

GRAIN SORGHUM IN CHICKEN DIETS: EFFECTS OF
TANNIN LEVEL AND TANNIN BINDERS ON BROILER AND
LAYER PERFORMANCE

MARY KIVALI AMBULA

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Signed: 
Mary K. Ambula

Date: 16/9/2002

This thesis is submitted to Graduate School with our approval as University supervisors:

Signed: 
Dr. George W. Oduho

Date: 18-09-2002

Signed: 
Prof. James K. Tuitoek

Date: 18/9/2002

DEDICATION

This work is dedicated to my brother Francis Ambula for his financial support for my education.

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Figure 2.1 Structure of tannins

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ANF	Anti-nutritional factor
B	Bentonite
BHT	Butylated hydroxytoluene
CE	Catechin equivalent
DM	Dry matter
DMD	Dry matter digestibility
d. o. c	Day old chick
ESG	Egg specific gravity
FAO	Food and Agriculture Organization
FE	Feed efficiency
FI	Feed intake
g	gram
GoK	Government of Kenya
h	Hour
Hcl	Hydrochloric acid
HHP	Hen house production
HPLC	High performance liquid chromatography
HTS	High tannin sorghum
LTS	Low tannin sorghum
LWT	Live weight
PEG	Polyethylene glycol
PVP	Polyvinylpyrrolidone
NDF	Neutral detergent fibre
NR	Nitrogen retention
NRC	National Research Council
SAS	Statistical analysis system
TAE	Tannic acid equivalents

ABSTRACT

The following study was designed to explore the possibility of using some of the indigenous sorghum cultivars grown in Kenya as alternative energy sources in chicken diets. The efficacy of polyvinylpyrrolidone (PVP) and bentonite when used at 0.25% and 0.5% to minimize the deleterious effects of tannins was also studied. Experiment 1 involved a 3-week feeding trial and investigated the effects of PVP when used at 0.25% and 0.5% on the utilization of high tannin sorghum (HTS) by broiler chicks. In experiment 2 the effects of sorghum inclusion level on dry matter digestibility (DMD), nitrogen (N) retention and tannin absorption were studied in a 3-week substitution assay. Experiment 3 was designed to study the effects of bentonite when used at 0.25% and 0.5% on the utilization of HTS by laying hens. In experiment 4 the effects of sorghum inclusion level on the performance of laying hens were investigated. The results of the first broiler experiment showed that diets based on maize, white sorghum and cream sorghum gave similar body weight gains and all were better ($P < 0.05$) than light brown or dark brown sorghum-based diets. Similarly, feed efficiencies were better ($P < 0.05$) for diets 1, 2 and 3 compared to diets 4, 5, 6 and 7. When 0.25% PVP was added to the HTS diet (diet 5) there was no significant improvement in chick performance ($P > 0.05$). However, the addition of 0.5% PVP resulted in significant improvement ($P < 0.05$) in body weight gain compared to the untreated dark brown sorghum. Overall, PVP did not completely overcome the deleterious effects of tannins. The results of the second broiler experiment showed that sorghum inclusion level had no effect ($p > 0.05$) on feed efficiency, weight gain, DMD and N retention. The results of experiment 3 showed that white sorghum (low tannin sorghum diet) supported better performance for all variables measured. Treating high tannin sorghum with 0.25% or 0.5% bentonite did not significantly improve the performance of the hens. The results of experiment 4 showed that sorghum inclusion level had no effect on layer performance. When all the results are taken together it is concluded that in diets with adequate energy and protein, tannin levels in the range of 2.7-3.2% CE have no adverse effect on broiler and layer performance. The use of PVP or bentonite to deactivate sorghum tannins is not necessary.

CHAPTER ONE

1. INTRODUCTION

1.1 General background

A critical health problem facing many households especially in third world developing nations is protein malnutrition. Overall, the level of protein intake in such countries is minimal and often does not meet the nutritional requirements of the people (FAO, 1991). One way of alleviating this problem is to increase the production and consumption of livestock products (meat, milk and eggs) that give protein of superior quality compared to plant protein. Ruminants are the animals of choice in many developing countries and are kept to produce meat and milk for home use while any surplus is released to the market for sale. Animal husbandry in such instances involves finishing animals on pasture and this requires more land and time to have the products ready for use. In a country such as Kenya with a rapidly rising population (presently 28.5 million as per 1999 census), there has been a reduction of grazing land as more of it has been taken up for human settlement. Moreover, a large proportion of Kenya's land-mass (>80%) is arid or semi arid. Consequently any increase in production of animal products and by-products can only be realized through intensive livestock farming under zero grazing or feedlot fattening in the relatively small high potential region (<20%). However, either of these options entails high investments that are not often accessible to many households. Another alternative is to increase the production of meat and eggs from chickens. Unlike ruminants, chickens adapt well to intensive management systems and are more efficient in utilizing feed (i.e. 4 kg feed/kg meat compared to sheep and cattle 7.5 kg feed/kg meat under feedlot). Furthermore, chickens require relatively less inputs and have a short life cycle and thus may be a good alternative source of animal protein for many households in developing countries.

Sorghum (*S. bicolor* (L.) Moench) is one crop that can be successfully cultivated in the arid and semi-arid lands. According to National Research Council (NRC, 1984) the composition of sorghum and maize is almost similar with sorghum having a higher protein content (10% vs. 8% respectively) while maize is slightly higher in energy (3350 kcal ME vs. 3288 kcal ME respectively). Compared to maize, sorghum has a higher content of macro minerals (Ca, P, K and Mg). Furthermore, sorghum is more resistant to drought and can withstand desiccation for longer periods compared to maize. In addition sorghum can be cultivated on a wider range of soils being able to tolerate a wide range of pH (4.5-8.5) as well as some degree of salinity (Doggett, 1988). According to the Kenya government report (GoK, 1996) the total land area under sorghum is approximately 200,000 ha with an average yield of 500-1700 kg per ha for the local cultivars, or 3800-6500 kg per ha for serena and other hybrids depending on locality and farming practices. Although the hybrids out-yield the indigenous cultivars, the majority of farmers still prefer the latter. Informal interviews with farmers around the Lake Basin region of Western Kenya revealed that the local cultivars are relatively cheaper to produce unlike the improved cultivars. They are also less prone to bird damage and one does not need to purchase new seed every planting season. However, increased production can only be possible if avenues of its utilization can be explored and expanded. One such avenue is to utilize the grain as energy source in chicken diets. Should this be the case then the pressure on maize as both the main staple food and as cereal grain in chicken diets will ease.

1.2 Livestock feed industry in Kenya

The livestock feed industry in Kenya has its beginnings with the sale of 'cereal balancers' (for dairy cattle and pigs) to the European farmers in the white highlands prior to independence. In addition to growing maize, wheat and barley, these farmers kept dairy and beef cattle as well as

pigs. Whole grain and/or milling by-products were used as energy supplements to cater for any deficits from pasture (Mbugua, 1988). By 1967, Kenya had an established feed industry with several millers producing a variety of livestock feeds such as dairy meal, poultry feeds (chick mash, growers mash, layers mash, broiler starter and broiler finisher mash), pig feeds (sow and weaner, creep feed, pig finishing meal), and calf pellets. At present Kenya has ten leading feed manufacturers and several other small-scale or 'cottage' millers. In Nakuru town alone (considered to be Kenya's leading agricultural town) there are well over ten feed millers all competing for their share of market sales (author's observation). On average the current feed capacity by the different millers ranges from 10,000-200,000 tons per year with an installed annual capacity of 700,000 tons. On the whole, production fluctuates regularly both in quantity and quality but with a general increase for all classes of livestock. Fluctuations in production are caused by the unavailability of raw materials and the market situation, which has not been very stable particularly for pigs and poultry. Maize, wheat and their by-products are the main sources of energy in commercial poultry feeds yet these cereals are also in demand as human food (Bartilol *et al.* (1988).

Poultry feeds

Of all the livestock feeds produced in Kenya, over 55% are poultry feeds. Feed is the single most expensive item in the production of meat and eggs from chicken. The reason for this scenario is that commercial chickens have to feed on well-balanced rations and cannot effectively subsist on non-conventional feeds. In addition, the majority of farmers do not mix their own feed on the farm principally due to lack of knowledge, capital and the necessary equipment. However, there is a slow change in this trend with more of the large scale producers trying to mix their own rations or blend these on the farm. There is a tremendous variation in the quality of poultry feeds produced by the feed millers or mixed by farmers at home (Bartilol *et al.*, 1988). The Kenya

Bureau of Standards (KBS) regulates livestock feed quality in the country. Although KBS spells out specifications for the minimum nutrient levels required in poultry feeds (protein, energy, amino acids, vitamins and minerals) the millers hardly adhere to these specifications. Furthermore, quality varies from one feed miller to the next and between batches by the same miller. Gichohi *et al.* (1988) reported that of all the farmers interviewed 48% complained of poor quality feed that resulted in low egg production and slow growth of broilers. The major cause of low or poor quality feed is the erratic fluctuation in the availability and cost of raw feed ingredients.

A complete or well-balanced poultry feed must have sufficient quantities of protein, energy, vitamins and minerals. The energy fraction makes approximately 60%, protein 30%, minerals and vitamins 10%. Vitamins and trace minerals are routinely added to the compound feeds as premixes while the major minerals (calcium and phosphorus) are supplied by limestone and dicalcium phosphate. Limestone is abundantly available in the country, but the premixes have to be imported and occasionally due to high foreign exchange rates, some of the small scale feed-millers do not use them at all, which is reflected in poor production. The protein component in poultry feeds is supplied by animal or plant sources. Animal sources are generally considered superior because they have a better amino acid profile and include fishmeal, blood meal, meat and bone meal. Plant protein sources include oilseed meals such as cotton seed cake as the major source and occasionally sunflower seed cake and soybean meal. Soybean is not produced in sufficient quantities in Kenya and large-scale feed millers rely on imports to meet the demand for feed formulation. The high crude fibre of sunflower seed cake limits its excessive use in chicken diets.

Cereals such as maize, rice, wheat and their milling by-products (bran, germ and middlings or pollard) supply the dietary energy fraction of poultry feeds. The three cereals (maize, rice and

wheat) form the staple food stuff for majority of people not only in Kenya but also worldwide. In the more developed nations especially USA and other European nations, there is often excess of maize and wheat for human consumption with a surplus that is available for livestock feeds. The situation in most developing countries is a complete reverse of the above. In Kenya for example, maize is not only the most important energy source in the feed industry but it is the staple food in many homesteads. As a consequence, there is a sharp competition between humans and livestock for the available maize. Rice and wheat are not produced in sufficient quantities for human consumption and frequently more have to be imported to meet the domestic demand. Furthermore, frequent droughts in recent years coupled with erratic changes in the pricing and availability of farming inputs (fertilizers and seed) have resulted in acute shortages of these basic cereals with hardly any surplus for feed manufacture. The feed miller relies on the milling by-products for the manufacture of poultry feeds which may be of lower nutritive quality than the whole cereal grain and whose availability depends on that of the grain (Bartilol *et al.*, 1988).

From the foregoing, it is apparent that Kenya has an established feed industry but quality feed production is limited by the unavailability of suitable raw feed ingredients, some of which must be imported which ultimately increases the feeding costs for poultry farmers.

1.3 The problem statement, hypotheses and objectives

Problem statement

The department of crop production in the Ministry of Agriculture has in the recent past tried to promote sorghum production in the country based on improved varieties. However, the adoption rate of the new hybrids by farmers has been very slow. Several reasons can be identified for this slow adoption rate. Compared to indigenous cultivars, the improved hybrids i.e. Serena, Seredo and Kari Mtama 1 among others have a lower resistance to drought, *Striga* weed, pests and

diseases and bird depredation. The indigenous sorghum cultivars are preferred because of their possession of tannin, which confers to them the positive attributes of resistance to pests and diseases and depredation by birds. Moreover, production costs of the indigenous cultivars are relatively low. Unlike the hybrids, one does not need to purchase new seed every planting season and yields are reasonable even without the use of fertilizer (Salasya *et al.*, 1996).

There are limited or few avenues for disposal of sorghum grain. The livestock feed industry would be a good alternative since sorghum is a potential substitute for maize as energy source for livestock. This study was aimed at exploring the possibility of using the indigenous high tannin sorghums grown in Kenya as alternative energy sources in broiler and layer diets. The results of the study are expected to provide a potential marketing outlet for such sorghums, while improving the quality of poultry feeds and encouraging broiler and layer production.

Hypotheses

This research focused on the possibility of using some of the indigenous sorghum cultivars grown in western Kenya as alternative grain energy sources in broiler and layer diets. The following hypotheses were tested:

- 1) Indigenous sorghum cultivars contain tannins that make them less suitable for use in commercial chicken diets.
- 2) The adverse effects of tannins can be minimized by means of a tannin binder such as PVP or colloidal clay such as bentonite.
- 3) High tannin sorghum (HTS) can be used in chicken diets without loss in production if the HTS is used at low inclusion levels.

Objectives

The objectives of the study were to:

- 1) Determine the tannin content of some of the indigenous sorghum cultivars grown in Kenya.
- 2) Examine the effects of polyvinylpyrrolidone (PVP) on the utilization of high tannin sorghum by broiler chicks.
- 3) Determine the optimal inclusion level of high tannin sorghum in broiler starter diets.
- 4) Evaluate the effects of tannin level on dry matter digestibility, nitrogen retention and tannin absorption in broiler chicks.
- 5) Examine the effects of bentonite on the utilization of high tannin sorghum by laying hens.
- 6) Determine the optimal inclusion level of high tannin sorghum in layer diets.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Sorghum as a potential feed for chicken

General information on sorghum

Sorghum (*S. bicolor* (L.) Moench) may be classified into four distinct groups: non-saccharine or grain sorghums, the saccharine or forage group, the broomcorn and grass sorghum (Bennett *et al.*, 1990). The grain sorghum group includes sorghums commonly known as durra, kafir, milo, hegari, kauliang and shallu all having minor morphological differences e.g. in panicle shape or colour of glume. The cultivated sorghums originated from the wild *Sorghum bicolor* subsp. *arundinaceum* and were first cultivated in Africa in the Ethiopia-Sudan region about 5000 years ago (Mann *et al.*, 1983). Sorghum is the fifth most widely cultivated crop after wheat, rice, maize, and barley and consists of two distinct sectors - a traditional, subsistence, small-holder farming sector and a modern, mechanized, high-input, large-scale sector. In the developed countries such as USA and some parts of Latin America sorghum is grown as a livestock feed. This may be attributed to widespread use of modern agriculture with resultant high yields averaging 3-5 t/ha.

