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SURVEY OF POLYUNSATURATED FATTY ACIDS, VITAMIN E AND SELENIUM IN LIVESTOCK AT PASTURE

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SUMMARY

A survey of sheep and cattle at pasture in 3 regions of South Australia showed that significant quantities of polyunsaturated fatty acids (PUFA) may pass through the rumen unhydrogenated, as a consequence of higher PUFA in the pasture, resulting in increased PUFA concentrations in the plasma. Animals were grazing predominantly dry feed during the autumn sample period and green pasture during the winter sample period, which was reflected in changing plasma vitamin E concentrations. Regional differences in the selenium status of sheep was evident.

Despite significant quantities of PUFA being absorbed by the animals in the survey they were at little risk of developing PUFA-induced myopathy due to the high concentrations of the antioxidants plasma vitamin E and blood selenium. The animals in the survey were observed to be in good physical condition throughout the sample period.

Keywords: polyunsaturated fatty acids, vitamin E, selenium, sheep, cattle.

INTRODUCTION

Nutritional myopathy in livestock can result from a combination of raised levels of dietary polyunsaturated fatty acids (PUFA) and depressed levels of vitamin E and selenium (Rice and Kennedy, 1988). These authors showed that significant quantities of PUFA were absorbed by housed cattle, depleted of vitamin E and selenium, when turned out to spring pasture. It was hypothesised that these animals were at risk to nutritional myopathy despite the high level of vitamin E in the pasture.

The present survey was undertaken to examine the relationship between selenium, vitamin E and PUFA in the blood of livestock during the dry autumn and wet winter seasons.

MATERIAL AND METHODS

Animals

In 1992 blood samples were taken from Merino heggets in 3 regions of South Australia (Mid-north, Adelaide Hills, Mallee) and from Angus cattle in the Adelaide Hills. The regions were chosen because they are important livestock producing areas of South Australia, but all have different geographic characteristics. The Adelaide Hills has an annual rainfall of 1000-1200 mm, with acidic soils, the Mid-north has red-brown alkaline soils with an annual rainfall of 400-500 mm and the Mallee has sandy-loam alkaline soils with an annual rainfall of 300-350 mm. Within each region 5 properties were included in the survey. At each property blood samples were taken from 10 animals in March when they were grazing predominantly dry feed and again in August when grazing green pasture. In August 10 lambs and 10 calves were also sampled.

At the time of blood sampling mixed herbage samples were randomly collected from the area grazed by the stock. The pasture sample was assayed for vitamin E, selenium and PUFA concentrations.

Blood and pasture samples

Samples were assayed for alpha-tocopherol using the high performance liquid chromatography (HPLC) technique described by Judson *et al.* (1991). Selenium was assayed fluorometrically using the technique described by Koh and Benson (1983). Polyunsaturated fatty acids were analysed using HLPC, essentially following the method of Babidge and Babidge (1994). Free PUFA were assayed in plasma samples. Plasma samples were protein precipitated with acetonitrile containing nonadecanoic acid (C19) as an internal standard. Fatty acids were extracted into ethyl acetate containing butylated hydroxytoluene as an antioxidant. Total fatty acids were assayed in pasture. Samples were digested with ethanolic potassium hydroxide containing ascorbic acid and Cl9 as an internal standard. The extracts were dried over magnesium sulphate and monodansylcadaverine was added before evaporating to dryness. The residue was taken up in a solution of dicyclohexylcarbodiimide and allowed to stand overnight. The solution was evaporated and taken up in 75% acetonitrile. An aliquot was injected onto the HPLC. Chromatographic conditions were as follows: Cl8 column; mobile phase a gradient of 75% to 95% acetonitrile containing dibutylamine pH 3 over 45 minutes; fluorescence detection Ex228/Em534.

RESULTS

All properties had animals with plasma concentrations of alpha-tocopherol above 1 mg/L (Table 1). On most properties lambs had a lower mean plasma vitamin E concentration than adult sheep. Within the Adelaide Hills cows had higher mean plasma vitamin E concentrations than sheep at both sampling times. Plasma vitamin E concentrations in calves varied markedly.

