AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
Batch 42

1. SUBJECT CLASSIFICATION
A. PRIMARY: Food production and nutrition — AL30-0000-0000
B. SECONDARY: Animal nutrition

2. TITLE AND SUBTITLE
Methods of mineral analysis for plant and animal tissues

3. AUTHOR(S)
Fick,K.R.; Miller,S.M.; Funk,J.D.; McDowell,L.R.; Houser,R.H.

4. DOCUMENT DATE: 1976
5. NUMBER OF PAGES: 80p.
6. ARC NUMBER: ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
Fla.

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)
(In English and Spanish; Spanish,90p.:PN-AAC-768)

9. ABSTRACT

A manual that describes in detail methods of analyzing cattle blood and tissue to determine its mineral contents. It is a workproduct of a research contract entitled "Determination of Mineral Deficiencies and Toxicities for Cattle in Latin America." The research objectives are to determine the locations of mineral deficiencies in cattle in selected areas of Latin America, assess the economic benefits of mineral supplementation for grazing cattle, evaluate supplementation methods, and publish research information to stimulate the widespread use of mineral supplements. The vast majority of cattle in Latin America receive little or no mineral supplements. This contributes to low yields in cattle production. No single factor has as much potential for increasing livestock production at a relatively low-cost input as does adequate mineral nutrition. This publication describes standardized methods of analyzing mineral contents. Several different procedures and methods are suitable for analyzing mineral contents. The methods described in this publication are offered as guides; in some cases they will need to be modified to fit experimental conditions or particular laboratories. The first section deals with collection, identification, and storage of samples (blood, liver, bone, forage). Next discussed are procedures for preparing samples at the laboratory; preparation of standard samples; determination of phosphorus by a colorimetric procedure; and mineral analysis by flame atomic absorption spectrophotometer.

10. CONTROL NUMBER
PN-AAC-873

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Chemical analysis
Minerals
Mineralogy
Plant tissues
Tests
Tissues

13. PROJECT NUMBER

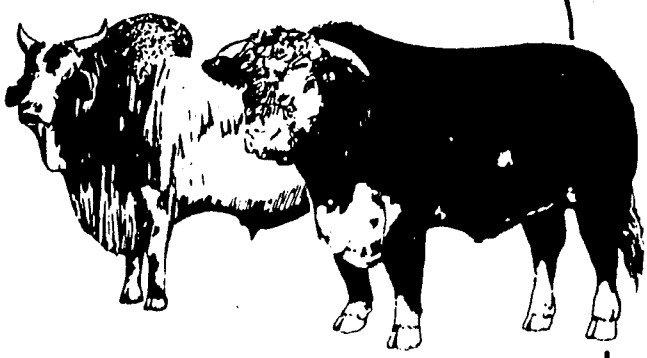
14. CONTRACT NUMBER
AID/ta-C-1153 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

AID/ta-C-1153 RCS
PN-AAC-873

Methods of Mineral Analysis for Plant and Animal Tissues



University of Florida Institute of Food and Agricultural Sciences
Center for Tropical Agriculture Animal Science Department

METHODS OF MINERAL ANALYSIS

FOR PLANT AND ANIMAL TISSUES

University of Florida
Institute of Food and Agricultural Sciences
Center for Tropical Agriculture
Department of Animal Science
In cooperation with the U. S. Agency for International Development

METHODS OF MINERAL ANALYSIS

FOR PLANT AND ANIMAL TISSUES

Prepared by

Karl R. Fick

Sarah M. Miller

John D. Funk

Lee R. McDowell

Richard H. Houser

Animal Science Department
University of Florida
Gainesville, Florida, U.S.A.

Latin American Mineral Research Program
Animal Science Department
Center for Tropical Agriculture
2001 McCarty Hall
University of Florida
Gainesville, Florida 32611
U.S.A.

In cooperation with the
U.S. Agency for International Development

1976

TABLE OF CONTENTS

Page

I. INTRODUCTION 101

II. SAMPLE COLLECTION IDENTIFICATION AND STORAGE. 201
A. Blood 201
1. Plasma 201-1
2. Serum 201-2
3. Precipitation of Plasma and Serum Proteins 201-2

B. Liver 201-3
1. Collection from Slaughtered Animals 201-3
2. Liver Biopsy Samples 201-3
a. Reference 201-3
b. Equipment and Supplies 201-4
c. Procedure 201-4

C. Bone 201-7

D. Forage 201-7
1. Collection Procedures 201-7
2. Drying 201-8
3. Grinding and Subsampling 201-8
4. Storage 201-8

III. SAMPLE PREPARATION AT THE LABORATORY 301
A. Cleaning Procedures 301
1. Crucibles 301
2. Glassware and Plasticware 301-1

B. Serum or Plasma 301-2
1. Equipment and Supplies 301-2
2. Calcium and Magnesium 301-2
3. Phosphorus 301-2
4. Zinc and Copper 301-2

C. Liver 301-3
1. Equipment and Supplies 301-3
2. Dry Matter 301-4
3. Pre-ashing or Wet Digestion 301-4
4. Ashing 301-5
5. Solubilization of Ash 301-5

D. Bone 301-6
1. Equipment and Supplies 301-6
2. Preparation 301-6

E. Forage 301-7
1. Equipment and Supplies 301-7
2. Dry Matter, Organic Matter and Ash 301-8
3. Solubilization 301-8

TABLE OF CONTENTS (cont.) Page

IV. PREPARATION OF STANDARDS 401
A. Procedure 401
B. Dilution of Samples and Standards 401-1

V. DETERMINATION OF PHOSPHORUS BY A COLORIMETRIC PROCEDURE 501
A. Reference 501
B. Reagents 501
C. Procedure 501
D. Calculations 501-1

VI. MINERAL ANALYSIS BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER . 601
A. Equipment and Supplies 601
B. Procedure 601
C. Macro-Element Determination 601-1
1. Sodium and Potassium 601-1
2. Calcium 601-2
3. Magnesium 601-2

D. Micro-Element Determination (Copper, Iron, Manganese, and Zinc) 601-2
E. Instrument Operation 601-4
1. Starting Procedure 601-4
2. Calibration Procedure 601-6
3. Turn-off Procedure 601-7
4. Lamp Change 601-7
5. Caution 601-8
6. Limitations 601-8

F. Reading Standards and Samples 601-8

G. Calculations of Results 601-9

VII. APPENDIX TABLES 701

VIII. REFERENCES 801

I. INTRODUCTION

The Center for Tropical Agriculture and the Department of Animal Science of the Institute of Food and Agricultural Sciences at the University of Florida, with the assistance of a contract from the Agency for International Development, are working collaboratively with Latin American institutions to promote mineral research in Latin America. The title of the contract is "Determination of Mineral Deficiencies and Toxicities for Cattle in Latin America" (AID-TA-C-1153). This research has the following objectives:
(1) to experimentally determine the locations of mineral deficiencies, adequacies, and toxicities in selected areas of Latin America, where grazing livestock predominate, (2) to establish, by experimentation, the biological response and economic benefit of mineral supplementation to grazing cattle, (3) to experimentally evaluate methods of mineral supplement administration for grazing cattle and (4) to publish and distribute research information in order to stimulate the widespread use of mineral supplements.

The project has been undertaken because a vast majority of the grazing cattle in Latin America receive little or no mineral supplementation. This contributes to low yields in cattle production throughout Latin America. No single factor has as much potential to increase livestock production in Latin America at a relatively low cost input as does adequate mineral nutrition.

The purpose of this publication is to describe standardized methods of mineral analysis procedures. There are a number of procedures and techniques suitable for analyzing minerals. Methods in this publication are offered as guides, and in some cases, they will need to be modified to fit the laboratory or the conditions of the experiments. Methods of sample collection identification, preparation, and analysis will be discussed. The atomic absorption technique is used for the determination of most minerals and a colorimetric procedure, for the analysis of phosphorus.

The authors acknowledge the following persons from the University of Florida for their ideas and help with this publication: C. B. Ammerman, J. E. Moore, J. H. Conrad, J. K. Loosli, R. L. Shirley, J. F. Easley, and J. E. Bell.

A large portion of the material has been adapted from procedures found satisfactory in accuracy and precision at the Animal Nutrition Laboratory, University of Florida, and taken from the following reference: R. L. Shirley, J. F. Easley, J. P. Feaster, C. B. Ammerman, and J. E. Moore. 1973. Equipment and procedures. Nutrition Laboratory, Department of Animal Science, University of Florida, Gainesville, Florida.

II. SAMPLE COLLECTION IDENTIFICATION AND STORAGE

The quantitative measurement of specific mineral content requires good sampling procedures, adequate identification, appropriate sample preparation, and application of suitable analytical techniques. No matter how accurate the analysis, the values obtained can be no better than the sample collected. Important considerations in sample collection include obtaining a representative sample avoiding contamination, and having the proper identification. Each sample should be related to a specific area, farm, or animal. All samples should be related to a questionnaire which describes the farm (table 1), animal (table 2), or forage sample (table 3). Sample containers or tags should be labeled with a permanent, waterproof and smearproof ink (i.e., Sharpie pen). Contamination errors are always a danger and may occur at any step until the final analysis is completed. Unclean containers, non-stainless steel instruments and air exposure are usually major sources of contamination in animal tissue samples, while the most important sources of contamination for forage are soil, and atmospheric dust, harvesting and grinding procedures. Contamination from the container used for blood and tissue samples can be minimized by soaking vessels overnight in a low-phosphate aqueous detergent solution. The containers (including caps) should be scrubbed with a brush and rinsed three times in tap water, three times in deionized water, three times with a 10% HCl solution and a final three rinses in deionized water. Since blood samples can become contaminated by anticoagulants and by the needle of the syringe used for collection, stainless steel needles are preferred. Tissue samples can be collected safely by careful dissection with stainless steel knives or scissors.

A. Blood

Representative samples of blood from cattle can be obtained conveniently by jugular puncture or tail bleeding with a needle and syringe or can be taken at the slaughterhouse once the animal's throat is cut. Needles of 18 gauge or

larger are recommended to prevent hemolysis. Ten to 50 ml of blood can be collected from each animal. Blood samples can be stored under refrigeration for several days or for longer periods if frozen. Whole blood should never be refrigerated or frozen if plasma or serum mineral concentrations are desired. It is necessary, therefore, to remove plasma or serum as soon as possible after the blood samples are taken. Deproteinized plasma or serum samples are more stable at room temperature for 2 to 4 weeks but may be frozen when a longer time lapse (over four weeks) between sample collection and analysis is expected.

1. Plasma: If blood plasma is desired, the collection tube should contain an anticoagulant and, as soon as the blood is collected, the container should be tipped (inverted) and thoroughly but gently mixed with the anticoagulant. Anticoagulants may be used in the following concentrations: heparin, .2 mg/ml blood; lithium and sodium citrates, 5 mg/ml blood; and lithium, sodium, and potassium oxalates, 1 to 2 mg/ml of blood. The anticoagulant of choice for mineral analysis should not cause hemolysis or contaminate the sample by adding an element which is subject for analysis. For example, sodium citrate and sodium and potassium oxalate should not be used if plasma is analyzed for sodium and potassium. There is some indication that the relatively fragile red cells of cattle are hemolyzed to some extent by heparin. Lithium citrate (20% w/v; .1 ml/10 ml blood) has given good results when used to prevent blood coagulation and may be an appropriate anticoagulant to use. Centrifuge the blood samples for 15 to 20 minutes at approximately 2,000 to 2,500 rpm. Centrifuge the samples within 24 hours after collection if possible. Remove plasma with pipettes being careful to lower pipette carefully so that cells will not be disturbed or aspirated. Expel plasma into a clean container. For macro-elemental analysis, pipette 1 ml into another test tube containing 9 ml 10% (w/v) trichloracetic acid (TCA) for precipitation of proteins. Stopper the plasma container, identify, mix by inversion or agitation, remove caps, centrifuge, and recap. Refrigerate or freeze for further use.

2. Serum: Serum collection is desirable in areas where refrigeration and centrifugation are not possible within 24 hours after sample collection. Collect 10 to 50 ml of blood from each animal in silicon-coated tubes. When blood samples are drawn with needles, best results may be obtained by using 15 gauge "California" bleeding needles and 20 ml tubes or vacutainers. Before obtaining blood sample, release the vacuum in vacutainers to prevent hemolysis. Allow the blood to clot and, as soon as sufficient serum is formed, usually about 8 or more hours, transfer the serum to another container. The transfer of serum can be accomplished using Pasteur glass pipettes with a 1 cc dropper bulb, being careful to lower pipette so that part of the clot will not be aspirated or red cell membranes ruptured. One ml of serum should be deproteinized as soon as facilities are available for macro-elemental analysis. The remaining serum sample can be labeled and refrigerated or frozen for future determination of micro-elements. Serum samples taken in the field should be kept cool in ice or refrigerated until taken to the laboratory. Pink serum samples are indicative of hemolysis and are unacceptable for mineral analysis; values for iron, zinc, magnesium, and potassium, in particular, may be high.

3. Precipitation of plasma and serum proteins: A protein-free filtrate must be used for the Fiske-Subbarow phosphorus analysis and macro-mineral atomic absorption analysis. Remaining serum or plasma will be used for micro-mineral analysis. If laboratory facilities are closeby, a good procedure for precipitation of plasma or serum protein is as follows:

a. Deliver 9 ml, 10% (w/v) trichloracetic acid (TCA) into labeled test tubes.

b. Pipette 1 ml of plasma or serum into this. The plasma or serum sample should be mixed immediately prior to pipetting.

c. Mix for 1 minute (capped) on vortex mixer. Let stand at least 10 minutes and centrifuge 10 minutes at 2500 rpm. Decant filtrate for long storage.