Sorghum does well in the tropics and subtropics and this is attributed to its ability to withstand drought and desiccation. It has a very efficient, well branched root system estimated to be twice that of maize with an endodermis that is high in silica which prevents the roots from collapsing in dry soil (Acland, 1975). The drought resistance of sorghum is further enhanced by its ability to roll its leaves or close the stomata on hot days, which minimizes water loss through transpiration. The presence of an abundant waxy covering on the leaves and stems also reduces

loss of water from the plant. Notwithstanding the economic and agronomic importance of sorghum as a subsistence grain crop for people living in the tropic and sub-tropic regions of the world, its potential nutritional benefits are limited by the presence of polyphenols (tannins) in some genotypes.

Basing on coat colour, sorghum may be classified into four groups: brown, yellow or cream, white and mixed (Rooney, 1987). Brown sorghum contains a pigmented testa, yellow sorghum has unpigmented testa, white sorghum has no testa while mixed sorghum has more than 10% white sorghum but less than 90% brown sorghum. The kernel structure consists of 3 parts: the seed coat, endosperm and germ. The structure of the kernel has an important bearing on many processing and food quality characteristics (Rooney and Murty, 1982), but the most deterrent to bird damage is related to seed coat colour (Bruggers and Jaeger, 1982). The seed coat consists of a fused pericarp and testa, while the size and colour of the pericarp plus the presence or absence of the testa is genetically determined. Sorghum seed colour is therefore influenced by pigmentation hence the pericarp may be white, tan, pink or brown. Testa pigmentation is associated with high levels of tannins and other polyphenolic compounds providing a natural protection against bird damage, moulds and fungi. The endosperm represents the largest portion of the kernel and is genetically determined. Sorghum cultivars may be floury or corneous depending on the ratio of soft to hard endosperm within the kernel. Generally tannin is contained in the pigmented testa of the sorghum kernel and by inference brown sorghums contain relatively high levels of tannins. Yellow or cream sorghums have little tannin while white sorghums have very little or no tannin. The tannins confer to the sorghum plant certain advantages principally resistance to diseases and moulds, preharvest seed germination and repellence to bird damage. However, too high a tannin level reduces the nutritional value of the grain as a feed ingredient

for livestock especially non-ruminants. The light coloured sorghum cultivars tend to have low levels of tannin and are more susceptible to bird damage.

McMillian *et al.* (1972) examined the bird resistance of several lines of sorghum and reported that the amount of bird damage was negatively correlated with the amount of tannin in the seed, seed colour and plant height. Sorghum seed develops in an open panicle and is therefore very vulnerable to attack by birds. Tipton *et al.* (1970) observed that during the milk stage the birds crush the seed to access the juices but after hardening the seed is removed from the glume and swallowed whole. In Kenya and most parts of Eastern Africa, the red-billed weaver (*Quelea quelea aethiopica*) poses a major threat to enhanced cultivation and production of sorghum grain among other cereals. Although cultivars with loose pendant heads, large glumes and awns are less susceptible to bird damage, cultivation of the bitter types of sorghum (high tannin sorghums) is imperative in areas with high bird pressure (Leonard and Martin, 1963).

Based on the tannin content and the genes that control it, sorghum grain cultivars have been further classified into three groups. Group I sorghums do not have a pigmented testa nor tannins although other polyphenols may be present. Group II sorghums have a pigmented testa, and contain tannins that cannot be extracted in methanol alone without addition of 1% HCL. Group III sorghums have a pigmented testa and a dominant S gene that results in the tannins being in both the testa and the pericarp. These tannins are readily extracted in methanol without addition of HCL (Asquith *et al.*, 1983).

Sorghum production in Kenya

Prior to introduction of maize along the coastal region by the Portuguese in 1530 A.D, sorghum was the staple food in most communities in Kenya (KIRDI, 1990). Cultivation of maize by the

European farmers effected a slow but steady shift from sorghum to maize in the Kenya highlands. Nationwide, the area under sorghum is approximately 0.12 million ha with an average yield of 0.5-1 t/ha (FAO, 1996). Notwithstanding the shift from cultivation of sorghum to maize, to date sorghum remains an important food security crop for communities living around Lake Victoria, some parts of the Rift Valley and Eastern province. Furthermore, sorghum plays a multipurpose role in the farming systems of the Lake Basin area where it is used for brewing *busaa*, as a supplementary starchy staple *ugali*, as a drought resistant “ insurance crop” and as a cash crop sold at local markets only. There are approximately one hundred sorghum cultivars grown in Kenya. Most of these cultivars are indigenous to the region, and are known by different generic names that describe them basing on place of origin, grain colour and panicle architecture. Indigenous sorghum cultivars are grown in Western Kenya primarily in Busia, Teso, Siaya, Homa Bay, Rachuonyo, Migori, Suba, Kuria and Kisumu districts. The majority of the cultivars are tall and late maturing with coarse brown grains high in tannin content and less susceptible to bird damage, weathering and moulds (Enserink, 1995). Overall, the sorghum cultivars may be classified into 4 groups based on coat colour. The red or brown cultivars have hard seeds with good storage qualities, are bitter and less susceptible to bird damage. The light brown cultivars are less bitter and are favoured for *ugali* and beer production. However, their storage qualities are poor hence they are consumed or sold soon after harvest. The white cultivars are sweet and most liked by birds. As a result this type is rare and the seed is very scarce.

The hybrids include ‘serena’, an improved brown cultivar introduced in the late 1960’s. ‘Serena’ was selected from a cross of “Dobbs”, is good for *ugali* and beer making but its seed is in short supply. Other hybrids such as ‘Seredo’ and ‘Kari Mtama 1’ are also grown but on small scale. On the whole, farmers prefer indigenous cultivars of sorghum and advance several reasons for this preference. The indigenous cultivars are said to have a higher satiety value and are more

tolerant to bird damage compared to the improved hybrids. Moreover, they give stable and reliable yields throughout the year and unlike the hybrids, one requires less flour of the indigenous sorghum to mix with that of cassava when making *Ugali* (Wanyama *et al.*, 1996).

Indigenous sorghums have varying colour shades that range from dark brown, brown, light brown or cream and white. The dark coloured ones tend to have high levels of tannin and give higher yields compared to the light coloured cultivars. Contrary to expectations, the improved hybrids Serena, Seredo and Kari Mtama I have not proved as popular as was envisioned. The relatively high bird damage, the extra cost associated with their production (seed and fertilizer), comparatively poor threshability and storability have limited the adoption and production of the improved hybrids in Kenya (Salasya *et al.*, 1996). Moreover, indigenous sorghum cultivars are more resistant to the parasitic weed *Striga hermonthica* and often out-yield maize and the improved sorghum cultivars in marginal areas of Western Kenya (GoK, 1982). However, limited commercial demand coupled with poor marketing channels are some of the problems that hinder the expansion and production of sorghum grain in Kenya (Ogecha, 1995).

2.2 Chemical composition of sorghum grain

Sorghum has a chemical composition very comparable to that of maize except that it has a higher crude protein and ash content but a lower lipid content than maize. Table 2.1 shows the chemical composition of sorghum and selected cereals.

Protein and amino acid profile

Generally proteins of cereal grains are classified into four types based on solubility as follows: albumins, soluble in water, globulins, soluble in salt solutions, prolamines, soluble in alcohol and glutelins, soluble in dilute alkalis. The albumin and globulin fractions of sorghum grain are the highest in nutritional value but are present in small quantities. Prolamine takes the greatest portion of the protein and is low in certain essential amino acids principally lysine, arginine, histidine, methionine and glycine but high in leucine. Glutelin is the second major protein fraction and is high in arginine, glycine and lysine (FAO, 1980). Earlier reports (Virupasksha and Sastry, 1968) indicated that prolamines and glutelins were the principal protein fraction of sorghum grain and that an increase in protein content of sorghum grain was correlated with an increase in prolamines fraction.

Table 2.1. Proximate analysis, total lipids composition, fatty acid composition of sorghum, maize, triticale and wheat¹

Proximate analysis (%)	Sorghum	Maize	Triticale	Wheat
Protein	11.9	8.9	18.6	16.1
Lipid	3.3	5.8	2.4	2.3
Starch	70.8	72.6	63.7	65.8
Crude fibre	2.8	3.1	2.6	2.0
Ash	2.0	1.3	2.5	2.2
<i>Lipid composition (%)</i>				
Neutral lipid	86.2	91.9	66.9	61.9
Glycolipid	3.1	2.1	16.0	21.6
Phospholipid	10.7	6.0	17.1	16.5
<i>FA² composition of total lipids (% by weight)</i>				
Myristic (C ₁₄ /0)	0.20	0.44	0.14	0.19
Palmitic (C ₁₆ /0)	19.48	13.37	16.57	7.16
Palmitoleic (C ₁₆ /1)	0.80	0.15	0.24	0.33
Stearic (C ₁₈ /0)	1.70	1.93	0.62	1.44
Oleic (C ₁₈ /1)	28.13	23.99	13.66	20.38
Linoleic (C ₁₈ /2)	44.73	59.29	63.78	57.66
Linolenic (C ₁₈ /3)	4.96	0.83	4.99	2.840
Fatty acid (%)	76.70	90.80	81.60	75.30

¹Source: Price and Parsons (1975)

²Fatty acids

Chavan *et al.* (1979) reported that low tannin sorghums tend to have a higher proportion of prolamine compared to high tannin sorghums. Moreover, sorghum has a very variable protein content. Hulse *et al.* (1980) presents a range of 4.9-18.2% crude protein by different authors worldwide. This variation in protein content may be due to genetic and/or environmental causes. Environmental factors such as location, chemical fertilizers, plant population and other chemical treatments e.g. use of herbicides will influence the protein content and amino acid profile of sorghum grain (Salunkhe *et al.*, 1977).

Waggle *et al.* (1967), observed that an increase in sorghum yields at a given soil nitrogen level is accompanied by a concomitant decrease in protein content in the grain and that a high rate of nitrogen application results in higher yields of higher protein content. Furthermore these same workers reported that as the level of protein increased with increased nitrogen application the amino acids do not increase proportionally. As a percentage of protein, proline, alanine, glutamic acid, phenylalanine, leucine and isoleucine increase while histidine, arginine, lysine, glycine and threonine decrease. Srinivasan *et al.* (1972) reported that of the 522 sorghum varieties from world collection, those with 12% protein or more had a positive correlation between protein level and isoleucine, leucine and histidine concentrations. However, for the sorghum varieties with less than 12% protein there was a negative correlation between lysine and glutamic acid, alanine, tryosine and phenylalanine. Overall sorghum grain is reported to be low in lysine, tryptophan and threonine (Salunkhe *et al.*, 1977), and leucine (FAO, 1980). In addition sorghum proteins differ from those of other cereals in that their digestibility is decreased by heating (Axtell *et al.*, 1982) and by the presence of polyphenols particularly tannins (Featherston and Rogler, 1975).

Lipids

Sorghum lipids like those of all other cereals are concentrated in the germ, aleurone and pericarp portions of the seed. Table 2.1 gives a comparative summary of the fatty acid composition of selected cereal grains. Sorghum grain oil is more unsaturated than corn oil, and contains more palmitic, palmitoleic, oleic and linolenic than corn oil (Hulse *et al.*, 1980). However, the fatty acid composition of sorghum has been shown to vary among different varieties (Price and Parsons, 1975).

Starch

This is the energy storage of plants and represents 60-80% of the total weight of cereal grains. Rooney and Pflugfelder (1986) noted that the starch of sorghum like that of other cereals is located inside the endosperm enmeshed in a protein matrix. This protein matrix is relatively stronger in sorghum than in maize. Because of this strong protein-starch matrix sorghum has the lowest starch digestibility compared to all other cereals. The presence and level of tannins further decreases the digestibility of sorghum grain in livestock feeds. Tanksley and Knabe (1984) observed that tannins interfere with starch digestion such that high tannin sorghum varieties were found to have 85% of the feeding value of maize in pig diets. The protein matrix is composed of glutelins in which starch granules and prolamine-rich proteins are embedded and may be continuous or incomplete (Rooney and Miller, 1982). Sorghum grains are classified as waxy or non-waxy depending on the protein content and continuity of the protein matrix in the endosperm. Waxy endosperm has smaller starch granules and the matrix is continuous while non-waxy endosperm has larger starch granules surrounded by a discontinuous matrix with less protein molecules. Furthermore, non-waxy sorghum starch contains 20-28% amylose and is more digestible unlike waxy starch, which is almost entirely amylopectin and is less digestible.

Crude fibre

Douglas *et al.* (1990) compared the chemical composition of sorghum grain with that of yellow maize and found that although there were minor differences in the neutral detergent fibre (NDF) levels, sorghum contained higher levels of acid detergent fibre (ADF). The ADF gives an estimate of the cell wall contents of the feed sample particularly cellulose, lignin and insoluble protein complexes and is negatively correlated to the digestibility of the feed. The NDF fraction is a measure of ADF plus hemicellulose and is negatively correlated to dry matter intake of the feed. High tannin sorghum was found to have higher levels of ADF than low tannin sorghum. This is possibly due to contamination by tannins that result in a highly insoluble protein-tannin complex (Janssen and Carré, 1985).

Minerals

From the nutrition point of view calcium and phosphorus are the most important minerals and these are routinely added to compound feeds as limestone and dicalcium phosphate respectively. Compared to maize, sorghum has higher amounts of minerals particularly calcium, phosphorus, magnesium and manganese but is lower in potassium (NRC, 1984). The mineral content of most grains can be increased by increased application of fertilizers (Bennett *et al.*, 1990). The tannin level in sorghum grain is negatively correlated to the amount of nitrogen uptake from the soil. In Kenya for example, sorghum is grown mostly by the less affluent rural communities that consider fertilizer application an unnecessary expense, thus the sorghum varieties grown tend to have very high levels of tannin (Salasya, *et al.* 1996).

