Journal of Animal and Feed Sciences, 6, 1997, 343–351

Seasonal variation of glutathione peroxidase, CK and AST in sheep in a low-selenium region

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(Received 27 December 1996; accepted 19 August 1997)

ABSTRACT

Blood glutathione peroxidase (GSH-Px; E.C. 1.11.1.9) activity, a measure of selenium status, as well as the plasma indicators of muscle damage creatine kinase (CK; E.C. 2.7.3.2) and aspartate aminotransferase (AST; E.C. 2.6.1.1) were evaluated in sheep in a region with low selenium levels during the spring and the summer, because this time may be critical for suffering selenium deficiencies. GSH-Px activity was highest during the spring, when mean values ranged from 74.16 and 90.51 UI/g Hb, descending drastically during the summer, below physiological levels ($P < 0.001$) with mean values ranging from 43.33 to 50.01 UI/g Hb. GSH-Px activity, within the deficiency range, registered during the summer did not originate muscle damage, since the muscle enzymes CK and AST were within their normal range.

KEY WORDS: enzymes, selenium, ewes

INTRODUCTION

The biochemical role of selenium was demonstrated by Rotruck et al. (1973) to be a component of the enzyme glutathione peroxidase (GSH-Px. EC 1.11.1.9). GSH-Px functions in cellular oxidation-reduction reactions to protect the cell membranes from oxidative damage caused by free radicals and peroxides (Flohe et al.,1973).

Selenium deficiency in sheep manifests itself in a variety of ways, the classical one being white-muscle disease, but it also brings about reduced growth rates, ill

thrift, infertility, suboptimal wool yields and periodontal disease (Sanson, 1990). These manifestations frequently occur in the same areas and chemical analyses of the pasture samples suggest that the conditions might be associated with low levels of selenium in the soil (Zachara et al., 1989).

Seasonal variations of Se level in pastures have been observed, being specially low or critical during the spring (Hunter et al., 1982; Blood et al., 1992), since at this time pasture was actively growing, and Se is in a lower ratio in the herbage. Other authors have also reported seasonal trends in the selenium status of animals, showing that tissue and blood selenium concentrations were at a minimum during the spring and the summer (Braun et al., 1991; Scholz, 1991; Capaul et al., 1992; Koh et al., 1992). This seasonal influence is specially relevant in regions with marginal to deficient Se level in the soils, where this mineral decreases drastically in the tissues of the animals with the occurrence of clinical manifestations of selenium deficiency (Wheatley and Beck, 1988; Zachara et al., 1989).

Determining the concentration of selenium in soils, pastures, as well as in blood and other animal tissues is both time consuming and costly. However, selenium concentration and glutathione peroxidase are highly correlated in the blood of sheep, with this enzyme being in turn a rapid and cheap indicator of selenium status (Mackintosh et al., 1989; Hamliri et al., 1990).

The objective of this experiment was to examine the selenium status of sheep in a region with low selenium levels (López Alonso,1995), during the spring and the summer, since this period may be a critical time for suffering selenium deficiencies. For this purpose whole blood GSH-Px activity, as a good indicator of the protection that the animal has against oxidative damage, as well as the plasma indicators of muscle damage, creatine kinase (CK) and aspartate aminotransferase (AST), were evaluated.

MATERIAL AND METHODS

Animals

Twenty pure native ewes of Gallega breed ranging from 2 to 4 years of age were selected at random from a flock in a low-selenium area of the province of Lugo (NW Spain). The animals were examined from April until September 1994. For the previous 3 months and during the experiment the ewes were kept on natural pastures (a mixture of white clover and rye-grass) without any supplementary feed, minerals or vitamins.

Blood samples were drawn, by jugular venepunction, at monthly intervals. Each sample was placed into heparinized tubes and tubes for serum separation,

and kept refrigerated for transportation to the laboratory. All samples were assayed within 12 h of sampling.