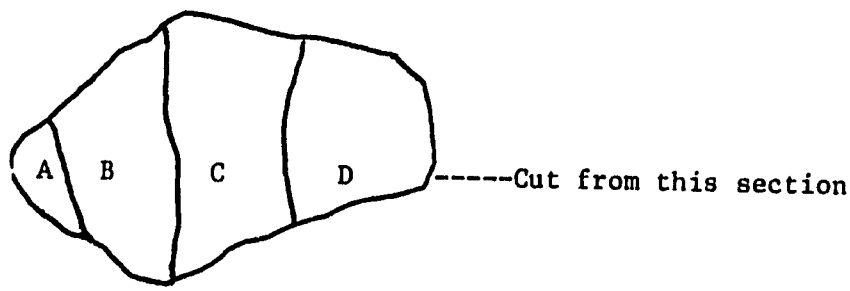
d. This filtrate represents a 1 → 10 dilution of the plasma or serum sample.

e. Standards should be diluted with TCA in the same manner at this time.

B. Liver

1. Liver collection from slaughtered animals: In order to prevent contamination, liver samples should be removed immediately from slaughtered animals as soon as the abdomen is opened. Every effort should be made not to touch the portion of the liver that is to be used as the sample. When possible, plastic gloves should be used. Approximately 50 to 100 g (fist size) of the right lobule (figure 1) of the liver is cut out with stainless steel scissors or a stainless steel knife.

Figure 1. Diagram of Bovine Liver



A. Caudate lobule (lobus caudate)

B. Left lobule (lobus sinister)

C. Middle lobule (lobus centralis)

D. Right lobule (lobus dexter)

The fresh liver may be stored frozen in labeled plastic bags or placed in 8 to 16 oz. polyethylene bottles containing 10% (v/v) formaldehyde until ready to prepare for analysis. In total volume, the size of the fresh sample would be about 4 cm^3. The polyethylene bottles should be approximately half full of 10% formaldehyde before the sample is introduced. Areas of the liver with heavy parasite damage should be avoided.

2. Liver biopsy samples:

a. Reference: Chapman, H. L., Jr., D. H. Cox, C. H. Haines and G. K. Davis. 1963. Evaluation of the liver biopsy technique for mineral nutrition studies with beef cattle. J. Anim. Sci. 22:733.

b. Equipment and supplies:

b-1 Some means to immobilize the animal (squeeze chute).

b-2 Clipper and extension cord (or razor to clip hair).

b-3 Wash bottle filled with 70% ethyl alcohol.

b-4 Scalpel or single-edge razor blade.

b-5 Trocar and cannula.

Trocar - Made of stainless steel with wooden handle. Overall length is 13½ in.; the blade is 9½ in. long and has a beveled point of approximately 1½ in.

Cannula - Made of stainless steel, 8 in. long with beveled edge on the distal end.

The inside diameter is approximately ¼ in., so that the trocar fits very snugly within the cannula when assembled.

b-6 100 ml graduated cylinder filled with 90% alcohol.

b-7 Wash bottle with deionized H_2O.

b-8 Cotton.

b-9 Paper towels.

b-10 Filter paper #40.

b-11 Sample bottles, labeling tape and pencil.

b-12 10% formaldehyde.

b-13 Wound dressing.

c. Procedure: Liver samples may be taken *in vivo* using the aspiration liver biopsy technique. It is an easy, simple operation with the main prerequisite being adequate means of immobilizing the animal. The procedure has been repeated every 28 days on the same animals, over a 2 year period, with no ill effects on animals. Aseptic conditions are important throughout the procedure. Another important consideration is to prevent contamination of the liver sample.

c-1 Rinse cannula and trocar with deionized water, then place the assembled trocar in the 100 ml graduated cylinder filled with 90% alcohol. Work the trocar in and out of the cannula several times to make sure it is well disinfected. Step c-1 is repeated each time just before the assembled trocar is inserted into the animal.

c-2 Immobilize the animal. Tranquilizers are unnecessary unless holding facilities are inadequate.

c-3 Hair is clipped over the 11, 12 and 13 intercostal areas, at approximately 6 to 8 inches ventral to the backbone (right side of the animal).

c-4 Area washed with 70% alcohol.

c-5 A vertical incision, approximately ½ in. in length is made through the hide parallel to the ribs, at approximately 6 to 8 inches ventral to the backbone in the 11th intercostal space (between second and third rib from the rear). The incision should be just large enough to admit the biopsy instrument. Preferably, use a single-edge razor blade to prevent injury to excited animals. In general, the use of anesthesia is time-consuming and unnecessary.

c-6 Insert assembled trocar, at about a 45° angle downward and gently but firmly, push through the connective tissue and diaphragm, directly into the liver.

c-7 Immediately after penetrating the diaphragm, the trocar should be removed from the cannula.

c-8 The cannula can then be pushed, gently but firmly, into the liver tissue with a rotating movement (liver penetration results in a characteristic feel and sound). This is continued until the instrument is entirely through the organ or resistance prevents

it from going any further.

c-9 Place thumb over the end of the cannula and gently withdraw the instrument through the liver (about 1½ in.), but not out of the liver. Change the angle of the cannula slightly and rotate the cannula inward and repeat steps (c-8 and c-9) two or three times to obtain a large sample. If cannula is pulled out of the liver in step (c-9), the cannula should be pulled out of the animal completely. If animal has not been injured and is not excited, the procedure may be repeated beginning at step (c-6).

c-10 When sufficient liver is in the cannula, pull it out of the animal, tilting the sample end to the level or slightly elevated plane as soon as possible and keeping the finger on the core of the cannula, creating a slight vacuum, so that liver sample is not lost. Push the liver sample out with the trocar and place it on #40 filter paper. The filter paper should not be removed from the box until ready since dust and dirt can easily contaminate the sample. Use the trocar to move the piece of liver around on the filter paper to remove the blood, then place biopsy in a labeled container.

c-11 After biopsy is obtained, apply wound dressing on the incision and release the animal. In screwworm areas, special repellents should be used in treating the wound.

c-12 The fresh biopsy may be stored frozen or preserved in 10% formaldehyde until ready to prepare for analysis.

Donor animals should be free of disease, older than 6 months, not pregnant, and at least one year on the farm to reflect mineral status. This technique is not suitable when a large sample is needed for mineral analysis. An average of .2 to .5 g of dry liver sample is obtained by an experienced person.

C. Bone

A sample of tail bones can be obtained with a knife or meat saw at slaughter. Remove two caudal vertebrae from the proximal end (large end) of a slaughtered animal. In smaller ruminant species, such as sheep and goats, the metacarpus (long bone attached to the hoof on anterior legs) can be taken at the time of slaughter. Bone samples can be stored frozen or placed in bottles containing 10% formaldehyde until ready to prepare for analysis.

D. Forage

1. Collection Procedure: In general, forage samples should be taken at least twice per year per farm. These samples should be collected near the end of the dry season and end of the wet season. Pasture samples should be collected only after careful observation of cattle grazing patterns. In some cases, it may be necessary to watch the animal graze and hand-pluck a sample to represent the animal's diet. Care should be taken to obtain samples representative of forage actually being consumed by the animal. Samples should not be taken from large clumps of grass which have obviously been rejected by the animals.

Separate samples of each of the major species should be taken. Samples can be collected in a cloth bag for immediate air-drying or in a plastic bag if oven-drying and can be carried out within several hours.

The sample should be collected with the aerial parts clipped using stainless steel scissors. The height of the cutting should be recorded and should represent the height that is being grazed. When possible, plastic gloves should be used. In sampling the aerial parts of the plants in a field, select at random at least ten or more sample sites from each field, depending on the size of the field. The combined samples should be a composite of the samples taken from different parts of a field and should weigh approximately 400 g fresh. Where wide differences between fields exist, each type of pasture or range (soil type + vegetation) should be sampled to establish the percentage of each of the major species.

2. Drying: Dry fresh forage in a forced-air oven at 45 to 60 C. Drying should be completed rapidly (i.e., within 8 hours or overnight). Use cloth bags rather than paper sacks. Allow samples to equilibrate with atmospheric moisture for 48 hours at ambient temperatures. Do not keep in airtight room or container during equilibration. Dry matter should be 88% or above.

3. Grinding and Subsampling: Sample contamination by grinding may result for certain trace minerals (i.e., copper, manganese, selenium, and zinc). If a sample is to be analyzed for copper, for example, grinding in a Wiley mill with brass screens may triple the copper content of a grass sample. Therefore, samples are best prepared for ashing by grinding in a clean Wiley (non-brass) mill with stainless steel screens, grinding with porcelain mortar and pestle or cutting with stainless steel scissors or knives. Air-dried samples are ground in a large Wiley mill to pass a 4 mm stainless steel screen, then subsampled and reground through a 1 mm stainless steel screen. Combine all material left in mill with the ground portion before subsampling. Take care to mix ground samples well before subsampling by placing them in a large plastic bag and rotating (bag should be no more than 1/3 full of dried ground sample with 2/3 air space).

4. Storage: Place about 30 g of the 1 mm ground material in a plastic bag (Whirl-Pak bag), seal tightly to prevent moisture changes in the laboratory during analysis, and label.

If additional analytical work is anticipated, and for precaution against accidental loss of the analytical sample, store up to 300 g of the coarsely-ground material in vermin and fumeproof containers. Make sure dry matter is 88% or above. If a preservative is found to be necessary to prevent mildew or insect growth, a naphtha or "moth" ball may be included in the container.

III. SAMPLE PREPARATION AT THE LABORATORY

Tissue and forage samples are oven-dried at 100 C and dry-ashed at 500 C. Non-liquid tissue samples generally require pre-ashing or wet digestion before samples are dry-ashed. The ash is solubilized in hot HCl and H_2O, filtered, and taken up quantitatively in 25 or 50 ml volumetric flasks with deionized water. Working standards are prepared to contain mineral concentrations comparable to the ranges expected in the sample solutions of μg/25 ml, μg/50 ml, mg/25 ml or mg/50 ml. For determination of the micro-elements copper, iron, zinc, and manganese, a multi-elemental background (matrix) solution is used for reference standards, which contains an ionic composition similar (within 25%) to that expected in the sample solutions. Further dilutions are made with an automatic dilutor when necessary. Mineral concentrations of the sample solutions are calculated according to sample weight and multiplied times 25 or 50 ml to obtain total quantity of mineral in the sample. When sample and standard solutions are diluted alike and absorbance values of the undiluted standards are used as references, no consideration of dilution factors is necessary in the calculation.

Accurate results are obtained only when careful attention is given to prevent metal contamination of the sample during collection, storage, and preparation for analysis. Deionized or triple distilled water should be used throughout sample collection and preparation. If copper analysis is to be made, distilled water should not be used where copper tubing has been in the distillation apparatus.

A. Cleaning Procedures

Careful attention should be given to cleaning procedures for hardware which will contact either samples or standards.

1. Crucibles: Crucibles should be "demineralized" overnight in dichromate-sulfuric acid solution, rinsed six separate times in water, rinsed three times

with 10% HCl and three times with deionized water. Dry crucibles in oven and store in plastic bags. Re-dry for several hours prior to use and remove to dessicator. Hereafter, handle only with tongs.

2. Glassware and Plasticware:

   a. Labware which has contained $LaCl_3$, blood, or its components should be rinsed in tap water prior to detergent soak to prevent precipitation. Labware which has contained MIBK (methyl isobutyl ketone) extracts should be pre-rinsed with acetone prior to detergent soak.

   b. Do not use labware on which chemicals or soap have been allowed to dry or bake without pretreatment with dichromate-sulfuric acid or hot HNO_3 solution.

   c. Soak labware overnight in a low-phosphate aqueous detergent solutio such as Sparkleen (Fisher Scientific Co.) or Acationix.

   d. Scrub with a brush, including caps, and rinse three times (rinse = fill and empty) in tap water; rinse in deionized water three times. (When dry, glassware can be visually checked at this point for residual film).

   e. Three rinses with (or an overnight soak in) 10% HCl should remove elements not soluble in the detergent and the residual sodium present in the soap.

   f. Finally, three rinses in deionized water are used.

   g. Plasticware is shaken out and dried as quickly as possible while covered with cheesecloth at room temperature.

   h. Glassware is dried in a drying oven or with acetone. Temperature should not exceed 100 C for calibrated glassware.

   i. All glassware and plasticware are stored immediately in plastic bags or sealed with parafilm or clean dry caps.

j. For ultra-micro analysis, labware should be rinsed in 10% HNO_3 immediately prior to use followed by deionized water rinses.

k. Dispenser tips for Eppendorf pipettes should be treated as in (j) above.

B. Serum or Plasma: Sample preparation varies for specific minerals.

1. Equipment and Supplies:

a. Centrifuge.

b. Standard repipet with 10 ml delivering capacity.

c. Plastic test tubes (17 x 100 mm) with airtight stoppers and racks.

d. Harvard trip balance.

e. Pipettes.

f. Labeling tape and pen.

g. Pipette filler.

2. Calcium and Magnesium: Approximate calcium level in serum or plasma is 10 mg % = 10 mg/100 ml = 100 µg/ml. Approximate magnesium level in serum or plasma is 2 mg % = 2 mg/100 ml = 20 µg/ml.

a. Dilute TCA filtrate 1 → 5 with 1% $LaCl_3$.

b. Final dilution factor is 50. Samples should fall on either side of 2 µg Ca/ml and .4 µg Mg/ml of serum or plasma.

