# PEROXIDE INTAKE AFFECTS THE RELATIONSHIP BETWEEN GLUTATHIONE PEROXIDASE ACTIVITY AND SOME PRODUCTION PARAMETERS IN RABBIT

MEZES M.[1], VIRÁG GY[2], BARTA M.[1], BERSÉNYI A[2], NOFAL R.[2]

[1] Department of Nutrition, Gödöllõ University of Agricultural Sciences, H-2103 Gödöllõ, Hungary
[2] Department of Rabbit Breeding, Institute for Small Animal Research, Gödöllõ, H-2101, Hungary

**Abstract** - The effect of subchronic alimentary lipid peroxide loading on the correlation between some production traits and glutathione peroxidase activity of blood plasma and red blood cell haemolysate was investigated in growing rabbits. It was found that the correlation was not significant among the calculated production trait parameters - feed intake, daily weight gain, feed conversion - and glutathione peroxidase activity in the case of healthy rabbits but moderate or close correlation was found in the case of lipid peroxide loading. The results suggested the effect of the lipid peroxide loading on the production traits in rabbits without clinical signs of toxicity.

## INTRODUCTION

Weak and moderate linear relationship have been reported earlier between glutathione peroxidase (GSHPx) enzyme activity of particular tissues and some production traits such as daily weight gain of growing rabbits (MEZES *et al.*, 1994a) and sperm motility of bucks (VIRAG *et al.*, 1992) in a New Zealand White population. Effect of pathological conditions like as acute enteritis (MEZES *et al*, 1986), high peroxide value of feed (MEZES *et al*, 1994b) or elevated temperature (MEZES *et al*, 1993) on the enzyme activity have also been demonstrated.
The effect of feeding oxidised oil on production traits - like as daily weight gain in the case of guinea pig (MIYAZAWA *et al*, 1986) and rabbit (SLIM *et al*, 1995) was described earlier.
Rabbits as model animals ideal for such kind of investigation because it was also found that rabbits are very susceptible to oxidative stress fed different high-fat diets (SLIM *et al.*, 1995).
The purpose of present study was to investigate the effect of subchronic alimentary lipid per-oxide loading on some production traits and also calculate the correlation between some performance traits and the glutathione peroxidase enzyme activity in normal and lipid peroxide loaded conditions.

## MATERIAL AND METHODS

### Experimental animals

The experiment was started at 10 weeks of age of New Zealand White (NZW) half sib broiler rabbits and lasted three following weeks. Twenty rabbits were divided into two groups. One group received experimental diet with high lipid peroxide value, the other given normal diet. Body weight, feed consumption and enzyme activity were measured at days 0th, 7th, 14th and 21st of the experiment. The gut weight was also measured at the end of the investigated period. Weight gain, feed consumption and feed conversion on the 1st, 2nd and 3rd weeks of treatment were calculated.

### Experimental diet

The peroxide content of the experimental diet was induced with adding of oxidised sunflower oil containing grinded corn at 2 % (w/w). The grinded corn was treated with 4 % (w/w) of sunflower oil and the mixture was oxygenated for 24 hours at 60 $^{o}$C.
The nutrient content also the peroxide- and acid value of the feed was determined according to the Hungarian National Standard methods (Hungarian Feed Codex, 1988).
The nutrient content of feeds are shown on Table 1.

**Table 1 : Nutrient content of feeds**

| Nutrient content | Control diet | Experimental diet |
|---|---|---|
| Dry matter | 90.50 % | 90.80 % |
| Ash | 8.90 % | 8.10 % |
| Crude protein | 19.97 % | 19.33 % |
| Crude fat | 3.40 % | 3.70 % |
| Crude fibre | 14.96 % | 12.14 % |
| Nitrogen-free extract | 43.91 % | 47.35 % |
| Acid value (meq /kg fat) | 12.30 | 99.20 |
| Peroxide value (meq/kg fat) | 40.30 | 249.05 |

**Determination of glutathione peroxidase enzyme activity**

Glutathione peroxidase (E.C. 1.11.1.9) activity was measured in blood plasma and red blood cell haemolysates. Blood plasma was separated by centrifugation at 1500 rpm, 4°C for 10 minutes. Erythrocytes were haemolyzed with nine-fold volume of redistilled water. Blood plasma and the RBC haemolysate protein content was determined by the biuret method (GORNALL and BARDAWILL, 1949). Glutathione-peroxidase activity was measured in presence of reduced glutathione and cumene-hydroperoxide as substrates using an end-point direct assay (MATKOVICS *et al.*, 1988). The enzyme activity was expressed in units reflecting the oxidation of reduced glutathione in nmoles per minute at 25 °C and was calculated to the protein content.

**Statistical analyses**

Effects of treatments were evaluated by analysis of variance, phenotypic relationship between enzyme activity and performance traits by correlation .