2.3 Plant tannins

Originally the term 'tannin' was used to describe substances in vegetable extracts employed in the conversion of animal hides and skins into leather (Goldstein and Swain, 1965). Later the

substances essential in the tanning process (tannins) were identified as polyphenolic compounds with variable and complex molecular weights. Research later showed that these polyphenolic compounds bind strongly not only to hide and skin proteins but also to other types of proteins and polysaccharides. Tannins are present in a large number of products of vegetable origin used as human or animal feeds. However, a number of adverse nutritional effects have been attributed to tannins especially when sorghum is incorporated in poultry diets (Nyachoti *et al.*, 1997).

Chemistry of tannins

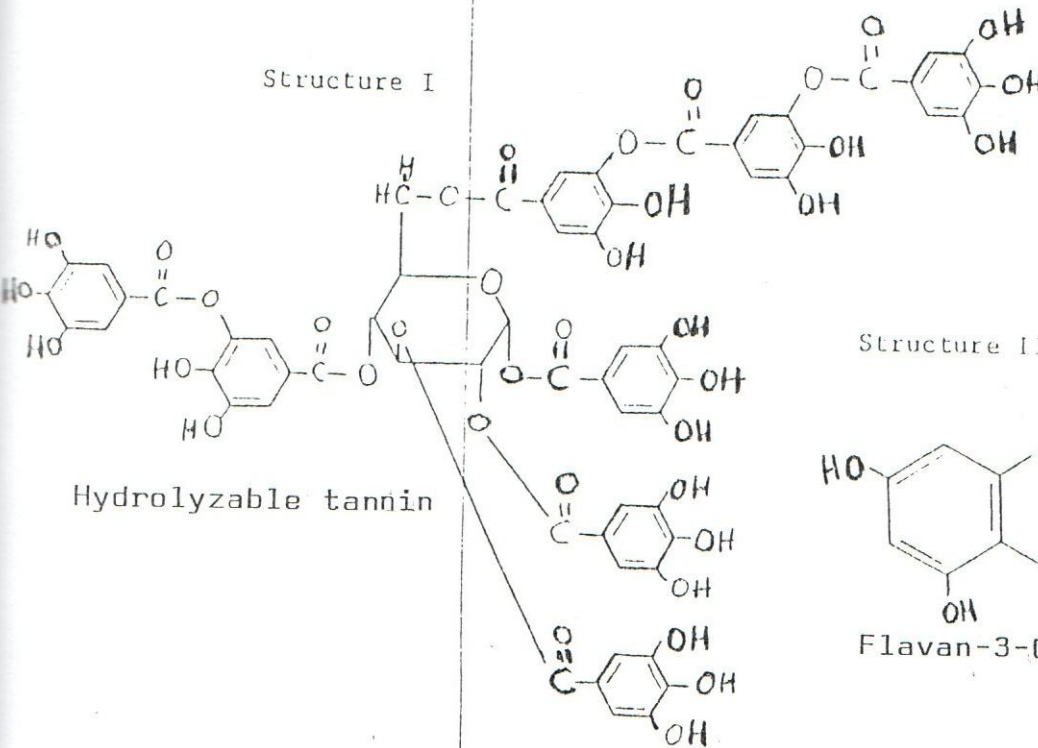
Bate-Smith and Swain (1962) defined tannins as naturally occurring water-soluble polyphenolic compounds with a molecular weight of between 500 to 3000 daltons, capable of precipitating alkaloids, gelatins as well as other proteins from aqueous solutions. Polyphenols referred to as tannins have a considerable number of phenolic groups that are capable of forming effective cross-links with other molecules. Vegetable tannins are usually divided into two groups (1) hydrolysable and (2) condensed tannins (Haslam, 1966). Hydrolysable tannins have a central carbohydrate core whose hydroxyl groups are esterified to phenolic carboxylic acids such as gallic acid, ellagic acid and hexahydroxydiphenic acid. Esters of the first two acids are known as gallotannins (e.g. of Chinese tree) while combinations of the other acids are referred to as ellagitannins (Fig. 2.1, structure 1). Tannic acid is a typical example of a gallotannin and contains 8-10 moles of gallic acid per mole of glucose. Such types of tannins are readily hydrolysed by acids, alkalis or enzymes yielding glucose or some other polyhydroxy alcohol, gallic acid or some phenolic acids related to it (Salunkhe *et al.*, 1990). The condensed tannins are mainly polymerized products of flavan-3-ol (catechin) and flavan-3, 4-diol or a mixture of both (Fig. 2.1, structure 2). Flavan-3, 4-diol belongs to the class of leucoanthocyanadins as they polymerize on acid and heat treatment to phlobaphene-like products and also to anthocyanidin. They are designated as proanthocyanidins. Flavan-3, 4-diol contains 3 asymmetric carbon atoms

and thus 8 stereoisomers. Condensed tannins are also referred to as flavolans or procyanidins. Acetate and phenylalanine are the two simple precursors required for the synthesis of flavonoids, the group of substances to which tannins belong. All flavonoids possess a typical C₆-C₃-C₆ structure. The precursors originate from carbohydrate and protein metabolism, respectively. Flavan-3-4-diol an immediate precursor of polymeric flavonols has a typical C₆-C₃-C₆ skeleton and is produced via chalcone, flavonone and dihydroflavanol intermediates.

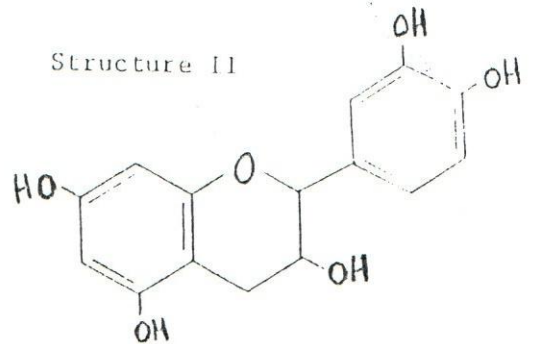
The exact metabolic routes and intermediates in plants for the formation of condensed tannins from flavonoid compounds are not yet clear although several enzymes that mediate the different steps in the condensation process have been identified (Mueller-Harvey and McAllan, 1992). Flavan-3-ols with a molecular weight of less than 3000 daltons are soluble compounds but those with higher molecular weights are insoluble and are more closely linked to the structural tissue of the plant (Salunkhe *et al.*, 1990). Structure 3 of figure 2.1 shows the final steps in the formation of condensed tannins in sorghum grain (Haslam, 1966).

Fig. 2.1 Structure of tannins

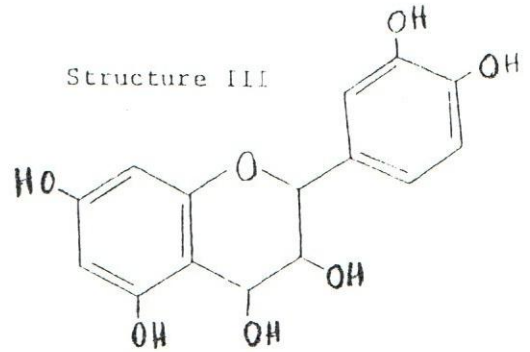
Structure I



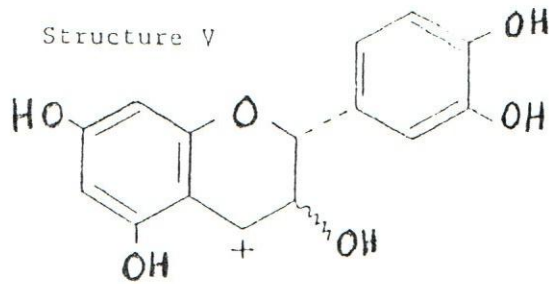
Structure II



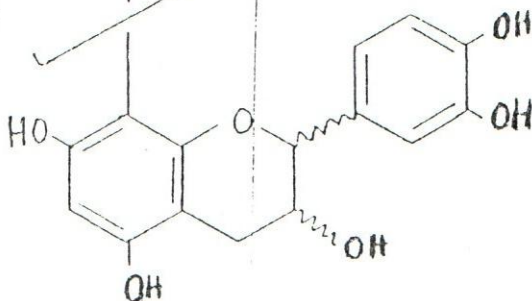
Structure III



Structure V



Structure IV



Condensed tannin

2.4 Occurrence of tannins

Tannins exist either in hydrolyzable or condensed forms (Butler, 1989) and are not known to play any function in plant growth and reproduction. However, they are thought to protect the plant from wild bird depredation (Bullard and Elias, 1980), pre-harvest seed germination and fungal and mould attack (Harris and Burns, 1973). Bate-Smith and Lerner (1954) examined a variety of plants for the presence of condensed tannins in leaves. They found out that condensed tannins are generally present in ferns. Their occurrence in monocotyledonous plants is common but scattered while in dicotyledonous plants they are mostly associated with plants of woody habits of growth. Some species contain both hydrolyzable and condensed tannins e.g. *Quercus* and green carob pods. Others e.g. field beans (*Vicia faba*), cocoa, persimon and sorghum among other plants, have condensed type of tannins.

Nutritional effects of tannins

The most readily apparent effects of tannin when fed at high levels (>2% CE) are reduced weight gain and increased feed conversion ratio (i.e. increase in the grams of food required per gram of weight gain). Some of the documented negative effects of tannins include reduced weight gain and poorer feed conversion ratio in chicks (Featherston and Rogler, 1975). Moreover, depressed growth (Armstrong *et al.*, 1973), reduced nutrient digestibility (Nelson *et al.*, 1975) and increased incidence of abnormal leg bone development (Elkin *et al.*, 1978; Ibrahim *et al.*, 1988) have been reported. A few studies have shown tannins to inhibit digestive enzymes. In studies with rats fed diets containing field beans high in tannin content, there was a reduction in the activities of trypsin and amylase but an increase in the activity of lipase (Griffiths and Mosley, 1980). This was interpreted to mean that dietary tannins stimulate increased secretion by the pancreas of all digestive enzymes but the affinity of tannins for lipase is less than for all other digestive enzymes.

Ahmed *et al.* (1991) reported increased pancreatic weights per unit live weight with increasing levels of tannin in the diets of broilers.

Tannins have the greatest affinity for proteins high in proline content, of large molecular weight with a relatively open structure (Hagerman and Butler, 1981). Furthermore, tannins have been shown to reduce the digestibility of protein in a sorghum-soybean layer diet (Herstad, 1979). In addition, Muindi and Thomke (1981) have reported negative effects of tannins on crude protein digestibility of sorghum grain fed to laying birds.

2.5 Analysis of tannins

Different standard compounds have been used to determine the tannin content of sorghum grain. They include catechin, gallic acid and tannic acid. Isolated sorghum tannins can also be used but due to the cost and unavailability of these other compounds catechin is the most widely used standard. The assays can be categorized into three groups: colorimetric, protein-precipitation methods and other methods.

Colorimetric methods

Vanillin-HCL or Catechin Equivalent assay

The vanillin-HCL assay is widely used as a method for quantitative measurement of condensed tannins in sorghum, fruits and other forage legumes (Burns, 1971; Price *et al.*, 1978). The assay is specific for flavan-3-ols, dihydrochalcones and proanthocyanidins (Sarkar and Harwordth, 1976). The principle is based on the substitution of vanillin for a specific phenolic hydroxyl usually the A-ring of the flavan-3-ols or flavan-3, 4-diols in a methanol-HCL solution to yield a red condensation product. This red compound is measured spectrophotometrically at 500 nm.

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Vanillin does not react in a stoichiometric way with the monomeric units in condensed tannins since the reactive sites in condensed tannins are not readily available (Salunkhe *et al.*, 1990), thus catechin (monomeric flavan-3-ol) is used as the standard. The tannin levels are therefore expressed as catechin equivalents. Alternatively if glacial acetic acid is used in the reaction the absorbance produced is approximately proportional to the concentration of terminal flavan-3-ol groups (Butler, *et al.* 1982). Hence the degree of polymerization of purified tannin samples can be determined as a ratio of monomer to polymer.

Folin-Denis or Tannic Acid Equivalent (TAE)

This assay is widely used to measure total phenol content in plant products and beverages. The principle is based on the reduction of phosphomolybdic-phosphotungstic acid (Folin-Denis reagent) to a blue colour complex by phenols in an alkaline solution (Singleton and Rossi, 1965). The assay is non-specific as it measures the amount of total phenolics and does not distinguish between tannin and non-tannin phenolics. Moreover, the Folin-Denis reagent reacts with other easily oxidized compounds such as ascorbic acid, xanthine, proteins and some amino acids (Lowry *et al.*, 1951). In addition, the Folin-Denis method does not yield stoichiometric results even after taking into account the number of hydroxyl groups (Goldstein and Swain, 1965). Tannic acid is used as the standard so tannin levels are reported as tannic acid equivalents.

Prussian blue assay

Like the Folin-Denis assay the Prussian blue method (Burns, 1963) measures the total content of phenolic hydroxyl groups and is therefore not specific to tannins. The assay is based on the reduction of the ferric ion (Fe^{+3}) to the ferrous ion (Fe^{+2}) by tannins and other phenolic compounds which then complex with ferricyanide to yield ferro ferri cyanide, a characteristic blue reagent known as Prussian blue. Tannic acid is used as the standard such that tannin levels are

reported as tannic acid equivalents. The Prussian blue method appears to be more feasible for the feed industry since a quick test can be done at the port prior to offloading of a shipment of sorghum. This involves adding a few milligrams of a ground sample of sorghum to a flask that contains dilute ferric chloride. A small volume of dilute potassium ferricyanide is added and if the sample contains little or no tannin the solution turns yellow. With increasing tannin content the solution turns green, turquoise then dark blue respectively.

Acid butanol assay

This is a functional group assay that is specific for proanthocyanidins (condensed tannins) particularly when used under conditions described by Bate-Smith (1973), the flavonoid subunits of the condensed tannins are oxidatively cleaved to yield anthocyanidin, which is then measured spectrophotometrically. The method measures the total number of subunits in the tannin fraction. Butler *et al.* (1982) described a method that can be used to estimate the degree of polymerization of condensed sorghum tannins by using a modified vanillin assay to measure the flavan-3-ol of end groups in combination with the acid butanol assay. This similarly determines the total number of subunits in the tannin molecules.

