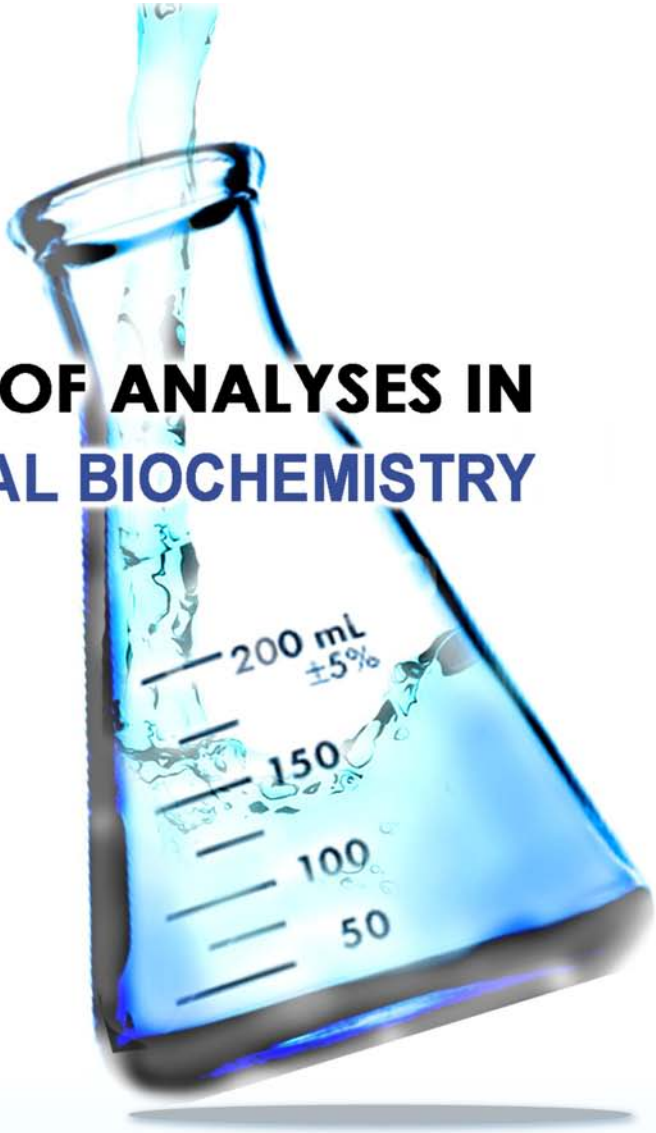
TECHNIQUES OF ANALYSES IN NUTRITIONAL BIOCHEMISTRY



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TECHNIQUES OF ANALYSES IN NUTRITIONAL BIOCHEMISTRY

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Published by BP Undip
Semarang
November 2009

Book : Techniques of Analyses in Nutritional Biochemistry; Retno Murwani (author); 1st Ed; Badan Penerbit Undip Semarang; 2009
xii + 184 pages; 16.5 x 23.5 cm
ISBN : 978-979-704-838-9

Title:

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Preface

This book is written to document empirical experience during the course of various research grants that the author has received and carried out, as well as to document experience of more than 23 years of teaching as a lecturer. This book is written as part of World Class University Program for Book Writing launched by Diponegoro University in the year 2009. The author is one of twelve university lecturers who are assigned to write the book in English language (SK Rektor No.520/SK/H7/2009).

This book is written to help students and other readers in carrying analyses either on their own, or as a reference and guide in understanding analytical methods written in scientific journals to support their research. The book consists of ten chapters, covering several major and minor nutrients as well as phytonutrient such as polyphenol. It is beyond the scope of this book to cover all nutrients known today. However, the principles and techniques can be applied to almost any nutrients. Each chapter is introduced with a paragraph that gives a sample phrase which can reflect the need of a nutrient analysis in the context of a scientific research that has been published in scientific journals, or on a nutrient label of feed or food products. This introduction is meant to ease the

reader to relate to information or an instruction found in many published works with the materials given in each chapter. After reading this book the readers will hopefully be able to understand written analytical procedures and execute the procedures into practice in nutrition analyses.