3. Phosphorus (as Phosphate): Approximate phosphorus as PO_4^{-3} level in serum or plasma is 3 to 5 mg/100 ml (30 to 50 µg/ml). Use 1 ml TCA filtrate for phosphorus analysis. Samples should fall on either side of 4 µg P/ml of serum or plasma.

4. Zinc and Copper: Approximate zinc level in serum or plasma is .8 µg/ml (.5 to 1.2 µg/ml) and copper is typically present at concentrations of .7 to 1.4 µg/ml.

Fernandez and Kahn (1971) indicated that zinc and copper may be determined by atomic absorption on serum samples diluted with deionized water (instrument will have to be equipped with a "high solids" burner head). For copper, serum

is diluted 1 → 2 and for zinc 1 → 5. Since serum, after the dilutions with deionized water, is more viscous than water, low recoveries are obtained if aqueous standards are used. To overcome this, glycerol is added to the blank and standards to match approximately the viscosity of the diluted serum. Copper standards are diluted to appropriate concentration levels with 10% (v/v) glycerol solution and zinc with a 5% (v/v) glycerol solution.

Low serum dilutions in water as indicated for copper and zinc may clog the aspirator valves and the regular burner head of the atomic absorption instrument; in addition, the bulk ion concentrations present provide solution matrix effects which may yield inaccurate values. These potential problems may be eliminated by ashing one or more ml of serum and solubilizing the ash in 5% HCl (5 ml 5% HCl/ml of serum ashed). The standards should be prepared to contain approximately similar concentrations of the bulk ions.

C. Liver

Liver is cut into small fragments ¼" x ¼" x ¼" with a stainless steel knife or scissors. If the liver was stored frozen, it should be cut while still partially frozen. If the tissue is preserved in formaldehyde, the preserving solution should be measured and analyzed for elements which may be leached out. If possible, analyze samples within 2 months of formaldehyde preservation.

1. Equipment and Supplies:

   a. Analytical balance.

   b. Drying oven.

   c. Muffle furnace.

   d. Automatic dilutor (American Optical or equivalent).

   e. Porcelain crucibles, 100 ml.

   f. Volumetric flasks, 25 and 50 ml.

   g. Plastic test tubes, with airtight stopper and racks.

   h. Volumetric pipettes.

   i. Stainless steel knife or scissors.

j. Solution storage bottles, preferably polyethylene.

k. Corrosion-resistant hood and blower.

l. Electric hot plate.

m. Nalgene (paddles) stirring rods.

n. Filtering funnels and funnel rack.

o. Sharkskin filter paper.

p. Colorimeter and cuvettes for phosphorus determination. (Handle cuvettes with care to prevent scratching or staining of the glass).

2. Dry Matter: Select crucibles with a good finish (smooth glaze to prevent difficulty in removing mineral solution during solubilization). Clean crucibles as previously discussed in preparation of glassware. Dry crucibles in drying oven (100 to 105 C) at least 2 hours. Remove crucibles from oven and cool in desiccator for 2 hours. Weigh crucibles to four decimal places on an analytical balance. Record all weights in a manner similar to enclosed analytical sheet (table 14). Weigh about 25 g of the "wet" liver fragments, if possible. Duplicate each sample at this point. Weigh crucible and "wet" liver sample, place in 100 C oven overnight or at least 16 hours. Remove from oven and cool in desiccator for 2 hrs. Weigh crucible and dry tissue. It is a good practice to run two or three blank crucibles along with the samples throughout the complete analysis to account for contamination.

3. Pre-Ashing or Wet Digestion: Crucibles, containing the sample of dry tissue (approximately 5 g), are placed on a hot plate under a fume hood and pre-ashed with drop-wise additions of concentrated HNO_3 until the sample is a smooth soupy consistency and no longer bubbles up with additions of acid. Samples will "souffle" if this procedure is hurried and too much acid is used. Spattering will occur if temperature of the hot plate is too high. Start at a setting between 200 and 300 C and raise or lower the temperature as needed. Samples will "souffle" in the furnace if charring is incomplete.

4. Ashing:

Crucibles containing the dry pre-ashed samples are placed in a muffle furnace and the temperature (200 C to begin) is raised 100 C every hour until no higher than 500 C is reached. Ashing proceeds overnight. Remove crucibles from furnace after they have cooled partially, to a desiccator to cool for 2 hrs. Weigh crucible and ash.

5. Solubilization of Ash: The crucibles containing the ash residue of the 25 g "wet" liver samples are placed on a hot plate for acid hydrolysis. Wet the ash with a few drops of deionized water; add about 5 ml 50% HCl. Evaporate the HCl solution to about ½ the volume and add 10% HCl to 2/3 the volume of the crucible. Rinse the sides of the crucible with the addition of 10% HCl. Evaporate the solution to about 10 ml and add deionized H_2O to 2/3 the crucible volume. Evaporate the solution to about 5 ml; do not allow the crucible to go dry. Acid concentration per 25 ml volume is estimated to be about 5% or approximately .6N HCl.

Remove the crucible from hot plate. (If the procedure is to be stopped at this point, allow the crucible to cool and cover it with strips of waxed paper). Rinse the funnel containing "Sharkskin" filter paper with 10% HCl into a beaker. Place the funnel into the neck of 25 ml volumetric flask so that air can easily escape when replaced by liquid. Use the acid remaining in the crucible and a Nalgene stirring rod paddle to clean up and down the sides of the crucible. Pour the solution along the paddle into the filter paper. Add about 5 ml deionized water and repeat scraping and pouring. Rinse the filter paper, crucible, and paddle with two 3 ml portions of deionized water; allow the filter paper to drain between each rinsing. Remove the filter paper and rinse the funnel and tip as funnel is removed. Make to volume by rinsing neck of flask with deionized water and mix by inversion at least eight times. Pour into storage bottles. These should have a large enough mouth to permit pipetter of dilutor or a pipette to enter (polyethylene bottles are preferable if Na is to be determined).

D. Bone

1. Equipment and Supplies:

a. Stainless steel knife.

b. Cheesecloth.

c. Soxhlet extractor.

d. Drying oven.

e. Analytical balance.

f. Muffle furnace.

g. Mortar and pestle.

h. Porcelain crucibles, 100 ml.

i. Corrosion-resistant hood and blower.

j. Electric hot plate.

k. Nalgene (paddles) stirring rods.

l. Volumetric flasks, 50 ml.

m. Filtering funnel and funnel rack.

n. Sharkskin filter paper.

o. Solution storage bottles, preferably polyethylene.

p. Plastic test tubes with airtight stoppers and racks.

q. Automatic dilutor (American Optical or equivalent).

r. Colorimeter and cuvettes for phosphorus determination (Handle cuvettes with care to prevent scratching or staining of the glass).

2. Procedure: The bone is stripped of all soft tissue. Dry fresh bone at 100 C for 24 hours, cool, wrap in cheesecloth and attach label (in pencil). Ether extract (with petroleum ether) in Soxhlet extractor for 48 hours. (Soxhlet extraction should syphon over about once every hour). After extraction, spread bone to dry (approximately 3 hours) or until the odor of ether is no longer detectable. Obtain dry weight at 100 C after 24 hrs. Ash the bone at 600 C overnight (temperature should be raised gradually). Obtain ash weight to calcu-

late percent ash of dry, fat-free bone sample.

Solubilization of the moisture-free weighed bone ash is similar to that described for liver except that the ashed bone is ground with mortar and pestle prior to sampling. The weighed bone ash is slightly wetted with deionized water and 10 ml concentrated HCl are added carefully; and the hot hydrolysis continued with nearly full crucibles of 10% HCl, followed by the addition of water. Evaporation is stopped before calcium phosphate crystals form. The digestion procedure should continue for several hours to ensure complete solubilization of the oxides and phosphates. Two g of bone ash may be solubilized and transferred quantitatively to 50 ml volumetric flask for analysis of calcium, phosphorus, magnesium, and zinc. Solutions must be 5% acidity. All dilutions should be less than pH 2 at time of analysis. Crystallization can occur with time (4 weeks) in diluted samples and the solution pH rises to more than pH 4.

E. Forage

1. Equipment and Supplies:

a. Analytical balance.

b. Drying oven.

c. Muffle furnace.

d. Automatic dilutor (American Optical or equivalent).

e. Porcelain crucibles, 100 ml.

f. 50 ml volumetric flasks.

g. Plastic test tubes with airtight stoppers and racks.

h. Volumetric pipettes.

i. Solution storage bottles, preferably polyethylene.

j. Funnel rack.

k. Nalgene (paddles) stirring rods.

l. Filtering funnels.

m. Sharkskin filter paper.

n. Electric hot plate.

o. Corrosion-resistant hood and blower.

p. Colorimeter and cuvettes for phosphorus determination. Care must be taken in washing and storage of cuvettes to prevent scratching or staining of the glass. Protected tube racks (rubber, plastic-coated, etc.) are necessary.

2. Dry Matter, Organic Matter and Ash: Select crucibles with a good inside finish (smooth glaze to prevent difficulty in removing mineral solution during solubilization). Clean crucibles as discussed above in preparation of glassware. Dry crucibles in drying oven (± 100 C) for at least 2 hours. Remove crucibles from oven and cool in desiccator for 2 hours.

Weigh crucible to four decimal places on an analytical balance. Record all weights in a manner similar to the enclosed analytical sheet (table 14). Weigh about 5 g of air-dry sample into crucible while on the balance pan (mix sample well before weighing 5 g aliquot). Duplicate each sample at this point. Weigh crucible and air-dry sample, place in 100 C oven overnight or for 16 hours. Remove from oven and cool in desiccator for 2 hrs. Weigh crucible plus dry matter, then place in muffle furnace, raising temperature in increments of 100 C until a maximum temperature of 500 C is reached. Maintain at 500 C for at least 8 hrs. Remove crucibles from furnaces after they have cooled partially, place in desiccator to cool for 2 hrs. (If crucibles cool completely in furnace, reheat to 100 C for 2 hrs. before placing in desiccator). Weigh crucibles and ash. It is a good practice to run two or three blank crucibles along with the sample throughout the complete analysis to account for contamination.

3. Solubilization: The solubilization of ash residue of the 5 g forage sample and the transfer to volumetric flasks is similar to that described for liver. After adding the 50% HCl, 10% HCl, and deionized water, evaporate to not more than 10 ml and transfer the ash solution to 50 ml volumetric flasks. Acid concentration per 50 ml volume is estimated to be not more than 2% or about .2N HCl.

IV. PREPARATION OF STANDARDS

A. Procedure

Standards should be prepared to duplicate as closely as possible, the concentrations of the minerals expected in the undiluted sample solutions. For any given mineral, its concentration in the sample must first be estimated. The similar standard is prepared by diluting a highly concentrated solution of the desired element to match. Both samples and standards are diluted alike to the appropriate analytical range for analysis of the element by atomic absorption spectrophotometry or colorimetry procedures. Once the definite range of mineral concentrations has been established for a group of samples, the working standards can be made up to include the range expected for undiluted sample solutions. Typical mineral concentrations in serum (or plasma), liver, bone, and forages are shown in table 4. Approximate elemental composition of liver (5 g dry tissue/25 ml) and forage (5 g dry sample/50 ml) in solution is presented in tables 5 and 6, respectively. The stock standard solutions may be prepared by using atomic absorption standard solutions available commercially or reagent grade chemicals. Table 8 provides detailed information regarding the calculations and preparations of stock standard solutions.

It is desirable, when possible, to prepare the concentrated stock solutions of each element in appropriate concentration so that they may be diluted by pipette into volumetric flasks to fit the range of undiluted sample solutions. These dilutions are known as working standards. Working standard solutions required to resemble serum (or plasma), liver, and forage sample solutions are presented in tables 7 to 13. If possible, working standards should be prepared to the same volume as the undiluted sample solution (i.e., 50 ml for forage samples). The range of the working standards should encompass the highest and lowest sample concentrations with a total of at least five standards for each mineral. The working standards and samples are then diluted with an autodilutor to the appropriate concentration for analysis.

When highly concentrated stock solutions are prepared with reagent grade compounds, accuracy of mineral content may be established by carrying through the dilution process and comparing the final dilution with certified atomic absorption standard solutions.

The chloride form of elements is used for the stock solutions in order to avoid introducing an unmatched anion into the standards. The nitrate forms are recommended when nitric acid solutions are required for subsequent injection of sample solution into the graphite furnace for flameless trace elemental determinations (chlorides are volatile at temperatures used).

B. Dilution of Samples and Standards

When diluting samples and standards, an automatic dilutor should be used whenever possible since repeatability is very important in this method of analysis. The available pump sizes for the dilutor are the only limiting factors to the extent the dilutor can be used. If the volumes to be pipetted fall below or beyond the capacity of the dilutor, pipettes must be used. If at all possible, avoid pipetting less than 5 ml with a glass pipette due to calibration error in the smaller volume pipettes.

The usual procedure is to prepare the working standards using pipettes and volumetric flasks which will match volume and acidity in sample solutions. Set the dilutor for the desired dilution and dilute all of the samples and standards without changing the setting. If another dilution is necessary, change the setting for diluent as required and complete dilutions without altering the settings. Be sure to mix each diluted sample well with a vortex mixer or by inversion before sampling it for a further dilution.

Highly concentrated acid solutions should not flow through the dilutor, as corrosion of pump parts will result.

Auto-diluting instruments used in mineral work should be thoroughly rinsed before and after use by running through at least 200 ml of deionized water, followed by 200 ml of 1% Acationix detergent, 100 ml 10% HCl, and a final rinse of

200 ml deionized water. Delivery of large bubbles requires a repeat of the dilution; small bubbles are inconsequential.