The blood selenium concentrations showed differences between regions (Table 1). Hoggets in the Mid-north region had a blood selenium concentration of around 2 μmol/L, while hoggets in the Mallee had mean blood selenium levels up to 5 μmol/L. Within the Adelaide Hills region, animals from some farms had a mean selenium concentration less than 0.5 μmol/L blood. Selenium concentrations were lower in the second sampling in all regions except the Mallee. In all regions lambs had lower mean whole blood selenium concentrations than the hoggets. None of the animals used in the survey had been given any form of selenium supplement.

The plasma linoleic acid (C18:2(c9,12)) did not have any trend between or within regions (Table 1). When lambs were sampled, they had similar values to the hoggets. Within the Adelaide Hills cattle had a lower linoleic acid concentration than sheep at both sampling periods (Table 1). Calves had similar mean plasma concentrations to the yearling animals.

The analysis of plasma linolenic acid (C18:3(c9,12,15)) showed that on most properties there was an increase in mean concentration at the August sampling (Table 1). In the Mallee and Adelaide Hills lambs had a higher mean plasma linolenic acid concentration than yearling animals, but in the Mid-north the yearling animals were higher. Within the Adelaide Hills cattle had a lower mean linolenic acid concentration than sheep at both sampling periods.

Linolenic and linoleic acids made up approximately 70% of the total fatty acid composition of the pasture sample assayed. A summary of the range in vitamin E, selenium and PUFA concentrations in the pasture appears in Table 1.

The pasture dry matter percentage (Table 1) is indicative of the type of pasture the animals were grazing at the time of sampling. It can be seen that in the first sample period animals in the Mallee and Mid-north were grazing dry pasture while in the Adelaide Hills some groups of sheep and cattle had access to green pasture.

DISCUSSION

The survey showed that in all regions sampled, the pasture grazed by the animals contained significant quantities of PUFA. Increased plasma PUFA concentrations in stock were generally associated with increased concentrations of the corresponding acids in the pasture (Table 1). The presence of PUFA in plasma suggests that PUFA are passing through the rumen unhydrogenated. Rice and Kennedy (1988) found that in cattle turned out to pasture the linoleic acid levels decreased as the linolenic levels increased. The PUFA concentrations they obtained were also higher than those in the present study, but they measured total PUFA and not the free form as was measured in the present study. In our study both linoleic and linolenic acid levels were usually higher in green pasture. In the Mallee and Mid-north regions animals had slightly higher mean plasma free fatty acid concentrations during the second sampling period (Table 1), although these values were in the normal range of 0.2-1.0 mmol/L for sheep (Russel and Doney 1969). Unseasonal autumn rains in the Adelaide Hills allowed animals access to some green pasture as well as dry pasture during the first sampling period, so comparisons between plasma PUFA levels of livestock grazing dry and green pasture could not be made within that region.

At both sample times there was little difference between the plasma vitamin E concentration of sheep from three regions, even though some of the March pasture samples had low vitamin E concentrations. This shows the importance of measuring the alpha-tocopherol level in the animal as the level in the feed is not always a good indicator of the status of the animal. The level of vitamin E in the pasture was higher during the second sampling than the first when animals were grazing dry feed. At both sampling periods sheep and cattle had apparently normal vitamin E concentrations with mean values over 1 and 2 mg alpha-tocopherol/L plasma respectively. Rammell and Cunliffe (1983) reported that these values are the lower normal values for each species although Hidiroglou *et al. (1992)* suggested that plasma alpha-tocopherol concentrations above 4 mg/L are indicative of adequate vitamin E status in cattle. In the August sampling period, when lambs were included, there was no difference in mean plasma vitamin E concentration between hoggets and lambs. When calves were sampled during August there was a large variation in the mean vitamin E values between farms. This may have been due to the stage of development of the calf at sampling. The animals that were still suckling had a lower plasma vitamin E concentration than animals that were either weaned or were consuming pasture.

