CHAPTER ONE

INTRODUCTION

Discovered of phytate has its beginning in1855-1856 when Harting isolated small particles, nonstarch grains, from several plant seeds.He considered these small particles to be a source of essential reserve nutrients for seed germination and plant growth (Rose 1912). Later in 1872 Pfeffer further characterized the grains isolated by Harting into three group, the third group was found in all of the 100 of different seeds that he examined, he found that the third group was free of nitrogen but contained calcium, magnesium, and phosphorus, he named this group of grains (globoid) . Schulze and Winterstein (1896) suggested inosite –phosphoric acid as the proper name for the globoid. Research interest in phytic acid continues to increase, as evidenced by the number of research publication,reviews, symposia, and technical presentation at various national and international meeting. A comprehensive review was published on phytate in cereals and legumes to develop a succinct summary of what was known at that time (Reddy et al 1986)Phytic acid (P.A) or myo-inositol 1, 2 , 3, 4 , 5 , 6 hexakis (dihydrogen Phosphate) is widely distributed in nature. It is the principal form of phosphorus in many plants. The term phytin indicates a calcium - magnesium salt of phytic acid. De Boland et al (1975.), stated that phytate in food stuffs could be a mixture of inositol polyphosphates. The term phytate includes commercial phytic acid and phytate containing phosphate derivatives lower than hexaphophate, Marfo et al (1990) mentioned that phytate general term used to describe hexaphosphate esters of inositols Phytic acid and it is metabolites as they occur in seeds and grains have several important role for the seeding plant. Because phytic acid and its salt(s) usually occur simultaneously in many seeds, researchers often do not make a distinction between these tow forms.Phytate are the primary storage form of both

inositol and phosphate in all seeds and grains . Most notably phytic acid functions as a phosphorus store as and energy store, as a source of cations and as a source of myoinositol (a cell wall precursor)Large amount of the hexaphosphat are present in grains, usually as the calcium or mixed ca++ - Mg++saltphytin (Mullaney2012). Phytic acid isfound in all plants seeds and among roots and tubers. (Marfo *et al*,1990).Phytic acid is the principal storage form of phosphorus in plant seed (Reddy, 1989). Phytic acid has been termed as anti – nutrient due to its ability to bind minerals protein and starches, either directly or indirectly and thus alter their solubility functionality and absorption .Many studies have demonstrated that phytic acid reduces minerals bioavailability in both animals and human (Sharon and Thompsson,1997) Phytic acid complex with essential dietary minerals such as calcium, Zinc, iron and Magnesium to make them biologically unavailable for absorption.Trinder (1960) found that phytic acid increased the cholesterol content in whole blood serum in rats. Lower serum lipids have been associated with higher intakes of the phytic acid, and in addition of phytic acid to high cholesterol diet reduced both serum cholesterol and triacylglycarols.

In broilers phytic acid showed lower feed consumption, severely reduced growth and leg problems as a sign of sever P-deficiency, (Punna and Roland ,1999).In most instances phytic acid is associated with the protein of the plant, in all cases there was a negative effect of the addition of up to 10 mg of Phytic Acid to any portion containing 10 mg of N after incubation for 1 hour at room temperature. This presents strong evidence that phytic acid protein interactions negatively affect protein digestibility is in vitro,there is a negative relation between phytate intakes and glycemic index (Blood glucose response) of cereal and legume food consumed by healthy humans.The investigators postulated that phytate was inhibiting amylase activity by complexing calcium ion.The highest retention of phytate

phosphorus in chicks (79.4%) was obtained when both phytate was present in the diet. Phytate is the main natural source of phosphorus in animals feeds of plant origin, but this phosphorus is not available for absorption unless the phosphorus groups are removed from the inositol molecule (Edward 1993).

Many studies have demonstrated that Phytic Acid reduces minerals bioavailability in both animals and humans. The degree to which animal is made unavailable for absorption depend on both the relative concentration of Phytic Acid and minerals as well as the strength of binding zinc, because it forms the most stable and insoluble complete with Phytic acid

The studies in rats and humans indicated that zinc utilization and growth was inhabited by Phytic acid(Frossard et al ,2000)Studies have been designed to develop ways to eliminate phytic acid from food by soaking / or extracting the food (phytic acid is soluble in acid solution) or by enhancing fermentation, thus creating phytate hydrolysis product which have weak minerals binding preparation as a means of improving the utilization of phytate by swine and poultry (Omara 2002) .The quality of phytic acid in a cereal product depends on large extent on the milling process and the extent to which bran and germ are separated from the endosperm (Ahmed ,1993) Soaking is very effective in the elimination of Phytic Acid, since 90% of Phytic acid is soluble.In home food preparation techniques can reduce the phytic acid of these food, simply cooking of food will reduce the phytic acid to some degree.Phytic acid it is often considered as an anti - nutrient because it binds minerals in the digestive tract, making them less available to our bodies, so the Paleo community keeps telling you to avoid, yet these same anti - nutrient properties can also help in the prevention of chronic disease. However recent evidence indicate that phytic acid has health full effects. One of the best knownproperties of phytc acid is anti oxidative ability by binding and thereby

inactivate Fe ion in solution.(Graf and Eaton,1990).In mammalian organisms phyic acid has been implicated in starch digestibility and blood glucose response in the prevention of dystrophic calcification in soft tissues and kidney stone formation and in the lower of cholesterol and triglycerides. During recent years,several alternative method for reducing the a aforementioned negative impacts of phytic acid on the environment and poultry performance have been devised, such strategies include the use of low phytate grains like corn and barley, but they are not available in Sudan.

There for the present study was conducted to investigate the reducing of phytic acid compound from poultry diet by using simple processing methods among the seeds(Sorghum bicolor, the main dish in poultry diets 50% - 75% from the ingredient of the diets)Moreover the study has been designed to develop ways to eliminate Phytic Acid from poultry diet by using a simple technical and methods such as dehulling - soaking -germination –vitamin C and storing methods .The main objectives of the study are to make phosphorus available to birds from sorghum phytate, and to shed light on phytic acid as a controversial component and to answer the big question whether phytic acid is an anti- nutrient or not? And in spite of importance of it is noticed that this study will be conducted for the first time in Sudan.

CHAPTER TWO

LITERATURE REVIEW

2. 1 Identifiers

Phytic acid or Phytate when in salt form discovered in 1903. A saturated cyclic acid is the principal storage form of phosphorus in many plants tissues especially bran and seed. It can be found in cereals and grains (Mullaney et al ,2012). Catabolites of phytic acid are called lower inositol polyphosphates Example are penta - (IP5), tetra - (IP4) and tri phosphate (IP3). Phytic acid and it is metabolites as they occur in seeds and grains have several important roles for the seeding plant. Most notably, phytic acid function as a source of cations and as a phosphorus store as an energy store as a source of cations and as a source of myoinositol (a cell wall precursor). Phytic acid is the principal storage form of phosphorus in plant seeds (Reddy 1982) .In animal cells myoinositol poly phosphates are ubiquitous and phytic acid is the most abundant, with its concentration ranging from 10 to 100µM in mammalian cells, depending on cell type and development stage (Sasakawa 1995) .This compound is not obtained from the animal diet, but must be synthesized inside the cell from phosphate and inositol, which in turn is produced from glucose, usually in the kidneys. The interaction of intra cellular phytic acid with specific intracellular proteins has been investigated in vetro and these interaction have been found to result in the inhabitation or potentiating of the physiological activities of those proteins.The best evidence from these studies suggests an intracellular role for Phytic Acid as a factor in DNA repair by non homologous end joining, other studies have also suggested intracellular phytic acid may be involved in m RNA export from the nucleus to the cytosol. There are still major gaps in the understanding of the molecule and the exact path ways of phytic acid unknown, As such the exact physiological roles of intracellular phytic acid are

still a matter of depate (Shears 2001).The term phytin indicates a calcium - magnesium salt of phytic acid , De Boland *et al* (1975), stated that phytate in food stuff could be mixture of inositol polyphosphates .The term phytate includes commercial phytic acid and phytate containing phosphate derivatives lower than hexa phosphate .Marfo *etal* (1990) mentioned that phytates, a general term used to describe hexaphosphate esters of inositols.

2.2 Nature of Phytic Acid

Phytic acid (PA) or phytate complex with essential dietary minerals such as calcium, zinc, iron and magnesium to make them biologically unavailable for absorption.The primary role of phytic acid maybe to store phosphorus and inositol, which are gradually utilized during germination. The phytic acid is mainly stored in the aleurone layer and to a lesser extent in the germ. Thus, milling and /or decortications greatly reduces the amount of phytic acid.Phytate is widely distributed in plants, especially in mature legumes, cereals, grains and oil seeds (Reddy *et al*, 1989). It is also found in roots, tuber, and nuts (Marfo *et al* 1990), phytate is absent in bananas, onions, celery, citrus, mushrooms and lettuce (Harland and Harland 1980). Phytic acid is also found in outer layer of monocotyledons seeds, while phytic acid was found in globoids of dicotyledons seeds. In cereal grains phytic acid is spread in both bran and germ except for corn where it is almost entirely in the germ. In wheat O'Dell et al (1972), found 87.15% of the phytic acid associated with aleurons layer-12.9 in germ 2.2% in the endosperm for sorghum, Doherty et al (1981) reported that phytate ranged from 1.72 to 4.07 mg/g dry weight, the mean of phytic acid content in Sudanese sorghum is 271, 313 mg/100g oil seeds contain the greatest level of phytic acid in nature.Phytic acid can be hydrolyzed by phosphatase enzymes known as phytases (Cosgrove 1966).The enzyme phytase (Myo- inositol, hexa phosphate

phosphohydrolase EC (3,1 – 3,8), dephosphorylates phytic acid on successive steps terminating with formation of inositol and free orthophosphate . and there by destroys the chelating ability of phytic acid (Peers 1953).Phytase has wide distribution in plant and animal tissues. It was also found in several species of Fungi, Yeast, certain bacteria and in some monogastric and ruminant micro flora.Phytase was completely inactive or a little soon phytate in dormant seed. Harland and Harland (1980), the optimum temperature for hydrolytic activity of the enzyme was reported to be 50c. degree. While (Peer1953) reported 55c. degree for wheat phytatethe optimum PH of enzyme was found to range from 5-15 to 5.6

2-3 Occurrence of phytic acid in foods and its interaction with food components

Phytic acid is found in cereals and legumes at levels ranging from 0.4% to 6.4% by weight (Shears 2001). In most seeds PA is the primary phosphate reserve, according to 60- 90% of the total phosphorus- Edwards,(1993). Phytic acid has been termed as an anti - nutrient due to its ability to bind minerals, proteins and starches, either directly or indirectly and thus alter their solubility, functionality, digestibility and absorption.At a normal PH range found in foods, the six phosphate Phytic Acid highlyreactive with other positively charged particles such as minerals and proteins .This interaction is a PH dependent, minerals Ions may bind with one or more phosphate group in one or more molecules forming complexes of varying solubility and stabiliti*es* .Proteins positively charged at a PH below their isoelectric PH, can binddirectly to phytic acid through electro static attractionAt an intermediate PH above the iso electric PH binding between PhyticAcid and proteins is mediated through multivalent cations such as calcium (CA) since both particles are negatively charged.At high PH, the Phytic Acid cation protein complex may dissociate with phytic acid precipitating as cation

phytic Acid . Binding to the phytic acid molecule is not necessarily electrostatic since starch binding may also occur through the formation of hydrogen bonds or indirectly associated.This binding with subsequent changes in nutrient digestibility and availability is thought to be responsible for both the adverse and beneficial effects of phytic acid.

2-3-1 Bioavailability of Minerals

For several decades, concerns have been raised about the role of phytic acid in reducing minerals bioavailability. Because dietary phytic acid is a ubiquitous plant constituent present in nuts, cereal, legumes, and oil seeds,current trends in food choices merit are examination of this issue. Recommendations for increasing consumption cereals and grains as the foundation of the food guide pyramid by the U.S. The effect of phytate on mineral bioavailability has been focus of numerous research studies and the subject of extensive reviews .Harland(1989).,Paullaud and Reimbach(1998).Phytic acid has a potential for binding positively charged proteins , amino acids and /or multivalentcations or minerals in food (Figure 3). The resulting complexes are insoluble, difficult for humans to hydrolyze during digestion, and thus, typically are nutritionally less available for absorption, phytate forms chelating conjugates with nutritionally important minerals such magnesium, calcium, copper, zinc, cobalt and manganese. Solubility is a prerequisite for absorption of most minerals although solubility at neutral pH has been shown to be less important for calcium absorption. Heaney et al (1990). Humans lack sufficient intestinal phytase to degrade the complexes.As much as 30-97% of the intake of phytic acid (0.3-3.7g/d) may be undigested before it reaches the colon and this phytic acid may be have protective or adverse effects on colonic health. Several factors determine the effect of phytate on mineral bioavailability:pH size and valence of the mineral. Mineral and phytate concentrations and ratios and food

matrix that includes the presence of enhancers and/or inhibitors.The effects off processing and degree of phosphorylation of phytate depend on the methods of the reduction and others factors. The effect of pH on mineral-phytate and protein-mineral-phytate interactions has been reviewed Champpagne and Phillippy (1989) As the pH increase, and under sufficient phytate concentrations, phytic acid becomes more ionized and begins binding cations. pH also affects the charge of peptides. On the acidic side of the isoelectric pH of the peptide, the negatively charged amino group. On the alkaline side of the isoelectric pH of the peptide where carboxy group are negatively charged, binding occurs through positively charged mineral ions.This can occur in processing or during digestion . For example, at higher pH, zinc was more associated with phytate in soy bean protein isdlates than at acidic pH (pHfor soy protein is 4.5).A higher pHenvironment would typically occur in the intestine. However, Champpagne and Phillippy (1989) report that high intraluminal gastric pHleads to the formation ingestion of soy protein isolate. Phytate concentrations have to be sufficiently high to exert a substantial effect on mineral bioavailability in the diet.Harland and Oberleas(1987), published the phytate values in approximately 200foods, the inhibitory effect of phytic acid on mineral absorption is often dose responsive .Genetically modified corn that reduced the phytic acid to one –third of the parent strain improved iron absorption by 50% .Mendoza et al (1998) . It is not clear whether phytic acid concentration per se or the ratio of phytate to minerals in the food stuff or diet is dominant in influencing mineral bioavailability, it is possible that these factors have different impacts for different nutrients.The molar ratio of phytate to zinc has been reported to be a major factor in influencing bioavailability of zinc to rats from breakfast cereals. Morris and Ellis (1981).Many studies have demonstrated that phytic acid reduces bioavailability in both animals and humans (Graf and Eaton, 1990). The degree to which a minerals is made unavailable for

absorption depends on both the relative concentrations of phytic acid and minerals as well as the strength of binding (Sandberg et al ,1993). Zinc because it forms the most stable and insoluble complex with phyticacid appears to be one of the minerals most affected by high phytic acid concentrations Studies in rate and human indicated that zinc utilization and growth was inhibited by phytic acid/ zinc molar ratio greater than 10. Since Calcium combined with zinc forms an even more insoluble complex with phytic acid.Later investigators have also used the (phytic acid) - calcium - zinc- molar ration, with limits of 3.5 mol / kg, BW in rats (Harland , 1989). There are limitations to the use of these ratios since the effect of phytic acid on minerals bioavailability is dependent on many other factors. There are several reasons that could explain why phytate;mineral molar ratios in a food or meal cannot reliable predict mineral bioavailability.There is not a fixed stoichiometric relationship between phytate and any particular mineral, because it depend on the presence of multiple ions, pH temperature and ionic strength. Furthermore, the intestinal milieu altars these factors. The pH alters along the digestive tract, and endogenous secretions can dilute the phytate;mineral ratios.The specific environment at the site of absorption determines absorption but cannot be readily measured

2-3-2 Phytate Carbohydrate - Glycemic Index Interaction

Yoon et al (1983) observed a negative relation between phytic acid intake and

glycemic index (Blood glucose response) of cereal and legume foods consumed

by healthy human. Removal of phytate from navy bean flour increased glycemic index compared to the whole bean flour, low glycemic index may aid diabetics to control blood glucose. The investigators postulated that phytate was inhibiting amylase activity by complexing Calcium ion. In vitro hydrolysis of either wheat or

bean starch in cubated with human saliva was retarded when Na phytate was included, but digestion was restored when Ca was added with the Naphytate.Yoon et al (1983)However, it is unable to confirm the in vitro effect of phytate when using purified a - amylase and strict control of PH in the incubation medium. (Sandberg et al 1987), found that same degree of starch digestion by ileostomy patients consuming either a high phytate extruded bran product or the raw product having lower phytate content by virtue of endogenous phytase action. The hard – to- cook phenomenon of legumes, primarily beans, may be related to phytate by way of Ca ions (Rickard and Thomposon, 1997). The condition is thought to develop from storage conditions which permits partial hydrolysis of phytate in the raw bean. This partial hydrolysis results in release of Ca ions which then forms cross links between acidic structures in pectin other mechanism that do not involve phytate may also contribute to the hard - to - cook phenomenon.

2-3-3 Phytate / protein interactions

In most instances, phytate is associated with the protein of the plant. There is over whelming evidence that native phytate as well as phytate added to purified diets decreases zinc homeostasis.Phytate forms strong complex with some proteins which resist proteolysis.In vitro digestibility of protein was tested using the following food sources: faba beans, peas, whole flour, protein concentrate, protein isolate, lactalbumin, casein, serum albumin and zein. In all cases there was a negative effect of the additions of up to 10 mg of phytic acid to any portion containing 10 mg of N after incubation for one hour at room temperature.This presents strong evidence that phytic acid protein interactions negatively affect protein digestibility in vitro

2-3-4 Phytate: Calcium Interaction

Other investigators (Sandberg et al, 1993) showed that in the presence of phytate and added calcium, interference with mineral absorption occurred as a result of the formation of insoluble complex under normal circumstances themineral binding strength becomes inositol hexa phosphate. Using 15 ileal fistulated pigs for this experiment, and measuring phytate hydrolysis of calcium in rape seed diet decreased phytate hydrolysis in the colon of pigs, but not in the stomach or small intesting, the latter being the major absorptive site for most nutrients.

2-3-5 Phytate: Phosphorus Interaction

The highest retention of phytate phosphorus in chicks (79.4 %) was obtained

when both phytase and 1.25 (OH)2 D3 were present in the diet (Edward, 1993). Phytate is the main natural source of phosphorus in animal feeds of plant origin, but this phosphorus is not available for absorption unless the phosphate groups are removed from the inositole molecule. This may be accomplished by intrinsic feed phytase, or by intestinal phosphatase.Phytate phosphorus is plants occur as mixtures of phytic acid complexes. Most foods of plants origin continent of 50 – 80% of their total phosphorus as phytate. Biological preparations containing phytase have been shown to increase the availability of feed phosphorus in chickens when incubated with the feed at 1000 unit of phytase / kg of food.The administration of phytase and 1.25 (oh 2) D3 increased ever further the effective utilization of phosphorus as measured by growth and bone ash of chicks.This is an important consideration, phosphate from animal wastes are one of the various problems because they eventually contaminate water reservoirs. In a study using trout as the rest fish, feeds were selected for their low phytate content to minimize stream pollution by phosphates (Ketola et al, 1993).

2.4 Phytic acid in sorghum

Sorghum bicolor is also know as great millet,durra, jowari, or milo is a grass species cultivated for its grain. which is used for food,both for animals and human, and for ethanol production.Sorghum originated in northern Africa ,and is now cultivated widely in tropical and subtropical regions. Sorghum is the worlds fifth most important cereal crop after rice, wheat, maize, and barley.

Table (2.1)The nutritional value of sorghum per 100 grams is:

Energy	1.418 kj :339 kcal
Carbohydrates	74.63g
Fiber	6.3g
Fat	3.30g
Protein	11.30g

Sorghum is used largely in world, over 300 million people depend on it.Sorghum reported to be antiabortive, cyanogentic, demulcent, diuretic. Emollient, intoxicant, sorghum is a folk remedy for cancer, epilepsy, flux and stomachache, the root of sorghum is used for malaria in southern Rhodesia, the seed has been used for breast disease and diarrhea.Per 100g ,the seed is reported to contain 342 calories, 10.0 g protein, 3.7 g fat, 2.2 fiber, 1,5 ash ,22mg calcium, 242mg P, 8mg Na, 44mg K, 3.8 mg F, 0.033mg thiamine, 3,90 mg niacin. (Miller.1980)

DM rang	71 - 96.3%
CP rang	8.7 - 16.8%
E E rang	1.4 - 6.1%
CF rang	4 - 3.4% %
Ash rang	1.2 - 7.1%

The sorghum phytic acid content fell within the range previously of 300 to 2000 mg/100g whole grain flour (Emmambux and Taylar .2009). In other study phytic acid ranged from 875.1 to 2219.9 mg/100gin sorghum and ranged from 851.6 to1419.4 mg/100g in millet.

2-5 Phytic acid in foods

During the past years, attention has been focused on phytic acid as an antinutritional factor in the diet of humans because of their inability phytate.The low bioavailabilityof the minerals bound in the phytic acid can lead to deficiencies in human populations where staples like wheat, rice, maize and sorghum are the major or the only source of nutrition. In the case of live stock production execration of phytic acid can lead to accumulation of phosphorus in soil and water, and subsequently to eutrophication of fresh water streams and near costal seawaters, low phytic acid mutant seeds can potentially reduce these problems, many low phytic acid mutants have been produced in most of the important crop. The negative effects of phytic acid in food on human healthy to be most pronounced in people on marginal subsistence diets that consist mainly of seeds/grain/fruits.Over two billion humans, largely located in developing countries, have micronutrient deficiencies FAO/WHO.(1992).Considerable scientific literature indicates that the strong chelation capacity of phytic acid can decrease the uptake of elements such as calcium,ironandzinc inmonogastricdigestivetracts .Thompson.(1993).,Nwokolo and Bragg(1977) ., Zhou et al (1992). The following foods contain no detectable phytate, apricots, canned, banana, citrus pectin, fruits cocktail, canned, solids and liquid, grapefruit, kale, peaches.Nuts are one of the richest natural source of phytic acid, almonds can contain up to 9.4 grams of phytic acid in every 100 grams, while walnuts my supply up to 6.7 grams and cashews almost 5 grams of whole grains contains a far higher concentration of phytic acid

than refined grains since most of the phytic acid in cereal grains is found within the outer bran layer. Wheat bran contains up to 7.3 grams of phytic acid in every 100 grams, and wild rice supply amounts ranging from 0.4 to 2.2 grams per 100 grams of the food. Sesam seeds have 1.4 to 5.4 grams and beans have 1 to 2.2 grams of phytic acid in every 100 gams, (Michelle ,2014).In sudan some studies in sudanese sorghum (sorghum bicolor), indicated that Fetarita Gadarif (as know locally) contained big amounts of phytic acid ,when it was compared with other Sudanese sorghum such as (Fetarita Gazera,Dabber and Hageen), table (1).

Table 2.2: Relation ship between total phosphorous(TP)and phytate phosphorous(PP)in sorghum grain

Cultivar	MeanTP(mg/100g)	MeanPP(mg/100g)	PercentagPP/TP
Dabber	2.2	1.0	89
Fetarita Gadarif	3.2	2.0	92
Fetarita Gazera	2.1	0.1	87
Fetarita Hageen	2.1	1.0	85

Table 2.3:Seeds/Grains/Fruits content phytic acid concentrationof one percent or more on a weight basis. Data obtained by Lott at al (2000).