The pipette tip must be wiped with lab tissue or rinsed between each sampling.

Tables 9 to 13 provide detailed information regarding the preparation of concentrated stock solutions and working standards, as well as the approximate dilutions for both standards and samples.

The acid concentration of samples and standards should agree closely as a difference may adversely affect the comparative readings on the atomic absorption unit. The working standards should agree with acid concentration in the original sample solutions. When samples and standards are treated alike, the final concentration reading will be comparable.

V. DETERMINATION OF PHOSPHORUS BY A COLORIMETRIC PROCEDURE

The lanthanum dilution made for calcium determination may be used for the colorimetric determination of phosphorus. The presence of $LaCl_3$ in solution has been tested in the colorimetric method and found to provide perfect duplication with acidified standard solutions. In fact, the acid concentration in the $LaCl_3$ dilution assures maintenance of a low pH to retain $PO\equiv_4$ in solution for the methodology of the Fiske-Subbarow analysis.

A. Reference:

Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375 (Modified for a 10 ml total volume). If desired; Chen, P. S., T. Y. Toribara, and H. Warner. 1956. Micro determination of phosphorus. Anal. Chem. 28:1756.

B. Reagents

1. Ammonium molybdate solution: 2.5% in 5N H_2SO_4.

   Dissolve 25 g ammonium molybdate $[(NH_4)_2\ MoO_4]$ crystals in 200 ml H_2O. In a one liter flask containing several hundred ml H_2O, add 150 ml concentrated H_2SO_4. When cool, quantitatively transfer the molybdate solution and bring to one liter volume with water. Stable indefinitely.

2. Amino-Naphthol-Sulfonic acid (ANS): (Available from Harleco Reagents or Fisher Scientific Co.).

   Dissolve one packet (pre-measured unit) of 1-Amino 2-Naphthol 4-Sulfonic acid dry mixture, according to package directions, in 100 ml warm to hot water. Cool and filter into amber storage bottle. Store in refrigerator and remove to room temperature before using. Stable about 2 to 3 weeks.

   (Chen, Toribara, and Warner method uses ascorbic acid reducing agent).

C. Procedure (Standards and blank solutions are treated exactly as samples).

1. Into matched colorimeter tubes, deliver .5 or 1 ml of sample solutions. (Hycel cuvettes, round, 19 x 105 mm, Scientific Products Co.).

2. Add 8 ml H_2O for .5 ml or 7.5 ml H_2O for 1 ml aliquots.

3. Add 1 ml molybdate solution. Mix thoroughly and let stand a few minutes.

4. Add .5 ml ANS solution and mix by inversion. Colorimetric readings should not begin before 20 min. after ANS addition to first sample and should be read within 1 hr.

5. Read % transmittance (T) at 825 mμ. Convert to absorbance or optical density (O.D.). Use red phototube or red filter.

D. Calculations

Calculate phosphorus as mg/100 ml (mg %) in serum or plasma. Calculate phosphorus in liver and forage as ppm or percent of dry matter. Standard values should be plotted on graph paper (concentration vs. optical density) to determine linearity and accuracy of the points. Phosphorus concentration of the samples may be read directly off the graph or calculated using a regression equation based on the concentration (y) and optical density (x) of the standards. Omit inaccurate or non-linear points.

When samples and standard solutions are diluted alike and optical density of the undiluted standards are used as reference, no consideration of dilution factors is necessary in the calculations and phosphorus concentrations may be determined as follows:

$$(\mu g/\text{volume});\ \text{ppm} = \frac{(a + bx)\ 1}{\text{dry sample wt.}}$$

$$(mg/\text{volume});\ \% = \frac{(a + bx)\ .1}{\text{dry sample wt.}}$$

Where b = regression slope

a = intercept

x = optical density

Phosphorus readings of standards and forage samples (5 g dry sample/50 ml) may be recorded on a data sheet such as found in table 15.

When standards are not diluted in the same manner as samples and the read-out is in absorbance units, a linear regression equation can be used to calculate µg of element per ml of solution analyzed. In case of liquid samples (i.e., serum or plasma), the concentration must be multiplied by the dilution factor to equal total µg of phosphorus present in the original sample. In solutions of ashed material (i.e., liver), the concentration of the solution analyzed must be multiplied by the ml of original solution of ashed material and by all subsequent dilutions to equal total µg of phosphorus present in the original sample. This value must be divided by the original sample dry weight in g to yield µg/g or ppm of element in the sample. Phosphorus readings (% T), optical density conversions, concentration (µg/ml), dilution factors, dry weight of sample, and calculations for samples as well as the readings for standards may be recorded on a data sheet, such as found in table 16.

VI. MINERAL ANALYSIS BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY

A. Equipment and Supplies:

1. Perkin-Elmer, model 306 (or equivalent). Vent system.
2. Compressed acetylene.
3. Compressed air.
4. Lamps for each element.
5. Atomic absorption standard solutions (Harleco or Fisher Scientific).

B. Procedure:

Atomic absorption spectrophotometry (AAS) is similar to emission spectrometry except that it measures energy as it is absorbed by atoms rather than as it is emitted by atoms. In flame emission, the sample element is raised to a high-energy (excited) state, and the radiation energy emitted as the atoms return to their minimum energy state is measured. In atomic absorption, dissociated atoms in their minimum-energy state absorb radiation energy to increase their energy state, and the energy absorbed is measured. Each element has its own characteristic emission spectrum and its own characteristic absorption spectrum. Energy emission and absorption do not always occur at the same wavelength lines. Energy wavelength lines, where both emission and absorption occur and which are useful in atomic absorption, are referred to as resonance lines.

The technique of atomic absorption analysis utilizes both energy emission and energy absorption. Generally, hollow cathode lamps, in which the cathode is constructed of the element to be determined, are used as a light or energy source. The emitted light is directed through a flame into which the liquid sample to be analyzed is aspirated. Atoms in the flame which are chemically unbound and in their minimum-energy state absorb the light at certain wavelength lines. The degree of absorption is proportional to the concentration of the element in the aspirated sample. Electronic detectors measure the changes in

the amount of light or energy which has passed through the flame. Signals coming from the detectors may be expanded and appear, according to instrumentation, either as percent absorption, as absorbance units, as direct readout in concentration units, or as discrete peaks on a stripchart recorder. Detection limits are described as the smallest amounts capable of being analyzed which produce a signal twice that of background noise. Sensitivity is defined as the amount of element required to produce 1% absorption or .0044 absorbance units.

Atomic absorption spectrophotometry has been considered to be relatively free of interferences. With wider application and increased sensitivity, however, certain physical characteristics of aspirated solutions have produced atomic absorbance signals for the analyte which differ from those obtained for known amounts in uncomplicated or "simple" aqueous solutions. Solutions of biological material are always complex and unpredictably variable.

Slavin (1968) suggested that, when the sample solution is such that total dissolved solids are less than .1% of the solution, simple known standards in a like solvent will give satisfactory results. When dissolved salts fall between .1 and 1% of solution, matching the solutions of known standards to within 25% of the major constituents in the sample solution will provide solutions similar enough in physical properties for accurate results by flame AAS. The most practical method for reduction of total dissolved solids is to dilute the sample, but this benefit is lost when the analyte concentration approaches detection limits for the element. Therefore, specious matrix effects, on biological material in solution must be suspected and measured when dilution to less than .1% dissolved solids is not possible for the analyte of interest:

C. Macro-elemental Determination:

1. Sodium and Potassium: Standard solution dilutions for these elements will match sample dilutions. Since enhancement of sensitivity is not necessary for these samples, no concern is given to ionization effects

in the flame. It has been found essential to carry through three or more blank crucibles to establish sodium contamination and to store solutions in polyethylene containers if analysis must be delayed.

2. Calcium: Dilutions of the sample solutions for calcium determination may be variable. It is customary, therefore, to make the final dilution for analysis about 1% $LaCl_3$. This concentration of lanthanum will control 200 μg/ml phosphate ion in the flame. Phosphate ion is a serious interferent in calcium analysis by flame AAS.

3. Magnesium: At the dilutions usually required for magnesium determinations, phosphate or silicate ions are not likely to be present in sufficient quantities to require the presence of lanthanum, but the latter may be present and should be matched in standard solutions.

D. Micro-elemental Determination by Flame AAS: copper, iron, manganese, zinc.

Tables 5 and 6 may be used as a guide for possible ionic concentrations which might occur in liver from cattle and sheep (5 g dry tissue/25 ml) and various forages (5 g dry sample/50 ml). Results from this laboratory and others confirm certain effects of solution ionic matrices on measurement of micro-elements by flame atomic absorption spectrophotometry (Billings, 1965; Koirtyohann and Pickett, 1966a and b; Slavin, 1968; Kahn, 1968; Hell and Ramirez-Munoz, 1970; West, Fassel, and Kniseley, 1973; Legget and Westerman, 1973; Giron, 1973; Baker and Smith, 1973; Ball and Gottschall, 1975).

At the concentrations of zinc, manganese, and iron found in many liver and forage solutions, dilution of the sample solution is often possible. When the dilution is 1 → 10 (forage) and 1 → 100 (liver) (tables 5 and 6), total solution ionic strength (including acid) may be reduced to less than .1%. A few undiluted sample solutions may be less than .1% total dissolved solids. These solutions may be analyzed with simple aqueous standard solutions. In general, however, total dissolved solids for liver (5 g dry tissue/25 ml) and forage (5 g dry sample/50 ml) fall between 2 to 5 and .5 to 1%, respectively. When micro-elemental

concentrations in the undiluted sample solutions are too low for dilution (i.e., concentrations at or below analytical detection limits), the degree of specious absorbance due to solution matrix must be determined. Two procedures follow:

1. Method of Billings, 1965. The sample solution is aspirated and absorbance readings are compared to that of a comparable acid blank solution at a nearby non-absorbing wavelength. Suggested lines (Å) were: manganese, 2576.1; copper, 2961; iron, 2510.8 or 2472.9. None were found for zinc. The method of standard additions will not correct or disclose matrix interferences.
2. Detection of constant bias can also be disclosed by measuring serial dilutions of the sample solution against simple standard solutions or by solubilizing serial weights of the sample in the same volume. Any change in the calculated dry sample concentration indicates a solution matrix interference. The significance of the interference on the analysis must be judged by the analyst in view of the application of the data.

There are several approaches to obtaining reliable data:

1. Use the deuterium arc background correction system, which is highly recommended for zinc analysis.
2. Separate the micro-elements from the high-salt matrix. This is laborious but has been successful with chelation-extraction procedures or with ion-exchange elution methods.
3. Prepare the micro-elemental standards to correspond to the sample macro-elemental matrix.

Since samples in undiluted solutions may exceed 1% total dissolved solids, matching a matrix to within 25% is often feasible. Determination was made on 1 liter of reagent grade matrix solution for elemental contamination by reagent grade compounds. A chelation-extraction procedure with Methyl Isobutyl Ketone (MIBK) and Ammonium Pyrrolidine Dithiocarbamate (APDC) or oxine indicated that

copper, zinc, and manganese contributed by the compounds would not be detected by the flame analysis. Iron could be a significant contaminant. Iron analysis by flame AAS, however, is relatively not as sensitive as copper, zinc, and manganese and the blank should fall below detection limits.

In tables 9, 10, and 13, instructions for preparation of micro-elemental standards are given. The mixed matrix known standard solutions should be used in analysis where the sample solution cannot be diluted at least 1 → 10. When the sample solution can be diluted to a total salt content <.5%, "simple" known standard solutions may be used.

The linearity of iron absorbance is limited, and baseline fluctuations make an instrumental calibration for concentration readout difficult. It is recommended that iron be measured by absorbance at an analytical range of 1 - 5 μg/ml and that sample solutions be diluted where possible. When using a concentration readout mode, use the integrate 10 setting for copper, manganese, and zinc. The reduced atomization rate of high dissolved solid solutions delays peak absorbance. Any significant changes in standard readings with time suggest further reduction in aspiration rate. It would be wise to aspirate 5% HCl or to ream the needle valve with the copper wire periodically to remove salt deposits. Do not use the three-slot burner head for trace elemental analyses of biological solutions.

E. Instrument Operation:

Space does not allow a discussion of all the information that is needed for proficiency in use of an atomic absorption spectrophotometer. Detailed instructions supplied by the manufacturer of the instrument must be followed carefully. The following steps are used at the Nutrition Laboratory, University of Florida, Gainesville, FL, U.S.A., with a Perkin-Elmer Model 306.

1. Starting procedure:

   a) Insert black plug (from the voltage regulator installed under the counter) into the wall outlet.

   b) Place appropriate hollow-cathode-lamp into receptacle unit. Allow

10 to 15 min. "warm-up."

c) Push "power button" on; it should be lit white. For potassium analysis, push "filter button" on; it should be lit green.

d) Using the source control knob, adjust milliamperage on meter behind lamp to correspond to operating milliamperage (designated for 303-306-403) on lamp label.

e) If a blue flame is to be used, insert a single-slot burner head. If a yellow flame is to be used, insert the three-slot burner head. If potassium is to be run, use the three-slot burner head. Burner heads are removed or inserted when cool by loosening or tightening the lock nut at the base of the head. Do not use force--this should be accomplished easily.

f) Check floor jug for run-off capacity. Check loop in plastic hose. This should be full of water and the hose inserted into 3 to 5 inches of water in the jug.