Protein precipitation methods

Protein-binding assays are based on the ability of tannins to form complexes with protein. Their main advantage is that they can either be used to determine the tannin content of a sample or to determine the potential biological activity of tannins (Hagerman and Butler, 1981). The main limitation with these methods is that the level of pH influences binding and also different proteins react differently with any one type of protein (Amory and Schubert, 1987). The two commonly used precipitation methods are the haeme analysis of Bate-Smith (1973) and the bovine-serum-albumin (BSA) method of Hagerman and Butler (1978). In the haeme analysis tannins precipitate

the haemoglobin while the non-precipitating analysis haemochrome in the remaining solution is determined colorimetrically. The method requires freshly drawn blood since commercial haemoglobin was found to give unsatisfactory results. Furthermore, this method does not work well with high tannin sorghum. In the bovine-serum (BSA) precipitation assay (Hagerman and Butler, 1978) tannin extracts are added to a BSA solution. The protein-tannin complex is then dissolved in a detergent system of 1% sodium dodecyl sulphate and 5% triethanolamine. Ferric chloride is added to the mixture after which the tannins/phenolics present in the dissolved complex are measured spectrophotometrically.

Other methods

More detailed information on the structure and nature of polyphenolic compounds and tannins can be obtained by use of high performance liquid chromatography (HPLC), mass spectral analysis (MS), droplet countercurrent chromatography or centrifugal partition chromatography (Okuda *et al.*, 1989). Although no individual compounds could be identified within the fraction of condensed tannins, Mueller-Harvey and McAllan (1992) were able to identify condensed tannins, gallotannins and some low molecular-weight phenolic compounds in aqueous extracts of certain tropical browse species using HPLC. Putman and Butler (1989) demonstrated that it was possible to separate high molecular weight sorghum procyanidins having a relative degree of polymerization of up to 13 monomeric units using a reversed phase HPLC.

Rapid screening test for tannins is another method that has been used to screen for tannins. This is a rapid and simple method for visual estimation of tannin content in sorghum grain. It is based on the development of a blue complex of potassium ferricyanide with ferrous ions. Ferrous ions are produced during oxidation of tannin and other phenols by ferric ions. Since the test is easily performed with a minimum of equipment and technical skill, it is ideal for either field or

laboratory screening of large sorghum populations or quick evaluation of sorghum grain shipments at the elevator (Price and Butler, 1977). All the methods described above particularly the chromatographic ones require skill and are very costly. However, the most commonly used method for assaying tannin levels in feed stuffs is the vanillin-HCL method.

2.6 Technological treatment for reducing tannin content

Physical removal (dehulling)

Several methods have been proposed to reduce the level of tannin in different feed stuffs or to minimize their biological effects. In legume seeds and sorghum grain the tannin is confined to the pigmented testa just beneath the pericarp of the kernel. Physical or mechanical removal of the hull (dehulling) reduced the tannin in faba beans and sorghum (Eggum *et al.*, 1983). Chibber *et al.* (1978) demonstrated that mechanical dehulling of the grain could reduce 80% of sorghum tannins. The main disadvantage of this method is that it results in loss of protein (approximately 45%) which ultimately reduces the amount of amino acids especially lysine, histidine and arginine. Removal of the pericarp using 20% aqueous solution of sodium hydroxide has been shown to minimize the level of tannin while leaving the endosperm and germ intact (Blessin *et al.*, 1971; Chavan *et al.*, 1979).

Deactivation by soaking in water and alkalis

Soaking tannin containing feed stuffs in water or alkaline solutions has been shown to either minimize the level of tannin or make the tannins nutritionally less active. Price *et al.* (1979) reported that soaking the grain in water or alkaline solutions reduced the amount of assayable tannin in sorghum. Similarly, Chavan *et al.* (1979) observed that soaking sorghum grains in aqueous sodium hydroxide then washing out the alkali improved the nutritional value, and increased *in vitro* protein digestibility of the grain. By soaking the whole grain in alkali solutions

followed by hot water washing Armstrong *et al.* (1974) observed that most of the tannins were removed thus making high tannin sorghum to be equivalent to non-bird resistant type. Other extraction methods include treatment with magadi soda (Muindi and Thomke, 1981; Nyachoti *et al.*, 1998). Germination in wood ash (Doggett, 1982), soaking in aqueous solutions of various alkalis for at least 12 hours (Reichert *et al.*, 1980; Banda-Nyirenda and Vohra, 1990), exposure to dilute aqueous ammonia (Price *et al.*, 1978; Butler, 1982) have been found to reduce tannin concentration in sorghum grain. However, these methods require that the sorghum grain to be washed thoroughly before being used to formulate feed for animals.

Reconstitution (high moisture storage) for 1-3 weeks reduces assayable tannin content and improves the nutritional value of high tannin sorghum for rats (Reichert *et al.*, 1980), chickens (Mitaru *et al.*, 1983) and pigs (Mitaru *et al.*, 1984). The moisture content of the grain is raised by adding distilled water (25 w/w) prior to storage at 25⁰ C followed by addition of small amounts of acetic and propionic acids to deter growth of moulds. The actual mechanism of tannin deactivation is not clear but Mitaru *et al.* (1983) speculated that the process involved polymerization of the tannin molecules. Polymerized tannin molecules are highly insoluble or are too large to bind and precipitate protein. Furthermore, germination of high tannin sorghum grain for 72 hours was shown to reduce assayable tannin content by 70% (Chavan *et al.*, 1979). Presumably during germination the tannins are leached out which improves the digestibility and nutritional value of sorghum (Daiber, 1975). It is likely that soaking grains containing tannins in water or alkali solutions resulted in a change of content and/or structure of tannins thus rendering them less effective in interfering with protein digestion.

Dietary supplementation

Jambunathan and Mertz (1973) reported that supplementing rat diets containing high tannin sorghum (3.08% CE) with L-lysine significantly improved weight gains, increased feed consumption and improved the Protein Efficiency Ratio (PER). Additionally, increasing the dietary nitrogen content of a high tannin sorghum was found to overcome the detrimental effects of tannins when the supplemental nitrogen was added as intact protein (Rodriguez *et al.*, 1983; Rogler *et al.*, 1985). These workers speculated that the added proteins bind and deactivate the tannins thus minimizing the possibility of their interfering with digestion or being absorbed in the gastrointestinal tract of the animal. Elkin *et al.* (1978) observed that supplementing high tannin sorghum diets with D,L-methionine improved nitrogen retention and dry matter utilization in chicks which was not the case for low tannin sorghum. Armstrong *et al.* (1973) and Featherston and Rogler (1975) found that D,L-methionine supplementation of brown (high) tannin sorghum diet alleviated the detrimental effects of tannin on body weight gain and feed efficiency in chicks. Potter and Fuller (1968) reported that tannic acid was hydrolysed to gallic acid which was then O-methylated and excreted in the urine as 4-O-methyl gallic acid. They suggested that the supplemental D,L-methionine acted as a methyl donor in the deactivation of the tannins thus overcoming their growth depressing effects.

Use of tannin binding compounds

Addition of chemicals with a high affinity for tannins may help to minimize the detrimental effects of tannins. Using competitive binding assays, proteins have been shown to differ in their relative affinity for tannins. Selective binding of polymers with low nutritive value will result in less harmful nutritional consequences than random non-selective binding. Loomis and Battaile (1966) developed a technique in which insoluble polyvinylpyrrolidone (PVP) was used to remove phenols from what they described as H-bonded complexes with protein in plant tissues, and so

obtained active soluble enzymes. Later, Jambunathan and Mertz (1973) reported that the weight gain and protein efficiency ratio (PER) of rats were improved when 5% PVP was added to diets containing high tannin sorghum (2.08% and 3.08% CE). In contrast, when a similar amount of PVP was added to diets containing low tannin sorghum (0.3% CE) negative performance was observed. Non-nutritive synthetic polymers such as PVP, polyethylene glycol (PEG 4000), polyoxyethylene sorbitan oleate (Tween 80), Lissapol (a non-ionic detergent) or gelatin may be used to reduce the harmful effects of tannins. When the efficacy of these polymers was compared in chick bioassays, PVP was found to be more effective in increasing the availability of methionine and improvement of the relative nutritional value of protein in the high tannin sorghum diet (Ford, 1977).

Similarly, Featherston *et al.* (1982) demonstrated that supplementation of high tannin sorghum (1.74% TAE) with 1% PVP improved feed consumption, nitrogen retention and dry matter utilization of broiler chicks. Lewis (1993) described PVP as a white, free flowing amorphous powder compatible with a wide range of hydrophilic and hydrophobic resins, and binds strongly to tannins. Armstrong *et al.* (1973) and Ford (1977) showed that the adverse effects of dietary tannins were overcome by addition of PVP. Tannins are more readily bound to PVP and PEG than to proteins, thus inhibiting their interference with protein digestion and cannot be absorbed from the gastrointestinal tract. Furthermore, supplementation of high tannin sorghum diets with 0.25% and 0.50% NaHCO₃ improved body weight gain and nitrogen retention in broilers (Mbanda-Nyirenda and Vohra, 1990). Colloidal clays such as aluminium silicate or bentonite may also be used to bind dietary tannins. Although it has not been documented in literature, it is likely that bentonite due to its strong thixotropic properties (Lewis, 1993) has an affinity for tannins and will bind them in any aqueous solution. This study sought to verify the possibility of using PVP and bentonite as tannin binders in high tannin sorghum diets for broilers and laying hens respectively.

2.7 Use of sorghum grain in chicken diets

Grain sorghum in broiler diets

There is a wide variation in results given in literature on the effects of sorghum grain on performance of broilers. While most workers reported negative effects for all the variables measured a few others failed to notice any such effects. Connor *et al.* (1969); Rostagno *et al.* (1973); Armstrong *et al.* (1973); Elkin *et al.* (1978, 1990); Featherston and Rogler (1975); Luis *et al.* (1982); Douglas *et al.* (1990) and Nyachoti *et al.* (1998) reported a decrease in body weight gain, feed consumption and feed efficiency. Other researchers reported depression only in body weight gain (Chang and Fuller, 1964) or feed efficiency (Petersen, 1969). However, other feeding trials did not show any detrimental effects of feeding sorghum grain to broilers (Smith *et al.* 1989). Variations in methods employed in tannin analysis as well as the composition of diets used and dietary tannin concentration partially account for the differences reported in literature.

Elkin *et al.* (1978) and Giles (1980) reported an increase in the incidence and severity of leg abnormality in broiler chicks. In this respect, the possibility of tannins interfering with mineral absorption or collagen formation cannot be ruled out. Elkin *et al.* (1978) observed that there may be a tannin-induced alteration of the organic matrix of the bone. Moreover, Mitaru *et al.* (1983) found a low correlation between the incidence of leg abnormality and dietary tannin level, suggesting that other factors may be involved. Although the physiological mechanism associated with leg abnormalities is not clear, Ibrahim *et al.* (1988) noticed that supplementation with phosphoric acid or dicalcium phosphate corrected the problem. It is noteworthy to mention that leg abnormalities are caused by many other factors principally mineral and vitamin deficiencies. Deficiency of manganese, zinc, choline, pyridoxine and biotin may result in distortion of the long bones, rotation of the shanks and a swelling of the hock joints (chondrodystrophy). More work

needs to be done to elucidate the actual effects of dietary tannins on bone development of broiler chicks.

Grain sorghum in layer diets

Literature gives more information on the effects of high tannin sorghum in broiler chicks than in laying birds. Several studies have dealt with the effects of tannic acid (hydrolysable tannin) on performance of laying flocks as opposed to condensed tannins such as those of sorghum grain and these should not be equated. Potter *et al.* (1967) reported a significant decrease in egg production when 2% tannic acid was added to layer diets. In addition there was an increase in olive green discolouration and mottling of the egg yolks. Weber (1970); Fry *et al.* (1972); Armanious *et al.* (1973) and Hughes (1973) made similar observations. Many feeding trials involving sorghum in layer diets do not indicate the level of tannin in the experimental diets which makes it difficult to know the minimum level of sorghum tannin that will elicit negative performance in laying flocks. Hammond (1942); Ali and Yeong (1977) did not specify the tannin levels but reported that sorghum could replace maize in layer diets without adversely affecting egg production and feed efficiency. However, Malik and Quisenberry (1963) reported that sorghum reduced egg production, egg weight and feed efficiency of layers fed a sorghum-based diet. Armanious *et al.* (1973) reported that diets containing high tannin sorghum (1.2% and 2.4% CE) had no significant effect on egg production, egg weight and yolk mottling but that yolk colour progressively declined at higher tannin levels. In addition layers fed high tannin sorghum exhibited the greatest loss in body weight. Furthermore, Sell *et al.* (1983) reported that a dietary tannin of 4.25% CE depressed egg production but had no effect on egg weight and internal egg quality (albumin weight, yolk colour and mottling).

From what has been reported in the literature, it is not yet clear at what dietary tannin level broiler performance or egg production and internal egg quality will not be affected when high tannin sorghum is used in chicken diets. The effectiveness of tannin binders such as polyvinylpyrrolidone (PVP) and bentonite to ameliorate the adverse effects of dietary sorghum tannins has not been established. This work was undertaken with the aim of establishing the maximum tannin concentration that is permissible in the diets of broiler chicks (7-28 days) and of laying hens during peak egg production (32-40 weeks). The effectiveness of using PVP and bentonite (at 0.25% and 0.5%) as tannin binders in chicken diets containing high tannin sorghum was also investigated.

CHAPTER THREE

3 EXPERIMENTS WITH BROILER CHICKENS

3.1 Experiment 1

Hypotheses

- Indigenous sorghum cultivars contain tannins that limit their use in broiler chick diets
- The deleterious effects of tannins can be minimized by use of a tannin binder such as PVP

Objectives

- To determine the effects of tannin level on the performance of broiler chicks
- To examine the effects of PVP on the utilization of high tannin sorghum (HTS) on broiler chicks

Materials and methods

Birds and housing

Two hundred commercial day-old broiler chicks of mixed sex (Arbo Acres) were obtained from a local hatchery (Kenchic Ltd.) and used for the experiment. The chicks were kept under conventional brooding conditions, receiving 24 h light and at a temperature range of 30-34⁰ C during the first 7 days of life and fed a standard maize-soybean diet. At the end of the first week, chicks were individually weighed and one hundred and five of them within the middle weight range with an average initial weight of 97 g were wing-banded and assigned to 7 treatments in a completely randomized design. Each treatment consisted of 3 replicate cages of 5 chicks per cage such that a raised floor cage measuring 84 cm x 78 cm x 43 cm with 5 chicks formed an experimental unit. A kerosene stove provided supplemental heat to maintain brooding temperature

for the first 28 days, after which room temperature was maintained within a range of 19⁰ to 22⁰ C.

