Improving the nutritive value and utilisation of non-conventional protein feed resources in smallholder village chicken production systems

By

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List of Abreviations

AA	Amino acid
ACIAR	Australian Centre for International Agricultural Research
AF	Aflatoxin
AFB_1	Aflatoxin B_1
AIDS	Acquired immunodeficiency syndrome
ANF's	Anti- nutritional factors
ALP	Alkaline phosphatase
AOAC	Association of Official Agricultural Chemists
AOCS	American Oil Chemists' Society
AST	Alanine aminotransferase
BC	Bentonite clay
BD	Basal diet
BRDU	Bromodeoxyuridine
BWT	Body weight
Ca	Calcium
BWG	Body weight gain
Cfu/g	Colony-forming units per gram
CI	Confidence interval
CP	Crude protein
cP	Centipioses
CPI	Cumulative performance index
DM	Dry matter
DNA	Deoxyribonucleic acid
DE	Digestible energy
Digestible TSAA	Digestibility of total sulfur amino acid
EBEB	Extruded black eyed bean
FC	Feed conversion
FCR	Feed conversion ratio
Fgdb	Feed gain day bird
FI	Feed intake
FAO	Food and Agriculture Organization of the United Nations
FRB	Feed resource base
GE	Gross energy

GGT	Gamma-glutamyltransferase
GOT	Glutamic- oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
GSH-Px	Glutathione peroxidase
H&E	Haematoxylin and eosin
HIV	Immune deficiency virus
AIDS	acquired immunodeficiency syndrome
HSCAS	Hydrated Sodium Calcium Aluminosilicate
IBD	Infectious bursal disease
IgA	Immunoglobulin A
IIAM	National Research Institute of Agriculture, Mozambique
INE	National Institute for Statistics
ME	Metabolisable energy
MIMOC	Minerais industriais de Moçambique
Min	Organic trace mineral mix
Mortpct	Mortality percentage
$NaHCO_3$	Sodium bicarbonate
NCD	Newcastle disease
N	Nitrogen
NRC	National Research Council
NSP	Non-starch polysaccharides
OH	Hydroxyl
OTA	Ochratoxin
PRA	Participatory rural appraisal
RBEB	Raw black eyed bean
RNH	Raw nhemba
ROBEB	Roasted black eyed bean
RONH	Roasted nhemba
SADC	Southern African Development Community
SID	Standardized digestibility of amino acids
SFRB	Scavenging feed resource base
SOD	Superoxide dismutase
TI	Trypsin inhibitor
TIU	Trypsin inhibitor unit
TIA	Trypsin inhibitor activity

TME_n	Metabolizable energy
TME	True metabolisable energy
TMEn	True metabolisable nitrogen
TMUC	Mucosa tissue
TSOD	Superoxide dismutase
TUM	Turmeric
TMP	Turmeric powder
UA	Uric acid
USA	United States of America
$Vit.D_3$	Cholecalciferol
VilLngth	Villi length
Vilwdth	Villi width

Abstract

Improving the nutritive value and utilisation of non-conventional protein feed resources in smallholder village chicken production systems

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F. Dos Anjos

Poultry production is one of the most important activities for creating wealth in developing countries. This study was conducted to assess methods of improving the nutritive value and utilization of cowpeas, and ameliorating the negative effects of aflatoxins in chicken feeds.

Farmer perceptions on feed resource availability and utilization of non-conventional feed resources for indigenous chickens were investigated in three Mozambican districts (n=240). Scavenging was the major source of feeds for chickens and two out of five of the respondents experienced feed shortages during the dry season (from May to October/November). Nearly 90 % of the respondents were willing to use novel protein sources (e.g. houseflies, earthworms and snails) as chicken feed. Household leftovers were identified as the major supplemental feed source, followed by kitchen waste, then crushed grain and maize bran, suggesting protein is deficient in chicken diets. Most often, the kitchen scraps include foods that are spoiled with mould or damaged by insects and are not used in human consumption. Farmers had no specific biases against the use of non-conventional feed ingredients for chickens. Households in mountain zones were more likely to use maize bran for feeding chickens than those near dams (odds ratio 8.26). Educated farmers were three times more likely to feed chickens with maize bran (odds ratio 3.01). Topography highly influenced household's likelihood of experiencing feed shortage. Farmers in mountains zones were 2.3 times more likely to experience feed shortage than the farmers in dam areas. Households headed by females were 1.2 times more likely to experience feed shortage than households headed by men.