## RESULTS AND DISCUSSION

**Table 2 : Blood plasma and RBC haemolysate glutathione peroxidase activities (U/g protein) at 0th, 7th, 14th and 21st days of alimentary lipid peroxide treatment (mean ± S.D.)**

| Days | blood plasma | | RBC haemolysate | |
|---|---|---|---|---|
| | normal | peroxide loaded | normal | peroxide loaded |
| 0 | 1.30 ± 0.57 | 1.41 ± 0.40 | 1.53 ± 0.52 | 1.94 ± 0.71 |
| 7 | 2.11 ± 1.27 | 2.98 ± 1.17 | 2.41 ± 0.90 | 3.22 ± 1.38 |
| 14 | 0.70 ± 0.23 | 1.76 ± 0.31 | 2.37 ± 1.33 | 2.06 ± 0.55 |
| 21 | 3.10 ± 0.54 | 1.09 ± 0.33 | 2.62 ± 0.65 | 2.09 ± 0.74 |

**Enzyme activities**

Blood plasma and RBC haemolysate enzyme activity values in the groups consuming normal or high peroxide content diet at the start and on the subsequent weeks of treatment are shown on Table 2. Main significant variation of blood plasma enzyme activity was due the sampling time as presented in Table 3. In the case of group receiving high peroxide content feed the sampling time can be considered as the duration of the treatment. Since the glutathione peroxidase activities are highly elevated at the end of the first week and then continuously decrease (Figure 1) this could be explained as a defence reaction against lipid peroxide loading (MEZES *et al.*, 1994b) first and possible enzyme inhibition and/or destruction later because of the continuous peroxide formation (BESTERVELT *et al.*, 1995). The statistically significant and close treatment type x sampling time interaction seems to support this theory. One of the possible causes of the differences among the weeks of the treatment at the group receiving normal diet would be the recently showed circadian and circannual oscillation of the lipid peroxide formation (SOLAR *et al.*, 1995).

**Table 3 : Significance of the sources of variation, considering the blood plasma enzyme activity values**

| Source of variation | SS | df | MS | F | P-value | F crit. |
|---|---|---|---|---|---|---|
| Type of food | 0.0004 | 1 | 0.0004 | 0.0008 | 0.9775 | 3.9909 |
| Period of treatment | 21.074 | 3 | 7.0248 | 13.976 | 4E-07 | 2.7482 |
| Interaction | 26.714 | 3 | 8.9046 | 17.715 | 2E-08 | 2.7482 |

**Production traits**

Total feed consumption for 20 days was significantly ($P<0.1$) lower in the group consuming high peroxide content diet as was compared to the control one. Total body weight gain, overall feed conversion rate and the

weight of gut on 21st day of the experiment did not differ statistically as it is summarised in Table 4. The absolute value of weight gain was higher in lipid peroxide loaded group. The possible cause of that interesting result would be the different nutrient content of high peroxide content feed - in this respect absolutely higher fat and carbohydrate content - which affect the energy content of feed as well. The positive effect of isoenergetic oil supplementation was also found on the production traits in rabbits using low per-oxide content corn oil (Van MANEN *et al.*, 1989).

**Figure 1 : Average blood plasma enzyme activity values according to the groups consuming normal (n) and peroxided (p) food on the 1st, 2nd and 3rd weeks of treatment**

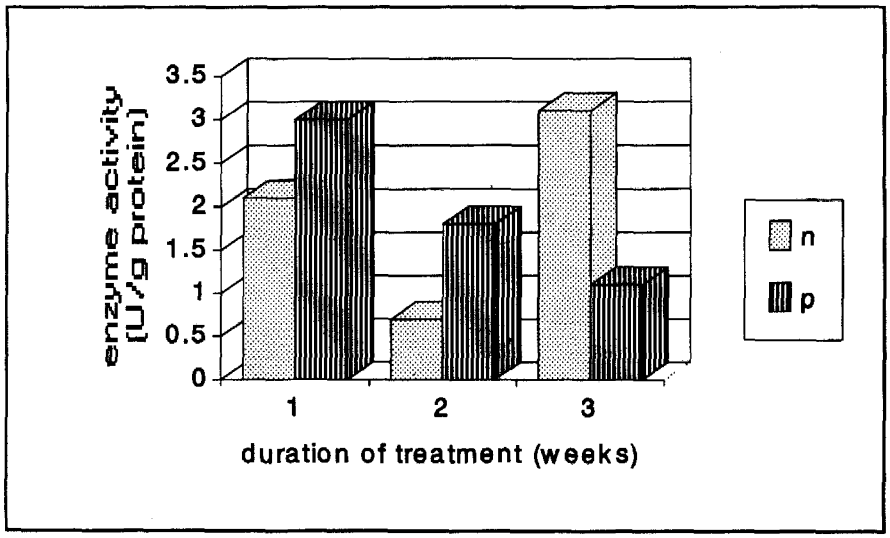



**Table 4 : Effect of subchronic alimentary lipid peroxide loading on some production traits of rabbits as calculated for the whole period of the experiment (mean ± S.D.)**