Data collection commenced on day 8 and was terminated on day 28.

Diets and feeding

Four sorghum cultivars of different seed coat colour (white, cream, light brown and dark brown) were obtained from sorghum growing areas of Western Kenya and used in the experiment. The four sorghum cultivars and maize were used to formulate diets designed to meet the nutrient requirements of broiler chicks (NRC, 1984). Two additional diets were included to test the effects of a tannin binder such as PVP when added at either 0.25% or 0.5% respectively (Table 3.1). Performance parameters i.e. feed consumption, feed conversion efficiency and body weight gain were measured weekly. Throughout the experimental period, feed was provided twice per day at 9.00 hours and at 16.00 hours such that there was feed in the feed troughs at all times. Water was provided at all times.

Table 3.1. Composition of experimental diets used to test the effect of sorghum cultivars¹ and PVP on the performance of broiler chicks (7-28 days)

Ingredient	Diets						
	1	2	3	4	5	6	7
Maize (7.6% CP ²)	60.00	-	-	-	-	-	-
White sorghum (8.4% CP ²)	-	60.00	-	-	-	-	-
Cream sorghum (8.4% CP ²)	-	-	60.00	-	-	-	-
Light brown (9.1% CP ²)	-	-	-	60.00	-	-	-
Dark brown (10.2% CP ²)	-	-	-	-	60.00	59.75	59.50
Soybean meal (45.6% CP ²)	27.00	26.50	26.50	26.50	26.50	26.50	26.50
Fishmeal (53.8% CP ²)	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Corn oil	2.50	3.00	3.00	3.00	3.00	3.00	3.00
Iodized salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Broiler premix ³	0.20	0.20	0.20	0.20	0.20	0.20	0.20
PVP ⁴	-	-	-	-	-	0.25	0.50
Calculated composition							
CP (%)	19.60	19.81	19.81	20.23	20.89	20.87	20.84
ME (kcal ME/kg) ⁵	3042	3025	3025	3025	3025	3025	3025
Methionine (%) ⁵	0.47	0.45	0.45	0.45	0.45	0.45	0.45
Lysine (%) ⁵	1.57	1.54	1.54	1.54	1.54	1.54	1.54
Tannin (% CE)	0.024	0.59	0.94	2.71	3.54	3.46	3.38

¹Sorghum-based diets: 2 = White sorghum (0.98 % CE); 3 = Cream sorghum (1.56 % CE); 4 = light brown (4.52 % CE); 5 = dark brown (5.90 % CE); 6 = Diet 5 + 0.25 % PVP, 7 = Diet 5 + 0.5 % PVP. ²Determined analysis

³Supplied per kg diet: Vit. A, 8,000 IU; Vit. D₃, 1,600 IU; Vit. E, 6.4 mg; Vit. K₃, 1.6 mg; Vit. B₂, 4.8 mg; Pantothenic acid, 8 mg; Niacin, 24 mg; Vit. B₁₂, 0.008 mg, Folic acid, 0.8 mg; Choline chloride, 120 mg; Lysine, 0.4 mg; Methionine, 0.096 mg; Manganese, 53.6 mg; Iron, 19.2 mg; Zinc, 53.6 mg; Copper, 11.2 mg; Iodine, 1.6; Selenium, 0.08 mg; BHT, 96 mg.

⁴Polyvinylpyrrolidone

⁵Adapted from NRC (1984) - ME (kcal) - maize 3350; sorghum 3288; soybean meal 2440; fishmeal 2820; corn oil 8800. Lysine - maize 0.24%; sorghum 0.21%; soybean meal 2.93%; fishmeal 4.83%. Methionine- maize 0.2%; sorghum 0.16%; soybean meal 0.65%; fishmeal 1.78%.

Chemical and statistical analyses

Proximate analysis for protein content in the cereal grains and in the compounded diets was determined by standard procedures as outlined in the Official Methods of Analysis (AOAC, 1995). Data were subjected to analysis of variance for a completely randomized design using the Statistical Analysis System (SAS, 1997). Where applicable, means were compared at 5% level using the Least Significant Difference.

3.2 Experiment 2

Hypotheses

- High tannin sorghum can be used in broiler chick diets without loss in production if it can be used at low inclusion levels
- Tannin level has no effect on dry matter digestibility, nitrogen retention and tannin absorption in broiler chicks

Objectives

1. To determine the optimal inclusion level of HTS in broiler chicks
2. To examine the effects of tannin level on dry matter digestibility, nitrogen retention and tannin absorption in broiler chicks

Materials and methods

Day-old broiler chicks (Arbo Acres) were obtained from a local commercial hatchery (Kenchic) and used for the experiment. The management of the chicks during the first 7 days of life was as described for the chicks of experiment 1. At the end of 7 days the chicks were weighed and the middle-weight group of 90 chicks were wing-banded and randomly distributed among six diets. The diets (Table 3.2) were formulated to meet the nutrient requirements of growing broiler chicks.

Collection of fecal samples (using the total collection method) was done on three days 19, 20 and 21. Two pens from each treatment were selected at random and a polyethylene plastic sheet spread beneath each cage to collect the feces. A known amount of feed was weighed and given to the chicks and after every 24 h the feces were collected, weighed and kept in air-tight bottles for freeze drying. The modified vanillin-HCL method (Price *et al.*, 1978) and Kjeldahl method were used to determine amounts of tannin and nitrogen in the fecal samples respectively. The values for dry matter intake, nitrogen retention and tannin absorption were computed by simple difference. Collection of data on feed consumption, feed efficiency and weight gain as well as statistical analysis of the data was as described for experiment 1.

Table 3.2. Composition of experimental diets¹ (% , air dry basis) used to determine the effect of sorghum inclusion level on broiler chick performance (7-28 days)

Ingredient (% of diet)	Diets					
	1	2	3	4	5	6
Maize (7% CP)	63.0	39.0	30.7	22.3	14.0	...
Brown sorghum ²	...	25.0	33.3	41.7	50.0	64.0
Corn gluten meal	27.5	27.0	26.5	26.0	26.0	25.5
Fishmeal (55% CP)	5.0	5.0	5.0	5.0	5.0	5.0
Limestone	2.0	1.0	1.0	1.5	1.5	2.0
DCP	1.0	1.0	1.5	1.5	1.5	1.5
Corn oil	1.0	1.5	1.5	1.5	1.5	1.5
Iodized salt	0.3	0.3	0.3	0.3	0.3	0.3
Broiler premix ³	0.2	0.2	0.2	0.2	0.2	0.2
Calculated composition						
CP (%)	23.59	23.79	23.66	23.52	23.69	23.63
ME (kcal ME/kg)	3372	3346	3338	3320	3312	3305
Methionine (%)	0.84	0.82	0.81	0.79	0.79	0.77
Tannin (% CE)	0.025	1.25	1.66	2.08	2.50	3.20

¹Maize and sorghum substituted on energy and protein basis to get isocaloric and isonitrogenous
Dark brown sorghum (tannin level 5% CE)

³See footnote² Table 3.1

3.3 Results

Experiment 1

The results of the composition of the formulated diets are shown in Table 3.1 above. The addition of 0.25% and 0.5% PVP to high tannin sorghum diet reduced the amount of assayable tannin by approximately 2.9% and 5.73% respectively. Results of the effects of grain source and a tannin binder (PVP) on broiler performance are shown in Table 3.3.

There was a marked difference between the first three diets and the last four diets for all the variables measured. Feed intake was affected by treatment such that chicks fed diets 1, 2, and 3 had similar ($p>0.05$) but higher feed intake compared to diets 4, 5, 6 and 7 which in turn were similar ($p>0.05$). Chicks fed diets 1, 2 and 3 attained similar body weight gain ($p>0.05$) which again was significantly higher ($p<0.05$) than diets 4, 5, 6 and 7. Diets (4, 5 and 6) had similar body weight gain ($p>0.05$) but this was lower ($p<0.05$) than that of diet 7. Feed efficiency was higher ($p<0.05$) in diets 1, 2 and 3 compared to that of diets 4, 5, 6 and 7, which showed similar feed efficiency ($p>0.05$). Although addition of 0.5% PVP to diet 7 improved weight gain ($p<0.05$) compared to 0.25% PVP added to diet 6, tannin binding did not significantly ($p<0.05$) improve feed consumption or feed efficiency among chicks fed on these diets. Overall, performance of chicks on maize, white sorghum and cream sorghum was better ($p<0.05$) than that of chicks on either light brown, dark brown or dark brown sorghum treated with PVP.

Experiment 2

The composition of the experimental diets used in experiment 1 is shown in Table 3.2 above. This composition was within the range recommended by NRC (1984). However, methionine was in excess of the requirements for broiler chicks. Use of corn gluten meal and fishmeal instead of soybean meal as sources of protein resulted in the increased levels of methionine. Table 3.4 gives a summary of the results of the effect of sorghum tannins on dry matter digestibility, nitrogen retention and tannin retention in the broiler chick.

Table 3.3. Effects of tannin level and PVP on 21-day feed intake, weight gain and feed conversion of broiler chicks

	Diets ¹							SEM ²
	1	2	3	4	5	6	7	
Tannin (% CE)	0.024	0.59	0.94	2.71	3.54	3.46	3.38	
Feed intake, g/bird	1256 ^a	1228 ^a	1231 ^a	1039 ^b	921 ^b	1003 ^b	999 ^b	± 43.8
Weight gain, g/bird	504 ^a	517 ^a	473 ^a	256 ^c	267 ^c	289 ^c	350 ^b	± 25.9
Gain/Feed	0.40 ^a	0.42 ^a	0.39 ^a	0.21 ^b	0.23 ^b	0.23 ^b	0.30 ^b	± 0.018

^{a,b,c}Means in the same row with different superscripts are significantly different ($p < 0.05$)

¹Diets: 1 = maize; 2 = white sorghum; 3 = cream sorghum; 4 = light brown sorghum; 5 = dark brown sorghum; 6 = Diet 5 + 0.25% PVP; 7 = Diet 5 + 0.5% PVP

²Standard error of the mean

Table 3.4. Effects of sorghum tannins on DM digestibility, N retention and tannin absorption in broiler chicks

	Diets ¹						SEM ²
	1	2	3	4	5	6	
Tannin (% CE)	0.025	1.25	1.66	2.08	2.50	3.20	
Feed intake, g/bird	917 ^{ab}	901 ^{ab}	961 ^a	949 ^a	865 ^b	877 ^b	± 39.71
Weight gain, g/bird	370 ^a	364 ^a	334 ^a	361 ^a	343 ^a	358 ^a	± 18.62
Gain/feed	0.40 ^a	0.40 ^a	0.35 ^a	0.38 ^a	0.39 ^a	0.40 ^a	± 0.021
DMD ³ , %	85.6 ^a	84.5 ^a	85.2 ^a	82.5 ^a	83.0 ^a	81.6 ^a	± 4.09
Nitrogen retention, %	61.80 ^a	62.30 ^a	61.08 ^a	59.80 ^a	61.45 ^a	60.10 ^a	± 2.88
Tannin absorption, %	-	25.30 ^b	31.80 ^a	34.40 ^a	28.95 ^a	28.57 ^a	± 3.92

^{a, b} Means in the same row with different superscripts are significantly different ($p < 0.05$)

¹Diets: 1 = Maize 63 %; 2 = Maize 39 % + 25 % HTS; 3 = Maize 30.7 % + 33.3 % HTS; 4 = Maize 22.3% + 41.7 % HTS; 5 = Maize 14 % + 50 % HTS; 6 = 64% HTS.

²Standard error of the mean

³Dry matter digestibility

3.4 Discussion

In experiment 1, there was approximately a 17 to 27% reduction in feed intake associated with increased tannin levels, which agrees with certain sections of literature. Mitaru *et al.* (1983) obtained a 19.4% reduction in feed intake with dietary tannin of 2.76% CE. However, at lower dietary tannin levels (1.3% CE and 1.57% CE respectively) Jacob *et al.* (1996a) and Nyachoti *et al.* (1998) failed to observe any negative effect of tannins on feed intake by broiler chicks. The 6.63% reduction in feed intake reported by Jacob *et al.* (1996b) may be attributed to the slightly higher tannin level examined (2.39% CE). The marked reduction in feed intake reported in the current study is probably due to the high levels of dietary tannins tested (i.e. 2.71 to 3.54% CE). In experiment 2, tannin levels of 1.25 to 3.2% did not adversely affect feed consumption. A possible explanation for this is the increased total protein and consequently methionine level of diets used in this experiment. Increased methionine level has been shown to alleviate the deleterious effects of tannins on feed consumption and other performance variables in broiler chicks (Armstrong *et al.*, 1973). Although taste acuity in chicken is not well developed, it seems likely that both the astringency and bitter taste of high tannin sorghum affects feed intake. This might explain the high reduction in feed intake observed among broiler chicks fed a starter diet containing 3.54% CE.

Deactivating tannins by adding PVP at either 0.25% or 0.5% gave a slight improvement of 8.45% and 8.9% in feed consumption, respectively. This is slightly higher than the value obtained by Featherston *et al.* (1982) who reported a 5.26% increase in feed intake when 1% PVP was added to diets containing 1.74% tannins expressed as tannic acid equivalents. Differences in tannin measurement and diet composition make it difficult to compare these results. The observed improvement in feed consumption when PVP was added to the diets is probably due to a

reduction in the formation of tannin-protein complexes with the resultant reduction in astringency and improved intake. It is logical to infer that PVP whether used at either 0.25% or 0.5% gives a similar effect on feed intake. There was a 26 to 50% reduction in weight gain among broiler chicks fed diets containing high levels of tannins in experiment 1, although no effect was observed in Experiment 2. Literature is more consistent with the effects of dietary tannins on body weight gain. Several workers have reported a negative effect on weight gain when high tannin sorghum is incorporated in broiler chick diets (Mitaru *et al.* 1983; Elkin *et al.* 1990). Reductions in body weight gain of up to 40% have been reported with diets ranging from 2.76% to 3.6% CE. However, other studies have not found negative effects on weight gain (Jacob *et al.*, 1996a; Nyachoti *et al.*, 1998). It is important to note that the diets used by the preceding authors contained relatively low levels of tannins (1.3% and 1.57% CE respectively), which possibly explains the lack of negative effect on weight gain. There appears to be a positive correlation between dietary tannin level and the magnitude of reduction in body weight gain. The highest dietary tannin level tested in experiment 1 was 3.54% CE which resulted in a 50% reduction in body weight gain.