Chemical composition, amino acid digestibility and the true metabolisable energy of cowpeas and pigeon peas- under various processing (heat and enzyme) treatments, as well as effects on growth performance, growth of internal organs, and gut health were investigated through feeding trials.

The precision-fed cecectomized rooster assay was used to determine amino acid digestibility and true metabolizable energy of cowpeas. Crude protein (CP) content was higher ($P<0.05$) for raw nhemba cowpea (228 g/kg) in comparison to raw black-eyed beans (207 g/kg). Except for tryptophan, amino acid contents were higher ($P<0.05$) in nhemba than in black-eyed beans. Trypsin inhibitor levels in nhemba averaged 6700 TIU/g, whereas the black-eyed beans contained 2200 TIU/g. Both roasting and extrusion increased the CP content of cowpeas. Extruded and roasted black-eyed beans contained higher ($P>0.05$) amino acid concentrations compared with raw black-eyed beans. Heat treatment had no effect ($P>0.05$) on the levels of methionine, threonine, proline, alanine, valine or leucine. The amino acid content of nhemba was reduced ($P<0.05$) by heat treatment. Heat treatment reduced ($P<0.05$) the concentration of trypsin inhibitors to below 2000 TIU/g. Roasting had no effect ($P>0.05$) on amino acid digestibility in black-eyed beans, but increased amino acid digestibility of nhemba by 3.4 %. True metabolizable energy (TME_n) was significantly increased (3535 versus 3164 kcal/kg) by extrusion.

Body weight gains, feed conversion and gut morphology demonstrated that, despite the overall lower nutritional value of local legumes compared to soybeans, extruded cowpeas with enzymes, or roasted pigeon peas, could improve bird production and gut health.

The effectiveness of diatomaceous earth (DE), bentonite clay (BC) and turmeric (TUM) in ameliorating the toxic effects of aflatoxin B_1 (AFB_1) was assessed in growing chickens. Addition of AFB_1 to the BD depressed ($P<0.05$) BWG and feed intake (FI) when compared to control chickens. The addition of BC to the AFB_1 diet reduced the severity of the histological lesions caused by aflatoxins. Body weight gain (BWG), feed intake (FI) and feed: gain of chickens fed the adsorbents (BC or DE) alone were not different ($P>0.05$) from those of control chicks. In contrast, chicks fed the 2.0 mg AFB_1/kg diet alone had significantly depressed ($P<0.05$) BWG and FI when compared to control chicks. Addition of 0.50 % BC did not improve ($P>0.05$) feed intake and growth rate of chickens fed the AFB_1 diet.

Chickens fed a control diet plus BC and either DE or TUM were as healthy as the control chicks. Bentonite clay gave a higher ($P<0.05$) body weight gain than the control chicks. Compared with chicks fed AFB_1 alone, the addition of TUM into the AFB_1 diet was not effective in preventing or reducing the increase in relative liver or kidney weight. Addition of a combination of both BC and TUM to the AFB_1 diet prevented the increase in relative liver and kidney weights caused by AFB_1. In contrast, the addition of a combination of DE and TUM to the AFB_1 diet was not effective in reducing or preventing the increase in the weight of these

organs caused by $AFB_{1.}$ Chickens fed the AFB_1 diet supplemented with combinations of BC and TUM or DE and TUM had lower (*P<0.05*) concentrations of serum calcium compared to control birds but similar (*P>0.05*) concentrations of Alanine aminotransferase (AST), Gamma-glutamyltransferase (GGT), and uric acid (UA) to that of chicks fed AFB_1 only. In conclusion the addition of TUM alone into the AFB_1 diet also did not demonstrate an ameliorating effect. The addition of the combination of BC and TUM to the AFB_1 diet was not as effective in reversing the effect of AFB_1 on BWG as the combination of DE and TUM. When BC and DE were fed in combination with TUM, the results showed a reversal in the comparative individual effectiveness of BE and DE in their ameliorating effect on BWG.

