. 2001. Protección y recuperación de los pastizales de la XÍI Región. Servicio Agrícola y Ganadero - Región de Magallanes y Antartica Chilena. Centro Regional de Investigación Kampenaike. I Simposio Internacional de la Federación de Ovejeros y Cabreros de América Latina (FOCAL).

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11:1

Gutiérrez O., Savón, L., Ojeda, A., Fajardo, H., Cair,o J.G., Crespo, G., Mejías, R., García-López, R., Oramas, A., Fraga, S., Alvarez, A., Monteagudo, F. & Viamonte, M.I. 2009. Fuentes minerales cubanas: impacto en la alimentación de monogástricos y rumiantes. Premio Academia de Ciencias de Cuba.

Martínez de Acurero, M., Capó, E., Chicco, C.F., Godoy de León, S., Acurero, G., & Quintana, H. 1993. Digestibilidad *in vitro* de la celulosa utilizando diferentes fuentes de fósforo. Zoot. Trop. 11:3.

NRC 2005 Mineral Tolerances of Animals. 2 Ed. National Academy of Sciences. Washington, DC.

Orskov, E.R. 1991. Manipulation of fibre digestion in the rumen. Proc. Nutrition Society 50:187

Ramírez-Pérez, A. & Meschy, F. 2005. Requerimiento de fósforo de los microorganismos ruminales: Una revisión. INCI. 30:11

Sakomura, N.K. & Rostagno, H.S. 2007. Métodos de pesquisa em nutrição de monogástricos. Universidade Federal de Viçosa. 282 pp.

Sindirações. 2005. Compêndio Brasileiro de Alimentação Animal. Ed. Sindicato Nacional da Industria de Alimentação Animal. São Paulo, SP. 204 pp.

UECAN. 2008. Unión de Empresas Combinado Avícola Nacional. Informe de datos Técnicos de Producción. Cuba. 30 pp.

Uzcátegui, L. & Rodríguez N. 2007. Evaluación de la roca fosfórica en la alimentación de cerdos en fase de engorde. APPA-ALPA-Cusco, Perú. Sitio Argentino de Producción Animal.

Received: November 13, 2012