In experiment 2 tannin levels of up to 3.2% CE had no negative effect on body weight gain, possibly due to the same reason alluded to earlier. The addition of PVP at 0.25% and 0.5% resulted in 8.2% and 31% increase in weight gain, respectively compared to the untreated diet. This finding agrees with the observation made by Featherston *et al.* (1982). It can therefore be concluded that PVP when used at a lower level (0.25%) is less effective in alleviating the negative effects of tannins on body weight gain of broiler chicks. Hence for effective deactivation of sorghum tannins, PVP should be added at levels higher than 0.25% for example 0.5% or more.

A negative effect of dietary tannins on feed efficiency was observed only in experiment 1. A 23 to 42.5% reduction in feed efficiency was observed among chicks fed diets with high tannin levels. Nyachoti *et al.* (1998) reported a 6.5% reduction in feed efficiency among chicks fed a diet containing 1.57% CE. The highest dietary tannin level in experiment 1 was 3.54% CE, which gave a 42.5% reduction in feed efficiency. In experiment 2 the highest dietary tannin level tested was 3.2% CE, which had no effect on feed efficiency. Similar reasons advanced for the effect of tannins in experiment 1 account for the observation in feed efficiency. The addition of PVP at 0.25% did not have any affect on feed efficiency, but when used at 0.5% there was 30.4% improvement in feed efficiency.

Although it is possible that PVP when used at a slightly higher level (0.5%) improves the utilization of grain sorghum by broiler chicks, it does not completely remove the adverse effects of tannins on feed efficiency. Moreover, using PVP is most likely to inflate feeding costs and reduce profit margins in a broiler enterprise. In addition, there was no negative effect attributable to dietary tannins on apparent dry matter digestibility (DMD) or nitrogen (N) retention. This is possibly due to alleviation of any deleterious effects of tannins by methionine. More recently, Nyachoti *et al.* (1998) obtained a 9% and 14% reduction in DMD and N retention, respectively among chicks fed sorghum-based diets containing 1.57% CE tannin content. This reduction was probably due to the lower methionine content of the experimental diets used in the study cited above (0.5% vs. 0.82% used in the current study). Work by Chang and Fuller (1964) showed that methionine was the main source of methyl groups for the O-methylation of gallic acid, a principal metabolite of tannins. The current results indicate that different inclusion levels with resultant different tannin content (1.25 to 3.2% CE) gave similar DMD and N retention. It seems likely that there were sufficient methyl groups that aided in the removal of tannins (as 4-O-methyl-gallic acid) thus diminishing their effect in binding at the absorptive sites along the gastrointestinal tract.

The results of this study suggest that tannin levels in broiler chick diets should be limited to below 2.71% CE in diets containing 20% protein and 0.45% methionine. However, at increased dietary protein (23%) and methionine (0.8%) levels, tannin levels of up to 3.2% CE will not adversely affect broiler performance.

CHAPTER FOUR

4 EXPERIMENTS WITH LAYING HENS

4.1 General

Aspects related to the care and management of the birds, data collection, chemical and statistical analyses, and measurement of egg specific gravity were common to both experiments and hence were considered together.

Materials and methods

Birds and housing

For each of the experiments, two hundred pullet chicks (Isabrown) were obtained from a commercial hatchery and raised for the trials. Vaccination against Marek's disease was done at 1-day-old at the hatchery and against Newcastle disease at 7 days of age. From day old to the start of the experiment, birds were raised in a deep litter house and received 12 h natural light provided by translucent sheets evenly distributed across the roof. At 30 weeks of age (experiment 1) and 32 weeks of age (experiment 2) all birds were individually weighed and 45 hens (experiment 1) and 63 hens (experiment 2) within the middle range weight were wing-banded, and transferred from litter floor to the raised-floor pens. The birds were allowed a 14-day acclimatization period before being assigned to dietary treatments. Each treatment consisted of three replicate pens with three hens per pen. Hence a pen measuring 84 cm x 78 cm x 43 cm with 3 hens formed the experimental unit. During the period of data collection the hens were maintained at a relatively constant room temperature of 22° C and a 12 h natural daylight.

Measurement of egg specific gravity

Egg specific gravity (an indirect measure of shell strength) was determined by the floatation method as described by Snapir and Perek (1969). Briefly, salt solutions ranging from 1.060 mg/ml to 1.100 mg/ml in specific gravity at intervals of 0.005 were prepared by adding 3 litres of water to the following amounts of salt: 276 g for 1.060, 298 g for 1.065, 320 g for 1.070, 342 g for 1.080, 390 g for 1.085, 414 g for 1.090, 438 g for 1.095, 462 g for 1.100. Freshly laid eggs were sampled (3 from each replicate) and immersed in each one of the salt solutions and specific gravity recorded where floating was first observed.

Chemical and statistical analyses

The protein content of the diets was determined by standard procedures as outlined in AOAC (1995). The tannin content of the sorghum cultivars was analysed using a modified version of the HCL-vanillin assay described by Price *et al.*, (1978). Two grams of finely ground sorghum was placed in a 125 ml conical flask, 50 ml analytical grade methanol added, and the flask covered with a cork stopper. The flask was shaken thoroughly every few minutes then left to stand at room temperature (24⁰ - 28⁰ C) for approximately 24 hours. One ml of the supernatant was pipetted into each of the two test tubes. Five ml of 2% vanillin-4% HCL were added to one of the test tubes and five ml of 4% HCL to the other. After 20 minutes the optical density (500 nm) of the solutions was read on a Beckman 25 spectrophotometer. The difference in the two optical densities (4% HCL acting as the blank) was then compared to a catechin standard curve, prepared following the recommendations of Price *et al.* (1978). The concentration (mg/ml) of catechin equivalents was determined for each sample and converted to percent tannins.

Data were analyzed by one-way analysis of variance according to a completely randomized design using the computer package of the General Linear Model of Statistical Analysis System

(SAS, 1997). Where applicable, means were compared at 5% level using the least significant difference.

4.2 Experiment 1

Hypotheses

- Indigenous sorghum cultivars can be used as grain energy sources in layer diets
- The adverse effects of sorghum tannins can be minimized by use of a colloidal clay such as bentonite

Objectives

1. To determine the effects of high tannin sorghum (HTS) on the performance of laying hens
2. To examine the effects of bentonite on the utilization of HTS by laying hens

Diets and feeding

From 0 to 8 weeks the birds were fed a chick starter diet calculated to contain 20% protein and 2900 kcal ME/kg. Thereafter the pullets were fed a grower diet calculated to contain 17% protein and 2900 kcal ME/kg. At 18 weeks of age the ration was gradually changed to layers mash designed to contain 16% protein and 2850 kcal ME/kg. All diets were conventional maize-soybean, fed in mash form and provided for *ad libitum* consumption. Feeding (in the raised floor pens) was achieved by use of modified aluminium feed troughs (58 cm x 16 cm x 11 cm), fastened along one side of the pen. Five dietary treatments were formulated to meet nutrient requirements of laying hens (NRC, 1984). Diets 1, 2 and 3 differed in their grain energy source, while diets 4 and 5 were included to test the effects of bentonite on high tannin sorghum (Table 4.1). At 32 weeks of age, the hens were assigned to the 5 dietary treatments. Data on feed intake, feed conversion and egg quality parameters, computed on weekly basis, began at 32 weeks of age

and continued for the next 6 weeks. Hens were weighed individually at the beginning and at the end of the experiment and this was used to calculate weight gain. To determine feed wastage, a polyethylene sheet was spread beneath any one of the replicate pens (selected at random) in order to collect spilled feed. The actual feed intake was obtained by comparing the difference between the amount of feed offered and that of the spilled feed after a 24 h period. Throughout the experimental period, feed was offered twice a day such that half was given at 09:00 h and the other half at 16:00 h. This ensured that there was feed in the feeders at all times. Drinking water was provided at all times.

4.3 Experiment 2

Hypothesis

- High tannin sorghum can be used in layer diets without loss in production at low inclusion levels

Objective

- To determine the optimal inclusion level of HTS in layer diets

Diets and feeding

Seven diets were formulated to meet the nutrient requirements of laying hens (NRC, 1984). Diet 1 served as the control while the other diets were included to test the effect of substituting varying levels of high tannin sorghum (5% CE) for white maize in layer diets (Table 4.2). Feeding, determination of feed wastage, watering and data analysis was as described for experiment 1.

Table 4.1. Composition of experimental diets used to test the effect of high tannin sorghum and bentonite on the performance of laying hens (32-40 weeks)

Ingredient (% , air dry basis)	Diets				
	1	2	3	4	5
Maize	62.00	-	-	-	-
Sorghum ¹	-	64.50	64.50	64.25	64.00
Soybean meal	19.00	16.00	16.00	16.00	16.00
Fishmeal	6.50	6.50	6.50	6.50	6.50
Limestone	7.00	7.00	7.00	7.00	7.00
DCP ²	2.20	2.70	2.70	2.70	2.70
Corn oil	3.00	3.00	3.00	3.00	3.00
Iodized salt	0.20	0.20	0.20	0.20	0.20
Layer premix ³	0.10	0.10	0.10	0.10	0.10
Bentonite	-	-	-	0.25	0.50
Calculated composition					
CP (%)	16.70	17.10	17.10	17.09	17.09
ME (kcal ME/kg)	2880	2815	2815	2815	2815
Methionine (%)	0.36	0.32	0.32	0.32	0.32
Tannin (% CE)	0.025	0.63	2.41	2.34	2.27

¹Diet 2 = White sorghum (LTS, 0.98% CE); Diet 3 = Dark brown sorghum (HTS, 3.74% CE);

Diet 4 = Diet 3 + 0.25%; Diet 5 = Diet 3 + 0.5% bentonite

²Dicalcium phosphate

³Layer premix supplied per kg diet: Vit. A, 6400 IU; Vit. D₃, 1600 IU; Vit. E, 2.4 mg;

Vit. K₃, 1.6 mg; Niacin, 16 mg; Vit. B₁₂, 0.0048 mg; Pantothenic acid, 4.8 mg; Riboflavin, 2.8 mg; Vit. B₆, 4 mg; Folic acid, 0.4 mg; Choline chloride, 160 mg; Cobalt, 0.8 mg; Manganese, 50.4 mg; Iron, 18.4 mg; Zinc, 50.4 mg; Iodine, 1.6 mg; Copper, 11.2 mg; Selenium, 0.08 mg; DL-Methionine, 0.096 mg; Carophyll (red and yellow), 32.5 mg.

Table 4.2. Composition of experimental diets used to determine the optimal substitution level of high tannin sorghum for laying hens (34-42 weeks)

Ingredient (% , air dry basis)	Diets						
	1	2	3	4	5	6	7
Maize	62.00	50.00	40.00	32.25	24.50	14.50	-
HTS ¹	-	14.50	24.50	32.25	40.00	50.00	64.00
Soybean meal	19.00	16.00	16.00	16.00	16.00	16.00	16.00
Fishmeal	6.50	6.50	6.50	6.50	6.50	6.50	6.50
Limestone	7.00	7.00	7.00	7.00	7.00	7.00	7.00
DCP ²	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Corn oil	2.50	3.00	3.00	3.00	3.00	3.00	3.50
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix ³	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calculated composition							
CP (%)	17.58	16.62	16.74	16.84	16.93	17.04	17.10
ME (kcal/kg diet)	2988	2978	2964	2954	2943	2929	2931
Methionine (%)	0.36	0.34	0.34	0.33	0.33	0.33	0.32
Tannin (% CE)	0.025	0.72	1.12	1.61	2.00	2.50	3.20

¹High tannin sorghum (5.00% CE)

²Dicalcium phosphate

³As for Table 4.1 above

4.4 Results

The results of experiment 1 are presented on Table 4.3. The addition of bentonite at 0.25% and 0.5% to diet 3 reduced the amount of assayable tannins by 2.9% and 5.73% for diets 4 and 5, respectively. All birds experienced an increase in weight gain, the highest gain being in diets 2 and 3 which was significantly poorer than the other three diets (1, 4 and 5). Feed consumption was better ($P < 0.05$) in diet 3 compared to all the other diets. Feed efficiency (kg feed/kg eggs) was significantly poorer in diets 1 and 5 compared to diets 2, 3 and 4, which in turn were similar. A significantly higher percent hen-housed production was observed in diet 2 compared to diets 1, 3, 4, and 5, which were statistically the same. There were no significant differences for egg weight or egg specific gravity for all the diets tested. There was no consistent trend of the effects of bentonite on the utilisation of high tannin sorghum by laying hens.

The results of the effect of varying the inclusion level (experiment 2) of high tannin sorghum on the performance of laying birds are presented on Table 4.4. All diets supported a similar level of egg production. There were no significant differences observed for egg weight and egg specific gravity among all diets tested. There was no consistent trend, which could be associated with the level of dietary tannins with respect to feed intake and feed efficiency. Feed intake was however lower ($p > 0.05$) for diets 1, 4, 6 and 7 compared to diets 2, 3, and 5.

Table 4.3. Effects of tannin level and bentonite on the performance of laying hens

	Diets ¹					SEM ²
	1	2	3	4	5	
Tannin level	0.025	0.63	2.41	2.34	2.27	
Daily weight gain, g/bird	2.9 ^b	3.7 ^a	3.9 ^a	2.9 ^b	2.9 ^b	0.98
Daily feed intake, g/bird	99 ^b	96 ^b	106 ^a	103 ^a	105 ^a	3.20
Kg feed/kg eggs	4.61 ^a	3.03 ^c	3.83 ^c	3.85 ^c	4.54 ^a	1.40
Hen-house production, %	62.30 ^b	76.30 ^a	66.00 ^b	65.30 ^b	67.30 ^b	3.20
Egg weight, g	64.40 ^a	62.30 ^a	62.70 ^a	62.00 ^a	62.00 ^a	1.85
Egg specific gravity (mg/ml)	1.087 ^a	1.090 ^a	1.090 ^a	1.092 ^a	1.093 ^a	0.003

^{a,b}Means in the same row with different superscripts are statistically different ($p < 0.05$).