It is my hope that this book will be useful not only for students in animal science faculty, but also for other students and readers who need a guide to carry out nutrient analyses. Grateful acknowledgement is due to the financial support from World Class University Program for Book Writing, Diponegoro University, year 2009, and Prof. Dr. Retmono, who is a Professor in English Education, as the appointed expert for the proof reading of the English language.

Author
Retno Murwani
November, 2009

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Chapter I

Introduction to Nutrition Analysis

In human, nutrition is defined as **"***the science of foods, the nutrients and the substances therein, their action, interaction and balance in relation to health and diseases".* Another nutrition definition is "*the sum of the processes involved in the taking in of nutrients and their assimilation and use for proper body functioning and maintenance of health. The successive stages include ingestion, digestion, absorption, assimilation, and excretion"* (AHMD, 2007). *In short Nutrition is* **"***the science of food at work in the body". Food for animals or livestock is termed as feed*. Therefore, adopting this definition of nutrition for animal, animal nutrition can be defined accordingly as *the science of feed at work in the body of an animal*. According to WHO of the United Nations, health is further defined as "*A state of complete physical, mental and social well-being and not merely the absence of disease and infirmity".* Animal nutrition deals with the science of feed at work in the body to support optimal productivity. The productivity can be further grouped according to the purpose of the animal being raised, i.e. as livestock for human consumption, as companion animals, sports (such as race horses), work (such as buffalo in rice producing countries), or contest. Productivity of livestock animals is different from those for work and other purposes. However, they all deserve to have proper nutrition in order to achieve optimal physical and physiological development. Further, recent development

Chapter II

Units of a Nutrient Concentration

INTRODUCTION

In a study of provitamin A β-carotene supplementation in a diet, the distribution in the body, and the activity of the enzyme involved in β-carotene metabolism in chicks, a publication under Materials & Methods described the following procedures (Murwani, 2002):

> *One-day-old male leghorn chicks were given a vitamin A deprived diet ad libitum (Table 1). They were kept under controlled light and warm temperature, and water was provided on free access. After feeding the chicks on vitamin-A deprived diet for 10 days to lower and equalise hepatic retinol storage, 28 chicks with similar average body weight were assigned into four treatment groups to receive diet containing: 1) 20% gluten protein supplemented with 1.5 μg β-carotene/g diet, 2) 20% gluten protein supplemented with 15 μg β-carotene/g diet, 3) 20% casein protein supplemented with 1.5 μg β-carotene/g diet, 4) 20% casein protein supplemented with 15 μg β-carotene/g diet.*

Another publication (Murwani, 2001) described the assay of an enzyme involved in the conversion of β-carotene into retinol in chick intestinal mucosa with the following procedures :

> *ARAT was assayed by measuring the formation of labeled [^{3}H] retinyl palmitate after incubating radioactive 50,000 cpm [^{3}H] retinol and 10 nmol unlabeled retinol with unlabeled palmitoyl-CoA in the presence of microsomal ARAT. The assay procedure were basically the same as that described by Helgerud et al., (1982, 1983), and modified so that radioactive retinol*

Chapter III

Principles of Spectrophotometer

Nutrient analysis in feed or food is an important step in composing a good diet to provide all nutrients needed for normal growth and development. It is also important in studying or the fate of nutrients during metabolism as has been described in Chapter I. Macro and micro nutrients in feed or food (animal product is part of food for human consumption), or in certain parts of body organs can be determined qualitatively or quantitatively by various methods. Quantitative analyses can be done with the aid of analytical equipment called Spectrophotometer. Spectrophotometer is an analytical instrument which works on the basis of simple colourimetric principles i.e. absorbance of light by colour solution. Thus this chapter begins with an understanding of the light and colourimeter principles. Consequently, spectrophotometry as the science of spectrum of a compound and as the basis of quantitative determination of a nutrient has a broad and growing application.

WHITE LIGHT / SUNLIGHT

Light that is emitted by the sun and seen by human eyes as white light consists of various colours. If a beam of light passes through a prism, a range of colour light (purple, blue, green, yellow, and red) will be emitted from the prism (see Figure 3.1).