Note: Failure to follow these steps may result in mixing chamber explosion.

g) Consult the sheet or standard operating book for instrument settings of element desired:

g-1 Set slit width as required.

g-2 Set knob to VIS or UV as required.

g-3 Adjust wavelength, using the gain setting and right-hand portion of energy meter. Return needle to dark green portion.

h) Turn on switches for air compressor and hood. Open valve of air line. Empty the water trap on the air line if it contains any water (the valve cannot be opened until the air-pressure returns to zero). When air-pressure dial on control box reads 30 psi and line valves read above 30 psi, air-pressure is optimum. With aspiration tubing out of liquid, let the compressor run a few minutes. Brass flow

meter ball should be about 10 to 10.5 for blue flame and 9 to 9.5 for yellow flame.

i) Close control box acetylene toggle valve. Open valves on acetylene tank, until control box dial reads 10. Open the control box valve, turn gray lighter switch forward or use flint friction lighter. White ball on control box should be 10.5. Insert aspirator tubing into beaker of fresh deionized water (no particles).

j) The instrument is now ready for calibration to obtain readout in concentration units, or measurements can be made without calibration in absorbance units.

2. Calibration procedure:

a) On left-hand control board, set knobs at ABS mode and DAMPING position 1, as per instructions.

b) On right-hand control board:

b-1 Make sure both curve correction knobs are in the "zero" position, (i.e., fully counterclockwise) on ONSET and MAGNITUDE knobs.

b-2 Set the lower right-hand knob to ABS position.

b-3 Insert aspirator tubing into blank solution. Hold down AUTO-ZERO button until digital readout is .000.

b-4 Providing accuracy and linearity of standards have been ascertained, place aspirator tubing into appropriate standard solution, preferably in the range of unknown solutions.

b-5 Turn lower right-hand knob to CONC position. Unlock and set knob above it to read the same as the standard being aspirated and lock it in.

b-6 Turn lower right-hand knob to AUTO CONC position. Hold down AUTO CONCENTRATION button until readout is the same value as in (b-5). Adjust decimal if necessary.

b-7 Rinse aspirator tubing in water beaker and place in blank solution again.

b-8 On left-hand control board, switch to INT 2 or INT 10 mode.

b-9 Hold AUTO ZERO button until readout is .000.

b-10 Insert tubing back into calibrating standard solution and touch READ button; when button relights, readout is complete.

b-11 Check other standard solutions covering the expected range of unknowns for linearity and accuracy. Use correction where necessary.

b-12 Insert tubing into unknown solution, touch READ button, obtain readout, and record.

b-13 Additional instructions for absorbance measurements, use of curve correction, nitrous oxide-acetylene operation, and measurements by strip-chart recording may be found in the Perkin-Elmer manuals.

3. Turn-off procedure:

a) Close acetylene valve on control box.

b) Close both valves at acetylene tank.

c) Open acetylene valve on control box to bleed the line.

d) Close valve in air line.

e) Turn down both main switches - air compressor and hood - to "off."

f) Push "power button" off (unlit).

g) Return source knob to "zero."

h) Remove wall outlet plug.

i) Change acetylene tank when pressure is down to 100 lb.

4. Lamp change: Turn off the acetylene at control box. Push "power button" off. Return source control knob to "zero." Allow lamp to cool before removal. Return cool lamp to drawer and insert new lamp. Relight burner.

5. Cautions:

a) Never leave the room with the flame lit.

b) If electrical power failure occurs (lights go off, etc.), switch off acetylene control box valve immediately (otherwise the compressor will go off and you will be burning only acetylene).

c) Failure to turn on and open the air line at the start in step 1 (h) also results in discharge of smoke and soot.

d) Always turn on acetylene last when starting the operation and turn off the acetylene first when stopping operations.

6. Limitations:

a) Do not attempt to read samples below the detection limits of your solution matrices.

b) For ppb (i.e., < 1 ppm) in any other than pure solution, consult the literature.

c) For any solution containing a mixture of ions, make sure the element to be measured is in an "interference-free" condition.

d) Standard solutions generally below 2 ppm should be prepared fresh.

e) Acid concentration and/or any "masking agent," and/or ionic concentrations of elements present in excess of 500 μg/ml, and/or organic constituents must be matched in standard solutions and blanks.

F. Reading Standards and Samples:

Follow the procedure outlined in 2 (b), (calibration procedure), above. Monitor blank and various standards every 15 samples at intervals during the readings. Two readings of each standard are recommended. If "auto-zero" is adjusted, re-read standards. Aspirator tubing should be dipped in water beaker between all samplings to prevent cross contamination. If complete clogging of aspirator tubing occurs, a "zero" readout will be obtained. Almost invisible particles, resulting from improper filtration, substances present in non-ashed liquids, or fall-in can clog the needle valve. The copper wire filament pro-

vided by the manufacturing company may be inserted and the tubing and valve gently reamed. If aspirator tubing is jammed against the side or bottom of the vessel, or if it is not in the solution, a "zero" readout will result.

Readings are recorded on data sheets such as found in tables 17 and 18.

G. Calculation of Results:

Calculate minerals as µg/ml or mg/100 ml (mg %) in serum or plasma. Calculate mineral concentrations in liver and forages as either percent or ppm (µg/g) of dry matter.

Standard values, in either absorbance or concentration units, should be plotted on graph paper to determine linearity and accuracy of the points. Omit inaccurate or nonlinear points. Sample concentrations may be determined directly from the plotted standard curve or a linear regression equation can be used, based on the standard concentration (y) and absorbance (x). When sample and standards are treated in the same manner (i.e., forage samples), no consideration of dilution factor is necessary and the calculations are based on regression of concentration in undiluted standards (y) (i.e., for forage µg/50 ml or mg/50 ml) on absorbance (x) as follows:

$$(\mu g/\text{volume});\ \text{ppm} = \frac{(a + bx)\ 1}{\text{dry matter wt.}}$$

$$(\text{mg/volume});\ \% = \frac{(a + bx)\ .1}{\text{dry matter wt.}}$$

where: b = regression slope
a = intercept
x = absorbance

Mineral readings of standards and forage samples (5 g dry sample/50 ml) may be recorded on a data sheet such as found in table 17.

When standards are not diluted in the same manner as samples and the readout is in absorbance units, the linear regression can be used to calculate µg of element per ml of solution analyzed. For liquid samples (i.e., serum or plasma), this concentration must be multiplied by the dilution factor to equal

total μg of mineral present in the original sample. In solutions of ashed material (i.e., liver), the concentration of the solution analyzed must be multiplied by the ml of original solution of ashed material and by all subsequent dilutions to equal total μg of element present in the original sample. These figures must be divided by the sample volume in ml or sample dry weight in g to yield ppm (μg/ml or μg/g) of element in the sample. Mineral readings for animal tissues are recorded on a data sheet such as found in table 18.

TABLE 1 FARM IDENTIFICATION FOR MINERAL STUDIES

Date _____

1. NAME OF RANCH _____

2. LOCATION OF RANCH:

   2.1 County _____ ☐ 2.2 State _____ ☐

   2.3 Country _____ 2.4 Latitude & Longitude _____ 2.5 Altitude _____

3. NAME & ADDRESS OF OWNER _____

4. NAME & ADDRESS OF MANAGER _____

5. LEVEL OF TRAINING OF MANAGER _____ ☐

6. ACCESS INFORMATION:

   6.1 Reference point (city) 6.2 Road 6.3 Distance

   _____ _____ _____

   Road type (paved, gravel, etc.) _____

7. LAND USE:

	ha.		(%)	
7.1 Infrastructure	_____	☐	_____	☐
7.2 Agronomic crops	_____	☐	_____	☐
7.3 Natural pasture	_____	☐	_____	☐
7.4 Improved pasture	_____	☐	_____	☐
7.5 Forest	_____	☐	_____	☐
7.6 Total cultivated	_____	☐	_____	☐
7.7 Water	_____	☐	_____	☐
7.8 Total area	_____	☐	_____	☐

8. CLIMATE:

   8.1 Type _____ ☐ 8.2 Average temperature _____ ☐

   8.3 Average rainfall _____ ☐

   8.4 Dry season _____ to _____ (months)

9. SOILS:

   9.1 General soil characteristics pH _____ ☐

   Drainage _____ ☐ Color _____ ☐

   Other _____ ☐ _____ ☐

TABLE 1 (continued)

9.2 Types:

		Class		%	
_____					
_____	☐	_____	☐	_____	☐
_____	☐	_____	☐	_____	☐
_____	☐	_____	☐	_____	☐

9.3 Soil analysis data Yes _____ ☐ No _____ ☐

(Annex if possible)

10. WATER DATA:

10.1 Source _____

10.2 Analysis Yes _____ ☐ No _____ ☐
(Annex if possible)

10.3 Observations on water _____

_____

11. PHYTOGRAPHY:

11.1 Original (total ranch)

	ha.		%	
Forest	_____	☐	_____	☐
High plains	_____	☐	_____	☐
Medium plains	_____	☐	_____	☐
Low plains	_____	☐	_____	☐
Flooded areas	_____	☐	_____	☐

11.2 Original phytography of cultivated areas

Type	%		Type	%	
Forest	_____	☐	Low plains	_____	☐
High plains	_____	☐	Flooded areas	_____	☐
Medium plains	_____	☐	Others	_____	☐

12. PASTURES:

12.1 Name of forage		Stocking rate (head/ha.)		Average area (ha.)	
Native					
_____	☐	_____	☐	_____	☐
_____	☐	_____	☐	_____	☐
Naturalized					
_____	☐	_____	☐	_____	☐

Improved

_____ ☐ _____ ☐ _____ ☐

_____ ☐ _____ ☐ _____ ☐

_____ _____ _____

12.2 Are pastures periodically burned? Yes ____ ☐ No _____ ☐

When? _____ ☐

12.3 Grazing system Rotational _____ ☐ Continuous _____ ☐

If rotational, explain _____

_____

12.4 Are fertilizers on pastures? Yes ____ ☐ No _____ ☐

Kinds & Quantities _____

_____

Frequency _____

12.5 Toxic plants:

Name	Month	Estimated Mortality (%)
_____ ☐	_____ ☐	_____ ☐
_____ ☐	_____ ☐	_____ ☐

13. CATTLE:

13.1 Beef ____ ☐ Dairy _____ ☐ Combination _____ ☐

13.2 Principal breeds _____ ☐ _____ ☐

13.3 Classes:

	No. Animals			No. Animals	
Bulls	_____	☐	Male 1-3 yrs.	_____	☐
Cows	_____	☐	Female 1-3 yrs.	_____	☐
Male Calves	_____	☐	Fattening Cattle	_____	☐
Female Calves	_____	☐	Total Cattle	_____	☐

13.4 Is artificial insemination used? Yes ____ ☐ No ____ ☐

13.5 No. calves born this year _____ ☐

13.6 What is the average milk production? _____ (liters) ☐

TABLE 1 (continued)

13.7 Acquisitions of cattle in last year

Origin		Quantity		Class	
_____	☐	_____	☐	_____	☐
_____	☐	_____	☐	_____	☐
_____	☐	_____	☐	_____	☐
_____	☐	_____	☐	_____	☐
_____	☐	_____	☐	_____	☐

13.8 Fertility (calving %)

197 _____ ☐
197 _____ ☐
197 _____ ☐

13.9 Kill percentage

197 _____ ☐
197 _____ ☐
197 _____ ☐

13.10 Mortality

	No.	%		Cause	
197	_____	_____	☐	_____	☐
197	_____	_____	☐	_____	☐
197	_____	_____	☐	_____	☐

13.11 Breeding season and management

Continual _____ ☐ Controlled _____ ☐

_____ to _____ (month)

13.12 What is the weaning age? _____ ☐

13.13 What is the age heifers are bred? _____ ☐

13.14 At what age are steers marketed? _____ ☐

14. FACILITIES:

14.1 Mineral feeders

Kind _____ ☐

Covered Yes _____ ☐ No _____ ☐ Total No. _____ ☐

No./100 head _____ ☐ Size _____ ☐ Height (cm) _____ ☐

14.2 Handling facilities

No. corrals _____ ☐ Size of corral _____ ☐

Chute Yes _____ No _____ ☐ Squeeze Yes _____ No _____ ☐

Balance Yes _____ No _____ ☐

Electricity at corral Yes _____ No _____ ☐

15. HERD HEALTH

15.1 Vaccinations

Hoof & Mouth _____ ☐
Brucellosis _____ ☐
Rabies _____ ☐
Other _____ ☐

15.2 Parasite control

Internal

Types _____ ☐ _____ ☐ _____ ☐

Wormed Yes _____ ☐ No _____ ☐

When _____

Treatment _____

External

Types _____ ☐ _____ ☐ _____ ☐

Treatment _____

When _____

15.3 Are there any health problems (i.e., broken bones, weight loss, rough hair coat, infertility, etc.)?

Yes _____ ☐ No _____ ☐

Describe _____

_____

_____

16. LABOR FORCE:

Veterinarios _____ ☐ Foreman _____ ☐

Agronomos _____ ☐ Cowboys _____ ☐

TABLE 1 (continued)

17. ENERGY OR PROTEIN SUPPLEMENTATION:

What is given (describing: supplements, methods and quantities given) _____

_____

_____

_____

18. MINERAL SUPPLEMENTATION:

18.1 Which of the following minerals are fed?

Common salt _____ ☐ Iodized salt _____ ☐

Calcium & Phosphorus _____ ☐ Trace minerals _____ ☐

Other (i.e., injections) _____

18.2 Mineral supplement formulation

Element	%		Element	%	
NaCl	_____	☐	Mn	_____	☐
Ca	_____	☐	Zn	_____	☐
P	_____	☐	Fe	_____	☐
Mg	_____	☐	I	_____	☐
Cu	_____	☐	Se	_____	☐
Co	_____	☐	Other	_____	☐

18.3 Ratio of salt to other minerals _____ ☐

18.4 Average consumption/head/year _____ ☐

18.5 How often are fresh minerals given/mo.? _____ ☐

18.6 Are there any known mineral problems in the area? _____

_____

_____

19. OBSERVATIONS:

_____

_____

_____

20. NAME OF PERSON TAKING SURVEY (PRINTED)

_____

TABLE 2 SAMPLE IDENTIFICATION FOR ANIMAL TISSUES

Date _____

1. Ranch Identification (Name, exact location, manager or owner) _____

2. Origin of collected samples (i.e., Name and location of slaughterhouse) _____

3. Sample Identification*

	Ranch	_____	_____	_____	_____
	Animal ID	_____	_____	_____	_____
(specify)	Tissues	_____	_____	_____	_____
	Date	_____	_____	_____	_____
	Sample	_____	_____	_____	_____

4. Animal Description
   a) Identification Number _____
   b) Breed _____
   c) Age _____
   d) Class: Steer _____ Heifer _____ Bull _____ Dry Cow _____ Lactating Cow _
   e) Weight _____
   f) Condition or Finish: Good _____ Fair _____ Poor _____
   g) Disease or Parasites (specify) _____

5. Other Observations _____

* It is essential that sample identification be the same on this form and the contain with the sample.