Dedication

To my mother Coleta Balane

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Filomena Dos Anjos (Mena)

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CHAPTER 1 - General Introduction

In Southern Africa, poultry production is largely categorized into subsistence and commercial systems. Commercial systems are run on a large-scale, with automated feeding systems, temperature-controlled housing, strict bio-security measures and high levels of management. In communal production systems, chickens usually scavenge for feed. Village chickens are an important component of rural livelihoods. The chickens provide meat and eggs consumed in these resource-limited households to provide the much needed proteins. The chickens are also an important source of household income (Mavale, 2001).

In Mozambique, for example, over 80% of Mozambicans depend on agriculture for their livelihood, with chickens representing the most important source of income for rural households, after cash crops. According to the National Institute for Statistics (INE), the national livestock population is estimated at 1.2, 3.9, 0.2 and 1.3 million head of cattle, goats, sheep and pigs, respectively. There are nearly 30 million poultry, with about 90% of these being chickens (INE, 2011). Chickens are widely available and accessible to the vulnerable members of the society, particularly, women, children, the elderly, and people affected by the acquired immunodeficiency syndrome (AIDS). Thus, with improved productivity, village chickens could play an integral role in improving the livelihoods of the resource-poor, as well as the vulnerable members of the community.

Despite their immense benefits to the livelihoods of rural households, their productivity is low. The major constraints to village chicken production include the low levels of management, poor agricultural extension services, high prevalence of diseases, high parasite infestation levels, and shortage of feeds.

In general, the scavenging feed resource base (SFRB) constitutes most of the feed input for the village chickens. Nutrients which cannot be optimally supplied by SFRB should, ideally, be provided as supplementary feeds. Establishing the contribution of the SFRB is, therefore, crucial as it assists in designing appropriate dietary supplementation programmes to increase village chicken productivity. The quantity and quality of the SFRB for chickens varies with location, season, altitude, climatic conditions, farming activities as well as social factors, management, and village flock biomass (Pousga *et al.,* 2006; Momoh *et al.,* 2010). The influence of these factors is, however, poorly understood.

Since most farmers cannot afford to purchase commercial supplementary feeds, appropriate and sustainable methods to utilize available non-conventional feedstuffs should be developed. At present, there is a scarcity of information on nutritional value and the potential of using cowpeas, and the negative impacts of mycotoxins on chicken meat and egg production.

1.1 Justification

The purpose of the project is to strengthen strategies for increasing poultry production to achieve substantial improvements in productivity and profitability by changing from scavenging to semi-scavenging. About 70 % of the rural community in Southern Africa own and keep village chickens. The flavour and texture of their meat is highly preferred. Thus, there is need to preserve indigenous chickens by sustainable utilization of these genetic resources.

The potential role of village chickens in nutrition, food security, and poverty alleviation is recognized. Unfortunately, there have been no comprehensive programmes that support village chicken production. As smallholder farmers depend on rainfed agriculture, changes in the quantity and quality of the scavenging feed resourced (SFRB) change correlates with seasons which may be worse due to climatic changes. Cereal grains rich in energy such as maize, sorghum, millet and their by-products are the major source of feed provided to village chickens. Therefore, protein is limiting in the diet of village chickens.

Feed quality can be drastically reduced by fungi and can become contaminated with mycotoxins that compromise the health status of birds, while also slowing the growth rates of birds. These toxins also pose a public health risk, via direct human consumption of grains and via consumption of poultry meat and eggs. The impact of these contaminants on village chicken production has not been determined and has received little attention.

Use of locally available adsorbents (bentonite, diatomaceous earth) and antioxidants (turmeric) could provide a low cost and readily available source of disease control materials for use in the feed for e village chickens. Therefore, by combining the use of adsorbents, antioxidants and locally-available unconventional protein sources in the feed for village chickens, a systematic approach to feed management emerges and improves opportunities for resource-poor farmers to not only improve rural household and community nutritional and food security, but also to establish agribusinesses to supply organic chicken meat products to the market.