Light is an electromagnetic radiation which consists of bands of certain wavelengths and so does sunlight which

Chapter IV

Determination of Lipid In Serum and Tissue

To study the effect of freshly made garlic powder on the serum concentration of lipid in broilers, a publication under Materials & Methods described the following procedures :

> *One hundred male Ross 308 chicks were divided into two equal groups. Group 1 received regular broiler diet supplemented with 2% garlic powder. Group 2 received regular broiler diet. All chickens were slaughtered on day 50, every carcass was weighed and its adipose tissue content was determined. At the time of slaughtering, a 5-ml blood sample was collected into a test tube from each chicken. Tubes were labelled and then centrifuged at 3,000 RPM for 10 min to collect blood serum samples. The sera were analyzed to measure total cholesterol and total triglyceride using enzymatic colorimetric methods (Dehkordi et al., 2009).*

We can note from the above procedure that blood serum samples were collected and the concentration of total cholesterol and tryglyceride were determined by colorimetric method or in another word by spectrophotometry that has been discussed in Chapter 2. The first step in determination of cholesterol in serum is preparation of serum samples from blood withdrawn from the chicken or other experimental animals such as mouse, rats, or other experimental livestock.

Chapter V

Determination of Protein in Blood Serum

To study the activity of the enzyme involved in β-carotene metabolism in chicks following supplementation with natural β-carotene, a publication under Materials & Methods described the following procedures (Murwani, 2002):

> *Protein concentration in intestinal mucosa preparation was measured following Lowry method using Bovine Serum Albumin as standard. The same microsome preparation was assayed for the enzyme activity which catalysed the esterification of retinol (as the result of β-carotene splitting) into retinyl ester.*

The above procedures described the determination of protein concentration of intestinal mucosa preparation. This determination is needed to express the activity of enzyme in Unit per mg protein. Another material and method can describe the determination of protein concentration in serum in order to see normality or abnormality which might occur following certain nutrient treatment.

Proteins make up 6–8% of the blood. The majority of protein found in serum consist of albumin, globulin, fibrinogen and prothrombin. Normal protein in chicken serum is 3-3.3 g/dl, and in human plasma is 7-7.5 g/dL. Protein in blood carry a variety of important functions i.e. circulatory, protection, and regulation. As circulatory function it transports biomolecules such as carbohydrate, lipid, protein, vitamins, minerals, hormones, enzymes, complement components, protease inhibitors, etc. As regulator, it regulates pH, temperature and osmotic balance. As

Chapter VI

Determination of Phosphor in Feed or Food

A study was conducted to evaluate the efficacy of 25-hydroxycholecalciferol [25-(OH)D_3] to minimize the development of tibial dyschondroplasia (TD) and improve phytate phosphorus retention in Ross cockerels during the starter period (Ledwaba and Roberson, 2003). Part of materials and methods was described below.

> *A total of 240 1-d-old male broiler chicks were used for each experiment. Ten chicks per pen were assigned randomly to each of 24 pens in an electrically heated battery brooder housed in a room without windows. Room temperature was maintained at approximately 23°C. Feed and water were provided ad libitum in all experiments. The compositions of the corn-soybean meal based diets are listed in Table 1. Dietary 25-(OH)D_3 premix was donated and delivered from commercial mill. Chromix oxide was used as an external indicator at 0.10% of the basal diet to determine phytate phosphorus retention. Feed phosphorus was determined colorimetrically with a spectrophotometer using the method described by Gomori (1942).*

In the material and method it is written that determination of phosphor in feed was done by spectrophotometry.

Phosphor is a macro mineral essential for bone formation. Phosphor combines with calcium to form calcium hydroxyapatite which is the constituent of bone and teeth.

