The effect of preserving liver tissue in formalin on the concentration of trace minerals in the liver

by

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I declare that this thesis for the degree Magister Scientiae (Agriculturae) at the University of Pretoria has not been submitted by me for a degree at any other university.

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"Commit thy way unto the Lord; trust also in him; and he shall bring it to pass."
Ps 37: 5

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List of Abbreviations

Cu	copper
Zn	zinc
Mn	manganese
Se	selenium
Co	cobalt
s.e.	standard error
s.d.	standard deviation
mg/kg	milligrams/kg
mg	milligram
kg	kilogram
mg/l	milligrams/litre
l	litre
ng/g	nanograms/kilogram
ng	nanograms
g	gram
ng/ml	nanogram/millilitre

ml	millilitre
MMA	methylmalonic acid
AAS	atomic absorption spectrophotometry
GPX	glutathione peroxidase
VLG	viral leukoencephalomyelitis of goats

Definition of terms

Deficient: Levels at which clinical or pathological signs of deficiency should be apparent (Underwood & Suttle, 1999).

Marginal: Levels at which subclinical effects may prevail, such as reduced immune response, or reduced growth rate (Underwood & Suttle, 1999).

Adequate: Levels sufficient for optimum functioning of all body mechanisms with a small margin of reserve to counteract commonly encountered antagonistic conditions (Underwood & Suttle, 1999).

High: Levels well above normal but not necessarily toxic (Underwood & Suttle, 1999).

Toxic: Levels at which subclinical, clinical or pathological signs of toxicity would be expected to occur (Underwood & Suttle, 1999).

Normal: Used where deficiencies are unknown, indicates normal background levels (Underwood & Suttle, 1999).

Abstract

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The concentrations of trace minerals (Mn, Cu, Co, Zn and Se) were examined after formalin preservation of 31 sheep and 5 impala livers over differing storing periods (one month, three months and six months). Under field conditions liver samples are often preserved in formalin until micro mineral analysis in the laboratory.

Analyses of trace minerals, expressed on dry basis, were done using atomic absorption spectrophotometry after wet ashing of the liver.
After one month of preserving of livers in formalin, the Mn concentration was significantly ($P<0.05$) lower than the concentration in fresh liver. It was found that at three months of preservation of livers in formalin the Zn and Co concentration were significantly ($P<0.05$) higher and the Mn and Cu concentration were significantly ($P<0.05$) lower compared to the concentration in fresh liver.

The Mn, Co and Cu concentrations were significantly ($P<0.05$) lower after six months of storage in formalin compared to fresh liver. The difference in the concentration between fresh liver and liver stored in formalin was small. It would not have any effect on the interpretation of the relative mineral concentration.

Mineral determinations using atomic absorption spectrophotometry was also done on the formalin in which the liver was preserved. There was a significant ($P<0.05$) increase in the mineral concentrations from pure formalin to formalin in which liver was stored for all the time periods. This was probably due to leaching of the minerals.

An additional investigation was also done to determine if there was a difference between the dry matter % of fresh (90.73 % DM) and formalinised (92.1% DM) liver. The dry matter % increased significantly ($P<0.05$) from fresh liver to liver that was preserved in formalin for all the time periods.

Samevatting

Die invloed van die preservering van lewer weefsel in formalien op die konsentrasie van mikro-minerale in die lewer

deur

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Die invloed van die preservering van 31 skaap en 5 impala lewers in formalien oor verskillende periodes (een maand, drie maande en ses maande) op mikromineraal konsentrasies (Mn, Cu, Co Zn and Se) in die lewer, is ondersoek. Onder veldtoestande word lewer monsters algemeen gepreserveer in formalien tot wanneer dit in die laboratorium geanaliseer kan word.

Die mineraal bepalings, uitgedruk op 'n droë basis, is gedoen met behulp van atoom-absorpsie spektrofotometrie nadat nat verassing op die lewer gedoen is.