TABLE 3 SAMPLE IDENTIFICATION FOR FORAGES

Date _____

1. Ranch Identification (Name, exact location, manager or owner) _____

2. Pasture where sample collected (Specify section of farm) _____

3. Samples collected (Collect at least one sample of each of the major species in this pasture)

4. Sample Identification*

Ranch				
Pasture				
Date				
Sample	a	b	c	d

5. Sample Description

Genus Species				
Common Name				
Variety				
Height when cut (cm)				
Stage of Maturity				
% of Pasture				

6. Soil Classification and Texture _____

7. Fertilizer (Specify type, date of application, and Kg/ha.) _____

* It is essential that Sample Identification be the same on this form and on the tag attached to the sample.

TABLE 4 TYPICAL MINERAL CONCENTRATIONS IN RUMINANT BLOOD SERUM (OR PLASMA), LIVER, BONE, AND FORAGES

Item	Mineral Concentrations[a]								
	Na	K	Ca	Mg	P	Cu	Fe	Zn	Mn
Serum (or Plasma), µg/ml	± 3,000	± 200	± 100	± 20	± 50	± 1	± 1.5	± 1	± .05
Liver, µg/g	± 4,000	± 10,000	± 300	± 600	± 10,000	± 200	± 500	± 150	± 5
Bone ash	1%	.05%	36%	.6%	17%	2-3ppm	± 15ppm	± 100ppm	2-3ppm
Forages, µg/g	80-1,000	± 1,500	± 3,500	± 200	± 1,650	1-40	10-1,000	4-500	5-1,000

[a]Values expressed on dry matter basis for liver, bone, and forages and fresh basis for serum (or plasma).

TABLE 5 APPROXIMATE ELEMENTAL COMPOSITION OF LIVER IN SOLUTION,
REPRESENTING 5 g DRY SAMPLE/25 ml VOLUME

			Elemental Composition, μg/ml										
Species	Dilution	HCl[a]	Na	K	P	Ca	Mg	Fe	Zn	Cu	Mn	%Salt	Reference
Cattle	None	18,000	734	2,340	2,480	43	125	65.2	30.8	17.8	2.5	2.38	Ammerman
	1→10		73.4	234	248	4.3	12.5	6.5	3.1	1.8	.2	.24	et al.
	1→100		7.3	23.4	24.8	.4	1.2	.6	.3	.2	----	.02	1974
Sheep	None	"	----	----	2,340	32.6	118	211	38.6	8.7	2.0	----	Watson
	1→10		----	----	234	3.3	11.8	21.1	3.9	.9	.2	----	et al.
	1→100		----	----	23.4	.3	1.2	2.1	.4	.09	----	----	1973
Cattle	None	"	----	----	-------	----	----	47.8	20	29.6	1.67	----	Lang,
	1→10		----	----	-------	----	----	4.8	2.0	3.0	.2	----	1974
	1→100		----	----	-------	----	----	.5	.2	.3	----	----	
Sheep	None	"	----	----	-------	40	117	98	36	136	1.96	----	Standish and
	1→10		----	----	-------	4	11.7	9.8	3.6	13.6	.2	----	Ammerman,
	1→100		----	----	-------	.4	1.2	1	.4	1.4	----	----	1971
Cattle	None	"	----	----	2,749	55	126	51	29	35.4	2.36	----	Standish
	1→10		----	----	275	5	12.6	5.1	2.9	3.5	.24	----	et al.
	1→100		----	----	27.5	.5	1.3	.5	.3	.3	----	----	1971
Sheep	None	50,400[b]	800[c]	2,200[c]	2,040	18.4	103	43.8	18.6	106.4	1.6	5.57	Nutr.Lab.
	1→10		80	220	204	1.8	10.3	4.4	1.9	10.6	.2	.56	Univ. of FL.
	1→100		8	22	20.4	.2	1.0	.4	.2	1.1	----	.06	
Cattle	None	18,000	486	1,940	-------	----	-----	54	26	38.6	2.1	----	NBS (Nat'l
NBS # 1577	1→10		48.6	194	-------	----	-----	5.4	2.6	3.9	.2	----	Bureau of
	1→100		4.9	19.4	-------	----	-----	.5	.3	.4	----	----	Standards)

[a] 1% HCl≈.1N = .1 g mol. wt./liter, 1% HCl = 3,600 μg/ml HCl, 5% HCl = 18,000 μg/ml HCl.

[b] .1N HNO_3 = .1 g mol. wt./liter = 6,300 μg/ml HNO_3, 5% HNO_3 ≈ .8N HNO_3 = 50,400 μg/ml HNO_3.

[c] Estimated values.

TABLE 6 APPROXIMATE ELEMENTAL COMPOSITION OF FORAGES IN SOLUTION, REPRESENTING 5 g DRY SAMPLE/50 ml VOLUME

			Elemental Composition, μg/ml										
Type Sample	Dilution	HCl[a]	Na	K	P	Ca	Mg	Zn	Fe	Mn	Cu	% Salt[b]	Reference
Pangola grass (10 weeks) Hay, FL.	None	7200	350	800	250	450	438	25	18	10	1	.95	Nutr. Lab. Univ. of FL.
	1 → 10		35	80	25	45	44	2.5	1.8	1	.10	.10	
Pangola grass Hay, Guyana	None	"	110	1620	170	310	100	4	11.5	5	.57	.95	"
	1 → 10		11	162	17	31	10	.4	1.15	.5	—	.10	
Pangola grass Hay, FL. (Scissor-cut)	None	"	150	135	145	250	65	10	80	55	.35	.95	"
	1 → 10		15	13.5	14.5	25	6.5	1	8	5.5	—	.10	
Bahia-Clover Hay, FL.	None	"	80	---	230	690	340	2.5	9.9	6.8	.52	—	"
	1 → 10		8	—	23	69	34	.25	1	.68	—	—	
Pasture, Argentine Bahia, FL.	None	"	—	---	80	630	190	1.9	16.5	25	1.6	—	"
	1 → 10		—	---	8	63	19	.19	1.65	2.5	.16	—	
Pellets, Cottonseed meal	None	"	46	1810	1220	325	625	6.4	9.6	2.1	1.22	1.12	"
	1 → 10		4.6	181	122	32.5	62.5	.64	1	.21	.12	.11	
Alfalfa, Wash.	None	3600	240	2220	280	1370	253	2.7	18.9	2.2	1.3	.76	Baker and Smith, 1968
	1 → 10		24	222	28	137	25.3	.27	1.89	.22	.13	.08	
Mixed forage, Wash.	None	"	182	2000	260	1390	253	3.13	11.6	15.2	1.33	.77	"
	1 → 10		18.2	200	26	139	25.3	.31	1.16	1.52	.13	.08	
Corn cobs, Wash.	None	"	18	580	53	30	26	2.19	14.5	.65	3.71	.43	"
	1 → 10		1.8	58	5.3	3	2.6	.22	1.45	—	.37	.04	

TABLE 6 (continued)

Type sample	Dilution	HCl	Na	K	P	Ca	Mg	Zn	Fe	Mn	Cu	% Salt[b]	Reference
		ELEMENTAL COMPOSITION, µg/ml											
Corn grain,	None	3600	13	630	364	30	133	2.71	2.52	.72	.67	.48	Baker and
Wash.	1 → 10		1.3	63	36.4	3	13.3	.27	.25	----	----	.05	Smith, 1968
Corn kernels,	None	7200	----	---	300	4	188	2.50	6.0	.75	.50	----	Nutr.Lab.
FL.	1 → 10		----	---	30	.4	18.8	.25	.6	----	---	----	Univ. of FL.
Citrus Pulp													
Ground	None	7200	100	1080	100	1540	120	1.77	13.5	.67	.97	1.02	Ammerman
Unground	None	"	110	1120	110	1470	120	1.14	9.4	.58	.60	.10	et al.
	1 → 10		10.5	110	10.5	150	12	.15	1.10	----	---	----	1973
Alder leaves,	None	3600	28	790	135	980	237	5.3	13.90	40.9	1.04	.58	Baker and
Wash.	1 → 10		2.8	79	13.5	98	23.7	.53	1.39	4.09	.10	.06	Smith, 1973
Douglas-fir	None	"	29	550	144	220	79	1.0	9.9	23.0	.29	.46	"
Needles, Wash.	1 → 10		2.9	55	14.4	22	7.9	.10	.99	2.30	----	.05	
Orchard leaves	None	7200	8	1470	210	2090	620	2.5	30	9.1	1.20	1.16	NBS Nat'l
NBS # 1571	1 → 10		.8	147	21	209	62	.25	3	.91	.12	.12	Bureau of Standards
Steer ration	None	"	45	90	340	225	126	10.8	43	2.7	1.8	.81	Nutr.Lab.
(Low Mn)	1 → 10		4.5	9	34	22.5	12.6	1.08	4.3	.27	.18	.08	Univ. of FL.
Molasses	None	"	288	4775	100	900	700	20	35	50	17.5	1.40	"
(Dry matter basis)	1 → 10		28.8	478	10	90	70	2	3.5	5	1.75	.14	

[a] 1% HCl ≃ .1N = .1 g mol. wt./liter, 1% HCl = 3600 µg/ml HCl, 2% HCl = 7200 µg/ml HCl.

[b] Total soluble salt includes Cl^- and H^+, calculated on a weight/volume basis.
Salts contributed by forage = Total % salt + .72 (2% HCl) or .36 (1% HCl).

TABLE 7 MACRO- AND MICRO-MINERAL LEVELS IN SERUM (OR PLASMA), LIVER, AND FORAGE. STOCK STANDARD ELEMENTAL CONCENTRATIONS REQUIRED TO MATCH SAMPLE SOLUTIONS

Serum (or plasma)	Na	K	Ca	Mg	P	Zn	Cu
Range, µg/ml	3,000	200	90-110	18-31	30-60	.5-1.2	.7-1.4
1 ml → 10, 10% TCA (w/v)	300	20	9-11	1.8-3	3-6	———	———
Working standard range, µg/ml	———	———	25-500	4-40	30-60	———	———
Stock standard concentration, µg/ml	13,000	15,600	10,000	10,000	2,000	1,000	1,000

Liver	Na	K	Ca	Mg	P
Range, µg/g, DM	4,000	10,000	200-460	400-600	10,000-14,000
Total µg/5 g sample, DM	20,000	50,000	1,000-2,300	2,000-3,000	50,000-70,000
Concentration/25 ml, µg/ml	800	2,000	40-92	80-120	2,000-2,800
Working standard range, µg/ml	———	———	50-1,500	10-400	500-7,500
Stock standard concentration, µg/ml	13,000	15,600	1,000	10,000	100,000

TABLE 7 (continued)

Liver	Fe	Zn	Cu	Mn	Co	Se	Mo
Range, µg/g, DM	180 - 376	84 - 132	100 - 300	8 - 12	.2 - 1	.5 - 5	2 - 4
Total µg/5 g sample, DM	900 - 1,800	420 - 660	500 - 1,500	40 - 60	1 - 5	2.5 - 25	10 - 20
Concentration/25 ml, µg/ml	36 - 75	17 - 26	20 - 60	1.6 - 2.4	.04 - .2	.1 - 5	.4 - .8
Working standard range, µg/ml	5 - 80	2 - 30	5 - 100	2 - 100	———	———	———
Stock standard concentration, µg/ml	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Forages	Na	K	Ca	Mg	P
Range, µg/g, DM	50 - 3,500	8,000 - 20,000	2,400 - 8,500	1,300 - 4,300	900 - 2,900
Total µg/5 g sample, DM	250 - 17,500	40,000 - 100,000	12,000 - 42,500	6,500 - 21,500	4,500 - 14,500
Concentration/50 ml, µg/ml	3 - 350	800 - 2,000	240 - 850	130 - 430	90 - 290
Working standard range, µg/ml	2.5 - 400	500 - 3,500	100 - 1,500	50 - 600	40 - 300
Stock standard concentration, µg/ml	500	50,000	10,000	10,000	2,000

TABLE 7 (continued)

	Fe	Zn	Cu	Mn
Range, µg/g, DM	10 - 1,000	4 - 500	1 - 40	5 - 1,000
Total µg/5 g sample, DM	50 - 5,000	20 - 2,500	5 - 200	25 - 5,000
Concentration/50 ml, µg/ml	1 - 100	.4 - 50	.1 - 4	.5 - 100
Working standard range. µg/ml	20 - 800	40 - 500	10 - 150	15 - 1,500
Stock standard concentration, µg/ml	1,000	1,000	1,000	1,000

Stock standard concentration for copper and zinc in serum and iron, zinc, copper, and manganese in liver and forage 1000 µg/ml commercial atomic absorption standards (Harleco, Fisher Scientific) are used.
Dilutions to provide 500, 100, and 10 µg/ml micro-elements may be useful.