Plant	Structure	%P.A
Sesame	Dry seed	4.71
Pumpkin/squash	Embryo	4.08
Rapeseed-canola	Dry seed	3.69
Coco beans	Dry seed	1.04

The higher inositol phosphate greatly reduced, so the available P is much greater. Sugiura et ai (1999). If more of the phosphorus in seed/grainswas absorbed by human and animals. the environmental problems related to P pollution would de reduced. One option is the use of *lpa* varieties to improve bioavailability of P and other minerals in crop seeds /grains /fruit. Theworld total tonnage for phytic acid in crop seeds / grains / is about 22.8millionmetric tones, without correction,the tone of crop seeds /grains /fruits was in the 2.8-4.1 billionmetric tone range. The P content was in the 8.3-12.1 million tone range,and the phytic acid content was in the 22.7-33.0 million metric tone range. Calculations showed that use of low phytic acid mutants of important crop plants could bring about considerable reduction in the phytic acid in crops. The cereals represented 69.5 % of the production but accounted for 77.3% of the total phytic acid.The legumes were the next largest category with 7.6% of the production and 13.0% of the phytic acid,mature seeds formed 17.2% of the production, but because water content is high for only 0.5% of the phytic acid, other dry seeds accounted for 5.7% of the production but 9.2% of the production,but 9.2%of the phytic acid. This reflects the fact that anumber of

high phytate- containing seeds are in this category.The crop seed with the concentration of phytic acid over 2% in the edible portion on aweight basis were sesame, pumpkin, and sunflower.

Table 2.4: Phytate contents of cereal, cereal products, and cereal-based foods

Cereal	Phytate%	References
Sorghum	1.35	Ravindran et al(1994)
Sorghum low tannin	0.57	Radhakrishnan et al(1980)
Sorghum high tannin	0.96	Radhakrishnan et al(1980)
Common millet	0.70	Ravindran(1991)
Prose millet	1.67	Lorenz(1983)
Pearl millet	0.52	Sripriya&Chand(1997)
Wheat	1.35	Frossard et al(2000)
Corn	2.22	Ravindrau(1991)
Oat	1.16	Ravindran et al (1994)

2-6: Phytic acid as an Anti - Carcinogen:

Recent investigations have begun to focus on possible beneficial physiological/ health effects of food phytates.Thepossible beneficial effectsof food phytate include lowering of serum cholesterol and triglycerides and protection against certain disease such as cardiovascular disease, renal stone formation, and certain types cancers. Thompson(1993). Rikard and Thomposon (1997),. Zhou and Erdman(1995).Graf(1983) has also reviewed medical and nonmedical applications

of phytic acid . The primary mechanism by which the beneficial health effects of phytates may be explained is the strong mineral chelating ability of phytates. For example, because phytic acid can chelate copper, iron, zinc, magnesium, and several other minerals,and many of these mineralsare essential cofactors for numerous oxdoreductases, several investigators have suggested that phytates may act as an antioxidant in vivoGraf(1983), the antioxidant activity has been suggested to be responsible for free radical scavenging action may lead to anticancer activity Shamsuddin et al (1997). Mineral-chelating ability of phytic acid has also been suggested to be responsible for antimicrobial(mold inhibition, for example) activity.Calcium and fluoride binding by phytic acid can insolubilize the minerals and, therefore ,may be useful in thedevelopment of dental enamels Reddy et al (1989),.Urbano et al(2000) . Experimental data supporting many of health benefits of phytic acid are inconclusive, at best. Careful,extensive and in-depth studies must be completed before making any recommendation on beneficial health effects of phytic acid. Adults consume about 1 gram of phytic acid per day from both animal and plant sources. Several mammalian tissues can synthesize about 4 gerams per day, testis, mammary gland, brain, liver and kidney.It is synthesized from D-glucose by the action of a phosphorylase on inositol – 1- phosphate which is derived from the cyclization of glucose – 6 – phosphate. Although plants routinely synthesize 1P6, the evidence that humans do is sparse. Nor can humans degrade 1P6, where as yeasts, fungi, plants, birds and some animal can. Dietary factors appear to have strong influences on the development of cancer of the large intestine cereals and legumes by virtue of the their higher phytate content (0.5 to 3.3% and 0.4 to 2.1 %, respectively) appear to be most protective.Most of the historical literature concerning phytate emphasize its mineral / chelating properties to the detriment of animal and human health. However, phytate by complexing iron may bring about a favorable reeducation in the formation of hydroxyl radicals

in the colon (Graf, 1993). Pretlow et al (1992) have suggested that phytic acid added at a 2% level to drinking water of rats may be used as an intermediate biomarker for rat tumor incidence. In a 36wk. study; F344 rats were injected with azoxymethane to induce colon cancer. Beginning stages appeared as aberrant crypts in unembedded segments of colon tissue. Untreated, these would progress to tumors in adose-response fashion. The higher the incidence of aberrant cryprs, the greater the tumor formation. Adding phytate to the drinking water reduced the incidence of tumor in rats denied phytate water was 83%compared with 25% in rats drinking phytate water. This study also showed that phytate in the drinking water administered after the azoxymethane injection could not prevent the formation of aberrant crypts, but it could prevent the next progressive step of tumor formation.Another pilot study (Shamsuddin et al 1997) was designed to discover if phytic acid effects could reverse or suppress initiating events. Eight month after four injection of azoxymethane (AOM) only 10% of rats drinking 2% phytate water developed lower intestinal cancer compared with a 43% incidence in the controls. There was also beneficial residual effects after discontinuing the phytate water regimen. Additional studies showed that by adjusting the phytate water toward a neutral PH there were greater therapeutic effects. Because commercially available phytate contains a small amount of the lowerinositol phosphates and intestinal phosphates dephosphrylate IP6 to lower forms. The authors (Shamsuddin 1992) speculated that a ready availability of Ins in the vicinity of dephosphorylation reactions might enhance the formation of lower inositol phosphate. These compounds were postulated as possibly the mediators of the antineoplastic effect animal drinking the mixture of 1% Ins and 1% IP6 fared better. This mixture was more effective in suppressing cell proliferation in both rats and mice. Drinking water containing high amounts (2%) of phytate (the animal would not drink the water with higher concentration of phytate. In tests with rats

and mice, azoxymethane (8mg/kg body weight) was injected intra muscularly for 12 weeks to determine whether IP6 prevented initiation events during carcinogenesis. After 6 month tumors were less frequent and smaller in animals drinking the phytate water. Further studies were designed and conducted (Shamsuddin 1992) to test the effect of phytic acid in human cancer cells (K-562 human erythroleukemia cell line). Repeated 1-hr treatment with 0.05-01 percent Na-IP6 was also used successfully to enhance natural killer cell activity in mice. Not only was it successfully, but it too was dose-dependent. In other laboratories (Sakamoto et al, 1997), the authors demonstrated growth inhibition and differentiation of H-29 cells.Human colon adenocarcinoma induced by 7-12-dimethylbenz – anthracene. Treatments with IP6 were effectively in both studies, in vitro a very rapid uptake of IP6 by malignant cells and a rapid conversion to IP3.Shamsuddin et al (1993) in a study designed to understand the anti-neoplastic action of phytic acid, added soluble IP6 to the drinking water of rats. The investigators found that the phytic acid was rapidly absorbed through the stomach walls and upper small intestine, was quickly dephosphorylated with in the mucosal cells and distributed to various organs as inositob and IP.The study was conducted using radioactive phytate. Radioactivity (though not phytate) was measured in urine, feces, blood, gastrointestinal tract contents and in various organs and tissues. It is well known that diets low in fat and high fiber confer protective effects against chronic diseases such as cancer and cardiovascular diseases. Colon studies in ileostomates suggest that as much as 4g phytic acid may escape degradation in the small intestine and enter the colon, thus phytic acid may have direct effect on the colonic mucus (Thompson, 1993).

2.7 Reduction of phytic acid

Therese a phytic acid in food and it matters. Researches have found that if we can reduce the phytic acid in our food we can improve our iron absorption markedly. A2003 study examined the change in iron absorption when phytic acid was removed from various grains. The phytate level in plant material is found to decrease during certain food processing,e.g milling. soaking (Chang et al 1977),germination, fermentation (Marofo et al,1990)Enzymic methods of phytic acid removal (fermentation and malting) were found to be more effective than physical extraction methods, i.e. dehulling, soaking and heating.Many attempts towards reduction of phytic acid through germination, incubation, cooking, fermentation and promising procedures involving activation of indigenous phytase and additional of microbial phytase.

2.7.1 Effect of germination method on phytic acid

Sprouting and germination are commonly used processing methods for improve the eating quality of cereals and legumes are often germinated (or sprouted) prior to their use in salads and other cooked dishes.The most effective enzymic method of removal of phytic acid is germination, In preparing alcoholic beverages from certain cereals such as barley, wheat,and sorghum, germination precedes the malting and fermentation steps. During germination, reserve nutrients are mobilized to provide for the growth of roots and shoots. Phytate being a major source of phosphorus, is hydrolyzed by endogenous enzymes to release inorganic phosphorus. phytate removal may be marginal (<3%) depending on the seed, germination condition, and the duration of germinationThe variable degree of phytate hydrolysis during germination is not only due to germination conditions, but also tovariable amounts of endogenous enzymes (phytases and phosphatases) capable of hydrolyzing phytate. Although not reported in many studies, the

temperature during germination may significantly influence the extent of phytate hydrolysis (especially by phytases), because most phytases have optimum temperature > 40C,since the activity of the enzyme phytase increase many folds during germination (Hulls et al, 1980). Phytate reduction during germination has been reported for wheat (Peers, 1953). For various varieties of bean (Tabekhia and Luha 1980); Corn (Change,et al 1977); and Lettuce seeds reduction of phytate due to germination may increase the bioavailability of germination reduction up to 75% of pea phytate and increased phytase activity 12 folds. germination also benefits in reduction some of the ant nutrients such as phytate and increase the availability of many essential minerals. germination increased crude protein contents and decreased phytat contents as a result of an increase in phytase activity during germination.Mahgoub and Elhag(1998) reported that germination method in sorghum (96 h) reduced the phytic acid from 68.3% to 86.8%. Marero et al(1991) indicated loss of 59.79 of corn phytate when germinated (96 h, 32C). Larsson and Sandberg (1992) found that more than 96% of phytic acid was lost when finger millet was germinated for about(96 h in 30C), but germinated pearl millet for abut 24 h in 30Creduced phytic acid 62.1% (Khetarpaul and Chauhan,1990) , germination rice 18 days in 14C reduced phytic acid 94.3% Kikunaga et al (1991)

2.7.2 Effects of incubation on phytate content

Chang et al (1977) reported that incubation of white bean for 10h at 60^0C reduced phytate over 60% in 24 hrs. Tabekhia and Luh (1980) mentioned that phytate concentration in the beans can be lowered by activation of phytase enzyme however, the process is slow, and may encounter a microbial growth problem during incubation. The decrease in phytate concentration in whole grains of wheat rice, barley and oats after hydrothermal treatment at conditions considered optimal

for phytase activity were examined during incubation in water or acetate butter (PH 4.8) at 55^0C for 24 hrs.

2.7.3 Effect of cooking method on phytate content

During cooking food is exposed to elevated temperatures for a certain period of time. Factors that influence cooking temperature and time vary considerably and included the following

1- Final desired texture of a cooked product, which varies somewhat with the consumer
2- Type of food being cooked.
3- Physical state and chemical composition of the food being cooked
4- Cooking medium,especially presence /absence of salts, acids, and alkalies in the cooking medium.
5- Cooking method (e.g., atmospheric versus pressure cooking)
6- Cooking equipment and heat and mass transfer rates during cooking
7- Amount of food that is cooked.

Regardless of cooking method, tissue is one of the desirable changes that takes place during cookingSoftening of the texture is partly due to loss of cell membrane integrity, cell membranes and middle lamellae contain pectic substances often insolubilized by cross-linkages afforded by divalent minerals such as calcium and magnesium. Phytates, being metal chelators, bind with calcium and magnesium and may, therefore, inhibit such cross – linkages by minerals that lead to weakened cell membranes and medile lamellae,resulting in shorter cooking time.High phytate peas were observed to have lower cooking time than their low –phytate counterparts (Mattson1964), similarly, Moscoso et al(1984) observed higher cooking time for food withlow phytate content, indicating that low phytate content

may promote more cross linking between middle lamellae. On the other hand several studies have failed to establish a clear relationship between phtate content and cooking time of food (Crean and Haisman 1963) , (Roseenbaum et al 1969).Perhapse part of the difficulty in explaining such mixed results is due a third posipility that the middle lamellar layers may coagulate more easily (in the absence of calcium and magnesium cross-linkages) , during the cooking , such collapse of the cementing material may lead to formation of larger and thicker layers surrounding the cells impeding the effective heat transfer, thereby increasing cooking time in low-phytate grain and legumes. Because phytate is heat stable, significant phytate reduction during cooking is not expected unless either the cooking water discarded or the food received additional processing treatment, such as soaking (soaking water discarded),germination, fermentation, etc**.**Another possible mechanism by which phytic acid reduction can occur during cooking is phytate hydrolysis due to activation of the endogenous phytases or phosphatases during the early part of the cooking phase. The presence of acid and alkalies durind cooking may also contribute to phytate hydrolysis. Typically, cooking allows a significant reduction up to 50% in most cases in phytate. Higher phytate reduction is possible when cooking is combined with certain other treatment such as cooking with soaking,cooking with pressure or cooking with soaking and pressure, cooking with pressure in 115Cand 3%Naclreduced phytic acid more than 91% Black-eyed beans Tabekhia and Luh (1980).Pressure cooking (autoclaving) typically caused higher phytate reduction than cooking under atmospheric pressure.Generally grains have high levels of phytic acid,a substance that reduces our absorption of minerals such as calcium, iron, zinc and magnesium. To reduce phytic acid in grain we must bake them using along rise time and good PH content. Crean and Haisman (1963) showed that phytate in dry peas is wholly waters soluble, but after cooking a small amount of phytate becomes insoluble in water as a result of phytic acid reduction

with calcium ions. 99.5% of the total phytic acid in dry mature beans is water soluble,in studying the cooking characteristic of the germinated legumes, a great reduction in phytic acid is the germinated legumes was found but no changes in phytic acid during cooking were observed. Phytate reduction through cooking for four bean varieties at 100^0C for 3hrs. was negligible , the rice and its flour phytic acid continents were 3.7 and 2.6 µ/g respectively. They observed and increase in phytate hydrolysis using increased volume of water and reduction particle size in cooking . There is no reduction of phytate reported when whole rice and its flour was cooking at 80^0c reduced phytase activity. Cooking food in cast iron pans increase iron consumption .A2007 study in Brazil showed that cooking tomato sauce in an iron skillet increase the amount of iron in the sauce and also increased iron statues among teen-aged .Cooking method as far as one can determine , that has not yet been systematically investigated, is the extent of phytate removal if one discarded and changed the cooking water several times, such treatment in addition to increased phytate removal may, of course , result in additional nutrient losses.

2.7.4 Effects of dehulling method on phytic acid content

The effectiveness of dehulling in removing phytate is dependent on the type of seed that is being processed as well as morphological distribution of seed phytate .For example , wheat and rice endosperms are almost devoid of phytate , as phytate is concentrated in the germ and aleurone layers (pericarp) of the cells of the kernel. On the other hand, in corn the majority of phytate (88%) is present in the germ .In pearl millet phytate is is distributed in germ and bran fraction , in legumes, the majority of phytate is distributed mainly in the cotyledons (mainly within the protein bodies) .Consequently , simple dehulling may be effective in removing significant amount of phytate ,only in seeds where most of the phytate is present in bran or seed coat .Removing the germ portion is an effective way to

remove a significant amount of phytate from corn , such methods are obviously not very effective in removing phytate from legumes. As the seed coat contributes substantial of weight to the whole seed weight , the removal of the seed coat can result in an increase in phytate content on aunit weight basis. In lgumes milling followed by protein bodies sepration air-classification may remove a substantial amount of phytate , such treatment may also contribute to significant protein losses .Dehulling of cereals, e.g. millet results in 27-53% reduction in phytate contents, the hulls contain 2-6 times as much as the dehulled grain (Lorenz, 1983) . But dehulling of sun flour increased the relative concentration of phytate by average of 20%. This suggested that the phytate might be characteristically in the kernel. Since the halls contribute to substantial portion of the whole seed weight, removing the hall will lead to increase in theconcentration of phytate on unite weight basis. In pearl millet , prose millet and sorghum dehulling method reduced the content of phytate more than 53% , (Lorenz 1983) , and Mahgoub and Elhage (1998).Milling of cereals removes the phytic acid , but this treatment also removes the major parts of the minerals and dietry fibers and can not therefore a nutritional solution to the problem, similarly , soakig or extracting in aqueous can remove up to two third of the phytic acid by the action of endogenous phytase activety , but loss of minerals.

2.7.5 Effect of soaking on phytate content

Dry cereals have tousgh seed coats that are not highly permeable to water. Hence, soaking is often used as apretreatment to facilitate grain processing . Soaking treatment may last for a short period (such as 15-20min) or for very long period (usually 12-16 h) .The amount of soaking medium may also change substantially depending on the type of grain and type of processing needed after the seed are soaked . In household situations ,cereals and legumes are typically soaked in water

overnight (12-14 h) , and if salts acids or alkalies are added to the soaked medium , the rate of water imbibitions can change significantly. For example, using alkali salts, such as sodium bicarbonate /carbonate , typically increases the water imbibitions rate ,because alkalies not only improve permeability of the seed coat but also loosen it. Phytate is water soluble, and therefore , significant phytate reduction can be realized when soak water is discarded.Soaking is very effective in the elimination of phytic acid in bean since 99% of phytic acid in water soluble In California small white beans,70% of phytate is water soluble, presoaked beans in water at 60^0C for 10 hrs. lowered the phytic acid concentration by 90% (Chang et al 1977) Many people make soaked flour breads to reduce phytic acid. The soaking temperature may have a significant effect on not only the rate of water imbibitions but also on whether endogenousphytases are active or not, If a soakingsteps carried out at temperature above 45Cbut below 60C , a significant percent of phytate hydrolysis can take place due to activation of endogenous phytases and acid phosphatases , for example , recently studiesshown that barley flour phytase was optimally active at pH 4.8 and temperature 57C , while the extracted phytase from the same flour was optimally active at pH5.2 and temperature of 47C, there is a significant positive correlation between phytase and acid phosphatease activities for cereals , cereal by –products and oil seeds Viveros et al (2000) also noted that the ratio of acid phytases to phytase activity ranged from 3.1 for wheat bran to 633 for beet pulp.

2.7.6 Effects of fermentation on phytate content

Many cereals and legumes are extensively used in the preparation of a variety of fermental foods , Reddy et al(1986) , Steinkraus (1983) . Microbes used in these fermentations may be natural micro flora commonly found in the cereal/legume that are fermented or especially cultivated cultures designed to bring a bout

specific change in the cereal/legumes that is being fermented . The type of microorganism , the fermentation condition used , and the starting amount of phytate present in the raw material significant affect the extent of the phytate removal, the efficiency of the microbial enzymes controls the rate and extent of phytate removal during fermentation Numerous studies indicate that phytate hydrolysis during fermentation significantly improves bioavailability of minerals (calcium , magnesium , copper , zinc ,and iron), phytate hydrolysis occurs throughout the different stages of bread making and obviously depends on the type of bread being made .Some of the factor that significantly affect phytate hydrolysis include the fallowing :

1- Flour type , freshness, extraction rate
2- Yeast presence/absence
3- Dough, pH water contrnt, fermentation time
4- Baking conditions leaving time, temperature
5- Additives calcium and magnesium salt , sodium bicarbonate.

The ability of different micro organisms to hydrolyze phytate has being investigated to some extent Reddy et al (1989). Lopez et al (2000), but much remains to be done. For example , to date no one knows which micro organisms produce the most efficient and economical phytases/phosphatases that can be used in these fermented foods .Molecular understanding of such phytase/ phosphatases in microorganisms may help in the development of treatments that can effect phytate removal from a certain food without the need for fermentation . Identification and development of a thermostable enzyme capable of withstanding temperature of 100 C or higher may be especially useful in this regard, because cereals and legume are often cooked otherwise exposed tohigh temperature (such as in extrusion processing) prior to consumption . The availability of thermostable

phytate hydrolyzing enzymes will enable the consumer/processor to include the enzymes in uniform manner with the food being processed as it is exposed to heat treatment, obviating the need to incur extra processing steps, with the availability of rapid and inexpensive molecular techniques, it is now possible to screen several enzymes simultaneously to permit identification of thermos table phytasesremoval is desired , the use of tailor made phytate hydrolyzing enzymes may prove to be the most robust, and economical means. Fermentation has a potential to reduce phytate content by degradation of phytate and hydrolysis of it is complexes and / or insoluble forms, principally

due to enzymatic hydrolysis of its phosphate groups.Marfo et al (1990)reported that 72 hrs. fermentation significantly decreased phytate concentration in foodstuff, ranging from 80 to 90% for rice, cassava and cocagam and for 52 to 65% for sorghum, maize, soybeans, cowpea and yam.(natural fermentation-12 to 72 h AT, reduced phytic acid more than 60%,Marfo et al.1990:Mahgoub and Elhage 1998)

2.7.7 Effect of storing method on phytate content

A number of studies indicate an a appreciable decrease in phytic acid content during storage. The extent of such reduction depend on the type of seed ,storage condition (especial relative humidity and temperature),and the age of the seed(fresh pods versus dry seeds) There are three possible mechanisms that may explain phytate reduction during storage : Firstly , phytate may form insoluble complexes with other food components (such as protein) and therefore , phytate extraction may be incomplete , resulting in low values . Secondly , if the water activity is sufficient and if the storage temperature is sufficient high , endogenous seed phytases / phosphatases may be activated, which may account for partial loss of phytate.Thirdly , at sufficiently high temperature and water activity , microbial growth on seeds (especially yeasts and molds) may permit phytic acid hydrolysisin

infected seeds due to microbial phytases /phosphatases to affect seed phytate content in any manner, perhaps with the exception of partial loss of solubility on extended storage The effect of storage on phytic acid content in cereal reduced the phytate from 0-65% , 67% in legume , according to temperature and humidity (Sathe et al,2002) .Environmental fluctuations, growing locations, types of soils , various fertilizer applications, and year during which a cultivar or variety is grown influences phytate content of seeds and grains.Bassiri and Nahapetian (1977) observed that wheat varieties grown under dry land conditions had a lower concentration of phytate compared with the ones grown under irrigated conditions.Nahapetian and Bassiri (1976) , Singh and Reddy (1977), Miller et al(1980),Feil and Fossati (1997), and Simwemba et al (1984) reported variation in the phytate content triticale's , wheat , rice, oats and pearl millet grown at different location and in different years .A variation in phytate content of cereals as a result of variety and location effects.

2.6.8 Effect of Radiation method on phytate content

Few studies have reported radiation to be effective in phytate removal from

seeds and grain .A closer examination of these studies indicates that the removal of phytate probably stems from the other treatment (such as germination, soaking,etc) given to the test samples. phytate reduction was <6% when soybeans (moisture content 7.5-30.5 % were exposed to-yirradiation dose ranging from 20-100 kGy , in the same study ,microwave treatment of the same soybeans (7.5 moisture content) caused a significant reduction (46%) in phytic acid.Microwave treatment causes the sample to heat up , while _yirradiation typically dose not raise the sample temperature significantly . Consequently , any reduction in phytic acid content as a result of exposure to radiation must be evaluated with caution.