Analytic procedures

GSH-Px was measured with a commercial kit (Ransel, Randox, UK), based on the method of Plagia and Valentine (1967). Enzyme activity was expressed in international units per gram of haemoglobin (UI/g Hb). Haemoglobin was determined by the standard cyanmethaemoglobin method (Hainline, 1958).

Serum creatine kinase (CK) and aspartate aminotransferase (AST) activities were expressed in international units per liter (UI/l) and were assayed using kinetic procedures with commercial reagents (Knickerborcker, SAE; Croma-test® Reagents).

Statistical analyses

One-way analysis of variance (Fisher's F test) was used to test for the changes of the analysed parameters, and for differences between seasons (Snedecor and Cochran, 1978). Differences were considered significant at $P<0.05$.

RESULTS

GSH-Px activity showed a strong seasonal variation (Table 1). An increase of GSH-Px activity was observed during the spring, from 74.16 ± 7.20 UI/g Hb at the beginning of the experiment, to a maximum value of 90.5 ± 8.66 UI/g Hb in June. In the following month this enzyme decreased drastically by 50%

TABLE 1

Changes of GSH-Px activity (UI/g Hb) and serum CK and AST activities (UI/l) during the study period, expressed as mean ± standard error

	Months					
	April	May	June	July	August	September
GSH-Px	74.16 ± 7.20	85.11 ± 15.44	90.51 ± 8.66	43.33 ± 4.88*	49.90 ± 6.10	50.01 ± 9.18
	39.0 – 140.3	9.5 – 200.9	55.5 – 198.1	14.4 – 78.0	10.4 – 111.6	3.7 – 137.0
CK	86.25 ± 11.66	64.38 ± 5.22	59.39 ± 5.09	116.59 ± 11.3*	81.38 ± 9.23*	81.76 ± 11.10
	45.4 – 208.0	33.0 – 111.4	24.8 – 94.9	53.6 – 198.1	45.4 – 155.2	31.3 – 208.0
ASAT	85.56 ± 2.65	81.31 ± 3.35	71.82 ± 2.74	72.44 ± 3.39	69.75 ± 3.06	64.25 ± 1.85
	62 – 104	61 – 104	55 – 92	50 – 99	55 – 104	56 – 82

* $P<0.05$

TABLE 2
Scales in whole blood GSH-Px activity and selenium concentration used to diagnosis selenium status in sheep (Sáez et al., 1995)

Selenium concentration μg/ml	GSH-Px activity UI/gHb	Selenium status
<0.05	<60	deficient
0.05-0.1	0-120	marginal
>0.1	>120	adequate