Die Mn konsentrasie was betekenisvol ($P<0.05$) laer in die lewers wat gepreserveer is in formalien vir een maand in vergelyking met die Mn konsentrasie in vars lewers. Na drie maande van preservering van lewers in formalien was die Zn en Co konsentrasies betekenisvol ($P<0.05$) hoër en die Mn en Cu konsentrasies betekenisvol laer as die konsentrasies in vars lewer. Die Mn, Co en Cu konsentrasies was betekenisvol ($P<0.05$) laer na ses maande van preservering van lewer in formalien. Die verskil in die konsentrasie tussen vars lewer en lewer wat in formalien gepreserveer is, was klein. Dit sal geen invloed hê op die interpretasie (of die mineraalvlak toksies is en of daar 'n tekort is) van die betrokke mineraal konsentrasie nie.

Mineraal bepalings op die formalien waarin die lewer gepreserveer was, is ook gedoen deur middel van atoom-absorpsie spektrofotometrie. Daar was 'n betekenisvolle ($P<0.05$) toename in al die minerale konsentrasies vanaf suiwer formalien na formalien waarin lewer gepreserveer is vir al die tydperke. Dit was moontlik a.g.v. loging van die minerale.

'n Verdere ondersoek is ook gedoen om te bepaal of daar 'n verskil was tussen die droë materiaal % van vars lewer (90.37 % DM) en lewer wat gepreserveer was in formalien (92.1 % DM). Die droë materiaal % het betekenisvol toegeneem vanaf vars lewer na lewer wat in formalien gepreserveer is, vir al die tydperke.

Introduction

'Most of the trace minerals can be measured accurately some of the time. Some of the trace minerals can be measured accurately most of the time. Most of the trace minerals are not measured accurately most of the time.' (Mertz, 1987).

The liver has been analysed for trace minerals more often than any other internal organ, mainly since variations in dietary uptake are more readily reflected in the liver, which acts as the main storage organ for some of the minerals (Theron *et al.*, 1973).
It has received special attention as a sample source, because the liver is the body's metabolic centre, and most minerals are integral portions of metallo-enzymes, which serve as catalysts for metabolic processes (Boyazoglu, 1976). Hepatic concentrations of trace minerals are commonly used to estimate trace mineral storage pools because dietary intake is rarely available and nutrient interactions affect availability or retention (Thomas *et al.*, 1994).

The liver is the main storage organ for copper and its copper concentration has been found to vary enormously (Widdowson & Dickerson, 1964). Low levels of the copper in the livers of cattle are the result of a primary deficiency, when the diet is inadequate, or a secondary (conditioned) deficiency, when the dietary intake is sufficient, but the utilization of the copper is impeded for example, by the interaction of molybdenum and sulphate (Ehret *et al.*, 1975).

Manganese is not concentrated in any particular organ or tissue. The concentrations in the liver are, however, higher than most other tissues and can be raised or lowered with varying manganese intake. The manganese storage capacity of the liver is limited when compared with the remarkable capacity of this organ to accumulate iron and copper (Widdowson & Dickerson, 1964; Ehret *et al.*, 1975).

The capacity of the animal to store zinc in any of its organs other than bones, is extremely limited so that animals do not normally carry large reserves of zinc. However, as a zinc deficiency develops, there is usually, but not invariable, a small decline in the concentration of zinc in the liver and certain other tissues (Underwood, 1966). High dietary levels of zinc give rise to large increases in bovine liver zinc concentrations (Ehret *et al.*, 1975).

Ehret *et al.* (1975) investigated the effect of formalin storage, over different time periods, on the copper, iron, manganese, zinc and magnesium concentrations in bovine livers. Theron *et al.* (1974) found no statistically significant differences for Cu, Fe, Mn, Zn and Mg concentrations after 22 days of formalin preservation.

The concentrations of Cu, Fe and Mg were also not affected by storage for six months in formalin, but statistically significant differences were detected in the concentrations of manganese and zinc after six months (Theron *et al.*, 1974).

Sullivan *et al.* (1993) found that tissue concentrations of Se and Cu remains unchanged by formalin-fixation and that the analysis of formalin-fixed tissues for diagnostic purposes can be recommended for Se and Cu.

Only a few studies have been done on this subject and under field conditions liver tissues are often preserved in formalin before trace mineral analysis in the laboratory. The aim of this investigation was to determine if the copper, cobalt, manganese, zinc and especially selenium concentrations in fresh and formalinised liver, from the same liver sample, differ significantly and if they are giving the same interpretation.