2.7,9 Effect of Vitamin C method on phtate content

Ascorbic acid (the active form of vitamin c) keep iron available for absorption through several mechanism, first it promotes acidic condition in the stomach and intestines, thereby providing optimal condition for iron absorption, second itchelates ferric iron and maintains it in stable complex even at higher PH ,finally it reduces ferric iron to it is ferrous form ,thereby preventing from precipitating as ferric hydroxide.Vitamin A or β-carotene also enhances iron absorption through formation of soluble iron complexes and to a certain extent it can reverse the effect of several inhibitors such as phytates and polyphenols .Ascorbic acid and meat can to some extent reverse the inhabitation of iron absorption by phytic acid .Vitamin C is strong enhancer of plant iron and can overcome the inhibitors in plant foods. One study found that various does of phytic acid reduced iron absorption by 10-50 %.But adding 50mg of vitamin C counteracted the phytate ,and adding 150mg of vitamin C increased iron absorption to almost 30%, it is important to resole iron deficiency because it can increase manganese accumulation in the brain (in iron deficiency manganese is absorbed instead of iron),the good news is that while phytate decrease both iron and manganese absorption , vitamin C increase iron absorption..The PH is another factor influencing the solubility of phytic acid ,solubility studies of the bran phytate prove that at gastric PH (approximately PH 2),Ca is actually not bound to phytic acid and this component dose not contribute to the solubility of the Ca ion (Siener *et al*,2001)Acidic anion complexes with the minerals like C and P result in an improvement in the digestibility of these minerals as reported by several workers .Snow *et al* ,(2004)reported that addition of citric acid to broiler diet improved the tibia ash without reducing weight gain or feed intake ,and phytase citric acid and 1-hydroxycholecalciferol improve phytate phosphorous utilization in chicks fed acorn –soybean meal .Vitamin C appears

strong to over com phytic acid .Some studies showed that adding 60 mg of vitamin c counteracted phytic acid load of meal .In other study 80 mg of ascorbic acid counteracted 2.5 mg of phytic acid Siegenberg *et a*l (1991) .Iron absorption from porridges based on flours from wheat, maize, oat and sorghum flour blend have been tested on humans. There results show that phytate degradation improves iron absorption from cereal porridges prepared with water but not with milk , and that addition of ascorbic acid actually is a better tool for enhancing iron absorption in baby food than addition of phytase (Hurrell et al 2003). One study showed that anti-iron phytate level in rice were disabled by vitamin C in collard greens.Research published in 2000 indicates that both vitamin A and beta- carotene form a complex with iron ,keeping it soluble and preventing the inhibitory effect of phytates on iron absorption.

2.7.10 Effect of Extrusion on phytate content

Extrusion cooking is a widely used technique in food manufacture, especially for cereal processing (such as breakfast cereals) , and is extremely versatile with respect to automation, production capacities, ingredient selection , and shapes and textures ofextruded products(Cheftel 1990). Food material is typically exposed to very high temperature andshear forces during extrusion . One may, therefore , expect a significant hydrolysis of phytate during extrusion processing . However , because the duration of exposure of food to high temperature and shear is short (usually only afew minute) , phytate reduction (<30%) during extrusion is usually

2.8 Seed phytate and phytase in animal diets

As early as 1949,the researcher Edward Mellanby , demonstrated demineralization effect of phytic acid by studying how grains with and without phytic acid affected dogs,Mellanby , discovered that consumption of high phytate cereal grain interfere

with bone growth , and interrupts vitamin D metabolism., high levels of phytic acid in the contex of adiet low in calcium and vitamin D resulted in rickets and a severe lack of bone formation . His studies showed that excessive phytate consumption use up vitamin D, when the dieds is rich in phytate perfect bone formation can only be procured if sufficient calcium is added to a diet containing vitamin D.Mellanby(1949)From the last few years the inclusion of microbial phytase in poultry diet has increased significantly mainly in response to heightened concerns over phosphorus pollution of the environment as cheaper means to make phosphorus available to birds from phytate.Phytate is the major form of the phosphorus , abundantly found in cereal grains , bean ,and and oil seeds meals used in poultry diet , but the monogastric animals like poultry birds are unable to utilize this source of phosphorus due to lack of endogenous phytase enzyme . Augstin et al (2003). Plant seeds such as corn and soybean are major components of live stock feed , including diets of nonruminant animals such swine and poultry. Phytate phosphorus isutilized inefficiently by monogastric animals , which can result in serious nutritional and environmental consequences. Diets of nonruminants must be supplemented with inorganic phosphate to meet animal growth requirements . Undigested phytate is excreted in manure , which typically is applied as fertilizer to agricultural fields. This practice can lead to elevated soil phosphorus level in areas of intensive animal production and the potential for phosphorus runoff into lakes and streams. High phosphorus levels can decrease water quality due to eutrophication , because phosphorus is the limiting nutrient for aquatic plant growth. Sharpley et al (1994).Phytate is also considered an anti-nutrient because it chelates essential minerals such as calcium , iron , , zinc, lowering their bioavailability in animal .Reddy et al (1898), Ravindran at al (1995).

Supplementation of animal diets with enzymes is an increasingly popular method for improving digestibility and nutrient utilization . The enzymes phytase catalyzes the removal of orthophosphate from phytate and other myo-inositol phosphate .Reddy et al (1989). Phytase as a feed supplement is available commercially as Natuphate(BASF). This supplement is derived from the fungus Aspergillus niger(NRRI.3135) , which produces high levels of the enzymes as an extracellular glycoprotein.Shich and Ware (1968),.Howson and Davis(1983). Almost 30 years ago supplementation of animal diets with phytase (E.C.3.1.3.8) from Aspergillus niger was shown to improve phosphorus availability . Nelson et al (1971). Numerous other supplementation studies have demonstrated the efficacy of the method for improving animal diets and lowering phosphorus excretion In recent years. Swick and Lvey (1992), .Yi ey al (1996).Simons et al (1990).One limitation of using phytase as a feed supplement is the cost associated with production and application of the enzymes. The countries where regulations controlling nutrient management have been implemented , such as the Netherlands , avoiding fines for excess phosphorus output can offset the expense associated with enzyme supplementation. However in areas where animal waste management remains less heavily ,regulated , enzyme costs have prevented widespread use . Another factor limiting use has been the inability of the commercially available phytase supplements to withstand the elevated temperatures associated with feed pelleting.To have impact on nutrient utilization , an enzyme present in the plant seed components of animal feed must retain activity until it is consumed and can act to release phytate phosphorus in the animal digestive tract . Improving the thermal and protease stability of phytases is an active area of research. Plant seeds already make up a major portion of animal diets and may provide a convenient delivery system for inclusion of supplemental enzymes .Crop plants supply asimple and inexpensive source of biomass and should compete well with other

method of enzyme production .To test the feasibility of this novel approach , recent efforts have been made to introduce a fungal phytase gene into the genomes of different plant species.

To determine the efficacy of feed supplementation with phytase produced in transgenic soybeans , a three weeks poultry feeding trial was conducted . Denbow et al (1998). Graded levels of phytase were added to a basal poultry diet as either milled transgenic soybean or as commercialavailable enzyme supplement, to generate transgenic soybean plants . Basal poultry diets contained a suboptimal level of monphytate phousphrus (0.2%) to which graded level of phytase were added (at 400,800, and 1200 units per kilo gram of diet) . Test diets were fed to broilers from age 1to 3 weeks. Each dietary treatment was fed to four pens of birds (eight birds per pen).except for the basal diet .which was fed to eight pens of birds , growthwas measured as body weight gain in the second and third weeks, phosphorus availability was determined using toe ash as an indicator of bone mineralization , middle toes were collected at the treatment of the experiment , dried , weight , and ashed. ,toe ash measurements were calculated as a percentage of dry weight , phosphorus excretion was measured as the phosphorus concentration in excreta per kg of diet consume (days 18 through 20).All data are presented as percentage of the results obtained for control birds(birds fed basal diets without supplementation) .Supplementing the diets with either sourceof phytase resulted in increased growth (measured as body weight gain) and increased phosphorus availability (measured as toe ash). These results indicated that transgenic soy beans expressing fungal phytase enhanced growth performance when used as afeed supplement. In addition phosphorus excretionin manure was reduced as result of supplementation withtransgenic soybeans or with(Natuphos). Even with the promising results obtained for recombinant phytase

expression in soybean , the use transgenic soybean as a source of phytase supplementation has several limitation , as components of animal feed ,soybean are generally addedin the form of the meal ,which has been roasted to inactivate anti-nutrients such as trypsin inhibitors.Successful introduction of a fungal phytase gene into several plant species has been reported, phytase- expressing plant were obtained using constructs containing the phytate Agene under control of constitutive and seed –specific promoters and a signal sequence to direct the protein to the plant endomembrane system for secretion .The stability of phytases as function of glycosylation has been addressed in other systems. Wyss et al (1999) ,.Han and Lei (1999). A fungal phytase with superior thermal stability has also been reported , and may represent an important advance in the utilization of phytase for food and feed application .Pasamontes et al (1997).

2.9 Degradation of phytic acid in the stomach and small intestine:

Few studies have been performed to determine hydrolysis of phytate in the stomach , It is likely that consumption of raw plant foods may lead to some hydrolysis in the stomach, as the enzyme phytase is present, and in particular, cereal phytase activity increases when the pH is lowered.Investigating hydrolysis of inositol phosphate in the stomach of slaughtered pigs showed that approximately 50% of the feed InsP6 was hydrolyzed Skoglund and Sandberg (1995).This hydrolysis was probably a result of activity of the native feed phytase , as raw barley was included in the feed. found little hydrolysis in the stomach of pigs in the absence of feed phytase .By removing some of the phosphate groups in the stomach , the inositol phosphates with a lower degree of phosphorylation formed are probable more easily hydrolyzed further by enzymes located in the intestine.Soluble sodium phytate is hydrolyzed by homogenized mucosal tissue from a wide range of species, Crushing the cell of the mucosa may, however ,

cause the release of intracellular phytase, which normally would have no contact with the intestinal contents .The situation in the intact intestine may, therefore , different .Using this type of methodology, Bitar and Reinhold (1972) claimed that humans also had a mucosal phytase, an observation that was later confirmed by .Davies and Flett. (1978) found mucosal phytase in the rat and showed that it was concentrated in the brush border , factors influencing the hydrolysis of InsP3-InsP6 by mucosal homogenates from the pig were recently reported by Hu et al, (1996)Activities toward substrates increased with a decreasing number of phosphate group . This suggests that hydrolysis in the intestine may be enhanced by earlier phytase activity that had led to production of some lower inositol phosphate in the stomach . Phytate disappearance from digesta has been demonstrated when soluble phytate was incubated in loops of rat duodenum ,jejunum , and ileum .Davies and Flett (1978). In all natural diets , however, calcium and magnesium are present with phytate is to be expected, the strength of which depends on the number of phosphate groups on the inositol molecule Xu et al, (1992) The main part of the phytate in the diet and ileal digesta is ,therefore , present as an insoluble complex.For determination of degradation of feed or food components in the stomach and small intestine , studies in ileostomized pigs Sandberget al, (1993). And humans have been performed Sandberg at al(1983) .The effect of dietary phytase and intestinal phytase on InsP6 hydrolysis was investigated Sandberg et al. (1987) , he found that 60% of the InsP6from raw bran were hydrolyzed during the passage through the stomach and small intestine and that hydrolysis products were foemed , but this did not occur when the subjects were fed extruded bran .During extrusion cooking , the intrinsic bran phytase was deactivated . Sanberg and Andersson (1988) extended , the study using phytase-deactivated wheat bran and found no hydrolysis of InsP6 and no formation of hydrolysis products . Further ,Sandberg and Andersson(1988) concluded that

dietary phytase is of significance for phytate hydrolysis in the stomach and small intestine , while intestinal phytase if present in humans , does not play a significant role. It was recently demonstrated that very low phytase activity occurs in the human small intestine , thus confirming that the human small intestine has very limited ability to digest InsP6, These conclusions were further supported by analysis of the specific isomers formed in the ileal content during hydrolysis , Apossible adaptation to increased phytate degradation in the small intensine after a longer period of high phytate intake was investigated (Sandberg et al ,1993). Nine ileostomy subjects consumed oat bran added to a low - fiber diet during a three-week period , no increased phytate degradation was found after 17 days of consumption , showing that no adaptation occurred during this period . Furthermor , a complete of dietary inositol penta-tetra , and triphosphates was obtained . In this ileostomy studies, the dietary level of calcium was relatively high (a round 30 mmol/ day). However, in another ileostomy studySandstrom et al, (1986), where degradation of phytate from soy bean flour, soy concentrates , and soy isolate was investigated, different dietary calcium levels were used. The calcium phytate molar ratios in this study were 20, 22, 28 and 4.5, independent of these molar ratios, an almost complete InsP6 , recovery was obtained , suggested that these differences in the dietary calcium level did not affected the degradation of InsP6 in the stomach and small intestine Sandberg (1996) .Similar results were obtained in pigs , indicating no effect of dietary calcium level on phytate degradation in the stomach and small intestine Skoglund and Sandberg (1997).

2.10 Degradation of phytic acid in colon:

The phytase level of microbial origin is very low in the small intestine of mammalians .Considering the large number of microorganisms present in the colon , degradation of the phytate by microbial phytase could be expected to occur in the

colon . The germ – free animal would be useful model with which to investigate the extent of phytate degradation by microbial phytase in vivo. In study by Wise and Giburt (1982) , the hydrolysis of phytate was compared in germ –free and conventional rats. In conventional rats fed high - and low – calcium diets 22% and 55% of phytate was hydrolyzed , respectively . Considering that negligible hydrolysis was observed in the germ – free rats , it was concluded that the micro flora had been responsibly for the observed hydrolysis .In direct support for bacterial hydrolysis was reported by Wise et al (1983).Hydrolysis products were found in the caecum, with increasing amounts in the colon and feces followed by phosphorus NMR, also found evidenceof phytate hydrolysis in the colon of pigs.A balance study in humans during three consecutive 24 day periods on white bread , brown bread, and whole –meal bread was performed to investigate the effect of dietary fiber on mineral absorption Andersson et al. (1983) . The phytate content was held constant during the three periods by addition of sodium phytate to the diet in periods with brown and white bread to the same level as in whole meal bread .

Insitol phosphates in the feces material were analyzed , a mean of 25-35% hydrolysis of InsP6 was found , although individual variation occurred .There was no significant differences between the dietary periods ,and there was no increased degradation after the third periods, and there was no increased degradation after third period compared with the first period.As the diet did not contain phytase activity , the results suggest that a degradation of phytate occurs in the colon of humans. In the feces samples from human subject consuming the white bread diet supplemented with high amounts of sodium phytate , constituting the main source of phytate in the diet , Moreover, the feces samples contained several different isomers of inositol tetraphosphates The calcium level of this study was rather high(30-40mmol/day) .In study with pigs, Sandberg et al .(1993) found that the

dietary calcium level markedly affected the phytate degradation in the colon .Total gastrointestinal degradation of InsP6 in pigs fed a rapeseeddiet (not containing phytase activity) was 97,77, and 42% (p>0.001) when calcium intake were 4.5.9.9 and 15g/day , while no difference in the phytate hydrolysis of ileal digesta were found between periods, Similar to these results, Skoglundet al .(1997) found an impaired InsP6 degradation in the colon when the pigs feedwas supplemented with calcium.

2.11 Phytate metabolism:

Phytate is natural organically bound compound present in most cereal grains (Maenz.2001)The Plinked with phytate is defined as phytate P,which is mostly unavailable to the monogastric animals including poultry(Oatway et al,2001) Because phytate P is poorlyuttlized by monogastric animals, their dietary P requirement is not fulfilled from dietary phytate P alone.Inorganic P is needed to supplelment with their feeds to meet dietary P requirement and to achieve optimal growth and production performance. Phytate has also been identified as an anti-nutritional factor . It can bind with other minerals, protein,and starch, preventing their absorpyion in the digestive tract and make them unavailable to the animals (Mazzuco and Bertechini,2014). Phosphorus in plant based feedstuffs principally remains boundas phytate form that is weekly digested and absorbed by monogastric animals. Distiller co-products such as dried distillers grains with soluble and brewers grains contain high concentration of total P with higher proporation of available P and low proportion of phytate P compared with cereal grains due to yeast fermentation (Deniz,2013) Many strategies like phytase addition to diets and the use of low phytate ingredients have been devoleped in an attempet to improve the P availability in monogastric animals . Several studies have demonstrated that low phytate P variety vegetable ingredients like maize and

barley is more available and better utilized by birds compared with the conventional varieties (Kahindi et al ,2017).For theavailabity of phytate P to the chickens,phytase must be hydrolyzed to inositol and inorganic P within the digestivetract (Sandberg,2002) . The degree of phytate utilization by poultry is quite variable ranging from 37 to 50% in poultry. Although birds are capable ofdegrading phytate of intestinal tracts and the intrinsic phytase activity in feeds of poultry are very lowthe supplementation of exogenous molecule.This necessitates the supplementation of exogenous phytase to the diets to improve the utilization of phytate . Onya-ngo et al .(2005) stated that dietary phytate prompted an increasein the diets did not decrease the endogenous losses. In a nutshell,the nature of phyic acid and it is importance for poultry necessitates the inclusion of phytase in the diet.

2.12Phytase:

Phytase (myo-inositol hexaphosphate phosphohydrolase) is an enzyme that hydrolyzes phytate in the digestive tract to inositol phosphate and in organic P

(Wyss et al .1999). There are two primary classes of phytases according to phosphate group position on the ring of myo_inositol , the first is 3_phytase that hydrolyzes the phosphate group from position 3. The second is 6_phytase which acts first at the position C6 (Zyla et al 2004) . In the background of animal nutrition , as feed additives , phytases may be created from intestine of animal, from intestinal bacteria from feed ingredients containing phytase or be added , as exogenous enzymes , as feed addedives in the feed (Nys et al .,1996). Extensive research in layers has been performed with the addition of phytase in diets of monogastric animals to improve phytate digestibility (AugSpurger et al 2007) .The phytase activity is very lowin the brush border membrance of the digestive tracts of monogastric animals (Maenz.2001). Therefor, the phytase enzyme is

included in diets to maximize the hydrolysis of the phytate molecule.Supplementation of phytase to wheat_bsed diets to maximize the hydrolysis of the phytate may alleviate the anti_ nutritional effects that are associated with phtate due to it is ability in phytate hydrolyse to release the digestive enzymes and bound nutrients (Bedford . 2000) . Microbial phytase usefulnessfor it is ability to release phytic acid bound phosphorus and improve bioavailability of phosphorus in poultry feedis well recognized (Cowieson etal,2006:Selle and Ravindran,2007).Various factors can have an influence on phytase efficacy ,including dietary –related factors, animal –relted factors , and phytase-related factorsas . The diet composition and due to the variation in composition, level and location of phytate, as well as the contribution of intrinsic phytase in some oilseeds and cereal , the rateof phytate hydrolysis by microbial phytase can differ to alarge extent in these plant- based ingredients (Akter et al .,2017). The release of P due to added phytase varies in different feed ingredients, for example addition of phytase increased the available P in corn , soybean meal, wheat, wheatmidds, barley, defatted rice bran and canola from 30.2 to 59%,34.9 to 72.4%, 30.7 to 46.8% , 29.1 to 52.1% , 32.2 to 71.3% , 33.2 to 48% , and 36.7 to 55.8%, respectively, with higher percentage of phytate degradation in canola (19%) compared to SBM(37.5%).This necessitates calculation of phytase equivalency for individual feeds for precise diet formulatin.Phytase is added in poultry diets to rlieve the effects of anti=nutritional for phytate and consecquently develop the performance . Microbial phytase supplementation to poultry diet is recognized to be the most effective means to utilize and release phytate -linked minerals.The ability to phytase to improve the availability of dietary nutrients in poultry depends upon the mineral content of the diets , specifically Ca and P . which can influence the effectiveness of phytase to hydrolyzed phytate in the digestive tract(Sandberg et al 1993) The Ca =phytate complexes are not easily

hydrolyzed by the phytase , resulting in unavailability of phytate P and bound Ca to poultry .Earlier research has demonstrated that high dietary Ca concentrations in diets decrease the availability of phytate P in laying hens. Poultry feed containing calcium can have agreat impact on phytase efficacy and phytate P utilization.Van der Klis et al , increasing dietary Ca from 30 to 40 g/kg in laying hens ,decreased degradation of phytate P from about 33 to 9% in the diet without phytase supplementation. Also, it has been detected that limestone particle size can have an effect on the phytase efficacy due to the high solubility in fine limestone particle size(Manangi and Coon .2007).Plumstead et al . (2008) stated that increasing Ca from 4.7 to 11.7 g/kg in broiler diets linealydecreased digestibility of ileal phytate Pby 71% while in the diets supplemented with phytase , the reduction in phytate P degradation was from 76 to 65%, with regard to the ratio between Ca and P , increasing the ratio of Ca :P may have abad impact on activities of phytase reduction the Ca :P in the diet from 2:1 to 1.2 :1 augmented phytase efficiency by around 16% and enhanced digestibility and performance in turkey.The optimum ratio between Ca and non-phytate that caused the lowest P excretion and highest P retention was 2.34:1, 2.53:1 for diets with 0.10%,0.28% phytate P . Increasing levels of dietary Ca decreased the phosphorus digestibility and phytate P hydrolysis with regard to Fe impact on phytse efficacy . Abudabos (2012) declared that the increasing dietary iron significantly decreased the activity of intestinal phosphatase in chickens .Also , Akter et al (2017)point out that high dietary Fe (100mg/kg) inhibited efficacy of phytase and subsequently lowered the nutrient utilization and overall performance of broilers. There are several sourse of phytase that are available in the markets as fungal-derived phytase for example from Aspergillus ficum, other sources for commercial phytase result from the bacteria E coli.Phytase (protein molecule) can be hydrolyzed by endogenous enzymes as protease was detected by Morales et al . (2011).There is ahuge variation in the

resistance of several commercial phytase against protease in the stomach.The effect of phytase addition in the diet of poultry on energy utilization is quite variable in different studies .Newkirk and Classen(2002) reported that phytase supplementation improve

2.13 Decreasing phytate from layinghens diet:

Recently many methods were used to decrease phytate content from layer diets.Mostof the published literature supports that dietary low in phytate can improve the poultry performance, fed efficiency and minerals contents , poultry feed containing amounts of calcium can have agreat impact on phytase efficiency and phytate P utilization. The common method was phytase enzymes. Phytase supplementation to poultry diet is recognized to be the most effective means to utilize and release phytic acid , linked minerals, also the decreased excretion of P will also reduce the environmental concerns . Using of phytase in the diets were improve energy, protein and phosphorus utilization in laers.Jalaudeen (2003) reported that the supplementation of phytase in Pdeficient layer diets numerically improve body weight. Metwally (2005) studied the effect of dietary Pconcentration with and without supplementation of phytase or dried yeast on the performance of Dandarawi laying hens and found that birds fed low available P (0.25%) diet supplemented with phytase enzymes, or dried yeast had higher final body weight . Silversides et al (2006) observed that the supplementation of phytase (34 to49 wks of age) had significant effecton body weight , when diets had reduced P and supplementd with xylanse , but phytase had no effects on body weight.Supplementation of phytase in layer diets has been shown to improve egg production performance and feeding efficiency, especially with diets containing low P. Sukumar and Jalaudeen(2003) showed that supplementation of phytase (200, 300,and 400FTU /kg diet) in P deficient layer diets numerically improved

hen-day egg production . Snow et al (2004) studied the minimum P requirement of one- cycle and two- cycle (molted) hens . They noted that egg production and egg mass were significantly reduced by all lower available P level and axcept 0.15% available P when compared to the 0.45% available P diet. Casartelli et al (2005) evaluated the effect of phytase in diets formulated with different P sources (Ca and sodium phosphate , micro-granulated di –Ca phosphate and triple super phosphate) .They showed that phytase supplementation significantly affected the egg production traits . In astudy with low-protein and energy diets, phytase increased egg production (Ponnuved et al,.2015) .However , Musapuor et al . (2005) noted that phytase supplementation did not influence egg production .Metwally (2005) reported that diets supplemented with phytase , or dried yeast had significant effects on egg number, egg mass and egg production (%) compared to the supplemented diets during the whole experimental period (32-48wks). Ahmadi et al (2007) investigated the effect of different dose of phytase supplementation on the performance and egg quality of laying hens. They noted that egg production was considerablyhigher in the phytase supplemented group than unsupplemented groups.Huglies et al (2008) studied the efficeacy of phytase in a40wk production trail in white Leghorn laying hens fed corn-soybean meal based diet and measured productin performance from 21 to 61 wks of age. Hens fed diet with phytase supplementation decreased total housed egg production at 61 wk of age compared with positive control diet .El-Deek et al (2009) noted that increasing corn gluten feed inclusion up to 20% with phytase addition (300FTU/kg) insignificantly decrease values of egg production weight and egg mass. In a similar way supplementing phytase at level of 20000FTU/kg diets had appositive impact on egg production in laying hens (Kim et al ,.(2017). Addition of phytase to a wheat –maize –soybean meal 0based laying hens improved egg production while maintaiing eggshell quality and egg content in older hens