¹Diets: 1 = Maize; 2 = White Sorghum (LTS); 3 = Dark Brown Sorghum (HTS);

4 = Diet 3 + 0.25 % bentonite; 5 = Diet 3 + 0.5 % bentonite

²Egg specific gravity (shell strength)

³Standard error of the mean

Table 4.4. Effects of sorghum inclusion level on the performance of laying hens

	Diets							SEM ²
	1	2	3	4	5	6	7	
Tannin level	0.025	0.72	1.12	1.61	2.02	2.50	3.20	
Daily feed intake, g/bird	88 ^b	101 ^a	99 ^a	91 ^b	102 ^a	92 ^b	95 ^b	3.01
Kg feed/kg eggs	2.27 ^b	2.40 ^b	2.52 ^b	2.64 ^b	2.01 ^b	3.37 ^a	2.70 ^b	0.30
Hen-house production, %	68.5 ^a	69.0 ^a	67.8 ^a	66.4 ^a	76.2 ^a	64.6 ^a	71.2 ^a	4.09
Egg weight, g	57 ^a	56 ^a	59 ^a	57 ^a	57 ^a	61 ^a	61 ^a	1.13
ESG ² (mg/ml)	1.083 ^a	1.087 ^a	1.085 ^a	1.090 ^a	1.083 ^a	1.087 ^a	1.083 ^a	0.003

^{a,b} Means in the same row with different superscripts are statistically different ($p < 0.05$)

¹Diets: 1 = Maize 62%; 2 = Maize 50% + Sorghum 14.5%; 3 = Maize 40% + Sorghum 24.5%; 4 = Maize 32.25% + Sorghum 32.25%; 5 = Maize 24.5% + Sorghum 40%; 6 = Maize 14.5% + Sorghum 50%; 7 = Sorghum 64%.

²Egg specific gravity (shell strength)

³Standard error of the mean

4.5 Discussion

Experiment 1 was designed to compare the effects on performance of layers fed diets containing either maize, low tannin sorghum or high tannin sorghum. Diets containing low or high tannin sorghum supported significant body weight gain (2.9-3.9 g/d). A low weight gain has been reported for hens fed diets containing high tannin sorghum compared to those fed a low tannin sorghum, which contradicts the present observation. This discrepancy may be attributed to the difference in the tannin content of diets used in the feeding trials. In the current study the highest dietary tannin level was 2.24% CE and this had no detrimental effect on weight gain. In their study Sell *et al.* (1983) using a dietary tannin level of 4.25% CE observed a significant reduction in weight gain. Tannins have been reported to interfere with the digestion, the uptake and utilization of nutrients in the feed. This interference ultimately affects cell accretion and proliferation thus reducing growth. Higher tannin levels in the diet are likely to aggravate this condition.

Literature does not give any information about the use of bentonite (aluminium silicate) as a tannin binder. However, it was chosen due to its use in the pharmaceutical industry as a binding agent to protect tablets from microbial degradation in the gastrointestinal tract. This was extrapolated to suggest that a similar phenomenon could happen when it is added to a high tannin sorghum-based diet, where it was expected to bind the tannins in the gastrointestinal tract of the bird. However, addition of either 0.25% or 0.5% of the bentonite to diets containing high tannin sorghum did not improve the performance of the hens. On the contrary, a higher amount (0.5%) tended to depress feed efficiency and egg production. Although the reason for this was not immediately known it is speculated that bentonite may have probably interfered with the absorption of other feed nutrients along the intestinal mucosa of the bird. Moreover, use of

bentonite in layer diets would most likely increase feeding costs and make the enterprise less profitable.

A low feed intake among hens fed maize-based and low tannin sorghum diets compared to the hens fed a high tannin sorghum-diet was observed. Feed intake is affected by a number of factors most of which are related to the physiological status of an animal, its environment, feed characteristics or an interaction of these factors. In addition to maintenance and production requirements, birds eat to meet their calorie demand. Armanious *et al.* (1973) noted a significant increase in feed consumption by hens fed a diet containing high tannin sorghum compared to those fed a control diet containing low tannin sorghum. This observation is in agreement with the findings reported herein. The increase in feed consumption may be attributed to the lower energy density of the sorghum-based diets (2815 kcal ME compared to 2880 kcal ME of the control diet). Elkin *et al.* (1996) observed that the ME content of sorghum decreases with increase in the tannin level. Despite this, hens are able to compensate for the decrease in ME by increasing their feed intake.

A low egg production was observed among hens fed high tannin sorghum, which agrees with what has been reported elsewhere in literature (Bonino *et al.*, 1980; Sell *et al.*, 1983; Jacob *et al.*, 1996b). These authors reported a significant reduction in egg production when diets containing high tannin sorghum were fed to layers. The actual mechanism by which tannins affect the process of egg formation is not clear, but the possibility of a systematic negative effect on enzyme activity and protein metabolism can not be ruled out (Tamir and Alumot, 1969). The cause of low egg production in hens fed a maize-based diet was not very clear but may probably be due to reduced feed utilization. The type of maize used to formulate this diet was feed grade off-colour maize, commonly used by feed millers in Kenya. The addition of bentonite at 0.25%

and 0.5% did not give an appreciable improvement in egg production and was therefore considered to be none beneficial.

There was no effect of sorghum tannins on egg weight or on egg specific gravity. This is in agreement with the observations made by Jacob *et al.* (1996b). Layers fed maize-based diet laid fewer eggs but of relatively larger size compared to layers fed sorghum based diets, although this was not statistically different from other treatments. Egg specific gravity, a measure of shell strength was not affected by dietary treatment suggesting that tannins do not affect the uptake and utilization of calcium by the bird. Although diet 1 (maize diet) showed a slightly lower value for egg specific gravity this may be attributed to the large size of eggs produced by birds fed this diet. It has been reported that there exists a negative correlation between egg weight and egg specific gravity, as well as egg size and egg number (Austic and Nesheim, 1990).

Using low tannin sorghum as grain energy source in layer diets may be a good alternative in situations where maize is in high demand as human food. However, because of the problem of severe bird depredation, and hence limited availability of white sorghum (0.98% CE), brown sorghum (3.74% CE) may be a suitable alternative to maize if it can possibly be included at moderate levels.

Experiment 2 was aimed at determining the highest level at which high tannin sorghum (5% CE), can be included in a diet of laying hens without adversely affecting their performance. Although there was no consistent trend across all the diets, the highest inclusion level (100%) did not affect egg production, feed consumption and feed efficiency of the hens. Furthermore, there was no effect observed on egg weight and egg specific gravity. In experiment 1, dietary tannin level of 2.24% CE affected egg production but not feed consumption and feed efficiency. This is

contrary to the findings reported by Jacob *et al.* (1996b), who observed a lower feed consumption and a poorer feed efficiency in layers given diets containing high tannin sorghum (dietary tannin level, 2.29% CE). Differences in diet composition, level of production and variety of sorghum used may account for the inconsistency noted in these findings.

The type of sorghum used in experiment 2 had a tannin content of 5% CE with the highest dietary tannin level of 3.2%, but this did not adversely affect egg production or any of the other parameters examined. In experiment 1 the type of sorghum used had a tannin content of 3.74% CE with the highest dietary tannin level of 2.41% CE, which affected egg production. The apparent discrepancy between Jacob's results and the current results may be attributed to the presence of other low molecular weight polyphenols besides tannins, which can be absorbed from the digestive tract and distributed to various tissues where they affect nutrient utilization (Jimenez-Ramsey *et al.* (1994). It is possible that sorghum cultivars with varying tannin levels contain different amounts of such polyphenols and will therefore show variation in their effect on chicken performance. Furthermore, as was pointed out by Elkin *et al.* (1996), tannins are not wholly responsible for poor performance of birds fed high tannin sorghum-based diets. When the results of both experiments are taken together, it can be concluded that high tannin sorghum (up to 5% CE) can be used in layer diets without loss in production.

CHAPTER FIVE

5 GENERAL DISCUSSION

5.1 Background

From the literature (Chapter 2) it was concluded that the level of tannins in sorghum is highly variable and that coat colour alone is not a suitable measure of the amount of tannins in the grain. Several sorghum cultivars exist in Kenya, and can broadly be grouped as white, cream, light brown and dark brown sorghum. However, quantitative measurement of tannins in the grain is the most reliable method of classifying sorghum into various groups as low, medium or high tannin sorghum. Of the three laboratory methods of tannin analysis, the modified vanillin-HCL method of Price *et al.* (1978) was found to be more consistent in ranking the sorghum cultivars and was adopted in this thesis.

5.2 Effect of sorghum tannins in broiler chicks

Effect on feed intake

With very few exceptions, tannins reduce feed intake in broiler chickens. However, this reduction will depend on such factors as energy density of the diet and the protein: energy ratio of the feed. In the current study attempts were made to make the diets as isocaloric as possible, and in the second experiment the amount of intact protein and methionine was higher than that of the first experiment. There was approximately 17-27% reduction in feed intake among chicks on high tannin sorghum diets. This is slightly higher than what has been reported in literature. Previous studies involving use of sorghum in broiler diets have given conflicting responses in terms of feed consumption. Studies by Chang and Fuller (1964) gave a numerically higher feed

intake for broiler chicks fed high tannin sorghum. However, since the composition of the diets was not given, it is difficult to compare the results. Jacob *et al.* (1996a) and Nyachoti *et al.* (1998) using starter diets with 1.3% CE and 1.57% CE, respectively failed to observe any negative effect of tannins on feed consumption by broiler chicks.

The high reduction in feed intake reported in the current study is possibly due to the high levels of dietary tannins tested. Diets used in this study contained between 2.71% to 3.54% CE, which are much higher than what is cited in literature. There appears to be a negative but direct correlation between the amount of tannin contained in the diet and the magnitude of reduction in feed intake. Although taste acuity in chicken is not well developed, it seems likely that both the astringency and bitter taste of high tannin sorghum does affect feed intake. Earlier Mitaru *et al.* (1984) observed that dietary tannins inhibit α -amylase leading to astringency and reduced palatability. This is especially evident from the sharp reduction in feed intake observed among broiler chicks fed a starter diet containing 3.54% CE.

Deactivating tannins by adding PVP at 0.25% and at 0.5% gave a slight improvement in feed intake of between 8.45% and 8.9%. This is slightly higher than the value obtained by Featherston *et al.* (1982) who reported a 5.26% increase in feed intake when 1% PVP was added to diets containing 1.74% TAE. Again since the composition of the experimental diets used by these authors is not given, it is difficult to compare the results. In general, chicken will increase their feed intake in order to meet their calorie requirements. The diets used in this study were formulated to meet the minimum requirements of growing broiler chicks. Hence the observed improvement in feed consumption when PVP was added to the diets is probably due to a reduction in the astringency effect of the tannins with the resultant effect of improved palatability.

Effect on body weight gain

Tannins generally reduce weight gain possibly through a decrease in the utilization of energy, protein and specific amino acids. With reduced availability of these nutrients, broiler chicks consuming tannin-based diets will meet their maintenance requirements but not their needs for tissue accretion. However, at increased levels of dietary protein and methionine, the deleterious effects of tannins on growth are minimized. This was evidenced in the second broiler experiment where slightly higher levels of intact protein and methionine counteracted the negative effects of tannins on growth of broiler chicks.

Overall, 26% to 50% reduction in weight gain among broiler chicks fed diets containing high tannin levels was observed (Ambula *et al.*, 2001). Literature is more consistent with the effects of high dietary tannins on body weight gain. Most researchers have reported a negative effect on weight gain when high tannin sorghum is incorporated in broiler chick diets. Mitaru *et al.* (1983), and Elkin *et al.* (1990) reported 34% and 41% reduction in weight gain with chick diets containing 2.76% and 3.6% CE respectively. Jacob *et al.* (1996a) and Nyachoti *et al.* (1998) using diets with 1.3% and 1.57% CE failed to observe any negative effect on weight gain. The tannin levels reported in this study were higher than those reported in literature. There appears to be a positive correlation between tannin level and reduction in body weight gain. The highest dietary tannin level tested in this study was 3.54% CE which gave a 50% reduction in body weight gain. Tissue cell accretion is positively correlated with feed intake hence a decrease in intake would most likely result in a decrease in growth. Moreover, reduced weight gain has been attributed to a decrease (due to sorghum tannins) in the utilization of energy, protein and specific amino acids (Douglas *et al.*, 1990). This appears to be the case in the work reported herein

where high tannin sorghum significantly affected feed consumption resulting in reduced body weight gain.

Effect on feed conversion

The effect of tannins on feed efficiency is more consistent compared to the other variables. Poor feed efficiency attributable to sorghum tannins has been reported by a number of studies. For example, 23 to 42.5% reduction in feed efficiency was observed among chicks fed diets with high dietary tannins. Armstrong *et al.* (1973) and Rogler *et al.* (1983) found that high tannin sorghum (1.57% and 1.97% TAE) reduced feed efficiency by 37.6% and 31.7% respectively. While Jacob *et al.* (1996a) using diets with 2.23% CE failed to observe any negative effect on feed efficiency, Nyachoti *et al.* (1998) observed a 6.5% reduction in feed efficiency among chicks fed a diet containing 1.57% CE. The highest dietary tannin level in this study was 3.54% CE, which resulted in 42.5% poorer feed efficiency whereas 2.71% CE caused 47.5% reduction in feed efficiency. Unlike body weight gain, there does not seem to be a linear correlation between tannin content and the magnitude of reduction in feed efficiency. However, it can be concluded that the tannins interfered not only with nutrient uptake but also utilization.

The addition of PVP at 0.25% did not have any effect on feed efficiency but when used at 0.5% there was 30.4% increase in feed efficiency. Although at higher levels PVP seems to bind the tannins more, it does not completely remove the adverse effects of tannins on feed efficiency. However, an economic evaluation is necessary to determine the feasibility of detoxifying sorghum tannins for incorporation in broiler chick diets. Moreover, chicks fed white or low tannin sorghum diets performed consistently better for all variables measured compared to treated or untreated high tannin sorghum diets. Although there were no statistical differences between maize and white sorghum, numerical values were higher for white sorghum. Results of

this study suggest that white sorghum (LTS) may be used as sole grain energy source in broiler starter diets. However, due to the problem of high depredation by birds, and hence lower availability, cream sorghum, which gives slightly higher yields than white sorghum may be used as sole grain source in chick diets. Alternatively dark brown or high tannin sorghum which is more readily available can be used after addition of a tannin binder such as PVP at 0.5%. However, since this is likely to inflate feeding costs and thus reduce profit margins, high tannin sorghum may be used but at a lower inclusion level.