Selenium status of sheep, as indicated by whole blood selenium concentration, differed between the 3

Table 1. The range in mean concentrations of vitamin E, selenium and PUFA in blood of livestock and in mixed pasture samples in 3 regions of South Australia

Region Species	Mallee Sheep	Mid-north Sheep	Adelaide Hills Sheep	Adelaide Hills Cattle
Plasma vitamin E (mg/L)				
March-yearling	1.0-4.3	1.9-2.9	2.4-2.6	4.5-9.5
August-yearling	2.1-3.4	2.0-2.5	2.2-2.8	5.4-8.5
August-lambs/calves	1.1-2.2	1.5-2.0	2.2-3.0	2.2-6.6
Blood selenium (μmol/L)				
March-yearling	4.3-4.9	1.9-2.9	0.5-1.0	0.2-0.8
August-yearling	3.9-5.5	1.4-2.9	0.4-0.8	0.2-0.3
August-lambs/calves	1.5-4.9	0.9-1.4	0.3-0.5	0.2-0.9
Plasma linoleic acid (μmol/L)				
March-yearling	25-40	18-33	21-35	9-23
August-yearling	17-76	19-26	19-53	9-20
August-lambs/calves	23-27	13-41	24-58	10-30
Plasma linolenic acid (μmol/L)				
March-yearling	1-12	5-19	12-32	7-18
August-yearling	15-47	15-61	17-27	13-19
August-lambs/calves	19-89	17-22	15-33	8-16
Plasma total free fatty acids (μmol/L)				
March-yearling	430	490	540	360
August-yearling	630	930	610	370
August-lambs/calves	1050	510	690	290
Pasture vitamin E (mg/kg DM)				
March-yearling	3-8	7-14	2-12	8-18
August-yearling	89-160	36-110	37-108	33-123
August-lambs/calves	70-139	80-90	26-113	33-166
Pasture selenium (μmol/kg DM)				
March-yearling	1.5-2.1	0.3-0.8	0.3-0.9	0.3-3.0
August-yearling	1.6-3.1	0.6-1.3	0.5-1.3	0.3-0.9
August-lambs/calves	0.5-2.7	0.7-2.6	0.8-1.3	0.3-1.2
Pasture linoleic acid (mmol/kg DM)				
March-yearling	4-22	12-21	3-22	10-14
August-yearling	14-45	10-14	6-17	2-13
August-lambs/calves	8-14	10-11	7-19	3-24
Pasture linolenic acid (mmol/kg DM)				
March-yearling	1-7	4-15	15-68	36-43
August-yearling	18-61	41-73	22-36	18-30
August-lambs/calves	27-71	34-44	18-56	23-135
Pasture total fatty acids (mmol/kg DM)				
March-yearling	17-57	46-64	56-80	81-129
August-yearling	37-98	64-105	55-62	51-61
August-lambs/calves	69-183	55-107	46-134	61-167
Pasture Dry Matter (%)				
March-yearling	76-90	86-89	22-76	17-56
August-yearling	21-25	22-31	26-59	20-46
August-lambs/calves	17-40	23-37	11-24	20-46

regions. Region differences in selenium levels would be due to differences in initial selenium levels in the soil, soil pH, rainfall, pasture composition and fertiliser practice. Blood selenium concentrations in sheep above 0.5 μmol/L and above 0.25 μmol/L in cattle are regarded as indicating adequate selenium status (Judson *et al.* 1987). Sheep in the Mallee and Mid-north had high blood selenium concentrations, in the order of 4 μmol/L and 2 μmol/L respectively. Within the Adelaide Hills, sheep were in the marginal range of 0.25 to 0.5 μmol/L, and some cattle were below the normal blood selenium concentration, indicating they may be at risk to selenium deficiency. There was no clear distinction in mean selenium concentrations between the March and August sampling.

The results indicate that PUFA were absorbed by the livestock at pasture, but the levels were within the documented range. The vitamin E status of the animals was above the level reported to prevent vitamin E deficiency disorders. There were regional differences in selenium status but in most cases animals were within the adequate range. Under the conditions in this survey it appears animals were at little risk of developing PUFA induced myopathy. However, it is important to assess both vitamin E and selenium to determine the cause of nutritional myopathy. This has been shown in southern Australia where low alpha-tocopherol and low selenium levels have been associated with the development of nutritional myopathy (Steele *et al. 1980;* Watson *et al. 1988).*

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