(Englmaierova et al ,. 2017) . This information may be due to our knowledge that phytase mobilized calcium , phytate and other nutrients . Then at the end of the laying period a higer concentration of calciumcan increase egg production and improve eggshell quality. Gao et al (2013) observed that the source of phytase in laying hens diets profoundly impact on egg production.Although by –product contain high concentration of available P and low phytate P . phytase supplementation with adiet containing DDGS may improve laying performance .Phytase may increase feed efficiency and or/ feed intake in layer diets .There was increase in feed intake by addition of phytase. In other study supplementation of phytase improve feed efficiency whithout affecting feed intake . Cabuk et al (2004) reported that phytase supplementation significantly increase daily feed consumption . Hughes et al (2008) observed that addition of exogenous phytase significantly increased feed consumption and feed conversion. Similar to weight gain and egg production in laying hens , feed consumption was also improved under the supplementation of phytase enzyme. Inclusion of phytase in the diets of laer chickens may improve egg quality traits depending opon the calcium. Phytase was associated with increased egg weight.Lim et al .(3003) noted that low phytate diets improve egg shell thickness in the period of 31-41 weeks of age, whereas high NNP diet lowerd the percentage of brokn and soft –shell eggs in the second 10 weeksof age .Low Calcium diet decreased egg shell strength and egg shell thickness in both periods . Phytase supplementation significantly increase egg shell quality. Musapuor et al (2005) studiey the effects of different dose of phytase (0.500 and 1000 FTU/kg diet), Ca (2.28 and 3.25%) and available P (0.175 and 0.25%) on phytate P utilization in lying hens .They observed (no beneficial effect on egg shell quality) because there are no data desciibing this point. Also supplementing phytase at level of 10000.20000or 30000FTU/kg diets did not affect egg quality of laying hens (Kim et al., 2017),which might be due to adequate

concentrations of P and Ca present in the diets .In a nutshell, the supplementation of phytase improve the egg quality traits at different doses and age of birds.Metwally (2005) reported that hens fed dietscontaining 0.45% had higher Ca and lowe P concentration in plasma than those fed the diets contaiing 0.25% PN .Supplementation of phytase or yeast in diets particulary increased both concentration of Ca and phosphorus in plasma Also, the latst auther indicated that supplementation of phytase increased the concentration of of tibia ash in hens fed 0.45% diet.Musapuor et al (2005) noted that in laying hens dietary phytase increased plasma P concentration. Dietary phytase significantly decrease plasma alkaline phosphotase activity , significantly interactions among phytase , Ca available P levels and plasma Calcium wewe noted . The interaction between phytase and available phosphorus on tibia P was found to be significant.Whereas , phytate binds some of the important minerals and decreases its availability for the consumption , phtase separatesthese minerals and makes them available for utilization . Silversides et al (2006) reported that phytase supplementation in laying hens diet increased serum phosphorus level from 5.17 mg/dl (with no phytase addition to diets)to 6.3 mg /dl with addition of 700U/kg of phytase .However , Kannan et al. (2008) shoed that various levels of phytase enzyme in layer diets had no significant differences in serum Calcium , P and alkaline phosphotase p among tareatmentgroups at 52 weeks of age .Similarly , Shehab et al (2012) reported that serum Calcium and P were not affected by supplementation of phytase . Phytate can form complexes with some minerals such as Ca, Mg , Cu , Zn ,Fe and K , consequently reducing their solubility , this confirms the beneficial effect of supplementation of phytase on tibia bone. Phytase enzyme can also release iron from inositol and thus improve iron reserve in hens (Abbasi et al .2015).

CHAPTER THREE

MATERIALS AND METHODS

The study was divided into three experiments.

The animal care and use protocol was approved by Department of Animal Production,Fuclty of Agricultural and Environmental Sciences.University of Gadarif.The samples of experiment were chemically analized in the National Center For Research -Khartoum, and Department of Animal Nutrition, Fuculty of Animal Production, Khrtoum University.

3. First Experiment

3.1 Reducing of phytic acid from Sudanese Sorghum bicolor(F.G) using simple techniques method (and the change in nutritive value before and after processing treatments);

In the first trail: Sudanese Sorghum , Sorghum bicolor (Fatareta Gadarif)was purchased from Gadarif State local market of (Dura).20 kg from the sorghum of the experimental was cleaned from damaged seeds and foreign objects. Then subjected separately to five treatments for processing using simple technique to reduced phytic acid .such as dehulling ,soaking ,germination, vitamin C and storing method in addition to control (un processed sorghum).Chemical composition of sorghum were analyzed before and after processing treatment to determine nutrients content and phytic acid in the grains(F.G)

3.2 chemical composition of unprocessed sorghum (F G)

The seeds were cleaned manually to remove broken seeds ,dust , and other extraneous materials .The cleaned grains were milled into fine flour with hammer mill (Gibbons Electric ,Essex K) to pass 0.4mm mesh size screen and were stored

at 4C before being used for their analysis. Then grain were chemicaly analyzed according to procedure of AOAC (1980) .The sorghum protein contents were determined by adopting standard AOAC(1995) method . Energy was calculated as described by Osbonrne and Voogt (1978).Minerals were determined in the sample by the dry-ashing methods described by Chapman and Pratt (1961). The amount of iron was determined using atomic absorption spectroscopy (Perkin-Elmer 2380) .Ammonium vanadate method of Chapman and Pratt (1982) Calcium was determined by a titration method described by Chapman and Pratt(1961) , hydrochloric acid extractability of minerals was performed according to the Champpange et al (1989).

3.3 Phytic Acid determination

The phytic acid contents was determined by the method described by Wheeler and Ferrel, (1971).

2ml of milled dried sample were weighted in 125ml conical flask. The sample was extracted with 50 ml of 3%. Trichoracetic acid (TCA) for 3 hours in mechanical shaker. The suspension was centrifuged for 5 min., and 10 ml aliquot of the supernatant were transferred to 40 ml tube 4ml of $FeCl_3$solution were added and the tube was heated in a boiling water path for 45 min., the cooled and centrifuged for 10-15 min the clear supernatant was decanted and the precipitate was washed twice by dispersing in 25ml of 3% TCA and was heated 10-15 min in a boiling water path re-centrifuged. Washing was repeated once using the water. The washed precipitation was dispersed in water and 3ml of 1.5 NnaOH were added with mixin mixing and the volume was made to 30ml distilled water. The tube was heated in a boiling water path for 330min., and filtered Whatman No.2 filter paper. .The precip

paper.Theprecipitate was washed with hot water. The precipitatewere washed from the paper using 40 ml hot 3.2 $NHNO_3$ into 100 ml volumetric flash. The paper was rewashed with water and the washing were collected in the same flask and completed to volume with distilled water 0.5 ml aliquots were taken and transferred into 10ml volumetric flask 2 ml of KSCN were added and completed to the volume by adding distilled water and immediately (within one min) the absorbed was read using spectrophones at 480 nm.

Calculations:

Standards curve of different $Fe(No_3)_3$ concentration was plotted against absorbency to calculate the Fe^{-3} concentration. The phytatephosphorus was calculated from the ferric ion (Fe^{+3}) concentration assuming 4:6 iron: phosphorus molar ratio

using 2.0 g dried sample .A standard curve was prepared expressing the results as Fe(No)3 equivalent ,phytate phosphorus was calculated from the standard curve assuming a 4..6iron to phosphorus molar ratio

3.4 Processing treatments:

3.4.1Dehulling

5.0 kg of cleaned cereals moistened by adding water before hulling to softening the surface of the grain and facilitating detachment of the pressure inside the machine ,the commercial machine combines two stages, dehulling and milling, the grain was passed through the machine capacity varies 200to 275 kg/h for hulling yield about 78%.The hulling grains were milled into fine flour with hammer mill (Gibbons Electric ,Essex K) to pass 0.4mm mesh size screen and were stored at 4C before being used for their analysis. The seed flour were chemically analyzed according to procedure of AOAC (1980) .The sorghum protein contents were determined by adopting standard AOAC(1995) method . Energy was calculated as

described by Osbonrne and Voogt (1978).Minerals were determined in the sample by the dry-ashing methods described by Chapman and Pratt (1961). Phytic acid was determined by the method described by Wheeler and Ferrel (1971) using 2.0 g dried sample . ..then 2ml dried of the milled sample were weighted to determined phytic acid.

3.4,2 Soaking:

5kg 0f the whole cleaned seeds was put in a pot filled with tap water The grain was removed from the water after 12 hour the lot was sun –dried, then milled into fine flour with hammer mill (Gibbons Electric ,Essex K) to pass 0.4mm mesh size screen and were stored at 4C before being used for their analysis , the processed seed flour were chemically analyzed according to procedure of AOAC (1980) .The processing sorghum protein content were determined by adopting standard AOAC(1995) method . Energy was calculated as described by Osbonrne and Voogt (1978).Minerals were determined in the sample by the dry-ashing methods described by Chapman and Pratt (1961). Phytic acid was determined by the method described by Wheeler and Ferrel (1971) using 2.0 g dried sample..then 2ml of the milled dried sample were weighted to determined phytic acid.

3.4.3 Germination:

According to the method of Koua Kou et al (2008) 3kg of the whole cleaned seeds was immersed in water over night .The grains were spread on trays lined with cloth .It was kept wet by frequent spraying water. After 96 hours ,the germinated grains were removed from the trays, sun –dried , then milled into fine flour with hammer mill (Gibbons Electric ,Essex K) to pass 0.4mm mesh size screen and were stored at 4C before being used for their analysis ,the processed seed flour were chemically analyzed according to procedure of AOAC (1980) .The processing

sorghum protein content were determined by adopting standard AOAC(1995) method . Energy was calculated as described by Osbonrne and Voogt (1978).Minerals were determined in the sample by the dry-ashing methods described by Chapman and Pratt (1961). Phytic acid was determined by the method described by Wheeler and Ferrel (1971) using 2.0 g dried sample..then 2ml dried of the milled sample were weighted to determined phytic acid.

3.4.4 Vitamin C addition:

added 1kg of the whole cleaned seeds was milled in a laboratory mill to obtain fine flour ,150mg of ascorbic acid was mixed with the sample well ,then the processed seed flour were chemically analyzed according to procedure of AOAC (1980) .The processing sorghum protein content were determined by adopting standard AOAC(1995) method . Energy was calculated as described by Osbonrne and Voogt (1978).Minerals were determined in the sample by the dry-ashing methods described by Chapman and Pratt (1961). Phytic acid was determined by the method described by Wheeler and Ferrel (1971) using 2.0 g dried sample..then 2ml of the milled dried sample were weighted to determined phytic acid.

3.4.5 Storing :

Sorghum cereal (fetareta gadarif) were stored for 12 month ,2kg of the seeds then milled into fine flour with hammer mill (Gibbons Electric ,Essex K) to pass 0.4mm mesh size screen and were stored at 4C before being used for their analysis , the processed seed flour were chemically analyzed according to procedure of AOAC (1980) .The processing sorghum protein content were determined by adopting standard AOAC(1995) method . Energy was calculated as described by Osbonrne and Voogt (1978).Minerals were determined in the sample by the dry-ashing methods described by Chapman and Pratt (1961). Phytic acid was

determined by the method described by Wheeler and Ferrel (1971) using 2.0 g dried sample..then 2ml of the milled dried sample were weighted to determined phytic acid.

3.5 Statistical Analysis:

Each sample was analyzed in triplicate , and the value were then averaged. Data was assessed by the analysis of variance (ANOVA) as described by Snedecor and Cochran (1987) and by Duncan – multiple range test at probability of $p<0.05$.

3.6 Second Experiment:

The Effect of Reducing Sorghum phytate on Broiler Performance:

The experiment was carried out during winter season . December twenty nine 2015 to second week of febury2016 in witch the ambient temperature ranged between 16 to 33,7 C° and relative humidity ranged between 21 to 33 %.The animal care and use protocol was obtained by, Faculty of Agricultural and Environmental Sciences . Gadarif University.

3.6.1 Experiment site and duration :

The experiment was carried out in full span open –sided deep litter house The house was located in east-west direction with flour walls .The dimensions of the hous

e were 30×12 meters for the length and width, respectively .The hight of the house was 3 meters at the apex .The materials used in the construction of the house include corrugated iron sheet ,red bricks, iron bares, wire net and cement. The walls of length axis (north and south sides) were rised to the height of 60 centimeters and the rest of the space was covered by rabbit wire. The house was cleaned and disinfected ,bedding of saw, dust was laid at each pen. Each pen was

provided with a feeder and drinker. The light was maintained for 24 Hours (12 hours naturally and 12hours artificially).Hundred of 100 watt power lamps were distributed among the pens at reasonable level to provide continuous light.

3.6.2 Experimental Diets:

Sudanese Sorghum (sorghum bicolor) locally known as Fetarita Gadarif as a source of energy in poultry diets in Sudan (The main dish of the diet (more than60to 75%of the gradient of the diets) was treated by using six methods (milling, germination, soaking, vitamin C addition and storing methods respectively.Sorghum was purchased from Gadarif State local market of (Dura) at price of 30 bounds/kg .Six experimental diets were formulated approximately isocaloric, isonitrogenous. These diets were formulated to meet the requirements of broiler chicks as outline by NRC (1984),then chemical analysis was carried out for the experimental diets (table 3. 5).

3.6.3 Experimental Birds:

A total of three hundreds 300 unsexed, day old broiler chicks (Hybro B) wereweighted and allotted randomly into 30 pens in groups of (10 chicks per pen and 5 pens per treatment) in complete randomized design .The initial body weight of all chicks (42to45g/bird) in each pen were adjusted to be approximately the same

3.7 Management and Data Collection

The experiment was divided in to two periods The first period (starting period) from (1 to 28) day old and the second period (finishing period) from (28 to 49) day old .The experimental diets were randomly assigned to

pens,and a number of 5 pens for each treatment as replicates (5 replicate/treatment) Feed and water provided *ad libitum.* In the starting period bodyweight,feed intake were weekly recorded .Weight gain and feed conversion ratio (F.C.R) were calculated for the individual replicate of each dietary treatment. Mortality was reported as occurred ,and clinical signs were observed and reported throughout the starting experiment period.

In the finishing period body weight,feed intake were weekly recorded .Weight gain and feed conversion ration (F.C.R) were calculated for the individual replicate of the each dietary treatment Mortality was reported as occurred .Leg abnormalities were determined by subjective evaluation of each birds . Only chicks showing amdium or severe degree of bowing were consider to be abnormal. At the termination of the experiment birds were fasted over night except from water to empty their digestive tract and to reduce the chance of carcass contamination during cleaning of ingested matter from the digestive tract.Twenty five chicks were randomly selected from each dietary treatment (5 birds/replicate) , leg –banded individually weighted and slaughtered .Blood samples drained from the cervical blood vessels of chicks during slaughter were collected into clean dry bottles and allowed to clot and sera were separated by centrifugation at 3000 r.p.m for 5 minutes and stored at -20 c until analysed..The birds were scaled in a pot of boiling water (50-55 c)and feather were plucked manually. The carcasses were washed and allowed to drain and eviscerated by ventralcut . The blisters were determined for each birds by breast, and each of chick was given ascore representing the number of blisters . Carcass and abdominal fat were weighted immediately.Dressing out percentage on carcass hot basis was calculated by expressing hot carcass weight to life weight . The carcasses were dissected in to meat and bone for determination of meat to bone ratio in the breast and legs.

3.8Chemical Methods

Sorghum seeds and experimental sample were chemically analyzed according to procedure of AOAC (1980), Phosphorous and calcium content were determined by the methods of the Chapman and Bratt(1961) . The phytic acid content was determined according to the method described by Wheeler and Ferrel (1971) , The level of serum lipids was measured by procedure of Frings *et al* (1970) , (appendix 3) The serum calcium level was determined as described by Trinder (1960) , calorimetric micro determination of calcium serum cholesterol was measured using the method of Kim and Goldbig (1969).

Total Cholesterol:

Cholesterol concentration (mg/dl) in serum was determined by enxymatic colorimetric method using a Kit Rondox(Laboratry- London)

1 .Phenol 28 mmol/L,4 – aminoantipyrin 0.5 mmoI/L, sodium cholate 35 peroxidase>0.8 U/ml. oxidation.

2. Cholesterol standard cholesterol 200mg/100ml.

Free and esterifies cholesterol in the sample, orgnateby means of the coupled reactions described below, hydrolysis and oxidation, acolored cooplex quininoneimine that can be measured by sepectrophotometery.

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{Oxdiase}]{\text{Cholesterol}} \text{Cholesterol+fatty acid}$$

$2H_2O$ + 4- Aminoantipyrine + phenol peroxide Quinoeimine + $4H_2O$.

To the three tubes blank, standard and reagent avolume of 1ml reagent was added, the blank tube contained only the reagent whereasto the sampletube. 0.01ml OD serumwas added, and the standard tube avolume of 0.01 ml standard was added

The tube were mixed and incubated for5 min. at room temperature. The absorbance sample were read by a spectrophotometer at 500 nm against the blank, and so the cholesterol concentration (C) in the sample is calculated using the formulate stated below

(C) Sample =Asample xCstandard.Serum in organic phosphorous level was determined by using akit (Randox Laborataries U. K)

Serum Gulcose Level:

Serum glucose concentration was determined by the enzymatic method using Kit (Mdi Europa GMBH, Hannover, Germany).

Principles:

Serum glucose was determinded by enzymatic oxidation in the presence of glucose oxidation. Thehydrogen peroxide formed reats, under catalysis with phenol and 4amino phenazone to form red-violet dye as indicaror.

$$\text{Gulcose} + O_2 + H_2O \xrightarrow[\text{Oxdiase}]{\text{Gulcose}} H_2O + \text{Gulconate}$$

Reagent 1Buffer solution was composed of 92 mmoi/Lof tris Buffer (PH 7.4) and 0.3 mmo/L of phenol.

Reagent2 (Enzymes) this reagent was compsed of 1500 IU/L of glucose oxidase and 1000 IU/L of peroxidase, and 26 mmoI/L of 4- aminophenazon (4- AP).

The meat of carcass were thawed in a refrigerator for 24 hours .Then approximately analyzed on dry matter basis for chemical components according to AOAC (1980).

Total protein:

Serum total protein was determined by the biuret reagent using akit(plasmetec Lab Products Ltd,.U.K) .

Test principle:

Colorimetric determination of total protein was based on the principle of B iuret reaction , protein in plasma or sserum sample forma ablue clored comlex when treated with cupric ions in alkaline solution. The intensity of the blue colour is proportional to protein concentration .Absorbancese of the samlp (A-sample) and stander (A-stander) were read in the spectrophotometer at 546nm.Serum total protein concentration (C) was estimated as follows:

$$C\ (g/dl) = \frac{\text{A samplel}}{\text{A stander}} \times 6$$

Serum phosphorus

Determined by a colorimetric method using a commercial kit (Spinract S.A.U., Spain).

Test principle

In nitric acid phosphate forms a coloured complex with molybdate and a reductant .

Serum was added to trichloroacetic acid for deproteinization. Then the supernatant was added to the reducatant and molybdate and the absorbance of the sample was read against a blank at a wavelength of 405 nm. Inorganic phosphorus concentration (C) was calculated as follows:

C(mg/dl) = 42.2X A sample.

Serum calcium :

Serum calcium concentration was determined by a colorimetric method using a commercial kit (spinreact, S.A.U., Spain)

Test principle

Calcium ions from a violet complex with chromogen (-O-cretholphthalein complex one -8- hydroxyquinoline hudrochloric acid) in an alkaline medium (2-amino-2-methy1-propan -1-01).

Serum was mixed with a buffered reagent and the absorbaince of the sample (A sample) and of the standard (A standard) were measured against a reagent blank at a wave length of 578nm and calcium concentration (C) was calculated as follows:

$$C(mg/dl) = \frac{\textbf{A sample X concentration of the standard}}{\textbf{A} \text{ standard}}$$

3.9 Experimental Design and Statistical Analysis

A complete randomized design was used and the data generated from the experiment were statistically analyzed by analysis of variance as out line by Steel and Torrie (1960) . Duncan s multiple range test was used to determine level of significant between treatment means at 5% level of probability. Duncan (1955).

Table (3.5) Composition of experimental diets of broiler (as fed percentages)

Parameters	control	dehulling	Germinating	soaking	Vita C	Storing
Sorghum	73.5	72.5	71	71	72	73.5
Ground nut	13	13	13	15	14.5	13
Sesame meal	**7**	**8**	**7.5**	**7.5**	**7**	**7**
Concentrate	**5**	**5**	**5**	**5**	**5**	**5**
Oyster shell	**1**	**1**	**1**	**1**	**1**	**1**
Salt	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
Total	**100**	**100**	**100**	**100**	**100**	**100**

*super concentrate content% CP 46%,fat2%, Energy Kcal /Kg2300%,lysin10.60, Methionine 2.49%, Ca8% ,Iron 200mg, Zinc 1000mg, Mg1400mg, Copper200mg ,Vita A200.00I.U ,Vita K 40mg, VitaB 100mg, Antbiotic 200mg ,Antioxidant100mg

Table (3.6): Calculated chemical analysis of experimental diet of broilers

Parameters	control	Dehulling	germinating	soaking	Vita C	Storing
MEk cal/kg	3114	3110	3111	3108	3114	3100
Crude protein	22.5	22.7	22.9	22.5	22.6	22.3
Lysine	1.2	1.4	1.4	1.3	1.3	1.2
Methionine	0.6	0.7	0.7	0.6	0.6	0.5
Phosphorus	0.7	0.6	0.5	0.6	0.6	0.6
Calcium	1.2	1.2	1.4	1.3	1.2	1.2
Ether Extract	4.6	4.4	4.5	4.5	4.6	4.4
Crud fiber	5.4	4.5	5.6	5.4	5.4	5.4

Calculated according to Ellis (1981).

3.10 Third Exeperiment:

3.10.1 The effect of reducing sorghum phytate on laying hens performance

3.11Experimental site (study area) and period:

This experiment was conducted at the poultry Unit of the Gagarif University.Fucalty of Agricultural and Environmental Sciences , Department of Animal Production, Gadarif , Sudan .

The experiment was carried out during Autum season, from the third of July 2018 to first week of Augustin which the ambiet temperature ranged between 10 to 39.6C and relative humidity ranged between 17 to 38 %.

3.12 Experimental stock :

Atotal of ninty Hy line chicken (17 weeks old) were used in the experiment. The chicken were purchased from Dr Moawya farm (ideal farm in Gadarif state) Then the flock was transferred to Poultry Unit in .Fucalty of Agricultural and Environmental Sciences department of animal production.The hens were weighted and allotted randomly into 18 pens in group of (5birds per pen and 3 pens per treatment) .

3.13Housing and feeding of the experimental birds:

The experimental birds were managed in open –side house in full span open –side deep litter house. The house was located in east-west direction with flour walls. The dimensions of of the house were 12x6 meters for the length and width, respectively. The hight of the house was 3meters at the apex.The materials used in the constration of the house include corrugated iron sheet, red bricks ,iron bares,

wire net and cement. The walls of length axis (north and south sides)) were rised to the height of 60 centimeters and the rest of the space was covered by rabbit wire.

The house was cleaned and disinfected; bedding of saw, dust was laid at each pen. Each pen was provided with a feeder and drinker. The lighting schedules were similar to guideline set in the Hy-Line W-36 Commercial Managgement Guide (Hy-Line International, 2003) and maintained on a16 h . L:8h D Lighting program for the entire period of the study. Each treatment was replicated five time (15 birds per treatment) in a complete randomized design (CRD). The flock were allowed 2 weeks of adaptation period. All birds were provided with laying diet containing 2800 kcal/kg diet , 17.00% CP, 1.45% calcium , and 0.47% phosphorous The feeding program consisted of layer basal diets was formulated to meet the birds dietary nutrient requirements (NRC , 1984) . Six dietary treatment groups were producted from the basal feed as follows

A= control without processing sorghum (normal contain of phytic acid) .