The results of experiment 2 indicate that except for feed intake, sorghum-based diets did not produce detrimental effects on broiler chick performance. This may be due to the lower dietary tannin levels of diets used in this experiment compared to those of experiment 1 where the highest tannin level was 3.54% CE while in experiment 2 this was 3.2% CE. Furthermore, diets used in experiment 2 contained higher levels of methionine compared to those of experiment 1 (0.82% vs 0.43% as recommended by NRC, 1984). It is possible that additional methionine was effective in minimizing the deleterious effects of tannins on chick performance. Armstrong *et al.* (1974) found that supplementation of bird resistant sorghum (2.66% CE) with 0.15% methionine gave 19.4% and 7.7% improvement in growth and feed efficiency in broiler chicks respectively. Several authors have shown that increased dietary methionine levels (0.15-0.18%) result in better performance of the broiler chicks (Armstrong *et al.* (1973); Featherston *et al.* (1982); Rogler *et al.* (1983; Elkin *et al.* 1978). It is possible that increased methionine content in chick diets with high dietary tannin aid in detoxifying the tannins. In the current study increased methionine content of the diets was attributed to the use of fishmeal and corn gluten meal rather than soybean meal as sources of protein.

Effect on DMD and N retention

Tannins reduce the nutritive value of diets by reducing dietary protein availability and digestive enzyme activity. As a consequence, dry matter digestibility and nitrogen retention are decreased. This negative effect is minimized through increased tannin excretion as 4-O methyl gallic acid in the urine. The methyl groups are derived from methionine and dietary protein. In the current study, no negative effect attributable to tannins was observed on dry matter digestibility and nitrogen retention. This is possibly due to alleviation of any deleterious effects by methionine. In a feeding trial where hybrid sorghum grains (0.16 to 0.77% CE) were fed to broiler chicks, Nelson *et al.* (1975) found a negative correlation (-0.63) between tannin content and dry matter digestibility (DMD) and amino acid availability. Similarly, Halley *et al.* (1986) obtained a negative correlation (-0.77) between tannin content and dry matter digestibility among broiler chicks fed diets with dietary levels of between 0.28% to 0.94%. However, since the composition of the diets used in the two trials are not given and the amount of methionine not known, it is difficult to compare the results. More recently, Nyachoti *et al.* (1998) obtained a 9% and 14% reduction in DMD reduction and N retention respectively among chicks fed sorghum-based diets with 1.57% CE. This reduction was probably due to the lower methionine content of the experimental diets used in their feeding trial (0.5% vs. 0.82% as used in the current study). Work by Chang and Fuller (1964) showed that methionine was the main source of methyl groups for the O-methylation of gallic acid, a principal metabolite of tannins. The current results indicate that different inclusion levels with resultant different tannin content (1.25% to 3.2% CE) gave similar DMD and N retention. It would appear as though there were sufficient methyl groups that aided in the removal of tannins (as 4-O-methyl gallic acid) and/or diminished their effect in binding at the absorptive sites along the gastrointestinal tract.

The approximately 30% tannin absorption across all the diets did not affect the performance of the chicks. It is speculated that for chick performance to be affected more than 30% tannin level would have to be absorbed into the blood stream of young broiler chicks. The results of the two experiments suggest that high tannin sorghum (dietary tannin level 2.7% and above) depresses broiler chick performance in maize-soybean chick diets. Furthermore, deactivating tannins by adding either 0.25% or 0.5% PVP to the diets is not beneficial and will most likely prove uneconomical. Moreover, since white sorghum (0.98% CE) gives better chick performance compared to maize, it can be used as sole grain energy source in broiler starter diets. However, since use of white sorghum is limited by inadequate availability of the grain due severe bird depredation, dark brown sorghum high in tannin content can be used at increased methionine levels. Hence in view of the increasingly severe inadequacy of maize occasioned by frequent droughts and high demand as human food, sorghum cultivars of tannin levels of up to 5% CE can be used as grain energy source but at increased protein and methionine levels (23% and 0.8%, respectively).

5.3 Effect of sorghum tannins on layer performance

Effect on weight gain

Both low tannin and high tannin sorghum when used as sole grain sources supported significant body weight gain in the present study (Table 4.3). The 34% increase in body weight gain among hens fed a high tannin sorghum (Ambula *et al.*, 2002) is in sharp contrast with what has been reported in literature. Whereas Bonino *et al.* (1980) obtained a significant reduction and Armanious *et al.* (1973) an insignificant reduction in weight gain among hens fed HTS, their diets were sub-optimal in energy and lysine respectively. Similarly, Sell *et al.* (1983) observed a decrease in body weight gain among hens fed high tannin sorghum (dietary tannin level 4.25%).

This observation may be attributed to the differences in diet composition and the amount of tannin content in the feeding trials.

In the current study the highest dietary tannin level tested was 2.41%, almost half that examined by Sell *et al.* (1983). Furthermore, in the feeding trials cited above no fishmeal was used unlike in the present study where its use boosted the amount of lysine and methionine slightly above levels recommended by NRC (1984). It is possible that differences in diet composition and dietary tannin levels accounted for the variation in the results obtained by different researchers. Moreover, tannins have been reported to interfere with the digestion, uptake and utilization of food nutrients, which ultimately affects cell accretion and proliferation thus reducing growth. Higher levels in the diet would further aggravate this condition. The cause of reduced weight gain among hens fed a maize control diet was not immediately clear although a possibility of lower feed intake and hence reduced nutrients for cell accretion cannot be ruled out.

Literature does not give any information about the use of bentonite as a tannin binder but this was chosen due to its use in the pharmaceutical industry where it is used as a bonding agent to protect tablets from microbial degradation in the gastrointestinal tract. This was extrapolated to suggest that a similar phenomenon could possibly occur when bentonite is added to a high tannin sorghum-based diet, where it was expected to bind the tannins in the gastrointestinal tract of the bird. Overall, bentonite reduced the amount of tannin by approximately 25%. However, addition of either 0.25% or 0.5% of the bentonite to diets containing high tannin sorghum (5% CE) did not improve the general performance of the hens. On the contrary a higher amount (0.5%) significantly affected feed efficiency. Although the reason for this was not immediately known it is probable that bentonite through its binding characteristics may have interfered with the absorption of other feed nutrients along the intestinal mucosa of the bird.

Effect on feed intake

There was a slight but significant increase (7%) in feed intake among hens fed a HTS diet compared to the control hens. This is in agreement with the findings of Armanious *et al.* (1973) and Jacob *et al.* (1996b) who noted a significant increase in feed consumption by laying hens fed a diet containing HTS (2.4% and 2.29% CE respectively) compared to those fed a control diet. However, Bonino *et al.* (1980) and Sell *et al.* (1983) did not observe any significant difference in feed consumption between hens fed either HTS or LTS diets. Feed intake is affected by a number of factors most of which are related to the physiological status of an animal, its environment, feed characteristics or an interaction of all these factors (Scott *et al.*, 1982). Although tannins have been associated with astringency and bitter taste that would possibly result in lowered palatability, this does not seem to be the case as demonstrated by the current results. Moreover, since taste acuity in chicken is not well developed the negative effect of reduced palatability on feed intake would therefore be ruled out.

Effect on egg production

Hens fed high tannin sorghum attained similar egg production as those fed a maize diet, but egg production was 20% higher among hens fed low tannin sorghum. The low egg production observed among hens fed high tannin sorghum agrees with what has been reported elsewhere in literature (Armanious *et al.* 1973; Bonino *et al.*, 1980; Sell *et al.* 1983). These authors reported a significant reduction in egg production when diets containing high tannin sorghum were fed to layers.

There was no negative effect of sorghum tannins on egg weight and egg specific gravity. This is in agreement with the findings of other workers (Armanious *et al.*, 1973; Bonino *et al.*, 1980; Sell *et al.*, 1983 and Jacob *et al.*, 1996b) who found no negative effect of dietary sorghum

tannins on egg weight or egg specific gravity. There is usually a negative correlation between egg number and egg size. Layers fed maize-based diet laid fewer eggs but of relatively larger size compared to sorghum fed layers although this was not statistically different from other treatments. Egg specific gravity, a measure of shell strength was not affected by treatment suggesting that tannins do not affect the uptake and utilization of calcium by the bird. Although diet 1 (maize diet) showed a slightly lower value for egg specific gravity this may be attributed to the large size of eggs produced by birds fed on this diet.

The results of this experiment suggest that although dietary sorghum tannin does not negatively affect egg weight and feed consumption of laying birds, feed efficiency and egg production are affected. Furthermore, addition of a tannin binder such as bentonite either at 0.25% or 0.5% does not improve the utilization of high tannin sorghum by laying hens. Overall, low tannin sorghum gave better performance for most of the parameters measured. Since the detoxification of tannins by addition of bentonite was not found to be beneficial, using low tannin (white sorghum) as sole grain energy source in layer diets may be a good alternative in situations where maize is in high demand as human food. However, because of the problem of severe depredation by birds, and hence limited availability of low tannin sorghum (white sorghum), high tannin (brown) sorghum may be the next alternative to maize and can be used as a partial grain energy source in layer diets.

Sorghum inclusion at all the levels tested did not have a negative effect on feed efficiency. Malik and Quisenberry (1963) substituting sorghum for corn at 50% reported a 10% reduction in feed efficiency. However, since the tannin levels tested were not given it is not possible to compare results. For one to be able to compare results effectively, the dietary tannin levels must

be given. Differences in diet composition, size of the hens and level of production may account for the variability noted for these results.

Of all the inclusion levels tested, substituting maize by 40% sorghum (dietary tannin of 2% CE) supported a fair though not statistically better performance in terms of egg production and feed efficiency. In addition, layers fed the control diet (maize) without any measurable tannin did not perform as well as expected. The diets used in this experiment were formulated to meet the nutrient requirements of laying birds as shown in Table 4.1. Hence the cause of low egg production was rather baffling and may probably be attributed to inherent errors in crude protein determination and/or other factors that were not immediately clear. Jacob *et al.* (1996b) substituted sorghum (dietary tannin 2.45% CE) for white maize and noted a significant reduction in egg production. In the current study the highest dietary tannin was 3.2% CE which did not adversely affect egg production or any of the other parameters measured. It is therefore not very clear at what dietary tannin level egg production and/or feed efficiency will or will not be affected when sorghum is used to substitute for white maize in layer diets. Further investigation is needed to establish this. There was no effect of sorghum tannins on egg specific gravity. This is consistent with the findings of Sell *et al.* (1983) and of Jacob *et al.* (1996b) who reported that dietary sorghum tannins (4.25% CE and 2.45% CE respectively) had no effect on egg specific gravity. This therefore implies that sorghum tannins do not necessarily interfere with calcium absorption, an important mineral required for egg shell formation and hence shell strength. Results of this study indicate that inclusion of up to 100 % high tannin sorghum (dietary tannin level 3.2% CE) does not affect layer performance although substituting high tannin sorghum for white maize at 40% gives a slightly though not statistically better egg production and feed efficiency. Results of the two experiments suggest that white sorghum (0.98% CE) can be used as sole grain source in layer diets. However, as previously mentioned due to high depredation by

birds hence low availability, high tannin sorghum (5% CE) can be used up to 100% of the grain portion. Consequently in countries such as Kenya where maize is the main staple food crop for the majority of people, use of sorghum by feed millers will allow the limited available maize to be used for human consumption, starch extraction or other purposes.

5.4 Conclusion

The preceding study has shown that the dark brown sorghum cultivars can be incorporated in chicken diets but at lower inclusion levels. Tannins will generally limit excessive use of sorghum particularly in chick starter diets. However, in layer diets the effects of tannins are not so severe. This is possibly because being adult birds, they are able to produce enough enzymes that assist in the metabolic deactivation of tannins. When all the results are considered together, the following conclusions can be drawn:

1. There are wide variations in the tannin content of indigenous sorghum cultivars grown in Kenya.
2. Of the three laboratory methods of tannin analysis, the vanillin-HCL was found to be more suitable in ranking the sorghum cultivars.
3. The use of tannin deactivating compounds such as PVP and bentonite to minimize the deleterious effects of tannins on broiler chick and layer performance is not necessary.
4. When diets have adequate energy and protein, tannin levels in the range of 2.7-3.2% CE have no adverse effect on broiler and layer performance.
5. There might be other factors besides tannins in sorghum grain that account for the inconsistent results reported in some sections of literature. More work needs to be done to elucidate this.

6 REFERENCES

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7 APPENDIX

7.1 Description of sorghum cultivars based on coat colour and tannin level

1) Brown/Red (27%)

N = 9	TAE (%)	CE (%)	% Total tannin
Mean	1.22	4.52	0.57
Range	0.93-1.69	3.22-6.20	0.48-0.66
SD	0.27	1.03	0.06
CV	22%	22.78%	10.5%

2. Light Brown (33%)

N = 10	% TAE	% CE	% Total tannin
Mean	0.82	3.06	0.50
Range	0.62-0.93	1.40-5.48	0.45-0.75
SD	0.10	1.26	0.043
CV	12%	41%	8.8%

3. Cream (39%)

N = 14	% TAE	% CE	% Total tannin
Mean	0.54	2.78	0.50
Range	0.40-0.77	1.26-5.44	0.29-0.62
SD	0.13	1.57	0.097
CV	24%	56%	19%

4. White (1%)

N = 2	% TAE	% CE	% Total tannin
Mean	0.37	0.82	0.53
Range	0.36	0.38	0.08-1.56
SD	0.01	0.05	0.07
CV	2%	6%	13%

SUMMARY OF %CE

Sample	Mean	Range	SD	CV%
Brown/Red	4.52	3.22-6.20	1.03	22.78
Light brown	3.06	1.40-5.48	1.26	41.00
Cream	2.78	1.26-5.44	1.57	56.00
White	0.82	0.08-1.56	0.05	6.00