Chapter VII

Determination of Calcium

A study was conducted to evaluate the efficacy of 25-hydroxycholecalciferol [25-(OH)D_3] to minimize the development of tibial dyschondroplasia (TD) and improve phytate phosphorus retention in Ross cockerels during the starter period (Ledwaba and Roberson, 2003).Part of materials and methods is described below :

> *Blood samples were obtained at 13 d of age via cardiac puncture from one chick per pen and were analyzed for each individual chick picked randomly as a representative of the pen. The blood was centrifuged for 15 min at 3,000 x g to extract serum and analyzed for serum phosphorus (Goldenberg and Fernandez, 1966) and calcium (Moorhead and Biggs, 1974) concentrations using the same spectrophotometer as for feed phosphorus analysis.*

Calcium is one of macro minerals which plays a central role in bone formation and maintanance in animals and human. In chicken, 94% of egg shell is composed of calcium carbonate. When dietary calcium is not sufficient it can lead to a decrease in egg production, weight, and specific gravity, feed consumption, body weight, bone density and strength. Dietary calcium therefore is essential for fast growing birds such as broilers and egg producing birds such as layers. In layers, there is a high demand for calcium during egg production. When this demand is not met it can lead to the occurence of

Chapter VIII

Determination of DNA and RNA from Cells or Tissue of an Animal

A study was conducted to investigate the developmental relationship between fatty acid composition in different lipid fractions and stearoyl-CoA desaturase (SCD) gene expression in steer muscles during growth. Under Materials & Methods part of the procedures is described below :

> *Twenty male Korean Hanwoo steers were used in this study. Steers were slaughtered at 6, 12, 18, 24 and 30-months old, respectively. Muscle samples were taken from longissimus dorsi between 5th and 6th lumbar vertebrae of steers. All samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Total RNA was isolated from loin muscle using Trizol reagent (Life Technologies Inc., Grand Island, NY, USA) according to the manufacturer's instructions. Following RNA isolation, the concentration and purity of the prepared RNA were analyzed by optical density measurements at 260/280 nm (Lee et al., 2005).*

The materials and methods above showed that RNA was determined by optical density measurement. Optical density or abbreviated as OD is the same as absorbance and indicates that it is measured spectrophotometrically (See Chapter 2).
DNA stands for Deoxyribo Nucleic Acid and RNA stands for Ribo Nucleic Acid. DNA and RNA are the genetic codes that determine the characteristic of an individual. Nucleic acids are found in the nucleus of animals, plants, and microbial

Chapter IX

Determination of Enzyme Activity

A study was conducted to measure the activity of intestinal phytase activity in broiler chickens as affected by dietary calcium and vitamin D. Under materials and methods the following procedures were described:

> *Hubbard x Peterson, male chicks were fed diets with or without 0.21 mg/kg 25-OH D_3 with varying dietary Ca concentrations (4 or 9 g/kg) supplied primarily from $CaCO_3$ from 7 to 21 d of age (five pens/diet, three birds/pen) in brooder battery cages. All birds were fed diets calculated to contain 0.20% nonphytate P. Diets contained Celite as an inert filler and as a digestive marker at a minimum of 10 g/kg diet to allow for determination of apparent phytate phosphorous (PP) hydrolysis. Dietary Ca and total P were determined by inductively coupled plasma-emission spectroscopy (ICP). Phytate-P content of diets was determined as described below. At 7 d of age, birds were weighed individually and allocated to experimental pens, such that BW differences were minimized. At 21 d of age, birds were euthanized by cervical dislocation. The duodenum and jejunum were rinsed in ice-cold saline, and the mucosa was scraped and frozen (-80°C) for future isolation of brush-border vesicles (BBV) and subsequent intestinal phytase determinations (Applegate et al., 2003)*

Enzymes are protein that speed biochemical reactions. Enzymes have many applications in the field of animal and

Chapter X

Determination of Total Phenolic Compound

Polyphenols or phenolics are groups of substance naturally found in the plant kingdom with chemical characteristics of having aromatic ring with one or more hydroxyl groups. They are located in the vacuole and tend to be water-soluble as they are found in association with sugars. Included in polyphernols are 1) simple phenols and their derivatives such as catechol, eugenol, hydroxycinnamic acids (p-coumaric, caffeic, ferulic and sinapic acids), and coumarins, 2) flavonoids such as flavonols and flavones, chalcone and aurone pigments, flavanones, and isoflavonoids, 3) anthocyanins, 4) Flavan-3-ols and flavan-3,4-diols, 5)Tannins which consist of hydrolysable tannins and condensed tannins (proanto-cyanidins) (Scalbert and Williamson, 2000; Bennick, 2002).