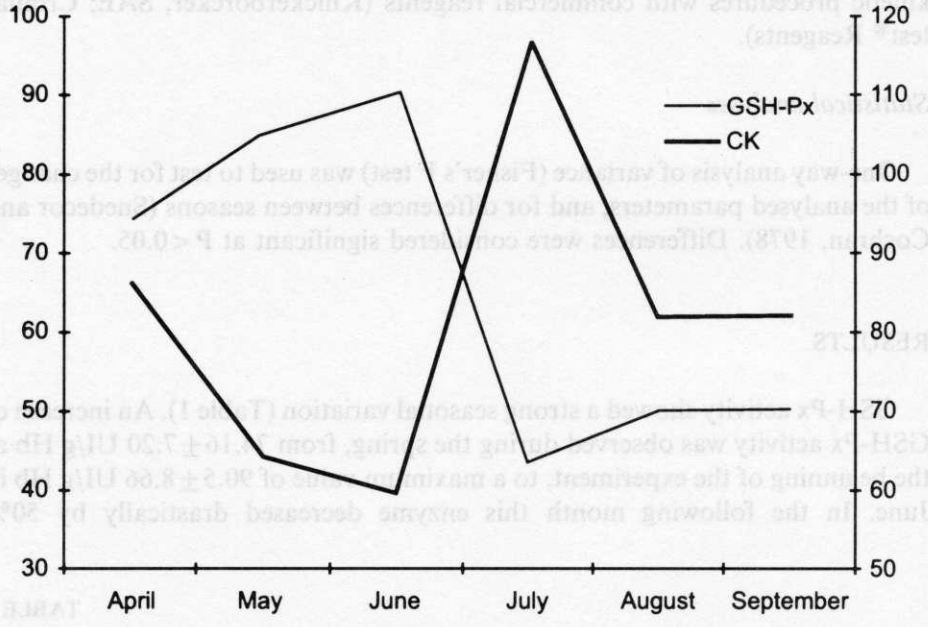

Figure 1. Changes of GSH-Px (UI/g Hb) and CK (UI/l) activities during the study period

TABLE 3
Mean values of GSH-Px (UI/g Hb), CK and AST (UI/l) activities during the spring and the summer, expressed as mean ± SE

	GSH-Px	CK	ASAT
Spring	77.29 ± 4.90	70.01 ± 4.80	79.57 ± 1.86
Summer	47.75 ± 3.96***	93.24 ± 6.46**	68.81 ± 1.68***

** $P < 0.01$
*** $P < 0.001$

($P < 0.05$), reaching the minimum value (43.33 ± 4.88 UI/g Hb). During the subsequent period GSH-Px activity showed a slight recovery, but remaining at deficient levels for sheep (Table 2). The wide range of values found during all the experiment indicate large individual variations of the GSH-Px activity.

Although in some cases the activity of the plasma indicators of muscle damage, CK and AST, was above the physiological levels (< 100 UI/l), the mean values were usually within the normal range (Table 1), except in July for CK ($P < 0.05$), where the mean value was 116.59 ± 11.39, coinciding with the lowest GSH-Px activity registered. During the whole experiment CK activity showed a marked seasonal variation, the evolution being inverse to the GSH-Px activity (Figure 1).

Finally, GSH-Px activity, as well as the plasma indicators of muscle damage, CK and AST, showed significant differences between both seasons (Table 3).

DISCUSSION

The majority of the selenium (75%) is in the erythrocytes as glutathione peroxidase (Oh et al., 1974). For this reason, variations in the level of selenium in the pasture, and therefore in the animal diet, are going to show up in the blood GSH-Px activity. Our data indicated a strong seasonal trend in GSH-Px activity; while in the spring this enzyme activity is marginal, during the summer it is below the physiological levels for sheep according to Saez et al. (1995). These results do not agree with Bedo et al. (1992) and Andrés et al. (1994), who have registered the lowest GSH-Px activities in the spring, with these observations coinciding with low selenium levels in the pastures. Nevertheless, we must consider that while selenium concentrations in serum and plasma correlated highly with the rate of oral or parenteral administration and respond quickly to changes in Se intake, whole blood selenium, as well as GSH-Px respond more slowly than serum (or plasma) to changes in selenium intake (Nicholson et al., 1991; Stowe and Herdt, 1992). This is because the majority of the glutathione peroxidase in whole blood is incorporated into the red blood cells at the time of erythropoiesis. So a complete response in whole blood Se, or GSH-Px activity, to a Se change in the diet will require therefore a time span equal to the average life span of the erythrocytes, which, in the case of sheep is approximately 120 days. This fact is specially evident when a Se supplementation is administered, since the whole blood selenium or GSH-Px need 8 weeks approximately to reach maximum activity (Wheatley and Beck, 1988; Hamliri et at., 1993). Considering this fact we could explain the adequate GSH-Px activity showed during the spring, which would correspond with high Se levels in pasture during the winter (Hunter at al., (1982), and then, how in the summer the GSH-Px activity was low, representing

a critical Se concentration in pasture during the spring in this area. Wheatley and Beck (1988) also reported that the GSH-Px activity decreased drastically at the end of the spring in a region with low levels of selenium. This fluctuation in GSH-Px activity suggests that Se status of ewes cannot be assumed to be constant throughout the year, and therefore, to diagnose a flock as marginal or adequate in selenium, or in need of supplementation, sampling must occur when the ewes are most likely to be in a deficient status. Several authors (Wheatley and Beck, 1988; Andrés et al., 1994) have also showed large individual variations, with animals included at the deficient, marginal and adequate categories of GSH-Px in the same herd.

