# THE EFFECT OF POLYETHYLENE GLYCOL (PEG) ON PROTEIN OUTPUT OF FREE RANGE ALENTEJANO PIGS

**M. I. Ferraz de Oliveira, M. Cancela d´Abreu and A. Freitas**

ICAM, Departamento de Zootecnia, Universidade de Évora - Polo da Mitra, Apartado 94, 7002-554 Évora Portugal

**SUMMARY -** The effect of PEG treated or untreated acorns fed to Alentejano pigs, on the protein output of the animals were studied. The animals had access to two sown pasture fields based on *Trifolium incarnatum* and *Lolium westerwoldicum* Four dietary treatments were tested with 5 pigs per treatment in a two by two factorial experimental design (factor 1: sown species; factor 2: acorn with or without PEG). Intake of acorns and protein faecal concentration were measured individually. Faecal output was estimated using an external faecal marker (dotriacontane, $C_{32}$).
Crude protein faecal concentration of animals fed acorns treated with PEG was significantly lower ($P<0,05$) then those fed untreated acorns, suggesting that the PEG may have increased the availability of dietary protein to the animals. However, the estimation of protein faecal output using the n-alkane $C_{32}$ was not significantly affected by the PEG treatment, even though, for the animals in the *Lolium westerwoldicum* pasture, there was a decrease ($P=0,063$) in the total excretion of CP when acorns were treated with PEG.

**KEY WORDS:** Alentejano pigs; protein output; PEG; acorns

## INTRODUCTION

The Alentejano pig, is a breed generally rose under free range conditions and fattened on “Montanheira”, (pasture under evergreen oaks) using the available natural feed resources, mainly acorns and pasture. Acorns have a high content in tannins (Cantos *et. al*, 2003), which are plant polyphenols with the capacity to form strong insoluble complexes with proteins and also other compounds such as starch and cellulose. Therefore, tannins have been reported to reduce protein digestibility and increase faecal nitrogen excretion in mammals (Mangan, 1988). The low Alentejano pig production in “Montanheira” associated with years of low pasture production, suggests that herbage as a relevant role on the detoxification of tannins from acorns (Almeida, 1986). PEG has a very high affinity to tannins and has been widely used to neutralise the deleterious effects of dietary tannins on nutrient digestibility, particularly protein.

Therefore, an experiment was designed to test the effect of PEG on the protein output of animals fed with acorns and with access to two sown pasture fields (*Trifolium incarnatum* and *Lolium westerwoldicum*). This experiment was part of a larger study, carried out to evaluate the effect of pasture intake on diet utilisation by Alentejano pigs.

## MATERIAL AND METHODS

Twenty castrated male Alentejano pigs with an average live weight of 90 kg were allocated to four homogeneous groups balanced by weight and placed in 4 paddocks with access, in each paddock, to a shed with five pens for individual distribution of acorns. Paddock one and two were sown with *Trifolium incarnatum* and paddock three and four with *Lolium westerwoldicum*.

Acorns from *Quercus rotundifolia* were collected from the ground and frozen. Acorns were distributed *ad libitum* to the animals, individually, in two meals per day (8:30 and 15:30) and refusals collected also individually. Animals in paddock one and three received acorns treated with polyethylene glycol (PEG 6000) (12,5g PEG/Kg acorns) and animals in paddock two and four received acorns without treatment. Therefore, four dietary treatments were tested with 5 pigs per treatment in a two by two factorial experimental design. Factor 1: pasture sown species; Factor two:

acorn treatment with or without PEG. The animals stayed in the 4 paddocks, with access to the same diet, for 33 days. During the data collection period, a faecal marker (dotriacontane, C32) spread on 60g doses of ground acorns was distributed individually in the first meal of the day. The data collection period comprised 5 days of adaptation to the faecal marker and 5 days of refusals total collection and faecal samples collection. Refusals were weighed, oven dried, ground (1mm Ø) and pulled per animal. Faecal samples were frozen, freeze dried, ground and also pulled per animal until needed for analysis.

Nitrogen was determined in all samples by the Dumas method in a LECO system (FP528). N-alkanes were extracted and determined by GC-FID as in Dove and Mayes (2006). Tannins in acorns were measured by a protein precipitation method, the radial diffusion assay (Hagerman, 1987). The extraction of tannins from acorn was done with a solution of acetone (70%). Intake of acorns was measured individually and protein faecal concentration was determined. Faecal output was estimated using dotriacontane as an external faecal marker. Diet composition was estimated by the n-alkane technique using the least-squares diet composition package EatWhat (Dove and Moore, 1996). Estimation of pasture intake was done by the n-alkane technique Dove and Mayes, 1991), considering the diet composition previously estimated. The data produced was subjected to a factorial ANOVA using the STATISTICA (data analysis software system), version 7 (StatSoft, Inc., 2004).