B= processed sorghum low in phytate by dehulling method

C= processed sorghum low in phytate by germination method.

D= processed sorghum low in phytate by soaking method.

E= processed sorghum low in phytate by Vitamin C addition method

F= processed sorghum low in phytate by storing method.

Table (3.7) : Percent inclusion rate (by weight) of dietary ingredients,used in experiment 3.

Ingredient	control	dehulling	germinating	Soaking	Vita C	Storing
Sorghum (F.G)	65.5	65	64	64	66	65.5
Grundnut cake	8	8	9	8	8	8
Sesame cake	10	10.5	11	9.5	10	9.5
Concentrate	5	5	5	5	5	5
Wheat bran	0	0	0	0	0	0
Oster shell	10	10	10	10	10	10
Salt	1	1	1	1	1	1
Laysine	0.3	0.3	0.3	0.3	0.3	.03
Methionine	0.2	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100	100

Table 3 .8: Calculated chemical analysis of experimental diets.

Component	MEkcal/kg	CP%	Ca%	P%	Lysine	Methionine
Rawsorghum	2799	17.3	3.81	0.42	0.85	0.45
Dehulling	2801	17.2	3.80	0.40	0.86	0.46
Germinating	2799	17.5	3.85	0.39	0.87	0.46
Soaking	2800	17.3	3.79	0.40	0.86	0.44
Vita C	2797	17.3	3.81	.041	0.86	0.44
Storing	2798	17.2	3.77	0.439	0.875	0.44

Calculated according to Ellis (1981).

3.14 Experimental Measurements:

Individual body weight of the birds was recorded at the beginning and end of the experiment.Eggproductionwere recorded daily with weekly measurement of feed intake ,egg weight , 5 eggs per cage (replicate) per week were randomly taken from each treatment group for egg quality measurements .Eggshell thickness and Haugh Unit . The average eggshell thickness was determined by measuring the thickness at the large end, small end and in the middle of the egg, and the mean of the three measurement were taken as the average shell thickness. Body weight ,feed intake were weekly recorded .Weight gain and feed conversion ration (F.C.R) were calculated for the individual replecat of the each dietary treatment Mortality was reported as occurred .Leg abnormalities were determined by subjective evaluation of each birds . Only chicks showing amdium or severe degree of bowing were consider to be abnormal. At the termination of the experiment3 birds from each treatment were fasted over night exept from water to empty their digestive tract and to reduce the chance of carcass contamination during cleaning of ingested matter from the digestive tract.Threehens were randomly selected from each dietary treatment (3 birds/replicate) , leg –banded individually and slaughtered .Blood samplesBlood samples drained from the cervical blood vssels of chicks during slaughter were collected into clean dry bottles and allowed to clot and sera were separated by centrifugation at 3000 r.p.m for 5 minutes and stored at -20 c until analysed.. The birds were scaled in a pot of boiling water(50-55) and feather were plucked manually. The carcasses were washed and allowed to drain . The blisters were determined for each birds by breast, and each of chick was given a score representing the number of blisters .The left middle toes of the birds within a pen after removed the soft tissue were booled, then fat content of samples

extracted by eigher for 4 h and dried to constant weight at 100 c for 4 h . The ash from toes was solubilized with nitric and perchloric acid , phosphorus and Calcium content were analyzed with epecttrophotometer respectively.

3.15 Chemical Methods

Sorghum grains and experimental sample were chemically analyzed according to procedure of AOAC (1980), Phosphorous and calcium content were determined by the methods of the Chapman and Bratt(1961) . The phytic acid content was determined according to the method described by Wheeler and Ferrel (1971) , The level of serum lipids was measured by procedure of Frings *et al* (1970) , The serum calcium level was determined as described by Trinder (1960) , calorimetric micro determination of calcium serum cholesterol was measured using the method of Kim and Goldbig (1969) .Serum in organic phosphorous level was determined by using akit (Randox Laborataries U. K). Yolk chemical composition and yolk cholesterol level were determined on day 50 of the experiment period . The analyses to determine egg cholesterol content were crried out according to the methodology proposed by Braganolo and Rodriguez-Amaya (2003) adapted as suggested by Mazalli et al , (2003), and cholesterol was determined using a commercial enzyme kit.The option for using the enzymatic method for cholesterol determination was based on the work of Nogueira and Bragagnolo (2002) and Mazzali et al , (2003) , who found that there were no cholesterol quantification differences between the enzymatic method , routinely used to determine serum cholesterol levels, and the chromatographic method, routinely used to determine cholesterol content). Cholesterol concentration (mg/dl)in serum was determined by enxymatic colorimetric method using a Kit Rondox(Laboratry- London)

1.Phenol 28 mmol/L,4 – aminoantipyrin 0.5 mmoI/L, sodium cholate 35 peroxidase>0.8 U/ml. oxidation.

2. Cholesterol standard cholesterol 200mg/100ml.

Free and esterifies cholesterol in the sample, orgnateby means of the coupled reactions described below, hydrolysis and oxidation, acolored cooplex quininoneimine that can be measured by sepectrophotometery.

Cholesterol ester + H_2O $\xrightarrow[\text{Oxdiase}]{\text{Cholesterol}}$ Cholesterol+fatty acid

$2H_2O$ + 4- Aminoantipyrine + phenol peroxide Quinoeimine + $4H_2O$.

To the three tubes blank, standard and reagent avolum of 1ml reagent was added, the blank tube contained only the reagent whereasto the sampletube. 0.01ml OD serumwas added, and the standard tube avolume of 0.01 ml standard was added

The tube were mixed and incubated for5 min. at room temperature. The absorbance at A od the standard and sample were read by a spectrophotometer at 500 nm against the blank, and so the cholesterol concentration (C) in the sample is calculated using the formulate stated below

(C) Sample =Asample xCstandard .

Serum in organic phosphorous level was determined by using akit (Randox Laborataries U. K).

3.16 Experimental Design and Statistical Analysis

A complete randomized design was used and the data generated from the experiment were statistically analyzed by analysis of variance as out line by Steel and Torrie (1960) . Duncan s multiple range test was used to determine level of significant between treatment means at 5% level of probability.

CHAPTER FOUR

RESULTS

4. Results of First Experiment:

4. 1 Results of the a proximate analysis ,minerals and calculated energy of the sorghum (F. G) is present in table (4.9) and (4.10). The result indicated that sorghum (F . G) had a high percentage of crude protein, Ether Extract and metabolizable energy .

4 . 2 Chemical composition of sorghum before and after processing were shown in table (4.11) , the result shown significant ($p>0.05$) change in some nutrient value of processed grains in energy, Ca ,P, and Fe, and slight change in protein content.

4.3 Determination of phytic acid

Results of determination of phytic acid in raw sorghum and processed sorghum (F.G) is present in table (4.12). The result indicated a significant decrease($p>0.05$) of phytic acid of all the processed treatments.

4.4 Results of the second experiment

4.4.1 Production performance of broiler chicks in starting period

Table (4.13) shows feed intake ,finally body weight gain and finally feed conversion ratio in starter period , the result indicated no significant effect in feed intake and body weight gain . Treatment had slight increase on feed conversion ratio The feed conversion ratio tended to be high in birds fed processed seeds than those fed on unprocessed sorghum .None of the treatment had significant effect on mortality rate during the starting period of the experiment.

Table (4. 9) : Chemical composition of sorghum (F.G) %

Chemical profile	Sorghum(F.G)
Dry matter	93.83
Crude protein	14.3
Ether Extract	4.58
Crude fiber	2.69
Nitrogen free extract	7.31
Metabolizable energy (Kcal/kg)	3423
Methionine	8.5mg/100g
Thiamin	0.38mg/100g
Lysine	117.6mg/100g
Niacin	3.8mg/100g

-Analysed values are means of duplicate sample, ME is calculated value by the equation of Carpenter and Clegg (1966).-NFE is calculated value

Table (4. 10) : Minerals content of sorghum bicolor (F.G)mg/100g

Chemical profile	Sorghum(F.G)
Calcium	12.8
Phosphorus	222
Magnisum	103
Mn	122
Fe	4.8

-Analysed values are means of duplicate sample.

-ME is calculated value by the equation of Carpenter and Clegg (1966).

-NFE is calculated value.

- Lab. National center for reseaech Khartoum

Table (4.11) :Effect of treatments on sorghum chemical composition before and after processing .(mg/100g):

Treatments	CP	Energy (kcal/kg)	Ca	P	Phytate
Raw sorghum	142.20	3100.50b	0.244	0.38a	889.20a
Dehulling	143.00	3101.00b	0.252	0.30b	445.06b
Germination	144.40	3113.33a	0.265	0.28b	87.90g
Soaking	143.70	3105.67b	0.250	0.28b	189.70e
Vitamin C	143.10	3101.00b	0.250	0.29b	122.40f
Storing	142.80	3100.80c	0.245	0.28b	360.00c
(SE±)	0.71	2.85	0.01	0.01	1.52
$LSD_{0.05}$	Ns	8.70	Ns	0.03	4.68

$LSD_{0.05}$: least significant difference at 0.05 level of significance (for mean separations and comparisons)

-

Table (4.12) :Effect of treatments on sorghum phytate mg/ 100g before and after processing

Treatments	Phytic acid content	Loss%	+SE
Raw sorghum	889.20a	0	0.98
Dehuling	445.20b	49.9	0.76
Germination	87.90g	90.1	1.03
Soaking	189.70e	78.6	0.87
Vitamin C	128.40f	86.2	1.09
Storing	360.00c	59.6	0.89

- Different small letters represent significant differences between treatments means in each column at 0.05

-SE: stander error of the means

4.4.2 Production of broiler chicks durind the finishing period :

Table (4.14) shows over all performance ,feed intake , final body weight gain and final feed conversion ratio .The results revealed that dietary treatments had significant effects($p<0.05$) in feed intake , and bird fed processed sorghum such as germinating,vita C and storing methods had significant high feed intake than birds fed unprocessed sorghum ,and dehulling one .Treatments had a significant increase ($p<0.05$) in over all weight gain. The weight gain tended to be statistically higher in birds fed processed sorghum .The highest body weight gain registed in birds fed in germinated sorghum Followed by birds fed on sorghum treated by vitamin C, then birds fed on stored sorghum respectively Chicks fed on processed sorghum had slight increase on feed conversion ratio than those fed on unprocessed sorghum .None of the treatments had significant effect on mortality rate during the finishing period of the experiment

4.4.3 Carcass Measurements;

The treatment had significant effect ($p<0.05$) on carcasses cut as shown in Table (4.15).The results indicated that feeding broiler on processed sorghum significantly($p<0.05$) increased the weight of breast , leg , wings ,back and neck

The results revealed that feeding broiler on processed seed had no significant effect ($p<0.05$) on dressing percentage.

4.4.4; Abdominal Fat:

The results of abdominal fat percentage indicated that there was no significant increase ($p<0.05$) by treatment as shown in Table (4.15) ,the slight increase in abdominal fat obtained by birds fed processed grains with germination method followed by birds fed by soaking, vita C ,storing and dehulling methods respectively

4.4.5 Meat Composition;

The results of meat composition is shown in table (4.16) . Results indicated that treatments had no significant effect($p<0.05$) on moisture contents, meat protein , and fat content , moreover birds fed on processed seeds had slight increase on ash content than those fed on unprocessed seeds.

4.4.6 Serum Composition;

The effect of treatments on serum composition of broiler shown in Table (4.17) . The results indicated a significantly($p<0.05$) decrease on cholesterol content in whole blood serum , obtained by birds fed on germinated sorghum Followed by birds fed on sorghum treated by vitamin C, and birds fed on stored sorghum respectively , The result shown a significant increase ($p<0.05$) in blood serum glucose obtained by bird fed processed seeds The result indicated a significant increase ($p<0.05$) effect on calcium and a significantly($p<0.05$) decrease on phosphorous content in whole blood serum in birds fed on processed sorghum grains.

4.4.7. Tabia Ash:

Table(4.18) showed the effect of processed sorghum on Tabia Ash. The results revealed that feeding broiler on processed seed had a significantly ($p<0.05$) increase on broiler Tabia Ash

4.5 Results of the third experiment:

4.5.1 Production performance of laying hens:

Table (19,20,21) , shows the over all feed intake, feed utilization, egg weight , percentage of hen day egg production.The results show no significant difference between different treatments in feed intake, egg weight, percentage of hen day egg

production and body weight. The result show asigneficant increase in feed utilization obtained by birds fed processed sorghum low in phytate . There were slight increase in egg production and percentage of hen day egg production obtained by birds fed processed sorghum

Theshell thickness (mm) and shell weight of layers fed processed sorghum (low in phytate) were almost similar and significantly higher than thickness of egg produced by birds fed the control diets, throughout most of the seven weeks duration of the experiment (Table 22, 23)

Haugh units were used as an indicater of egg freshness and it was found that the values of these units were significant high for fresh eggs produced by birds fed processed sorghum low in phytate.Table (4.24)

4.5.2 Egg shell Ash

Table (4.25) show egg ash and the content of calcium and phosphorus in shell . Treatment shown significant increase in ash and calcium content% obtained by birds fed germinating sorghum fllowed by birds fed(+Vitamin C), soaking, storing and dehulling sorghum respectivelly.

4.5.3 Toe Ash

Table (4.26) show asignificant increase in toe ash ,toe ash calcium and toe ash phosphorus % obtained by birds fed processed sorghum low in phytate with germination method followed by birds fed vitamin Cmethod,soaking,storing and dehulling methods respectiveily.

4.5.4 Egg Cholesterol

The effect of feeding processed sorghum low in phytate on Yolk cholesterol content (mg/100g)of the layers eggs shown in table (4.27). The result showed

asignigificant increase (p>.05) in chlosterol of egg produced by birds fed germinating sorghum and vitamin C flowed by bird fed soaking , storing and dehulling sorghum respectively as compered with control

4.5.5 Blood profiles

Table (4.28) show the effect of feeding processed sorghum low in phytate on blood profiles of laying hens, Treatments show asignificant increase (p>0.05) intotal protein and gulcose content ,

4.6 Economic appraisal:

The total cost, return ,net profit and profitability ratio per head of broiler chicks fed processed sorghum low in phytate for 7 weeks are shown in table(4.29). Chicks purchase management and feedcost values(IQD) were the major in put considered.The selling values of meat are total revenues obtained profitability ratio (1.42) of germination and vitamin C group.

Table (4.30) shows the effect of treatment on some economic efficiency parameters .Birds fed processed sorghum with germination method and vitamine C had the highest profitabilityratio compared to all other treatments

Generally , processed sorghum low in phytate increased the profitability ratio compared to un processed treatment.

Table (4.13) : performance of experimental chicks during starting period between (1 –28) day old

Treatments							
Parameters	**Unprocessedsorghum (control)**	**Processedsorghum (dehulling)**	**Processedsorghum (germination)**	**processedsorghum soaking**	**Processedsorghum (+ vita C)**	**processedsorghum (storing)**	**+_SE**
Initial weight	**45**	**43**	**44**	**45**	**43**	**45**	**NS**
Final weigh	**895**	**891**	**893**	**894**	**893**	**895**	**NS**
Feed intake g /bird	**1753.80**	**1778.20**	**1763.20**	**1743.60**	**1772.80**	**1772.00**	**NS**
Weight gain g/bird	**850.26**	**847.78**	**849.30**	**849.36**	**850.16**	**850.34**	**NS**
F C R	**2.06**	**2.09**	**2.07**	**2.1**	**2.08**	**2.08**	**NS**

-Means on the same row showing common superscripts are not significantly different at 0.05% level.

Table (4.14) : performance of the experimental chicks during the finishing period:

Treatments							
Parameters	**Unprocessedsorghum (control)**	**Processed sorghum (dehulling)**	**processed sorghum (germination)**	**processed sorghum (soaking)**	**processed sorghum(vita C)**	**processed sorghum (storing)**	**SE (±)**
Feed intake g /bird	**1939.56±32.58b**	**1877.00±50.37b**	**2369.44±66.14a**	**1883.90±68.35b**	**2490.60±51.56a**	**2015.36±53.86a**	**96.83**
Initial weigh	**895**	**891**	**893**	**894**	**893**	**895**	**NS**
Finaly weight	**1785d**	**1785d**	**12038a**	**1794**	**1987b**	**1896c**	**93.67**
Weight gain g/bird	**889.60±10.27d**	**893.82±13.32d**	**1144.90±36.25a**	**899.50±4.47d**	**1093.50±60.55b**	**1001.12±4.99c**	**13.30**
F C R	**2.01**	**2.09**	**2.06**	**2.09**	**2.1**	**2.01**	**NS**

*Values are means of 5 replicate each of 10 birds

*Means 0n the same row showing common superscripts are not significantly different at 0.05% level.

* SE : stander error of the means

Table (4.15) :Effect of diatry tretments on broiler carcass cut weight (gm), Abdominal fat% and dressing percentage**:**

Treatments							
Parameters	**Unprocessed sorghum (control)**	**Processed sorghum (dehulling)**	**processedsorghum (germination)**	**Processedsorghum (soaking)**	**Processedsorghum (vita C)**	**processedsorghum (storing)**	**SE (±)**
Breast weight	**410.10 ±1.84c**	**408.60±4.25c**	**474.0 ± 46.87a**	**436.36 ± 077b**	**436.80 ± 0.97b**	**477.76 ± 2.29a**	**1.59**
Leg and thigh weight	**421.52±1.46e**	**422.06±2.41e**	**484.70 ± 6.51a**	**447.02 ± 1.41d**	**477.92 ± 0.79d**	**454.84 ±3.29c**	**1.46**
Wings weight	**160.10±0.68c**	**161.56±1.43e**	**186.88 ± 1.02a**	**170.16 ± 1.19d**	**181.60 ± 1.25b**	**172.76 ± 2.23c**	**0.62**
Neck and back weight	**262.28±2.43d**	**263.38±1.39d**	**301.92 ± 6.15a**	**208.28 ± 0.74a**	**295.00 ± 3.69b**	**283.68 ± 2.59c**	**1.46**
Abdominal fat %	**2.7**	**2.8**	**3.0**	**2.9**	**2.8**	**2.8**	**NS**
Dressing %	**70.6**	**70.8**	**72.1**	**72.4**	**71.7**	**72.3**	**NS**

-Means on the same row showing common superscripts are not significantly different at 0.05% level.

-SE: stander error of the means

Table (4.16) : Effect of feeding processed sorghum on broiler meat chemical composition

Treatments							
Parameters	**Unprocessed sorghum (control)**	**Processed sorghum (milling)**	**processed sorghum (germination)**	**processed sorghum soaking)**	**processed sorghum (adding vita C)**	**processedsorghum (storing)**	**SE (±)**
Moisture	**71.1**	**70.2**	**69.8**	**70.7**	**70.3**	**70**	**NS**
C. P	**19.9**	**19.94**	**21.97**	**20.5**	**21.0**	**19.2**	**NS**
Fat	**2.0**	**2.2**	**2.5**	**3.0**	**3.1**	**3.2**	**NS**
Ash	**1.2**	**1.3**	**1.4**	**1.3**	**1.4**	**1.3**	**NS**

Table (4.17) : Effect of feeding processed sorghum on serum composition of broiler chicks

Treatments							
Parameters	**Unprocessedsorghum (control)**	**Processed sorghum (dehulling)**	**processed sorghum (germination)**	**processed sorghum (soaking)**	**processed sorghum (vita C)**	**processed sorghum (storing)**	**SE (±)**
Cholesterol (mg /dl)	**134.14±0.86a**	**126.76±0.93b**	**126.76 ± 2.57c**	**121.82 ± o72c**	**121.74 ± 1.36c**	**121.02 ± 4.56c**	**1.02**
Glucose (mg /dl)	**176.16±2.28b**	**181.04±1.60a**	**181.98 ± 2.97a**	**182.76 ± 3.27a**	**181.54 ±3.41a**	**182.80 ± 2.69a**	**1.24**
Calcium (mg/dl)	**10.86±0.27b**	**11.54±0.65a**	**11.92 ± 0.31a**	**11.78 ± 0.23a**	**11.70 ±0.40a**	**12.10 ± 1.73a**	**0.18**
Phosphorous (mg/dl)	**7.27±0.39a**	**6.46±0.21b**	**5.96 ± 0.96 ± 0.**	**6.00 ± 0.23c**	**6.04 ± 0.23c**	**6.00 ± 0.25bc**	**0.12**

-Means on the same row showing common superscripts are not significantly different at 0.05% level.

- SE stander error of the means

Table (4.18) : Effect of feeding processed sorghum on Tibia Ash

Treatments							
Parameters	**Unprocessedsorghum (control)**	**Processed sorghum (milling)**	**Processedsorghum (germination)**	**processed sorghum (soaking)**	**processed sorghum (adding vita C)**	**Processedsorghum (storing)**	**SE (±)**
Tabia Ash	**53.80 ± 1.09b**	**56.66 ± 0.61a**	**57.06 ± 0.59a**	**56.32 ± 1.45a**	**57.14 ± 0.48a**	**56.40 ±1.70a**	**0.49**

-Means on the same row showing common superscripts are not significantly different at 0.05% level.

-SE : stander error of the means

Table (4.19): Effect of processed sorghum (low in phytate) in daily feed intake - (g/hen) and feed utilization- mass of eggs in g/1g of feed).

Treatments	FI (g/hen)	Feed utilization
Unprocessed sorghum (control)	118	0.425c
Processed sorghum (dehulling)	118	0.427c
Processed sorghum (germinating)	121	0.455a
Processed sorghum (soaking)	120	0.445b
Processed sorghum (+ vitamin C.)	121	0.456a
Processed sorghum (storing)	119	0.467a
LSD (0.05)	NS	0.013
SE (±)	2.21	0.005
Probability	$p \geq 0.05$	$p \leq 0.01$
C.V. (%)	3.2	2.04

LSD (0.05): represents the least significant difference at 5% probability

NS: represents non-significant difference.

SE: Standard error.

C.V. (%): coefficient of variation.

* Means followed by different letters in the column are statistically different according to the least significant difference (LSD) method ($p \leq 0.05$).

Table: (4.20) Effect of processed sorghum (low in phytate) on egg weight(gm)

Treatments	Weeks						
	1	2	3	4	5	6	7
Unprocessed sorghum Control	45.65	46.12	46.51	47.32	47.80	48.71	50.21
Processed sorghum Dehulling	45.61	46.10	46.48	47.35	47.88	49.35	50.40
Processed sorghum Germinating	45.64	46.08	46.39	47.38	48.76	53.67	55.12
Processed sorghum Soaking	45.59	46.12	46.52	47.40	47.98	50.23	53.92
Processed sorghum +Vita C	45.65	46.09	46.53	47.41	48.82	54.31	55.20
Processed sorghum Storing	45.63	46.10	46.49	47.42	47.92	52.31	54.67
LSD(0.5)	NS	NS	NS	NS	NS	NS	NS
± SE	1.343	1.378	0.876	1.793	0.978	0.889	1.487

*Mean (s) having different superscript (s) in a column are significantly different ($p<0.05$) according to DMRT.

Table: (4.21) Effect of processed sorghum (low in phytate) on egg production

Treatments	Weeks						
	1	2	3	4	5	6	7
Unprocessed sorghum Control	66.6a	73.3a	75.3a	73.3a	80.9a	73.3ab	75.3b
Processed sorghum Dehulling	73.3a	66.6a	66.6a	75.3a	81.9a	81.9a	80.9ab
Processed sorghum Germinating	66.6a	75.3a	73.3a	73.3a	80.9a	84.7a	87.6a
Processed sorghum Soaking	75.3a	75.3a	75.3a	75.3a	75.3a	87.6a	85.7a
Processed sorghum +Vita C	66.6a	66.6a	73.3a	75.3a	75.3a	85.7a	88.5a
Processed sorghum Storing	73.3a	73.3a	75.3a	73.3a	81.9a	85.7a	84.7a
LSD(0.5)	NS	NS	NS	NS	NS	3.94	3.78
CV%	4.47						

*Mean (s) having different superscript (s) in a column are significantly different ($p<0.05$) according to DMRT.