Polyphenols can excert dual effect i.e. as functional phytonutrient and as antinutrient. As functional phytonutrient, many polyphenols have been shown to have antioxidant properties which are important in biological system. The antioxidant properties of isolated or pure polyphenols are used to enrich feed and food to improve nutritional status of animals and human. Naturally occurring poliphenol in feed materials such as sorghum can also be used (Murwani, 2008[a,b,c]). The anti-nutrient effect of naturally occurring poliphenolic compounds of sorghum is well known. It is detrimental to poultry when given in large amounts (Nyacoti et al., 1996). However, when it is used in small amount in the diet, it can exert its biological function as immunomodulator

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APPENDIX A

Atomic Weights of The Elements

ATOMIC WEIGHTS OF THE ELEMENTS

Element	Symbol	Atomic weight	Element	Symbol	Atomic weight
Aluminum	Al	26.98	Neodymium	Nd	144.27
Antimony	Sb	121.75	Neon	Ne	20.18
Argon	Ar	39.95	Nickel	Ni	58.71
Arsenic	As	74.92	Niobium	Nb	92.91
Barium	Ba	137.34	Nitrogen	N	14.01
Beryllium	Be	9.01	Osmium	Os	190.2
Bismuth	Bi	208.98	Oxygen	Ni	16.00
Boron	B	10.81	Palladium	Nb	106.4
Bromine	Br	79.90	Phosphorus	N	30.97
Cadmium	Cd	112.40	Platinum	Os	195.09
Calcium	Ca	40.08	Potassium	O	39.09
Carbon	C	12.01	Praseodymium	Pd	140.91
Cerium	Ce	140.12	Protactinium	P	231.04
Cesium	Cs	132.91	Radium	Pt	226.03
Chlorine	Cl	35.45	Radon	K	222
Chromium	Cr	52.00	Rhenium	Pr	186.2
Cobalt	Co	58.93	Rhodium	Pa	102.91
Copper	Cu	63.55	Rubidium	Ra	85.47
Dysprosium	Dy	162.50	Ruthenium	Rn	101.07
Erbium	Er	167.26	Samarium	Re	150.43
Europium	Eu	151.96	Scandium	Rh	44.96
Fluorine	F	19.00	Selenium	Rb	78.96
Gadolinium	Gd	157.25	Silicon	Ru	28.09
Gallium	Ga	69.72	Silver	Sm	107.87
Germanium	Ge	72.59	Sodium	Sc	23.00
Gold	Au	196.97	Strotium	Se	87.62
Hafnium	Hf	178.49	Sulfur	Si	32.06
Helium	He	4.00	Tantalum	Ag	180.95
Holmium	Ho	164.93	Tellurium	Na	127.60
Hydrogen	H	1.008	Terbium	Sr	158.93
Indium	In	114.82	Thallium	S	204.39
Iodine	I	126.91	Thorium	Ta	232.04
Iridium	Ir	192.22	Thulium	Te	169.93
Iron	Fe	55.84	Tin	Tb	118.69
Krypton	Kr	83.80	Titanium	Ti	47.90
Lanthanum	La	138.91	Tungsten	W	183.85
Lead	Pb	207.21	Uranium	U	238.03
Lithium	Li	6.94	Vanadium	V	50.94
Lutecium	Lu	174.97	Xenon	Xe	131.30
Magnesium	Mg	24.31	Ytterbium	YB	173.04
Manganese	Mn	54.94	Yttrium	Y	88.91
Mercury	Hg	200.59	Zinc	Zn	65.38
Molybdenum	Mo	95.94	Zirconium	Zr	91.22

APPENDIX B

Periodic Table

PERIODIC TABLE OF THE ELEMENTS
(IUPAC, 2008)