TABLE 8 CALCULATIONS AND PREPARATION OF STOCK STANDARD SOLUTIONS AND OTHER REAGENTS

1. Phosphorus

a. Phosphorus, 2,000 µg P/ml

KH_2PO_4 mol. wt., 136.09; 22.76% P

1 g KH_2PO_4 = .2276 g P = 227.6 mg P

1 g KH_2PO_4/liter = .2276 mg P/ml = 227.6 µg P/ml

2,000 ÷ 227.6 = 8.7873 g/liter

Dissolve 8.7873 g KH_2PO_4 (dessicator dry) in one liter deionized water (inclusion of 1 ml concentrated H_2SO_4 preserves solution indefinitely).

b. Phosphorus, 10 and 20% (w/v)

H_3PO_4 85%, mol. wt., 97.97; 31.63% P

31.63% P x .85 = 26.89% P = 268,900 µg/g (≃ ml)

1 → 10 dilution = 26,890 µg P/ml = 10% (w/v)

1 → 20 dilution = 13,445 µg P/ml = 20% (w/v)

To use this compound for a standard solution, specific gravity corrections must be used.

2. Sodium

a. Sodium, 500 µg/ml

NaCl mol. wt., 58.44; 39.34% Na

1 g NaCl = .3934 g Na = 393.4 mg Na

1 g NaCl/liter = .3934 mg Na/ml = 393.4 µg Na/ml

500 ÷ 393.4 = 1.2710 g/liter

Dissolve 1.2710 g NaCl (oven dry at 70 C) in 1 liter deionized water. Store in polyethylene container.

TABLE 8 (continued)

b. NaCl, 6.5 N

NaCl, mol. wt., 58.4; 1N = 23 g Na/liter

6.5N = 14.95% Na = 149,500 μg/ml

6.5 x 58.4 = 379.6 g NaCl/liter

c. NaCl, .6N

.6 x 58.4 = 35.04 g/liter

Weigh 35.04 g (dessicator dry) NaCl in one liter deionized water.

3. Potassium

a. Potassium, 50,000 μg K/ml

KCl, mol. wt., 74.56; 52.44% K

1 g KCl = .5244 g K = 524.4 mg K

1 g KCl/liter = .5244 mg K/ml = 524.4 μg K/ml

50,000 ÷ 524.4 = 95.35 g/liter

Dissolve 95.35 g KCl (oven dry at 70 C) in one liter deionized water.

b. KCl, 4.5N

KCl, mol. wt., 74.5; 1N = 39.1 g K/liter

4.5N = 17.60% K = 175,950 μg/ml

4.5N x 74.5 = 335.25 g KCl/liter

c. KCl, .4N

.4 x 74.5 = 29.80 g/liter

Weigh 29.80 g KCl (dessicator dry) in one liter deionized water.

TABLE 8 (continued)

4. Calcium

a. Calcium, 10,000 µg Ca/ml

$CaCO_3$, mol. wt., 100.09; 40.08% Ca

1 g $CaCO_3$ = .4008 g Ca = 400.8 mg Ca

1 g $CaCO_3$/liter = .4008 mg Ca/ml = 400.8 µg Ca/ml

10,000 ÷ 400.8 = 24.95 g/liter

Dissolve 24.95 g $CaCO_3$ (oven dry at 70 C) in 50% HCl as needed. When cool, bring to one liter volume with deionized water.

b. $CaCl_2$, 10%; dissolve 24.95 g $CaCO_3$ in 50% HCl as needed. When cool, bring to 100 ml volume with deionized H_2O.

5. Magnesium

a. Magnesium, 10,000 µg Mg/ml

Mg metal = 100% Mg; 10 g Mg metal = 10 g Mg = 10,000 mg Mg

10 g Mg metal/liter = 10 mg Mg/ml = 10,000 µg Mg/ml

Dissolve 10 g Mg metal in 10% HCl as needed. Bring to one liter volume with deionized water.

b. $MgCl_2$, 10% (w/v)

Dissolve 10 g Mg metal in 10% HCl. Bring to 100 ml volume with deionized water.

6. $LaCl_3$ solution, 5% Lanthanum

Dissolve 117.3 g La_2O_3 in 500 ml HCl (concentrated) and make to two liters with deionized water when cool. Use a three liter Pyrex beaker under the hood.

TABLE 8 (continued)

Add acid to the Lanthanum powder very slowly and carefully. The solution releases O_2 and Cl_2 rapidly and boils up.

7. Trichloracetic Acid (TCA), 10% (w/v)

Carefully dissolve 100 g TCA per liter in deionized water.

TABLE 9 WORKING STANDARD SOLUTIONS AND DILUTIONS FOR SERUM OR PLASMA SAMPLES FOR ANALYSIS OF CALCIUM, MAGNESIUM, PHOSPHORUS, ZINC, AND COPPER

Aqueous stock solutions:

KCl, .4N = 15,600 μg K/ml = 14,176 μg Cl/ml

NaCl, .6N = 13,800 μg Na/ml = 22,240 μg Cl/ml

$CaCO_3$ in HCl = 10,000 μg Ca/ml

Mg metal in HCl = 10,000 μg Mg/ml

H_3PO_4 (diluted) = 10,000 μg P/ml

Working standards:

Calcium: In 100 ml volumetric flask, make standards of 0, 25, 50, 150, 250, 350, and 500 μg Ca/ml. If desired, add 1 ml of potassium stock solution and 20 ml of sodium stock solution. Each standard will have 156 μg K/ml, 2760 μg Na/ml and approximately match the potassium and sodium concentrations of the undiluted sample. Dilute standards as samples in the following manner:

1 ml → 10, TCA (10%) → 1 ml → 5, 1% $LaCl_3$. Dilution factor of 50.

Standards should contain: 0, .5, 1, 3, 5, 7, and 10 μg/ml of calcium.

Magnesium: In 100 ml volumetric flask, make standards of 0, 4, 10, 20, 30, and 40 μg Mg/ml (add sodium and potassium, as above, if desired) and dilute as samples.

1 ml → 10, TCA (10%) → 1 ml → 5, 1% $LaCl_3$. Dilution factor is 50.

Standards should contain: 0, .08, .2, .4, .6, .8 μg/ml magnesium.

TABLE 9 (continued)

Phosphorus:

Dilute stock standard (2,000 µg P/ml) 25 ml → 500 ml = 100 µg P/ml working stock standard.

Working standards:

1 ml → 100 ml = 1 µg/ml

2 ml → 100 ml = 2 µg/ml

10 ml → 100 ml = 10 µg/ml

15 ml → 100 ml = 15 µg/ml

Follow the procedure for the colorimetric determination of phosphorus. Standards and blank solutions are treated exactly as samples.

Zinc and copper in serum (plasma):

Standards in glycerol solutions for serum dilutions in deionized water:

In 100 ml volumetric flask, prepare matrix solutions using:

Stock solution	Concentration of elements in µg/ml
1 ml K solution	156 K
20 ml Na solution	2,760 Na
1 ml Ca solution	100 Ca
.2 ml Mg solution	20 Mg
.5 ml P solution	50 P

Working standards for zinc analysis are prepared by adding zinc to above matrix solutions to provide the following concentrations: 0, 1, 2, 3, 5, and 10 µg Zn/ml. Make to volume with a 25% (v/v) glycerol solution. Dilute working standards (1 → 5), along with serum samples with deionized water. Standards will contain 0, .2, .4, .6, 1, and 2 µg Zn/ml of a solution containing approximately 5% (v/v) glycerol.

TABLE 9 (continued)

Working standards for copper analysis are prepared by adding copper to above matrix solutions to provide the following concentrations: 0, .5, 1, 1.5, and 2 μg Cu/ml. Make to volume with a 20% (v/v) glycerol solution. Dilute working standards (1 → 2) along with serum samples with deionized water. Standards will contain: 0, .25, .50, .75, 1 μg Cu/ml of a solution containing approximately 10% (v/v) glycerol.

Standards in 5% HCl (v/v) for ashed serum solutions (1 ml serum/5 ml HCl 5%):

Prepare a zinc and copper mixed standard with the following concentrations: 0, .1, .2, .4, 1, and 2 μg Cu/ml, and 0, .1, .4, 1, 2, and 3 μg Zn/ml in 5% HCl.

In 100 ml volumetric flasks containing the matrix solutions indicated previously, prepare the above mixed standard solutions. To each 100 ml flask, add the following stock elemental solutions (10 ml each):

Standard	Volumes	Stock elemental solution, μg/ml		Concentration of standard, μg/ml	
		Cu	Zn	Cu	Zn
A blank		0	0	0	0
B	10 ml	5	5	.5	.5
C	" "	10	20	1	2
D	" "	20	50	2	5
E	" "	50	100	5	10
F	" "	100	150	10	15

Make to volume with 5% HCl. Dilute standards 1 → 5 in 5% HCl to have the appropriate concentration range for Cu (0 to 2 μg/ml) and Zn (0 to 3 μg/ml), and a similar concentration of the bulk ions as in ashed serum solutions (1 ml/5 ml HCl 5%).

TABLE 10 WORKING STANDARD SOLUTIONS REQUIRED TO MATCH LIVER SAMPLES, 5 g DRY TISSUE/25 ml SOLUTION

Calcium

Prepare standards containing 0, .5, 1, 2, 4, 8, 10, and 15 μg Ca/ml in 5% HCl and 1% $LaCl_3$. Dilute samples 1 → 100 to equal 1% $LaCl_3$ as in standards.

Magnesium

Prepare standards containing 0, .1, .5, 1, 1.5, 2, 3, and 4 μg Mg/ml in 5% HCl and 1% $LaCl_3$. Dilute samples 1 → 100 to equal 1% $LaCl_3$ as standards. (This is the same as for calcium).

Phosphorus

From a working stock standard of 100 μg P/ml, prepare working standards of 0, 1, 2, 4, 6, 8, 10, 15, and 20 μg/ml in deionized water. Use the 1 → 100 1% $LaCl_3$ sample dilution and dilute further 1 → 10 in deionized water. This will put samples in a range of 2 to 2.8 μg P/ml. Follow the colorimetric procedure for phosphorus determination; samples and standards are treated alike.

TABLE 10 (continued)

STANDARDS FOR MICRO-ELEMENTS IRON, ZINC, COPPER, AND MANGANESE

Solution that will approximate concentrations of sodium, potassium, calcium, magnesium, and phosphorus in liver solutions (5 g dry tissue/25 ml 5% HCl).

Elements	µg/ml	Stock solution	ml required/liter	Stock solution sources
Na	828	13,800 µg/ml	60	.6N
K	2,184	15,600 "	140	.4N
Ca	50	1,000 "	50	10% Ca ($CaCO_3$ in HCl)
Mg	150	10,000 "	15	1% Mg (metal in HCl)
P	2,000	100,000 "	20	10% P (concentrated H_3PO_4)
			285 ml	
HCl	2.5%	10%	250	

The bulk ion solution should be extracted twice with 10 ml Ammonium Pyrrolidine Dithiocarbamate (APDC), 1% (w/v) and 10 ml Methyl Isobutyl Ketone (MIBK) water saturated, to remove contaminant elements prior to addition of the trace element standards.

Trace element solutions are added to the above bulk ion solution to provide in µg/ml:

	Blank	A	B	C	D	E	F	G
Fe	0	5	10	15	30	50	100	150
Zn	0	3	5	10	20	30	50	80
Cu	0	3	5	10	30	50	100	150
Mn	0	.1	.3	.5	.8	1	2	3

TABLE 10 (continued)

MICRO-ELEMENT STANDARDS

	Blank	A	B	C	D	E	F	G
Fe								
ml required/100 ml	0	5	10	15	3	5	10	15
Zn								
ml required/100 ml	0	3	5	10	2	3	5	8
Cu								
ml required/100 ml	0	3	5	10	3	5	10	15
	---- 100 µg/ml stock sln--				--1,000 µg/ml stock sln---			
Mn								
ml required/100 ml	0	1	3	5	8	10	20	30
	---------- 10 µg/ml stock solution ----------							

Use a 100 µg/ml stock standard for standards A, B, C for iron, zinc, and copper.

Use a 1,000 µg/ml stock standard for standards D, E, F, G for iron, zinc, copper.

A 10 µg/ml stock solution may be used to prepare all the manganese standards.

The standards for iron, zinc, and copper are diluted 1 → 10 with deionized water, along with the liver sample solutions.

Use undiluted standards and samples to analyze for manganese.