Table : (4.22) Effect of processed sorghum (low in phytate) treatments on shell weight (g).

Treatments	Shell weight (g) “mean ± SD”
Unprocessed sorghum (control)	5.55 ± 0.19
Processed sorghum (dehulling)	5.92 ± 0.13
Processed sorghum (germinating)	6.32 ± 0.27
Processed sorghum (soaking)	5.76 ± 0.35
Processed sorghum (+ vitamin C.)	6.34 ± 0.29
Processed sorghum (storing)	6.18 ± 0.28
Probability	$P \leq 0.01$

SD: represents standard deviation.

C.V. (%): coefficient of variation

Table : (4.23) Effect of processed sorghum (low in phytate) treatments on shell thickness (mm).

Treatments	Shell thickness (mm) "mean ± SD"
Unprocessed sorghum (control)	0.34 ± 0.01
Processed sorghum (dehulling)	0.34 ± 0.01
Processed sorghum (germinating)	0.37 ± 0.01
Processed sorghum (soaking)	0.36 ± 0.02
Processed sorghum (+ vitamin C.)	0.36 ± 0.01
Processed sorghum (storing)	0.35 ± 0.01
Probability	P ≤ 0.01
Period	Shell thickness (mm) "mean ± SD"
Week 1	0.34 ± 0.01
Week2	0.34 ± 0.01
Week 3	0.35 ± 0.01
Week 4	0.35 ± 0.01
Week 5	0.36 ± 0.01
Week 6	0.36 ± 0.01
Week 7	0.37 ± 0.02
Probability	P ≤ 0.01
Treatments X Period	
Probability	P ≤ 0.01
C.V. (%)	1.19

SD: represents standard deviation.

C.V. (%): coefficient of variation

Table : (4.24) Effect of feeding processed sorghum (low in phytate) on haugh unit and yolk height "mm".

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Treatments	HU	YH (mm)
Unprocessed sorghum (control)	86.23	7.50
Processed sorghum (dehulling)	86.00	7.50
Processed sorghum (germinating)	87.61	8.00
Processed sorghum (soaking)	86.34	7.50
Processed sorghum (+ vitamin C.)	87.42	8.00
Processed sorghum (storing)	86.56	7.50
LSD (0.05)	NS	NS
SE (±)	0.07	0.28
Probability	$p \leq 0.05$	$p \geq 0.05$
C.V. (%)	0.24	10.80

NS: represents non-significant difference.

SE: Standard error.

C.V. (%): coefficient of variance.

Table :(4.25) Effect of processed sorghum (low in phytate) shell, calcium and phosphorous ash (%).

Treatments	Shell ash (%)	Calcium ash (%)	Phosphorous ash (%)
Unprocessed sorghum (control)	85.89c	29.85c	0.24
Processed sorghum (dehulling)	85.96c	30.55b	0.24
Processed sorghum (germinating)	88.21a	33.20a	0.25
Processed sorghum (soaking)	86.92b	32.81a	0.25
Processed sorghum (+ vitamin C.)	87.01b	32.81b	0.24
Processed sorghum (storing)	86.61b	31.23a	0.24
LSD (0.05)	0.87	1.03	NS
SE (±)	0.34	0.41	0.01
Probability	$p \leq 0.05$	$p \leq 0.05$	$p \geq 0.05$
C.V. (%)	1.19	3.87	8.00

NS: represents non-significant difference.

SE: Standard error.

C.V. (%): coefficient of variance.

* Means followed by different letters in the column are statistically different according to the least significant difference (LSD) method ($p \leq 0.05$).

Table : (4.26) Effect of processed sorghum (low in phytate) toe ash and toe ash calcium and phosphorous (%).

Treatments	Toe ash (%)	Toe ash calcium (%)	Toe ash Phosphorous (%)
Unprocessed sorghum (control)	91.52c	24.62b	15.21
Processed sorghum (dehulling)	91.81b	24.81b	15.52
Processed sorghum (germinating)	93.2a	26.65b	15.86
Processed sorghum (soaking)	92.10b	24.92a	15.79
Processed sorghum (+ vitamin C.)	92.15b	24.92a	15.59
Processed sorghum (storing)	92.00b	25.23a	15.56
LSD (0.05)	0.35	0.36	NS
SE (±)	0.14	0.16	0.18
Probability	$p \leq 0.01$	$p \leq 0.01$	$p \geq 0.05$
C.V. (%)	0.45	1.66	2.65

NS: represents non-significant difference.

SE: Standard error.

C.V. (%): coefficient of variance.

* Means followed by different letters in the column are statistically different according to the least significant difference (LSD) method ($p \leq 0.05$).

Table (4.27): Effect of processed sorghum (low in phytate) on cholesterol content (mg/100g) of the layer eggs.

Treatments	cholesterol content (mg/100g)
Unprocessed sorghum (control)	1066c
Processed sorghum (dehulling)	1081b
Processed sorghum (germinating)	1100a
Processed sorghum (soaking)	1099a
Processed sorghum (+ vitamin C.)	1100a
Processed sorghum (storing)	1098a
LSD (0.05)	6.55
SE (±)	2.6
Probability	$p \leq 0.01$
C.V. (%)	0.72

NS: represents non-significant difference.

SE: Standard error.

C.V. (%): coefficient of variance.

* Means followed by different letters in the column are statistically different according to the least significant difference (LSD) method ($p \leq 0.05$).

Table (4.28) Effect of feeding processed sorghum (low in phytate) on blood profiles in laying hens

Treatments	Total Protein (g/l)	Glucose (mg/dl)
Unprocessed sorghum (control)	4.96d	173.20c
Processed sorghum (dehulling)	5.28c	179.11b
Processed sorghum (germinating)	5.88a	182.22a
Processed sorghum (soaking)	5.87a	181.33a
Processed sorghum (+ vitamin C.)	5.81b	182.00a
Processed sorghum (storing)	5.29c	180.55
LSD (0.05)	0.06	1.12
SE (±)	0.02	0.45
Probability	$p \leq 0.01$	$p \leq 0.01$
C.V. (%)	1.31	1.12

NS: represents non-significant difference.

SE: Standard error.

C.V. (%): coefficient of variance.

* Means followed by different letters in the column are statistically different according to the least significant difference (LSD) method ($p \leq 0.05$).

Table.(4. 29); Total cost ,revenues. Net,profit and profitability ratio of broiler chicks.

I tems	Control	Dehulling	germiating	soaking	Vitamin C	Storing
Chick purchase	5.0	5.0	5.0	5.0	5.0	5.0
Total feed cost	22	22.5	23.2	22.5	23	22
Management	5	5	5	5	5	5
Total cost production	32	32.5	33.2	32.5	33	32
Live weight	1783.20	1783.80	2018	1848	1993.46	1890.8
Dressing percentage	70.6	70.8	72.1	72.4	71.8	72.3
Average weight	1.26	1.27	1.45	1.34	1.43	1.37
Price /kg	35	35	35	35	35	35
Total revenue/chick	44.10	44.45	50.75	46.90	50.05	47.95
Totalrevenue/chick Total price	44.10 32	44.45 32.5	50.75 33.2	46.90 32.5	50.05 33	47.95 32
Total profit	11.90	11.95	17.55	14.40	17.05	15.95
Profitability	1	1.04	1.47	1.21	1.43	1.34

Total Cost Calculated Accord to (2014) Price of mean (S P) kg

Price kilogram of bird calculated accordind to (2014)

Table.(4.30): Total cost ,revenues. Net,profit and profitability ratio of laying hens.

Items	A	B	C	D	E	F
Total feed cost	37.58	38.22	38.50	37.80	38.83	37.00
Management	6	6	6	6	6	6
Total production cost	43.58	44.22	44.50	43.80	44.83	43.00
Egg production	36.72	39.20	43.20	41.63	43.00	41.16
Totalrevenue	73.44	78.40	86.40	83.30	86.24	82.32
Total revenue Total cost	73.44 43.58	78.40 44.22	82.26 44.50	83.30 43.80	86.24 44.83	82.32 43.00
Total profit	29.86	34.18	41.90	39.50	41.41	39.32
Profitability	1	1.31	1.40	1.32	1.39	1.31

Total Cost Calculated Accord to (2018) of mean price of eggs (S .P) /dosen

In price of 35 (S.P) calculated accordind to (2018)

A= control (raw sorghum)

B= processed sorghum (dehullin)

C= processed sorghum (germination)

D= processed sorghum (soaking)

E= processed sorghum (Vitamin C)

F= processed sorghum (storing)

CHAPTER FIVE

DISCUSSION

The moisture content of sorghum bicolor (F.G) was in the range obtained by AbdElnour (2001) who indicated that moisture content of Feterita and Dabar was 9.6-8.75 respectively. The crude protein content of sorghum bicolor (F.G) was in the range observed by Ibitaye *et al* (2012), Clement *et al* (2010) and, Hulse *et al* (1980) who found that the protein content of sorghum bicolor ranged between 8-16 % ,but lower than Elsayed (1999) analyzed the protein content of Tabat and Fetarita was 6.46-9.11 , and higher than the value reported byDillon(2007), and lower than that obtained by Mayada(2009).The metabolizable energy of sorghum (F.G) was in the range detected by Idris(2004) who found that carbohydrates content for sorghum was 80.7%.Minerals content was in range optimum by Hulse et al (1980) and Idris(2004).

The phytic acid content of sorghum (F.G) are close to those reviewed by Marfo et al (1990) who reported that phytic acid content of red sorghum was 886mg /100g. Greiner (2005) , and Konietzny and Koyode (2006) whom found that sorghum phytate ranged from 590 to 1180 and from 400 to 3500 mg/100g dwt . The result was high than the range obtained by Emmambux *et al* (2009) , Makokha *et al* (2002) and, Sathe(2002), these can be explained by that phytic acid content varied due to stage of maturity , climatic , condition s type of soil , amount of available phosphorous and milling fraction of the grains

The removal of phytate after processed treatments , showed that the highest loss of phytate ($p>0.05$) obtained by germination method (90.1 %) followed by vitamin C method (86.2%),soaking method (78.6%),storing method (59.6%) and dehulling

method (49.9%) respectively. In fact phytic acid in flour can be hydrolyzed by the enzyme phytase, and the optimum condition for phytase activity in PH range from 5.0 to 5.5 and temperature range 50 to 55c.Germination method in sorghum grains reduced phytate up t0 90.9 after 96h Wisal(2004). The result obtained was in line with Abdelrahman *et al* (2007) who reported that germination increase part of both major and trace minerals and also reduced significantly the phytic acid , germination is more effective way to remove phytic acid , and germination in 80-90£ removed 92% of phytate and releases vitamins and make grains and seeds more digestible . Vitamin C method reduced(P.A) 86.2%,vitamin C is strong enhancer of plant iron can over come the inhibitors in plant foods. One study found that various doses of phytate reduced iron absorption by 10 to 50 %.But adding 50 mg of vitamin C counteracted the phytate and adding 150 mg of vitamin C increased iron absorption to almost 30%, similarly, in the presence of a large dose of tannic acid , 100 mg of vitamin C increased iron absorption from 2-8% .Snedecor *et al* (1987) , this could be attributed to the fact that acidic anion complexes with the minerals like calcium and phosphorus result in an improvement in the digestibility of these minerals as reported by several workers. The results of soaked sorghum was in range of result obtained by Aotzc ,(2001) who reported that soaking of maize for 1 h at room temperature already led to the reduction of phytic acid by 51%, but lower than the report obtained by Mahgoub and Elhage (1998) whom reported that soaking of sorghum flour at room temperature for 24 h reducing phytic acid level by 16-21%.The reduction of phytic acid using storing method was in the same line with Sathe (2002) ,who reported that the decreased of phytic acid during storage from 0 to 65% in cereal according to temperature and humidity and from 2.5 to 76% in legume, and the reduction depend on the type of seeds , storage condition, and the age of the seeds.The processed of sorghum bicolor (F.G) by using five technical methods changed the

nutrient values of the seeds after processing . The high content of crude protein metabolizable energy and fat obtained by germinated seeds , the result in line with many workers , they observed increase in proteins during germination of cereals , this increase could be attributed to a synthesis of enzymatic proteins by germination seeds . (W H O , 1998) . Marero *et al* (1988) also reported that the increases in protein might be due to the fact some a mino acids are produced in excess of the requirement during protein synthesis and these tend to accumulate in free a mino acids pool . In the same line Kouakou *et al* (2008) indicated that the seeds of cereals during their germination develop a strong enzymatic activity . The results also in line with Ocheme and Chinma , (2007) who found that germination significantly increases the protein dry mater and ash content , while fat content and energy values of the flour sample showed a decrease , the protein increase not exceeding 14% of the starting protein content . This was found to be attributed to loss of dry weight through respiration during germination , in the same line Beal and Mehta (1985) reported that germination reducing up to 75% of phytate and increase phytase activity , but different from Martinez et al (1980) who reported that germination decreased the content of lysine , tryptophan and vitamins such as C , B , A and E .)

In this study there were no signs of disease observed and all experimental stock looked apparently healthy. The general behavior of the stock also was good. The ambient temperature during the experimental period fell within the thermoneutral zone has extracted no heat stress on the experimental birds.No mortalities were recorded among the different treatment groups throughout the experimental periods, this may be due to good hygienic situation of the experiment. In this study all birds were kept in clean disinfected environment following all hygien regulation program

In production performance of the experimental Chicks, as expected the treatments of sorghum was led to greater performance. Performance indices such as body weight , body weight gain, feed efficiency were higher with processed sorghum diets than unprocessed sorghum diets . Body weight gain differed significantly in the various treatments , the higher body weight gain obtained by birds fed on germinated sorghum followed by birds fed on processed sorghum with vitamin C, birds fed on stored sorghum ,soaked and dehulling sorghum respectively . The greatest increase of body weight gain synchronization inversely with the decreased of sorghum phytate . These can be explained by the finding of Doherty et al (1982) who reported that phytic acid reduced the growth. The results was in line with Harland (1936) who indicated that growth and zinc utilization was inhibited by phytic acid in rats and human, phytic acid interferes with enzymes need to digest food, including pepsin , witch is needed for break down of protein in the stomach and amylase, which is required for the break down of starch, and inhibits the enzyme trypsin which is needed for protein digestion in small intestine .The high feed intake obtained by birds fed on germinated seeds was explained by the find of Mamudu *et al* (2005)who reported that germination is causing profound changes in the seeds and derivatives , thus giving them especial flavor, on account of hydrolysis of starch ,derived porridge from cereal which has a low viscosity ,thus the porridge from cereal sprouts for children have a nutritional certain advantage: high energy density intake of macronutrients and micro level, thus may be increases feed intake and explained the highest body weight of birds fed on germinated seeds and this similar to that finding of Saford *et al* (1973) . The result was also on line with Makokha *et al* (2002) who reported that germination increases the rate of minerals available ,and soaking reducing phytic acid 43%. The high growth obtained by birds fed on processed sorghum with vitamin C this could be explained by the fact that acidic anion complexes with the minerals like

Ca and P result in an improvement in the digestibility of these mineral, ascorbic acid has PH reducing property and thus conducive for the growth ,the result in the same understanding of Siegenberg *et a*l (1991) who reported that adding 60 mg of vitamin c counteracted phytic acid load of meal .In other study 80 mg of ascorbic acid counteracted 2.5 mg of phyticacid. Snow et al (2004) reported that addition of citric acid to broiler diets improve the tibia ash without reducing the weight gain or feed intake. Leg abnormality, serum Cholesterol ,serum Glucose, and Tibia ash were the strong effect of reducing phytic acid from the poultry diets. The leg abnormality appear in birds fed in high amount of phytic acid than birds fed in low amount of phytic acid, this can be explained by the finding of Punna and Roland (1999) who reported that phytic acid reduced growth and caused leg abnormality as a sign of phosphorous deficiency . In the same direction (Atwal et al ,1980) reported that feeding rats with rape seed protein diets containing 1.24% phytate resulted in reduced growth rates and feed intake and efficiency of protein utilization of these animals . Moreover in early as(1949) the researcher Edward Mellanby ,discovered that consumption of high –phytate cereal grain interfere with bone growth ,and interrupts vitamin D metabolism resulted in rickets and a severe lack of bone formation (Mellanby,1949), so most people had a diets high in phytate cause mineral deficiencies for example richest and osteoporosis are common in societies where cereal grain are staple part of the diet. The result shown significant increase ($p>0.05$) in whole blood serum cholesterol obtained by birds fed on a high amount of phytate diet (in control), may be explained by the results of Szkudelski(1998) who reported that phytic acid increased the cholesterol content in the whole blood serum in the rats . It is different from that obtained by Sharon and Thomposon (1997) who reported that an addition of phytic acid to a high cholesterol diet reduced both serum cholesterol and triacyl glycerols Result shown significant increase ($p>0.05$) in Calcium of whole blood serum obtained by

birds fed on diet content a low amount of phytate , and shown decrease in phosphorous of whole blood serum ,may be explained by the finding of Sharon and Thompson (1997) who indicated that phytic acid reduced mineral bioavailability in both animal and humans. A similar observation was obtained by Punna and Ronald (1999). It is well known that the major phosphorus content of the grain is in the form of phytate , which has low available to monogastric animal, such as poultry (Simon et al.1990 :Summers,1997. Bed ford2000,:Lesson and Summers ,2001) .phytic acid not only reduce phosphorus availability for poultry ,but also reduce the available of other nutrients in poultry (Ravindran et al ,(1999):Punna *etal* ,2001:Shirley and Edwards ,2003), so poor utilization of phytate phosphorus by monogastric poses several problems for producer processed seeds .

As shown in the results of experiment three treatments of processing sorghum (low in phytate) was led to good performance on egg production achievement . The inclusion of all methods had asignificant effect on laying performance as compared with control parameters group . The significant increase in feed utilization, egg production , egg shell weight .egg thickness, Huge unit and yolk high. Morover there were slight positive increase on egg weight, feed intake and body weight obtained by birds fed on processed seeds (low in phytate), but these effect was not significant.The improved performance observed when layers hen fed processed cereals has been attributed to decrease phytic acid from the diet and thus lead to improve the production achevments .The results are consistent with those reported by Sukumar and Jalaudeen (2003), who asserted that layer diets low in phytate improved production performance and feed efficiency. In similar studies Ahmadi et al (2008) noted that diets low in phytate had apositve effects on the performance and edd quality of laying hens.

Treatments has asignificant effect($p>0.01$) on yolk cholesterol content of the eggsThe highest cholostrol level obtained by birds fed processing sorghum (low in phytate) as compared with control (unprossed sorghum-high in phytate). These results are consistent with those reported by Sharon and Thompson(1997) who indicated that addition of phytic acid to ahigh cholostrol diet reduced serum cholesterol and triacyl glycerols.

The significant effects in ash, calcium and phosphorus content of Shells and Toe which obtained by birds fed processing cereals low in phytate may be explaned byseveral studies, Harland (1989). Pauland and Reimbach(1998) whom imphasized negative influence of phytate in reducing minerals bioavailability such as calcium and phosphorus, and thus alter their absorption.

The economical evaluation of the experiment diets indicated that , the diet with germination and vitamin C methods showed the highest profitability ratio(1.41),and (1.40) and in both broilers and layers as compared to other experimental group . This might be due to the highest return of the weight gain in broilers and highest egg production in layers recorded by the group fed with processed sorghum low in phytate than the others group.

CONCLUSION AND RECOMMENDATION

The results of this study showed that:

1.Sorghum bicolor (F. G) the main dish of poultry diet in Sudan had a high amount of phytic acid

2. The processing of sorghum using simple methods such as dehulling ,soaking, germination, storing and adding of vitamin C had a good effect to reducing phytic acid from sorghum.

3.The finding of this study have shown some of the benefits attributed to the feeding of processed sorghum to the broiler chicks .Feeding processed seeds results in better performance of broiler chicks and layers hen.These benefits are attributed to the low amount of phytic acid in processed seeds

4. Germination of seeds was the best method to reduced phytic acid, and this treatment obtained highest performance than the other methods of experiment

5. Reducing of phytic acid using simple methods protect leg abnormality and richits

6.Processed sorghum low in phytate reduced the cholesterol levels inwhole blood serum of broiler chicks.

7. Processed sorghum low in phytic acid increased Tibia ash,Toe ash,shell ash, and Calcium, in broilers and layers

8. Finally, in a addition of the above mentioned benefits of feeding processed sorghum to reduced phytic acid content from seeds on broilers and layers performance ,the methods of processing was also reduced the other anti nutritional factor such as Tannin and poly phenlics and gluosinolates

Recommendation;

1.Used of some simple technique methods to eliminate phytic acid from the grains recommended to develop performance of broilers and layers

2.Physical methods always low in cost than others chemical methods for phytate elimination.

3.Uses of old cereal grains (stored grain) recommended because of their good affect in poultry diets and to reduce the pressure on human food.

4.All the methods of reducing the sorghum phytate in this study were recommended economic-wise, but the germination and vitamin C methods were more profitable.

5. Enzymatic methods (fermentation ,malting) mor affective than physicale methods(milling, cooking) to reduce cereals phytate.

Suggestion for future research:

1. More trails are needed to clarify the effect of processed sorghum low in phytate on performance of broilers and layers.
2. More other simple technique methods such as coocking, fermentation might had agood affect on broilers and layers performance.
3. Depth studies must be completed before making any recommendation on beneficial health effects of phytic acid.

Referances

Abasi M., Mojta Z., Mehdi G.,S.Kh.(2015).Is dietary iron requerment of broilers breeder hens at the late stage of production cycle influenced by phytase supplentation . J. AppI.Anim, Res., 43:166-167.

Abd Elnour , K.M .(2001).The Effect of decortications on wet milling and starch quality of sorghum and millet grain .M Sc, Thesis ,faculty of Agric. University of Khartoum, Sudan

Abdalla,AA.ElTinay ,A.H,Mohamed, B,E and Abdalla,.A.H,(1998).Effect of traditional processes on phytate and minerals content of pearl millet .Food.Chem.63:79-48**.**

AbdelrahmanS.M.H.B,Elmakki,W.H.Idris,A.B.Hasan,W.E.BabikerandA.H.El Yinay(.2007).Antinutritionalfactorcontentand hydrochloricacid extractability of minerals in pearl milet cultivars by germination. Int.J,Food Sci Nutr,58:6-17.

Abelson,P.H.(1999).A potentialphosphate crisis Science 283:2015 Ahmed,E.F.(1993) Comparative study of wet milling properties of some sorghum varieties .Msc.Thesis, Facultyof Agric. University of Khartoum .Sudan

Ahmadi A., Saki A., Tabatabata M M,. Siyar S. A. H .A. ,Zaboli K.H.Mirzaeis (2008). Effectof different sources of phytase supplementationOn theperformance and egg quality of laying hens Polt Sci.New South. Wales .Australia 12-14 ,pp76-78.

Akter M., Iji P.A., Grham H .(2017). Increasing zinc levels in phytaseSupplemented diets improves the performance and nutrient utilization ofbroilers. South.Afr.J.Anim. Sci. 47:648-660**.**

AOAC. (1980) Official Methods of Analysis Association of OfficialAnalytical Chemists Washington, DC.20044.po pox540.

AOAC. (1995) Official Methods of Analysis Association of OfficialAnalytical Chemists Washington, DC.

Aotzc and Others .(2001).Ahome-based method to reduce phytate content and increase zinc bioavailability in maize based complementary diets.International Journal of Food Sci. and Nutrition ,52:133-142

Atwal,A.S,Eskin,N.A.M.MC.Donald,B.EandVaisey-Genser,M,(1980).Theeffect of phytate on nitrogen utilization and zinc metabolism in young rates ,Nutr.Rep. Int 21:257-267.

Andersson ,H, Naaveri, B, Binghani, S,R,Englyst, H.N. and Cumming.J.H. (1983). The effects of breads containing similar amounts phytate but different a mount of wheat bran on calcium zinc and iron balance in man Br.J Nuter.50:503-510.