| Treatments | Total feed consumption (dkg) | Average daily weight gain (g) | Average feed conversion (kg/kg) | Gut weight on the 21st day (dkg) |
|---|---|---|---|---|
| normal | 343.67[a] ± 19.07 | 20.31 ± 4.87 | 8.46 ± 2.15 | 48.61 ± 6.06 |
| peroxide loaded | 318.61[b] ± 25.54 | 22.97 ± 4.70 | 6.93 ± 2.22 | 45.00 ± 3.11 |

a and b in the same column denote significant difference between values at P<0.1.

Interactions between treatment and week of experiment however can be seen (fig.2a and 2b) particularly at the group receiving high peroxide content diet although the differences were not statistically significant. In that group food consumption, daily weight gain decreased continuously. Furthermore, the worst feed utilisation was produced at the last week of the experiment.

**Figure 2a : Average body weight gain (dkg) in groups receiving normal (n) and peroxided (p) diet on the 1st, 2nd and 3rd weeks of the treatment**

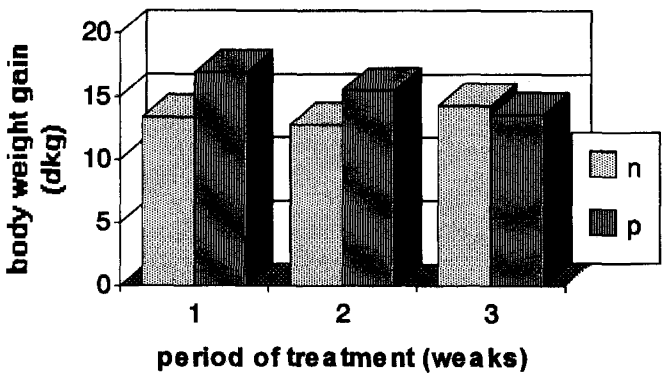
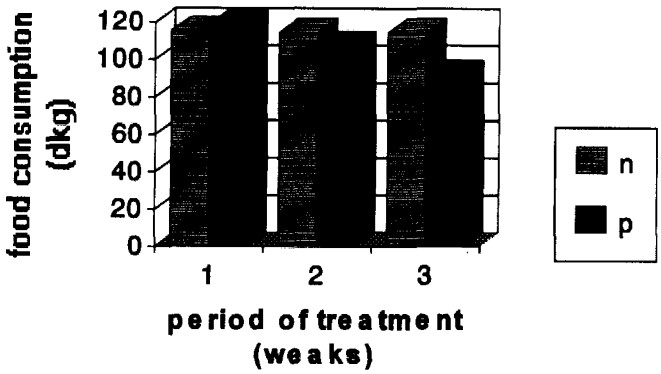


**Figure 2b : Average food consumption (dkg) in groups receiving normal (n) and peroxided (p) diet on the 1st, 2nd and 3rd weeks of the treatment**




## Interactions between production traits and enzyme activity

At the group receiving normal diet only weak to moderate, mainly not significant , correlations were found between production traits and enzyme activity values (data not presented). However at the group treated with high peroxide level diet and only on the 3rd week of the experiment moderate to strong correlations were found as it presented in Table 4.

**Table 4 : Correlation coefficients between production trait and enzyme activity values on the 3rd week of alimentary lipid peroxide loading**

| Treatment | Sample | Body weight gain | Feed intake | Feed conversion |
|---|---|---|---|---|
| normal | blood plasma | 0.07 ns | -0.02 ns | -0.13 ns |
| | RBC hemolysate | 0.23 ns | 0.53 P<0.1 | -0.07 ns |
| peroxided | blood plasma | -0.80 P<0.01 | -0.69 P<0.05 | 0.57 P<0.1 |
| | RBC hemolysate | -0.78 P<0.01 | -0.80 P<0.01 | 0.75 P<0.05 |

Although the consistence of these results is weak, the relationship between GSHPx activity and some productive parameters becoming evident after a certain period of peroxide loading is recently detected. At the group consuming high peroxide level diet stronger correlations were found not only as was compared to the values of the control group but also as it was compared to those which have been found in random normal populations which were applied in our previous investigations. The explanation of the increase of closeness of the linear relationship between GSHPx enzyme activity and production traits as an effect of oxidative stress required more experiments.

**Acknowledgement**: This work was supported by the National Scientific Research Fund (OTKA T 006474).

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