TABLE 11 WORKING STANDARD SOLUTIONS REQUIRED TO MATCH FORAGE SAMPLES, 5 g DRY WEIGHT/50 ml; PHOSPHORUS, SODIUM, POTASSIUM, CALCIUM, AND MAGNESIUM

Standard No. (2% HCl ≃ .2N)	Concentrations µg/ml for 100 ml solutions					ml stock solution required/100 ml					
	P	Na	K	Ca	Mg	P	Na	K	Ca	Mg	HCl
1 A	40	2.5	500	100	50	2	.5	1	1	.5	4 ml
2 A	60	10	1000	300	100	3	2	2	3	1	4 ml
3 A	80	25	1500	500	200	4	5	3	5	2	4 ml
4 A	120	50	2000	700	300	6	10	4	7	3	4 ml
5 A	160	100	2500	800	400	8	10	5	8	4	4 ml
6 A	200	300	3000	1000	500	10	30	6	10	5	4 ml
7 A	300	400	3500	1500	600	15	40	7	15	6	4 ml
8 A (blank)	0	0	0	0	0	0	0	0	0	0	4 ml

Make to 100 ml volume with deionized water.

Stock standard solutions:

P, 2000 µg/ml; Na, 500 µg/ml, 1000 µg/ml; K, 50,000 µg/ml

Ca and Mg, 10,000 µg/ml; HCl, 50% (v/v) or 6N

Pipette stock standards into 100 ml volumetric flasks. Store in bottles.

Notes: Phosphorus will be as single standard solutions since the phosphorus compound (KH_2PO_4) contributes potassium.

Sodium, potassium, calcium, and magnesium may be combined into multi-element working standard solutions.

Working standard solution concentrations correspond to those expected in undiluted forage solutions. Standard solution should be standardized or calibrated at appropriate dilutions against known atomic absorption standard solutions.

TABLE 12 DILUTIONS REQUIRED FOR ANALYTICAL RANGES, CORRESPONDING STANDARDS TO FORAGE SAMPLE SOLUTION DILUTIONS FOR SODIUM, POTASSIUM, CALCIUM, MAGNESIUM, AND PHOSPHORUS

Standard No.	Concentration µg/ml 1 → 10 dilution, deionized H_2O				Standard No.	Concentration µg/m 1 → 20 dilution, 1% $LaCl_3$
	Na	K	Ca	Mg		P
1B	.25	50	10	5	1C	2
2B	1	100	30	10	2C	3
3B	2.5	150	50	20	3C	4
4B	5	200	70	30	4C	6
5B	10	250	80	40	5C	8
6B	30	300	100	50	6C	10
7B	40	350	150	60	7C	15
8B	0	0	0	0	8C	0

Standard No.	Concentration µg/ml 1 → 100 dilution, 1% $LaCl_3$				Standard No.	Concentration µg/ 1 → 500 dilution, 1% $LaCl_3$	
	Na	K	Ca	Mg		Mg	K
1D	-	5	1	.5	1E	.1	1
2D	.1	10	3	1	2E	.2	2
3D	.25	15	5	2	3E	.4	3
4D	.5	20	7	3	4E	.6	4
5D	1	25	8	4	5E	.8	5
6D	3	30	10	5	6E	1	6
7D	4	35	15	6	7E	1.2	7
8D	0	0	0	0	8E	0	0

TABLE 12 (continued)

Solutions	1B - 8B	represent	a 1 → 10	dilution	of	stock	standards	1A - 8A
"	1C - 8C	"	a 1 → 20	"	of	"	"	1A - 8A
"	1D - 8D	"	a 1 → 10	"	of	solutions		1B - 8B
"	1E - 8E	"	a 1 → 5	"	of	"	"	1D - 8D

All dilutions may be performed with the autodilutor, along with forage sample solutions into Falcon polyethylene culture tubes (+ caps).

TABLE 13 PREPARATION OF MIXED MATRIX SOLUTIONS FOR FORAGE MICRO-ELEMENTAL STANDARD SOLUTIONS, STANDARDS, AND STANDARD DILUTIONS FOR ANALYSIS OF IRON, ZINC, MANGANESE, AND COPPER

A. Into a 1 liter volumetric flask, deliver:

1. Low matrix (.12% dissolved solids, .84% total salt)

40 ml KCl, 4.5N	Approx.	7,000 µg/ml	K
5 ml NaCl, 6.5N	"	750 "	Na
10 ml H_3PO_4, 5% (v/v)	"	1,340 "	P
20 ml $CaCl_2$, 10% (w/v)	"	2,000 "	Ca
10 ml $MgCl_2$, 10% (w/v)	"	1,000 "	Mg
200 ml HCl, concentrated	"	20% HCl (v/v)	

2. High matrix (.32% dissolved solids, 1.04% total salt)

100 ml KCl, 4.5N	Approx.	17,500 µg/ml	K
20 ml NaCl, 6.5N	"	2,990 "	Na
20 ml H_3PO_4, 5% (v/v)	"	2,700 "	P
50 ml $CaCl_2$, 10% (w/v)	"	5,000 "	Ca
40 ml $MgCl_2$, 10% (w/v)	"	4,000 "	Mg
200 ml HCl, concentrated	"	20% HCl (v/v)	

Make to volume with deionized H_2O. Invert 10 times to mix. Store in clean Nalgene reagent bottle.

When diluted 1 → 10 ml, the matrix solutions resemble those of 5 g forage/50 ml.

TABLE 13 (continued)

B. Micro-elemental standards:

For ranges in forage:

Copper, 10 - 150 μg/5 g sample	.2 - 3 μg/ml (50 ml)
Iron, 20 - 800 " "	.4 - 16 " "
Zinc, 40 - 500 " "	.8 - 10 " "
Manganese, 15 - 1,500 μg/5 g sample	.3 - 30 " "

Analytical standards may be freshly prepared with the autodilutor.

Analytical standards, to contain, in μg/ml

	A	B	C	D	E	F
Cu, 2 & 3	0	.2	.5	1	2	4
Fe, 2 & 3	0	.2	.5	1	2	4
Zn, 2 & 3	0	.2	.5	1	2	4
Mn, 2 & 3	0	.2	.5	1	2	4
Mn	0	.4	1	2	4	8

Above standards, A2 - F2, contain .2% HCl and are to be used for analysis of 1 → 10 dilutions of the undiluted sample solutions. For 1 → 5 dilutions of the undiluted samples, these standards should contain .4% HCl.
Above standards, A3 - F3, contain one ml mixed matrix solutions per 10 ml of solution and are to be used for analysis of undiluted sample solutions.

Stock multi-element standard solutions:

Stock multi-element standard solutions, A1 - F1 to be diluted 1 → 10 ml to make above analytical standard solutions, A2 - F2 or A3 - F3.

Store solutions in clean Nalgene bottles.

TABLE 13 (continued)

Stock standards A1 - F1 should be 2% HCl:

Pipette appropriate standard solutions and HCl and bring to 100 ml volume.

Stock	standard	B1	contains	2	µg/ml	each	of	Cu,	Fe,	Zn,	and	Mn.
"	"	C1	"	5	"	"	"	"	"	"	"	"
"	"	D1	"	10	"	"	"	"	"	"	"	"
"	"	E1	"	20	"	"	"	"	"	"	"	"
"	"	F1	"	40	"	"	"	"	"	"	"	"

An additional series, A1A - F1F, to contain 0, 4, 10, 20, 40, 80 µg/ml may be necessary for forages high in manganese.

Micro-elements are provided by diluting commercial atomic absorption standard solutions (1,000 µg/ml) accordingly.

TABLE 14. DATA SHEET TO RECORD WEIGHTS

Exp. No.________________ Date________________

________________________ Technician________________

			"Wet" weight		Dry weight			Ash weight		
nple ID	Crucible number	Crucible weight	Crucible + sample	Sample weight	Crucible + sample	Sample weight	% dry matter	Crucible + sample	Sample weight	% ash

TABLE 15. PHOSPHORUS ANALYSIS DATA SHEET (FORAGE)

Exp. No. ______________________ Date ______________________

Unit (μg or mg)* ________________ Technician ________________

Sample ID	% T	O.D.	Dry wt. g	**	STANDARDS *(y) - /50 ml	STANDARDS % T	STANDARDS (x) O.D.

Regression

b = ____________________

a = ____________________

**factor ____________________

% = 0.1 (mg/50 ml)

ppm = 1.0 (μg/50 ml)

Correlation coefficient

r = ____________________

Standard error of the estimate y on x

Sy·x = ____________________

TABLE 16. PHOSPHORUS ANALYSIS DATA SHEET

Exp. No. ____________________ Dilution __________________ Date ________________

Original solution ____________ Aliquot __________________ Technician __________

le	% T	O.D.	μg/ml graph	μg/ml regression	Final dilution	Dry wt. g	μg/g	P %	Standards μg/ml	Standards % T	Standards O.D.

Regression

b ______________

a ______________

correlation coefficient

r ______________

Standard error of the estimate Y on X

$S_{y \cdot x}$ ____________

TABLE 17. AAS MINERAL ANALYSIS DATA SHEET (FORAGE)

Exp. No.________________ Unit (μg or mg)* ___________ Date ___________

Mineral ________________ Approx. dilution___________ Technician______

Sample ID	Abs.	Abs.	Dry wt. g	**	STANDARD (x) Abs.	(x) Abs.	* (y) -/50 ml

Regression

b = ________________

a = ________________

** factor = ____________

%=0.1 (mg/50 ml)

ppm = 1.0 (μg/50 ml)

Correlation coefficient

r = ________________

Standard error of the estimate y on x

Sy·x ________________

TABLE 18. AAS MINERAL ANALYSIS DATA SHEET

Exp. No. ______________ Original solution ______________ Date ______________

Mineral ______________ Dilution ______________ Technician __________

ple	Readout		µg/ml		Total dilution	Dry wt. g	µg/g	STANDARD		
)	Conc.	Abs.	Graph	regression				µg/ml	Readout Conc.	Abs.

Regression

b = ______________

a = ______________

Correlation coefficient

r = ______________

Standard error of the estimate y on x

Sy·x ______________

REFERENCES

Ammerman, C. B., J. M. Loaiza, W. G. Blue, J. F. Gamble and F. G. Martin. 1974. Mineral composition of tissues from beef cattle under grazing conditions in Panama. J. Anim. Sci. 38:158.

Ammerman, C. B., F. G. Martin and L. R. Arrington. 1973. Mineral contamination of feed samples by grinding. J. Dairy Sci. 53:1514.

Atomic Absorption Newsletter. Vol. 1, 1962 through Vol. 14 (4), 1975. Perkin-Elmer Corp., Norwalk, Conn.

Baetz, R. A. and C. T. Kenner. 1975. Determination of trace metals in foods using chelating ion exchange concentration. J. Agr. Food Chem. 23:44.

Baker, A. S. and R. R. Smith. 1973. Preparation of solutions for atomic absorption analyses of Fe, Mn, Zn, and Cu in plant tissue. J. Agr. Food Chem. 22:103.

Ball, J. W. and W. C. Gottschall. 1975. Matrix interferences in the determination of iron by atomic absorption spectrophotometry. Atomic Absorption Newsletter 14:63.

Billings, G. K. 1965. Light scattering in trace element analysis by atomic absorption. Atomic Absorption Newsletter 4:357.

Chapman, Jr. H. L., D. H. Cox, C. H. Haines and G. K. Davis. 1963. Evaluation of the liver biopsy technique for mineral nutrition studies with beef cattle. J. Anim. Sci. 22:733.

Chen, P. S., T. Y. Toribara, and H. Warner. 1956. Micro-determination of phosphorus. Anal. Chem. 28:1755.

Coker, D. T. and J. N. Ottoway. 1970. Overexcitation interferences in atomic absorption spectrophotometry with an air acetylene flame. Nature 227:831.

Fernandez, F. J. and H. L. Kahn. 1971. Clinical methods for atomic absorption spectroscopy. Clin. Chem. Newsletter 3:24.

Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.

Giron, H. C. 1973. Comparison between dry-ashing and wet digestion in the preparation of plant material for atomic absorption analysis. Atomic Absorption Newsletter 12:28.

Hell, A. and J. Ramirez-Munoz. 1970. High solid samples in atomic absorption flame spectrophotometry. Anal. Chim. Acta. 51:141.

Kahn, H. L. 1968. A background compensation system for atomic absorption. Atomic Absorption Newsletter 7:40.

Koirtyohann, S. R. and E. E. Pickett. 1966a. Spectral interferences in atomic absorption spectrophotometry. Anal. Chem. 38:585.

Koirtyohann, S. R. and E. E. Pickett. 1966b. Light scattering by particles in atomic absorption spectrometry. Anal. Chem. 38:1087.

Lang, C. E. 1974. Phosphorus and trace mineral status of beef cattle in the Guanacaste region of Costa Rica. M. S. Thesis. University of Florida, Gainesville.

Legget, G. E. and D. T. Westerman. 1973. Determination of mineral elements in plant tissues using trichloracetic acid extraction. J. Agr. Food Chem. 21:65.

Manning, D. C. 1972. Using the Perkin-Elmer deuterium background correction system. Atomic Absorption Newsletter 11:112.

Slavin, W. 1968. Atomic Absorption Spectroscopy. John Wiley and Sons, N. Y., N. Y.

Standish, J. F. and C. B. Ammerman. 1971. Effect of excess dietary iron as ferrous sulfate and ferric citrate on tissue mineral composition of sheep. J. Anim. Sci. 33:481.

Standish, J. F., C. B. Ammerman, A. Z. Palmer and C. F. Simpson. 1971. Influence of dietary iron and phosphorus on performance, tissue mineral composition and mineral absorption in steers. J. Anim. Sci. 33:171.

Watson, L. T., C. B. Ammerman, J. P. Feaster and C. E. Roessler. 1973. Influence of manganese intake on metabolism of manganese and other minerals in sheep. J. Anim. Sci. 36:131.

West, A. C., F. A. Fassel, and R. N. Kniseley. 1973. Calcium phosphate solute volatilization interference in flame spectrophotometry. Anal. Chem. 45:2420.