AugstinBrenes, Viveros,A.,Arija.I,.Centeno,Pizarro,M…and Bravo.C.(2003). The effect of citric acid and microbial phytase on minerals utilization in broiler chicks .Anim. Food.Sci. Technol.110:201-219.

Bassiri, A,and Nahapetian.A.(1977). Diffirences in concentiration and interrelationships of phtate ,phosphorus, magnesium,calcium,zinc and iron in wheat varieties grown under dry land and irrigated condition,J.Agric.Food Chem 25:1118-1122.

Beal, Mehta T(1985). Zinc and phytate distribution in peas:Influence of heat treatment, germination, pH.substrate and phosphorus on pea phytate and phytase. J. Food .Sci.50:96-100.

Bitar,K, and Reinhold, J.G 1972, Phytate andalkalin phosphatate activites in intestinal muccosae of rat ,chickin, calf and man . Bio. Chem. .Acta.268:442-452.

Bedford ,M.R.(2000) .Exogenous enzymes in monogastric nutrition –their current value and future benefits Anim.Feed.Sci.Tec ,86:1-13

Braganolo N.Rodriguez-Amaya DB, (2003). Comparison of theCholesterol of chicken and quail eggs.J.Food Comp .Anls..16:147.

Cabuk. K.M. Bozkurt M. Kyrkpynar F. O.H.(2004). Effect of phytaseSupplementation of diets with diffirent levels of phosphorusOn performance and egg quality of laying hens.South.Afr. J.Anim. Sci. 34: 13-17.

Celement, I.M.Ibrahim,D.K.Joseph,I.Ivo,N,.Ibrahim. D.M, and Bruce, H.,(2010).Performance and economics of production of chickens food sorghum or millet as replacement for maize in the semi-arid zone of Nigeria.Agri.Bio.J.N.Anim.1,3:321-325.

Chang,R,Schwimmer,and Burr,H.K.(1977). Phytate removal from whole dry beans by enzymatic hydrolysis and diffusion J. Food Sci.42:1098-1101.

Chapman H D and Pratt F. P 1982 Determination of Mineral titration of analysis for soils plants and water .Edition Agriculture Division .California University U.S.A pp 169-170.

Chapman,B.M and Mahjan,L.(1988) Effect of Natural Fermentation on the Extractability of minerals from pearl millet flour. J food Sic 53 1576-1577.

Chapman,H.D and Peatt,F.P (1961).Ammonium Vandate molybdate Method for Determination of Phosphorus .Methods of analysis for soils plants and water .Edition Agriculture Division .California University .U.S.A PP184=203

Chapman,H.D,andPratt,F,P.(1961).AmmoniumVandate-molybdate methods for Determination of Phosphorus, Methods of analysis for soil, plant and

water.IEdition, Agriculture Division, California .University, U.S.A,pp,184-203

Champpagne,E.T and Phillippy ,B.Q.(1989). Effect of pHon calcium , zinc and phytate solubility and complexes following in vitro digestions of soy protein isolate .J.Food Sci .54:587-572.

Cheftel.J,C.(1990). Extrusion cooking operating principles ,research trends and food applications .I.In processing and quality of food high temperature/short time(HTST) Processing Gaurantee for High Quality Food with Long Shelflife.1.60-1.70 Elsevier Applid Science .London.

Cosgrove,D.J.(1966).The chemisty and biochemistry of inositol phosphate .Rev.Pure.Appl.Chem.16:209-224.

Crean, D.E.C.and Haisman ,D.R.1963.The interaction between phytic acid and divalent cations during the cooking of dried peas J.Sci. Food Agri.14:824-833.

Davies .N.T , and Flett. A,A,(1978). The similarity between alkalin phosphotase and phytase activity in rat intestine and their importance in phytate –induced zinc deficiency Br,J, Nutr. 39:307-316.

DeBoland,A,Garner, and O Dell.B.L.(1975). Identification and properties of phytate in cereal grains and oil seed products,J.Agri. Food. Chem.23:1186-1189.

Denbow,D,M.J and Grabau.E,A,.Lacy.G,H.,Kornegay,E,T,.Russell,D,R and Umbcck,O.F.(1998). Soybeans transformed with fungal phytase gene improve phosphorus availability for broilers .Poultry Sci.77:878-881.

Doherty,J.M Faubion.J,M .L.W Rooney.(1982).Sem-automated determination of phytate in sorghum and sorghum products.Cereal.Chem.,pp373-378.

Douglase,J.H,.T.W,Sullvan ,P.L.Bond and F.J.Strawem(1990).Nutriient composition and metabolizable value of selected sorghum grains varieties andyallow corn in broiler diets.Poultry .Sci.69:1147-1155.

Dillon , Sally L, (2007).Domestication to crop improvement . Genetic Resources for sorghum and saccharum. Annals of botany 100(5) 975-989.

Duncan,D B.(1955). Multiple rang and multiple F test .Biometric, 11:42

Eechkhout.W,and Depaape and M.1994.Total phosphorus phytase activety in plant feed stuffs.Animal Feed Sci. Technol. 47:19-29.

Feil, B and Fossati.D. (1997). Phytic acid triticale grains as affected bycultivar and environment .Crop. Sci. 37:916-921.

Edwards H M.,(1993) . Dietary 1.25 dihydroxy cholcalciferl supplementationIncrease natural phytate phosphorus utilization in chickens J.Nut:23;56-77.

EL.Deek A A., Mona M.(2009). Effect of dietary corn gluten feed and phytateSupplementation to laying hens diets .Egypt. Polt. Sci.29:21-38.

Ellis, N. (1981).The nutrients composition of Sudanese animal feeds. Bulletin (1);Northern and Central Sudan. Central Animal Nutrition Research Laboratory. Kuku Research Center. Khartoum- North, Sudan.

ELsayed ,A.F ,M(1999). Alternative dietary protein sources for formed tilapia, oreochromis spp. Aquaculture, 179(1-4),149-168.

Emmambux,M. Nand Taylor, R.N (2009) Properties of heat –treat men ted sorghum and maize meal and their prolamin proteins .J .Agric, Food chem.,57,1045-1050.

FAO/WHO.(1992).Preventingspecificmicronutrientdeficiencies.Theme.Paper. 6international Conference on Nutrition, Rome. Food and Agri.Organizationof the United Nations / world health organization.

Feil,B, and Fossati.D.(1997).Phytic acid in triticale grains as affected by cultivar and environment.CropSci.37:916-921.

Frolich,W, and Nyman M.1988. Minerals phytate and dietry fiber in diffirent fractions of oat grain.J.Cereal Sci 7:73-80.

Frossard E, Bucher,M, Machler F, Mozafar A. and Hurrell, R.(2000). Potential for increasing the content and bioavailability of Fe, Zn, and Ca in plant for human nutrition .J. Food .Agri. 80; 861-879.

Frings,Christopher.S. Dunn,MT .(1970). A colorimetric method for determination of total serum lipids based on the sulfo-phsphoro-vanillin reaction .American journal of clinical pathology volume 53,page 89-91.

Graf ,E and Dintzis F,R, (1982). Determination of phytic acid in food by high performance liquid chromatography, J.Agri. Food Chem. 30:1094-1997.Graf,E.1983.Applicatios of phytic acid.J.Amer.Oil Chem.Soc.60:1861-1867.

Graf,F,,and Eaton,J.W.1990. Antioxidant functions of phytic acid Free Radical, Bio,Med. 8:61-69.

Garaham.R.D,and Welch R.M.1996. Breeding for staple food crops with high micronutrient ,density .Washington D,C.International . Food .Policy,Resr.Institute.

Greiner R.Konietznyu (2006) Phytase for food Application .Food Techinol Biote chnol :44, 125-140.

Guallerto,D,G, bergman ,CJ, K .M and Weber, C,W. (1997). Effect of extrusion processing on the soluble and insoluble fiber anf phytic acid content of cereal brans, Plant Food. Hum. Nutri. 31:187-198.

Han,Y, and Lei.X.G.(1999).Role of glycosylation in the functional expression of an Aspergillas niger phytase in Pichia pastoris.Arch,Biochem Biophys.346:83-90.

Harland, B,F. (1989). Dietary fiber and mineral bioavailability .Nutri. .Res, 2;133-147.

Harland ,B,F.and Harland ,J,(1980). Fermentative reduction of phytate in ray white and whole wheat breads. Cereal Chem.57:226-229.

Harland ,B,F.and Oberleas,D .(1987).Phytate in food . Wrld.Rev,Nutri.Diet,52:235-259.

Harland ,S.C.(1936). The genetical conception of the species .Biolodical Review 11:83-112.

Heaney ,R,P.Recker ,R,R, and Weaver,C,M ,(1990). Absorbability of calcium sores .The limited role of solubility ,Calcif,Tissue.Intl,46:300-304.

Howson,S,J, and Davis, R.P.(1983). Production of phytate hydrolyzing enzyme by some fungi.Enzyem.Microb.Technal 5:377-382.

Hu,H,L,Wise,A, and Henderson,C,(1996).hydrolysis of phytate and inositol tetra-and penta phosphatese by the intestinal mucosa of the pigs ,Nutri,Res.16:781-787.

Hulse.J.H:Laing.E.M,and,Pearson,.E(1980).Sorghum,Millets:their composition and nutritive value.NewYork.Acadimic Press997p

Hughes A. L., Dahiya J.P Wyattc. H. L. (2008).The efficacy of Quantum T M phytase in 40 week production trail using white leg horn fed corn -soybean meal based diets .Poult. Sci.87;1156-1161**.**

Jalal M .A., Scheideler S. E. (2001). Effect of supplementation ofDifferent sourse of phytase on egg production parameters inLaying hens .Poultry Sci., 80:1463-1471.

Idris,W.H(2004) Effect of processing on the anti-nutritional factors and mineralsbioavailabilityofsorghum.PhD.Thesis.Faculty of Agric , University of Khartoum.Sudan.

Kannan D.Viswanthan K. Edwin S. C.Amutha R.A.R. Ravi R.(2008).Dietary inclusion of enzyme phytase in egg layer diet on retention of nutrients, serum biochemical characters and pexcretion .Rea.J.Agri.Biol.Sci.4:273-277.

Kasim ,A, B and Edwards H.M..(1998). The analysis ofinositol phosphate forms in feed ingredientsJ Sci. Food Agri. 76;1-9.

Ketola,H.G.Harland .B.F.(1993).Influence of phosphorus inraibow trout diets on phosphorus discharges influent water .Transition of the American fisheries society6:1120-1122.

Khan,n Zaman,R and Elahi,M. 1991. Effect of heat treatments on the phytic acid content of maize products J.Sci.Food Agric,54:153-156.

Khetarpaul .N. and Chauhan, B.M.1990.Effects of germination and pure culture fermentation by yeasts and lactobacilli on phytic acid and polyphenol content of pearl millet.J.Food. Sci. 55:1180-1182

Kikunaga.S..Katoh.Y.and Takahashi,M.(1991).Biochemical change in phosphorus compounds and in the activity of phytase and alpha-amylase in the rice grain during germination J. Sci. Food .Agri.56:357-343.

Kikunage,S, Takahashi, M,and Huzisige,H.(1985).Accurate and simple measurement of phytic acid contentsin cereal grain Plant Cell Phsiol 26:1323-1330.

Kim ,E. and Goldbig.M (1969). Serum cholesterol assay using a stable Lieberman-Bur chardreagent.Clinical. chem..15 (12)117-9.

Kim J.H., Pitatragne F.M., Jung H.HanG.f, Choi H.S,KiLD.Y**.(2017)**. Effect of super dosing phytase on produetive performance and egg quality in layin hens.Asian .Austral.J.Anim.Sci:doi:

Kouakou,B.K,K,S,Alens,D.Alexis,D.Adjehi,D,K.Marcelin and G.Dago (2008).Biochemical changes occurring during germination and fermentation of millet and effect of technological processes on starch hydrolysis by the crude enzymatic extract of millet. J. Applied .Sci ,Res 4:1502-1510

Larbi,A and M barek E.1985. Dietry fiber and phytic acid level in the major items consumed in Marocco. Nutr.Rep.Iitern.31:469-476.

Larsson,M. and Sandberg, A.S.(1992). Phytate reduction in oat during malting .J.Food.Sic..57:994-997.

Latta ,M and Eskin N,A,M. (1980). A simple and rapid colorimetric methodfor phytate determination .J .Agri. Food Chem 28: 1313-1315.

Lesson ,S, and J.D.Summers,2001.Nutrition of the chickens ,4th Edition pp,331-428(University books.P.O.Box 1326,Guelph.ontario,Canada. NIH

Lim H, S., Nanikung,H., Paik I. K (2003). Effect of phytase supplementation on performance of broilers fed with different of dietary calcium nonphytate phosphorus .Poult Sci. 82;92-99.

Lopez.H,W Ouvry,A,E,Guy,C.,Messager.A.,Demigne,,C and Remesy,.C (2000).Strain of lactic acid bacteria isolate from sour dough degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour .J.Agric.Food Chem.48:2281-2285.

Lorenz.K.(1983). Tannins and phytate content in proso millets (panicum miliaceum) Cereal.Chem. 60:424-426.

Lott,J.N.A.Ockenden.I,RaboyV,and Batten ,G,D,2000.Phytic acid and phosphorusincropseeds and fruits aglobal estimate .Seed.Sci.Res.10:1133.

Ibitoye,E,B. Olorde,B,R.,Jimh, A. and Abubakar, H. (2012).Comparative performance and organ relative weight of broiler chickens fed three sorce of energy diets.J.Anim.Pro.Advices2:5.233-238.

MaenzD.D(2001). Enzynatic characteristics of phytase as thye relate to their use in animal feeds in: Enzymes in faram animal , wolling ford,UK,pp.61-

Mahgoub ,S.E.O and S.A.Elhage,(1998).Effect of milling soaking ,malting heat treatment and fermentation on phytate level of flour Sudanese sorghum cultivars .food chem..61:77-80.

Makokha,A.O,Oniango,R.K,Njoroges.M,Kamaraok, (2002).Effect of traditional fermentation and malting on phytic acid and mineral availability from sorghum and finger millet grain varietirs grown in Kenya.University of Agriculture and Technology ,department of food science.Journal Articil, Research Support-Non-U.S Govt.

MameeshM,S, and Tomar,M.(1993). Phytate content of some popular .Kuwaiti foods Cereal Chem.70: 502-503.

Mamudu.H.B,Jideani and + I .Nkama , (2005) .Sugar content of pereal millet as diversed amount cultivars and affected by germination. J.Applied Glycosci,52 :331-335.

Marero,L.M,Payumo,E.M.,Aguinaldo,A.R.,Mastsumoto,I,andHomma,S.(1988) .Anttinutrtional factor in weaning foods prepared from germinated cereals and legumes .Lebensm .Wiss.U. Technol. 24:177-181.

Marfo.E.KSimpson.B.K Idowu,JS.andOke.O.L (1990)Effect of local food processing of phytate level in cassava,cocoyam,yam,maize,sorghum,rice and soy been .J.Agric.Food Chem.38:1580-1585

Martinez,A.B,R.K,Simposon,J.S.Idwu andR,Bressan,1980.Relation of lysine and tryptophan tothat of Z,during germination of grain of corn and it is

possible linkage with the growing cycle of theplant.Aresearch, Latinoam,Nutr,30:607-633.

Mattson, S.(1964).The cook ability of yellow peas a colloid chemical and biochemical study .Acta.Agri.Sci..2:185.

Mazalli M.R Saldaha T. Bragagnolo N (2003).Determination cholesterol Of egg yolk. Journal Applied poultry Research 62(1):49-54.

Mazzuca H., Bertechini A, G .(2014). Critical point on egg production :Causes, importance and incidence of egg shell breakage and Defects. Ciencia .Agrotecno.Logia, Lavras, 38:7-14.

Mellanby,E.1949.The Rickets-producing and ant-calcifying action of phytate.Journal of physiology,109:pp488-533.

McKenzie- Parnell,J,M and Guthrie .B.E. (1986). The phytate and minerals content of some cereals , cereal product, legume ,legume product , snack baras, and nuts available in New Zealand Biol. Trace. Element. Res. 10: 107-121.

Mendoza,C,V, F.E.Lonnerdal ,B,Young.K.A.Raboy.V, and BrownK,H, 1998. Effect of genetically modifided low phytic acid on obsorption of iron from tortillas Amer..J.Clinc. Nutri,68:1123-1127.

Metwally M. A .(2005).The effect of dietary phosphorus level with andWithout supplemental phytase or diet yeast on the performanceOf Dandarawi laying hens. Egypt Sci 26; 159-178.

Miller,G,A.Young,V,L, and Oplinger,E,S.1980.Effect of available soil phosphorus and environment on phytic acid concentration in oats .Cereal Chem.57:192-196.

Miller,G,A.Young,V,L, and Oplinger,E,S.(1980) Environmental and cultivar effects on oat phytic acid concentration Cereal Chem 57: 189-191.

Michelle Kerns. (2014) Journal of Food Science and techinology; Revelution of phytic acid and Enhancement of Bioavailable micronutrient in food grains.

Morris,E,R, and Ellis ,R. 1981. Phytate zinc molar ratio of breakfast cereals and bioavailability of zinc to rats ,Cereal Chem.58:363-366.

Moscoso ,W.Bourne .M.C,and Hood ,L.F.1984 .Relationships between the hard- to – cook phenomenon in red kidney bean and water and water – absorption , puncture force , pectin ,phytic acid and minerals .J.Food . Sci .49:1577-1583.

Mullaney,Edward.J:Ullah,Abul.H.J: (2012) Phytaseatrributes-catalytic mechanisms and application.United State Department of Agriculture-.Agricultural Research Service.Retrieved May 18.2012

Musapuor A., Pourrez. J., Samie A., Morad, Shahabake H. (2005). The Effect ofphytase and diffirent level of dietary calcium and phosphoruOn phytate utilization in laying hens Int.Poult, Sci.8:560-562.

MyadaAliasMustfa(2009).Evaluation of nutrients content of sorghum flour supplemented with wheat and pumpkin seed flour.MSc thesis.Faculty of Agriculture University of Khartoum Sudan

Nahapetian,A,and Bassiri,A(1976).Variationsin concentrations and interrelationships of phytate ,phosphorus,magnesium,calcium, zinc,and iron in wheat varieties during two yearsJ.Agri.Food.Chem24:947-950

Nelson, K.J and Potter, N,N.1979. Iron binding by wheat glutin , soy isolite zein , albumim and casein J. Food . Sci. 44:104-107.

Nelson ,T,S,Shich.T.R.Wodzinski ,R,J and Ware.J.H,(1971).Effect of supplemental phytase on the utilization of phytate phosphoruse by chicks,J.Nater.101:1289-1294

Nogueira GC, Bragagnolo N, (2002)Assessment of methodology fortheenzyematics. Assay of cholesterol in egg noodles .Food .Chem.79:267-70**.**

NRC. (1984) National Research Council. Nutrient Requirement of poultry ,8th ,rev. ed . Washington. DC .National Academy. Sci. Press

Nwokolo ,E.N and BraggD.G.1977.Influnce of phytic acid and crud fiber on the availability of minerals from four protein supplements in growing chicks.Can .J.Animal Sci.57:475-477

Ockenden,.I,Falk,D.E. and Lott,J.N.A.1997.Stability of phytate in barley and beans during storage. J .Agric.Food Chem.45:1673-1677.

Ocheme,O.B,andC.E.Chinma,(2007). Effect of soaking and germination on some physicochemicall properties millet flour for porridgeproduction.J .Food Technol,6:185-188.

Ochenden I, Falk, D,E, and Lott.JNA.(1997). Stability of phytate in barley and beans during storage.J. Agri. Food ,Chem. 45;1673-1677.

Odell, B,L,deBoland and Koirtyohann,R.(1972) .Distribution of phytate and nutritionally importants among the morphological copmpements of cereal grains J. Agric. Food Chem 20:718-712.

Omara.,Omar Elteyb (2000).Effect of feeding whole millet grain on broilers performance. MSc thesis .Faculty of Animal Production. University of Khartoum Sudan.

Osbonrne, D. R and Voogt ,P(1978). Calculation of caloric value In Analysis of Nutrients in foods Academic Press New York pp 23-34

Pasamontes,L,Haiker,,M,.Tessier,M ANDvanLoon,a,p.g.m 1997.Gene cloning puritication pattern and characterization of aheat 0stable phytasefromthefungus Asoergillus fungus,Appl,Environ.Micro.63:1696-1700.

Pauland, J, Pietsch,M and Reimbach .G (1998). Dietry phytate reduces magnesium bioavaialability in groing rats.Nutri.Res 18:1029=1039.

Peers F. G., (1953). The phytase at wheat. Biochem. J. 53 :102-105.

Plaami, S and Kumpulainen, J.1995. Inositol phosphate content of some cereal bassed food.J.Food. Comp. Analysis 8:324-335

Pfefler ,M.(1972) .Comprehensive study of aleurone grains, identification of globoid and approximation of its chemical nature.Johrb.Wiss,Bot 8:429-574.

Ponnuvel P, Nara Yankutty K. J. A, Anithap.(2015). Effect of phytase supplementation in low energy- protein diet on the production Performance of layer chicken. In.J .Vet.Sci.Biochem.87;1156-1161

Punna.s, and Roland.Sr.(1999). Variation in phytate phosphorus utilization within the same broilers strain .J.App.Poultry Research ,volume 8.10-15.

RadhaakrisnanM,R, and Sivaprasad,J. (1980). Tannin content of sorghum varieties and their role in iron availability J.Agri.Food Cheme.28:55-58

Ravindran,G 1991. Studies on millets, mineral composition and phytate and oxalate contents.Food,Chem.39:99-107.

Ravindran,V,Ravindran.G,and Sivalogan ,S.(1994). Total and phytate phosphorus contentsof various food and feed stuffs of plant grain.Food.Chem. 50:133-136.

Ravindran,V.S.Cabahug,G.Ravidran and W.L.Bryden .1999b.Influnce of microbial phytase on apparent ileal amino acid digestibility of feed stuffs for broilers. Poult.Sci.78:699-706.

Ravindran,V.Korneday,E,T,Denbow,D,M.Yi.Z. and Hulet .R.M.(1995). Response of turkey pouls to tiered levels of nonphytate phosphorus .Poultry.Sci.74;1843-1854

Reddy ,N,R. (1982). Milling and biochemical characteristics of mineral M,S.c Thesis.AlabamaA&M, University .Normal.AL.

Reddy.N.R.PiersonM.D and Salunkhe .D.K(.1986). Legume-Based Fermentation Food .CRC .Press, Boca..Raton ,Florida.

Reddy.N.R.PiersonM.D.Sathe and Salunkhe .D.K.(1989). Phytates in cereals andlegumes, Boca Raton.Floreda.CRC Press.

Rickard,S.E.and Thomposon,L.U(.1997). Interactions and biological effects on phytic acid in anti-nutrients and phyotochemicals in food .Shahidi.F.(FD).ACS.symposiumseris,662.AmericanChemical.Society.Washington.DC.pp294-312

Rimbach,G, and Pallaul,J.(1998). Phytic acid inhibits free radical formation in vitro but does not affect liver oxidant or antioxidant statusin growing rats .J.Nutr.128: 1950-1955.

Rose,A.R.(1912).A resume of the literature on inosite-phosphoric acid with special reference to the relation of that substanceto plants .Biochem.Bull.2:21-49.

Roseenbaum, T. M. and Baker ,B.E.(1969). Constitution of legumeinous seeds. VII, Ease of cooking field peas in relation to phytic acid content and calcium diffusion J.Sci. Food. Agri.20:709-712.

Sakamoto K,. Vuceniki, Shamsuddin. AM. (1993).Phytic acid, it is absorbed**And distributed to various tissues in rat. J .Nutr : 123:713-200.**

Sandberg ,A,S. Degradation of inositol phosphates at different sites,in dietary fiber and fermentation in the colon proceding of COST92,Espoo, Finland .june 15-17-(1995). Luxembourg,Office for official publication of the European Commmmunities 1996191-197.