1 **H** 1.0079	II 2											III 13	IV 14	V 15	VI 16	VII 17	2 **He** 4.0026
3 **Li** 6.941	4 **Be** 9.0122											5 **B** 10.811	6 **C** 12.011	7 **N** 14.007	8 **O** 15.999	9 **F** 18.998	10 **Ne** 20.180
11 **Na** 22.990	12 **Mg** 24.305	3	4	5	6	7	8	9	10	11	12	13 **Al** 26.982	14 **Si** 28.086	15 **P** 30.974	16 **S** 32.066	17 **Cl** 35.453	18 **Ar** 39.948
19 **K** 39.098	20 **Ca** 40.078	21 **Sc** 44.956	22 **Ti** 47.88	23 **V** 50.942	24 **Cr** 51.996	25 **Mn** 54.938	26 **Fe** 55.847	27 **Co** 58.933	28 **Ni** 58.69	29 **Cu** 63.546	30 **Zn** 65.39	31 **Ga** 69.723	32 **Ge** 72.61	33 **As** 74.922	34 **Se** 78.96	35 **Br** 79.904	36 **Kr** 83.80
37 **Rb** 85.468	38 **Sr** 87.62	39 **Y** 88.906	40 **Zr** 91.224	41 **Nb** 92.906	42 **Mo** 95.94	43 **Tc** {98}	44 **Ru** 101.07	45 **Rh** 102.91	46 **Pd** 105.42	47 **Ag** 107.87	48 **Cd** 112.41	49 **In** 114.82	50 **Sn** 118.71	51 **Sb** 121.75	52 **Te** 127.60	53 **I** 126.90	54 **Xe** 131.29
55 **Cs** 132.91	56 **Ba** 137.33	57-71 ***La** 138.91	72 **Hf** 178.49	73 **Ta** 180.95	74 **W** 183.85	75 **Re** 186.21	76 **Os** 190.2	77 **Ir** 192.22	78 **Pt** 195.08	79 **Au** 196.97	80 **Hg** 200.59	81 **Tl** 204.38	82 **Pb** 207.2	83 **Bi** 208.98	84 **Po** {209}	85 **At** {210}	86 **Rn** {222}
87 **Fr** {223}	88 **Ra** 226.03	89-103 ****Ac** 227.03	104 **Unq** {261}	105 **Unp** {262}	106 **Unh** {263}												

Atomic weights are based on $^{12}C = 12$ and conform to the 1987 IUPAC report values rounded to 5 significant digits. Numbers in { } indicate the most stable isotope.

*** Lanthanides**	57 ***La** 138.91	58 **Ce** 140.12	59 **Pr** 140.91	60 **Nd** 144.24	61 **Pm** {145}	62 **Sm** 150.36	63 **Eu** 151.97	64 **Gd** 157.25	65 **Tb** 158.93	66 **Dy** 162.50	67 **Ho** 164.93	68 **Er** 167.26	69 **Tm** 168.93	70 **Yb** 173.04	71 **Lu** 174.97
**** Actinides**	89 ****Ac** 227.03	90 **Th** 232.04	91 **Pa** 231.04	92 **U** 238.03	93 **Np** 237.05	94 **Pu** {244}	95 **Am** {243}	96 **Cm** {247}	97 **Bk** {247}	98 **Cf** {251}	99 **Es** {252}	100 **Fm** {257}	101 **Md** {258}	102 **No** {259}	103 **Lr** {262}

GLOSSARY

AAS	:	Atomic Aborption Spectrophotometer, an analytical instrument which ionizes mineral sample and the absorption of the ionized atom is measured spectrophotometrically.
Abdominal fat	:	Fat which is deposited in the abdominal cavity of broilers or commercial meat chicken.
Albumin	:	A type of protein that is soluble in water and in water half saturated with a salt such as ammonium sulfate. Serum albumin is a component of blood serum. Egg and milk also contain albumin. Seeds contain very small amounts of albumins.
Antibody	:	Protein which is secreted by specialized immune cells i.e. B cells. It protects the body against foreign antigen.
Antibody titers	:	The quantity of antibody produced by B cells in response to antigen.
Antigen	:	Spesific molecules of foreign microorganism or cells or tissue.
Aqueous phase	:	liquids that are water-soluble. Because they mix with water, they form a liquid phase which are not separable. For example, methanol is mixed with water, therefore they do not form a separate phase.
Arachidonic acid	:	is a carboxylic acid. Arachidonic acid is important because the human body uses it as a starting material in the

Index

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ISBN 978-979-704-838-9
9 789797 048389 >