Although in our study GSH-Px activity is below the physiological levels during the summer, within a deficient range, it did not cause important muscle damage, since CK and AST activities were usually within the normal range. This deficient GSH-Px activity may be compensated by an adequate level of vitamin E in the forage. In this study vitamin E status was not assessed. However ewes were grazing on fresh herbage, considered as an adequate source of vitamin E (Maas et al., 1984; Hamliri et al., 1993), suggesting that vitamin E status was adequate. It is well known that vitamin E and Se-GSH-Px activity are the most important components of the antioxidative defence mechanisms, capable of substituting for one another. In this sense, Garce and Clarck (1991) have showed that Se-responsive diseases do not occur at the same selenium status in different regions, suggesting that these differences in Se requirements may be partly explained by the varying vitamin E intakes. Nevertheless, it must be considered that during situations where an important oxidative stress occurs, such as stages of development, late gestation or lactation, infectious processes, etc., these two antioxidative systems could overload, with the appearance of clinical manifestations of selenium deficiency.

We have found differences in CK and AST activities between both seasons. Braun et al. (1993) and Andrés et al. (1994) have also described similar seasonal trends for these enzymes. This variations are, in their point of view, in relation with changes of the diet of the animals. The fact of finding CK activities slightly above the normal range when GSH-Px activity showed a marked descent indicates that this critical concentration of GSH-Px could cause slight subclinical peroxidative damage in cell membranes. As a consequence of this, alterations in the permeability of the membranes occurs, bringing about the liberation of this cytosolic enzyme into the blood stream, and which finally results in a slight increase in plasma CK activity. The AST evolution was different, showing the lowest activity during the summer, just when the GSH-Px activity was at the minimum value. This behaviour confirms that AST is not as specific as CK as marker of muscle damage, since it is widely distributed in different organs and tissues (Blood et al., 1992; Smith et al., 1994). The inverse changes of

GSH-Px-CK activities could also indicate that this later enzyme may be used as a good monitor of muscle damage. In this sense different authors have showed that CK is one of the most specific parameters of acute muscle damage in all species, reporting also that CK activity was highly correlated to muscle damage (Blood et al., 1993; Braun et al., 1993; Smith et al.,1994).

CONCLUSIONS

GSH-Px activity registered during the summer, within a deficient range, indicates that the animals were constantly at risk from selenium-deficiency diseases. However, this low activity did not originate muscle damage, since the enzymes CK and AST were within their normal range, indicating that it may be compensated by an adequate level of vitamin E in the diet.

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STRESZCZENIE

Sezonowe zmiany aktywności peroksydazy glutationu, kinazy kreatynowej i transferazy asparaginianowej u owiec z rejonu niedoboru selenu

Oznaczono aktywność peroksydazy glutationu (GSH-Px; E.C.1.11.19), jako wskaźnika zaopatrzenia organizmu w selen, oraz kinazy kreatynowej (CK; E.C. 2.7.3.2) i transferazy asparaginianowej (AST; E.C. 2.6.1.1), jako wskaźników uszkodzeń mięśni, u owiec pochodzących z rejonu o niskiej zawartości selenu. Oznaczenia wykonano wiosną i latem, bowiem te pory roku są krytyczne dla występowania niedoboru Se.

Aktywność GSH-Px była największa wiosną; średnie wartości wahały się od 74,5 do 90,51 UI/g Hb, obniżając się drastycznie w ciągu lata do poziomów poniżej fizjologicznych ($P < 0{,}001$), od 43,33 do 50,01 UI/g Hb. Nie stwierdzono jednak w tym czasie uszkodzeń mięśni, gdyż oznaczona aktywność enzymów CK i AST mieściła się w granicach norm fizjologicznych.