SandbergA,S, Anderson,H,Carlsson,N,G.and Sandstrom B(1987). Degradation products of bran phytate formal during digestion in the human small intestine. Effect of extrusion cookingon digestibility J.Nutr.117:2061-2065

Sandberg,A,SLarsen. T, and Sandstrom,B(.1993).High dietary calciumlevel decreass colonic phytate degradation in pigs fed a rapeseed diet. J. Nutr. 123:559-566.

Sandstrom,B,Andersson, ,H,Kivisto,B and Sandberg, A,S.(1986).Apparent small, intensital absotption of nitrogen and mineral from soy and meat protein bassed diets.J.Nutr. 116-22092218.

Sasakawa N, Sharif M , Hamuley Mr.(1995). Metabolism and biologicalActiveites of inositol peataphosphorus and inositol hexa kis Phosphate. Biochem . Pharmacol. 50:2:137-146.

Sathe.N.Rukma Reddy Shridhark.(2002).Food Phytates.International Stander Book Number 1-5,6676-8675,CRCPress,Boca Raton London New York Washington,D.C.

Savanberg U and Lorri.W(1998).Fermintationand Nutrition Availability .food cont-8 319-327.

Schulze,E.andWinterstein,E.(1896).Physiol.Chem.40:120.CRC.press Boca,Raton,FL

Shamsuddin,A.M,Vucenik,I, and Cole, K,E,.(1997).IP6: A novel anti-cancer agent .Life .Sci. 61:343-354.

Sharpley.A.N.Chapra.S.C.Wedepohl.R,Sims.J.T.Daniel.T.C.andReddy,K.R.(1994). Managing agricultural phosphorus for protection of surface water: issues and options.J.Environ. Qual.23:437-451

Sharon,E.Ricard and Lilian,U. Thomposn. (1997).Interaction and Biological effect of phytic acid. Department of Nutrition Sci.Faculty of Medicin.University of Tornto,ontarjo MSS 3E2.Canada.

Shears S B. (2001). Assessing the omnipotence of inositol hexakis phosvate**Cell Signalling 13.**3 :151-158.doc10-1016/508098-6568(01)

Shich.T.Rand Ware.J.H.(1968).Survey of microorganisms for the the the production of extracellular phytase Appl.Microbal.16:1348-1351.

Shirly,R.B and H.M.Edward,Jr.,(2003).Graded levelsof phytase past industry standard improves broiler performance .Poult .Sci.82:671-680.

Skoglund,E,Larsen,T, and Sandberg, A, S(1997). Comparison between steeping and pelleting a mixed diet at different calcium levels on phytate degadration in pigs , Can. J .Animal. Sci.77:471-477.

Siegenberg D, Baynes RD,Bothwell TH, Macfarlane BJ, Lamparelli RD ,Car NG, Macphail p, Schmidt U, Tal A,Mayet F . (1991) . Ascorbic Acid prevents the dose dependent inhibitory effects of polyphenols and phytates on nonheme- iron absorption. Am. J .Clin .Nutr,Feb:53(2):537-41.

Silversides F .G., Scoot T. A.Korvert D, R .ME, Hruby M.(2006). Astudy On the interaction of xylanase and phytase enzymes in wheat Based diets fed to commercial white and bron egg laying hens.Poultry Sci. ., 85:297-305.

Singh,B,and Reddy,N.R.(1977).Phytic acid and mineral compositions of triticales.J.Food.Sci.42:1077-1083.

Simons.P.C.M.Versteegh.H,A.J.Jongbloed..A.W.Kemme.P.A.Slump.P.Boss.K .D.,BeudekerR.F.and Verchoor.G.J.(1990).Improvment of phosphorus availability by microbial phytase in broilers and pigs.Brit.J.Nutri64;525-540.

Simwemba,C.G,Hoseney,R.C.Variano-Marston,E,and Z.K.(1984) CertainB-vitamin and phytic acid contents of pearl millet.J.Agric.Food Chem, 32:31-34.

Snedecor,G.W and W,G. Cochran.(1987) .Statistical Methods"Printing ,.The lawa State . University Press, Amcs, Jowa, U.S.A.

Snow,J.L.,D.H.Baker, and C.M.Parsons.(2004)Phytase citric acid and 1-hydroxycholecalci ferol improve phytate phosphorus utilization in chicks fed acorn-soybean meal diet. Poultry Sci, vol 83,no 7 pp 1187-1192

Sripriya,G., Antony,U, and Chandra. T,S.(1997).Changes in carbohydrates , free amino acids organic acids phytate and HCL Textraactability of minerals during germination and fermentation of finger millet .Food Chem 58;345-350..

Steel, R.G.D and J.H .Torrie, (1960).Principles and procedures of statisticA Biometrical Approach.McGraw Hill Co Inc. NY, USA.

Steinkraus.K.H.(1983). Handbook of indigenous fermented food .Marcel Dekker .New York

SugiuraS.H.Raboy.V,Young.K.A.DongF.M.andHardy.M.W(.1999).Availabilit y of phosphorus and trace elementsin low- phytate varieties of barley and corn for raibow trout.Aquaculture 170:285-29

Sukumar D. Jalaudeen A.(2003). Effect of supplemental phytase in diet on certain economic traits in layer chicken .In J.Anim. Sci 73:1357-1359..

Summers,J.D.1997.Precisiophosphorusnutrition ..J.Appl.Poult,Res,6:495-500.

Swick.R.,A and Lvey..R.J.(1992).The value of improving phosphorus retention ,feed,Manag.43:8-17.

Szkudels ki .T(1998). Phytic acid affects lipid metabolism in the rats. Department of Animal physiology and Biochemistry. Pozan Agri. University ,wolynska 35, 60-637. G. Anim. Feed Sci.

Tabekhia,M,M and Donnelly B.J.(1982).Phytic acid in durum wheat and it is milled products Cereal.Chem ,59:105-107.

Tabekhia,M.,M and Luh.B,S 1980.Effect of germination ,cooking and canning on phosphours and phytate retention in dry beans .J.Food.Sci.45:406-408.

Thompson,L.U.(1993).Potential health benefits and problems associated with anti-nutrients in foods.Food .Research .Inter,26:131-149.

Trinder.P.(1960). Colorimetric micro determination of Calcium in serum analyst 85:889-894.

T.S.Nelson (1967).The utilization of phytate –phosphorus by poultry: a review- poultry sci.46 pp862-871.

Urbano.G.Lopez,Jurado.M,Aranda,P.VidaiValverde,C,Tenorio,E,and PorresJ.(2000).The role of phytic acid in legumes anti-nutrientional or beneficial function.J.Physiol.Biochem.56:283-294.

Valencia,S, Svanberg, U,.Sandberg, A,S and Ruales, J. (1999). Processing of quinoa (Chenopodium quinoa,Wild): effects onin vitro iron availability and phytate hydrolysis. Int .J .Food Sci,Nutr.50 :203-211.

VinhL,T, and Dworschak E 1985. Phytate content of some foods from plant origin from Vietnam and Hungary Die.Nahrung 29:161-166.

Wheeler,E.L and Ferrel,R.E (1971) Amethod for Phytic Acid Determination in wheat and wheat fractions .Cereal Chemistry,48,312-320

WHO.1998. Complementary of young children in developing countries AreviewofcurrentscientificknowledgeWHO,Geneva,http://apps.who.int/bookorders/anglais/detart1.jspsession=1&codlan=1&codco1=93&codcch=141

Wikipedia, The free encyclopedia ,(2014). Sorghum ,page 1-4.

Wisal Hasan Idris Badi. (2004) Effect of processing on Anti-Nutrition Facter and minerals Bioavailability of Sorghum .Faculty of Agric University of Khartoum.Sudan

Wise.A,Richards, C,P and Trimble .M,LM (1983), Phytate hydrolysis in the gastrointestinal tract of the rat followed by phosphorus-31Fourier transform nuclear magnetic resonance spectroscopy App. Environ .Microbial.45:313-314.

Wise, A, and Giburt,D.J (1982). Phytate hydrolysis by germ- free,and conventional rats Appl. Environ..Microbial.43:753.s

Wyss,M,Pasamontes,L,.Friedlei,A,.Remy,R,Tessier,.M,.Kronenberger,A,Middendorf,A.Lehmann,M,.Schnoebelen,.L.Rothlisberger.U,.Kusznir,.E,Wahl,G..Mular..F,Lahin,H.W,Vogel,K,andVanlon,A.PG.M.(1999).Biophysical characterizationoffungalphytase(myo-iositolhexakisphosphatephosphohydrolases)molecularsize,glycosylationpatternandengineeringofproteolyticresistance.Appl.Environ,Micro65:359-366.

York JD,Odom A R ,Murphy R ,Ives ,E B,Wente SR.(1999).A phospholipase C-depent inositol polyphosphate Kinase pathway required For efficient messenger RNA exeport.Sci. Washington, D,C.285(5424):96-100.

Xu, P, Price J, Wise.A and Aggett,P,J.(1992). Iiteraction of inositol phosphate with calcium zinc and histidine.J.Inorg.Biochem 47:119-130.

Yi,.Z.Kornegay.E.T.Ravidran,VandDenbow,D,.M.(1996).Improving phytate phosphoruse availability in corn and soybean meal for broilersusing microbal phytase and calculation of phosphorus equivalency values for phytase.Poultry Sci.75:240-249.

Zhou,J.R and Erdman,JW,(1995).Phytic acid in health and disease, Crit. Rev.Food.Sci.Nutr.35:495-508.

Zhou,J.R .Fordyce.E.J.Raboy.V,.Dickinson,D.B.Wong.M,S,.Burans R.A.and Erdman J.W.(1992),Reduction of phytic acid in soybean product improve zinc bioavailability in rats.J.Nutr.122:2466-2473.

APPENDIXS

Appendix.1 Structure of phytic acid

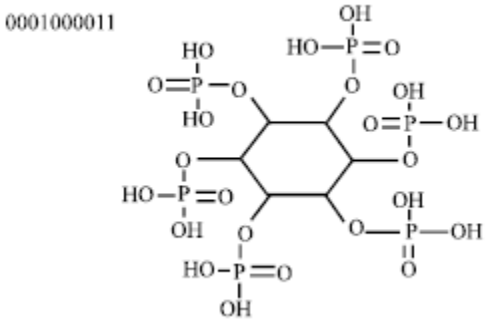


Fig : 1 Structure of phytic acid

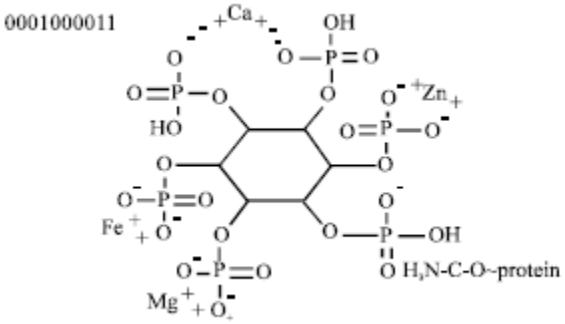


Fig. 2: Structure of phytic acid with the different possibilities to interact with both metal cations (minerals) as with protein residues

Appendix .2 Weekly Maximum and Minimum experimental Temperture during the period (3July - 7August 2018).source Gadarif Meteorological Station.

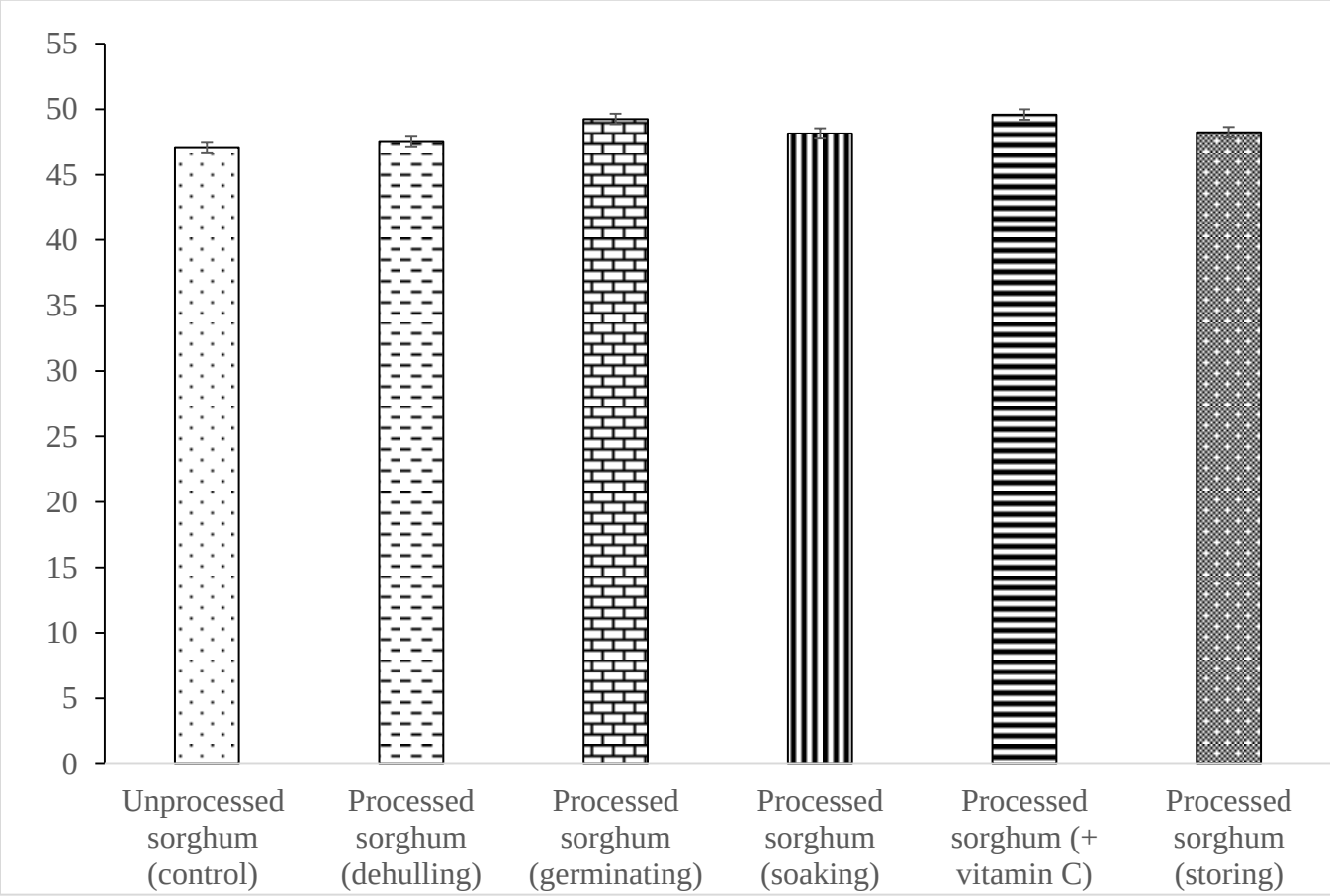
Week	Maximum temperture	Minimum temperature
1	40	21
2	39	17
3	35	17
4	39	16
5	37	15
6	38	17
7	39	17

Appendix . 3 : Methods for determination of the level of lipids

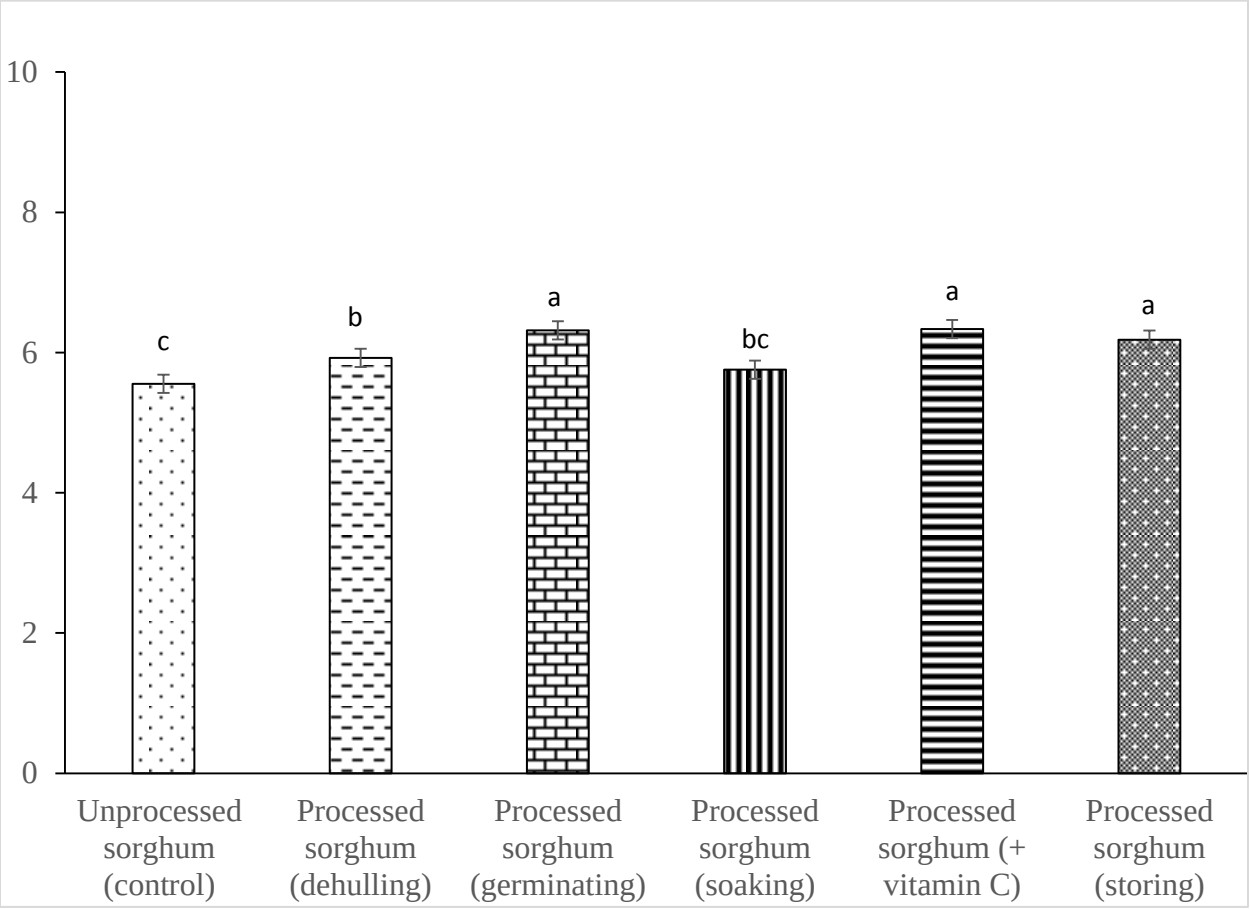
Methods for determination of the level of lipids ml of the serum was added to 2 ml concentration H_2SO_4 well mixed heated in boiling water path for 10 minutes, then cooled, 0.1 ml of the mixture was transferred into a clean test tube to which 5 ml of phosphovanline reagents was added. The tubes were incubated for 15 minutes at 37^0C. after Colling for 5 minutes, they were react at 540nm agents blank (Fringsetl. 1970)

Blank	0.1 ml concentration H_2SO_4
Lipids concentration (ml%)	Sample * 600
	Standard

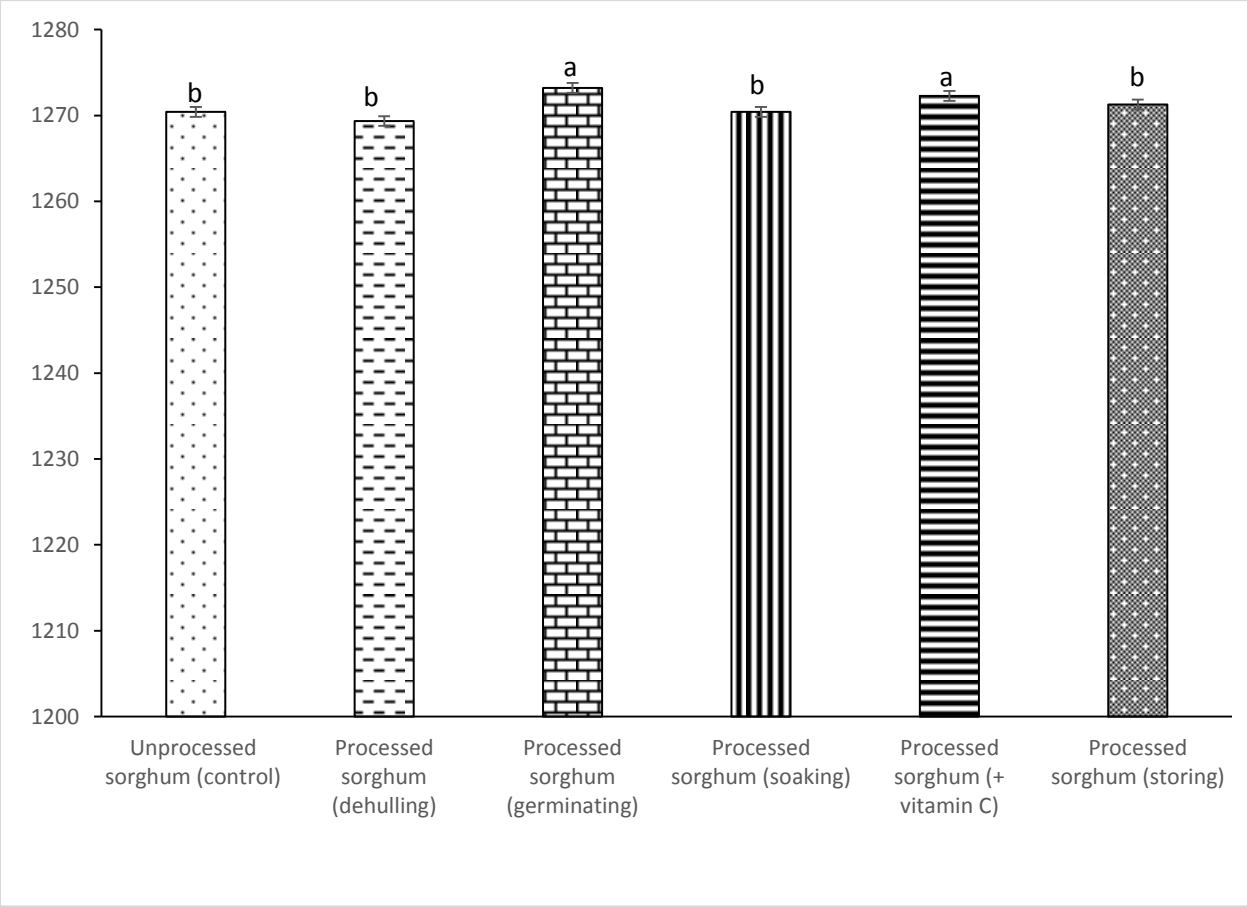
Appendix .3 Fig. 1. Effect of processed sorghum (low in phytate) treatments on egg weight (g). There were no significant difference ($p \geq 0.05$) between treatments



Appendix 4 . Effect of processed sorghum (low in phytate) treatments on shell thickness (mm).



Appendix . . 5. Effect of processed sorghum (low in phytate) treatments on body live weight (g).



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Appendix 6: Amino acid composition of sorghum (g/ kg)fresh basis

Sorghum	
Dry matter	870
Nitrogen	14.1
Arginine	3.4
Cystine	1.6
Glycine	3.5
Histidin	1.9
Isoleucine	4.2
Leucin	11.8
Lysine	2.1
Methionine	1.6
Phenylalanine	4.2
Serine	3.9
Threonine	2.9
Tryptophan	1.0
Tyrosine	3.8
Valine	5.3

Appendix 7 : Vitamin potency of sorghum (fresh basis)

Vitamin A(i.u./kg)	0.7
Vitamin E (i. u/ kg)	12.0
Vitamin B6(mg/kg)	3.2
Vitami B12 (mg/kg)	-
Thiamine(mg/kg)	4.0
Nicotinc acid (mg/kg)	41
Choline (mg/kg)	450
Riboflvin (mg/kg)	1.1
Pantothenic acid (mg/kg)	12