## RESULTS AND DISCUSSION

Dry matter (DM), organic matter (OM) and crude protein (CP) content of the feed resources available for the animals are presented in Table 1. All calculations were done on OM basis, since some animals had high amounts of ashes in the faeces indicating that variable amounts of soil were ingested. Acorns had 40,1g/kgOM of tannins measured as equivalents to tannic acid (radial diffusion assay).

As expected the CP content of acorns was low what resulted in a low CP intake due to the large percentage of acorns in the diet. Both pastures were in the first stage of development (vegetative state) and therefore with a quite high CP content even on the grass pasture. Pasture 2 had a greater quantity of clover and so its CP content was also the highest.

Table 1: Chemical composition of diet ingredients, acorns and pasture.

| | Acorns | Pasture | | | |
|---|---|---|---|---|---|
| | | Based on *Trifolium incarnatum* | | Based on *Lolium westerwoldicum* | |
| | | 1 | 2 | 3 | 4 |
| DM (g/kg) | 617,7 | 299,3 | 158,5 | 199,4 | 204,6 |
| OM (g/kgDM) | 980,5 | 595,5 | 761,1 | 743,7 | 777,3 |
| CP (g/kgOM) | 59,2 | 201,9 | 269,3 | 191,0 | 161,8 |

Individual intake of acorns (Table 2) was measured directly on the feed troughs and did not differ among experimental groups. Intake of pasture tend to be higher on the *Lolium westerwoldicum* than the *Trifolium incarnatum* based pasture, however this was not statistically significant, probably due to the high standard error observed (0,14). Estimated intake of OM and CP was not different among the four treatment groups.

Crude protein faecal concentration of animals fed acorns treated with PEG was significantly lower ($P<0,05$) then those fed untreated acorns. However, this did not result in a lower total protein excretion when PEG was used, due to an observed increase in the organic matter faecal output and a significant decrease on OM digestibility.

The CP balance (input-output) was negative in all treatments except for animals on *Lolium* pasture and acorns with PEG. The negative CP balance was probably due to the low CP content of diet (about 74g/kgOM) and also to the formation of insoluble tannin protein complexes. It is interesting to notice that the only treatment where the CP balance was positive is associated with the highest intake of pasture and, therefore, the highest CP intake (Table 2).

Tabe 2: Intake and output of OM and CP of animals fed acorns treated or untreated with PEG with access to two different pastures

| | **Pasture** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Based on *Trifolium incarnatum* | | Based on *Lolium westerwoldicum* | | | Probability of effect | | |
| | A+PEG 1 | A-PEG 2 | A+PEG 3 | A-PEG 4 | SE | pasture | PEG | Pasturex PEG |
| Intake of acorns kgOM/d | 3,2 | 2,8 | 2,8 | 2,9 | 0,14 | NS | NS | NS |
| Estimated pasture intake kgOM/d | 0,17 | 0,27 | 0,65 | 0,32 | 0,14 | NS | NS | NS |
| Estimated CP intake g/d | 241,8 | 232,7 | 265,1 | 223,9 | 20,15 | NS | NS | NS |
| Faecal CP gCP/kgOM | 224,8 | 291,3 | 203,2 | 304,6 | 15,65 | NS | 0,000 | NS |
| Faecal output gOM/d | 1236 | 848 | 1273 | 1028 | 120 | NS | 0,019 | NS |
| Protein output gCP/d | 269,9 | 241,3 | 257,6 | 305,8 | 19,18 | NS | NS | NS |
| Estimated OM digestibility (%) | 63,4 | 72,2 | 63,2 | 67,8 | 2,99 | NS | 0,041 | NS |

[1]A+PEG= acorns treated with PEG; [2]A+PEG= acorns untreated with PEG; [3]SE=Standard error

## CONCLUSIONS

PEG treatment did not affect the protein digestibility and reduced OM digestibility. Estimated intake of OM and CP was not different among the four treatments groups although intake of *Lolium westerwoldicum* based pasture tended to be higher. The very low total protein intake was probably responsible for the lack of effect of PEG on the total protein output. The reduction of OM digestibility associated with PEG treatment is difficult to explain and needs further investigation.

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