1.2 Objectives

The broad objective of this study was to assess the nutritive value of non-conventional protein sources for chickens and assess sustainable methods to control aflatoxins. The specific objectives were to:

1. Assess farmer perceptions on the availability of feed resources and adaptability to non-conventional available feeds;

2. Assess the nutritive value, and determine amino acid digestibility of cowpeas;

3. Determine the effect of heat treatment and enzyme inclusion of cowpeas and pigeon peas on growth performance and gut health of chickens;

4. Investigate the effect of roasting, extrusion, and enzyme incorporation of black-eyed beans on growth performance and gut health of chickens;

5. Assess the efficacy of Mozambican bentonite and diatomaceous earth in ameliorating the toxic effects of aflatoxins in growing chickens; and

6. Determine the efficacy of adsorbents and tumeric to ameliorate the effects of aflatoxins in chickens.

1.3 Hypotheses

1. Perceptions of farmers to availability of feeds for chickens are likely to be influenced by the availability of feed resources and adaptability to non-conventional available feeds;

2. The nutrition value and amino acid digestibility of cowpeas will vary with cultivars;

3. Heat treatment and enzyme inclusion of cowpeas and pigeon peas will improve amino acid digestibility, growth performance and gut health of chickens;

4. Roasting or extrusion of black-eyed beans, supplemented with exogenous enzymes, will improve performance and gut health of chickens;

5. Inclusion of bentonite and diatomaceous earth ameliorated the toxic effects of aflatoxins in growing chickens; and

6. Inclusion of bentonite, diatomaceous earth and curcuminoids or combinations of the three will ameliorate the toxic effect of aflatoxins in chickens.

CHAPTER 2 - Literature Review

2.1 Introduction

Throughout the world, chickens are produced in diverse and mixed farming systems. They are a source of meat and eggs in human diets at the household and community levels. Also, of all livestock, chickens are a "cash flow" component of the portfolio of agricultural assets for rural households. Compared to other livestock, chickens adapt to diverse climatic conditions, have a relatively low unit price – making it an accessible source of protein for the resource-poor, have a short reproduction cycle, and have a relatively high feed conversion ratio.

Chicken production can be categorized into intensive and scavenging systems. The intensive chicken production system usually is large, commercial scale, involving improved hybrid breeds and relies on a controlled, intensive, confined rearing environment and requires external animal health and feed inputs. The scavenging system dominates in rural communities, where more than 70 % of Africa's population resides. Provision of housing, feeding and veterinary care is limited. Scavenging chickens obtain some of their nutrients from by-products and leftovers of household food consumption (Anjos, 2013).

Indigenous chickens play other valuable roles within households. They can contribute significantly in alleviating poverty, securing food supply and promoting gender equality (Kitalyi, 1998; Guéye, 2000). Women are generally the main owners of chickens and they are also able to make decisions regarding their sales, consumption and gifts to guests (Kitalyi, 1998; Bagnol, 2005; Okitoi *et al.*, 2007; Mlambo *et al.*, 2011). Women and children own most of the chickens and are directly responsible for their management (Mengesha *et al.*, 2008; Okitoi *et al.*, 2007; Sayda, 2012).

Indigenous chickens have attracted attention as a vehicle for rural development (Hailemichael, 2007). There are, however, major constraints to increase productivity. These include high disease prevalences, poor housing and nutritional management, low and irregular availability of feed, and unreliable marketing systems. The objective of this chapter is to give an overview of the constraints facing scavenging chicken production systems, and to suggest potential interventions to strengthen the contribution of indigenous chickens to rural livelihoods.

2.2 Roles and functions of village chickens

The rearing of indigenous chickens is an integral part of the smallholder farming system in the tropics, where they are kept by the rural poor to fulfill multiple functions (Dessie *et al*., 2011). They make a great contribution to rural communities as a source of meat and eggs for home consumption, as a source of income for supporting the livelihood of the family (Alders *et al.*, 2007; Kebede *et al.*, 2012) and there are no cultural or religious taboos connected to production and consumption of chickens (Meseret *et al.*, 2011). In spite of being raised to be sold as a source of income and household consumption they have several roles and functions (Mapiye *et al.*, 2008; Mtileni *et al.*, 2009). Chicken management does not involve a lot of hard labour (Kingori *et al*., 2010). The roles and functions of chickens in communal production systems may vary with locations.

2.2.1 Chicken sales

Chicken sales offer the poor households a pathway out of poverty, income to purchase food items, to pay school fees, and a source of cash for grain milling services, and to purchase improved seed. In Nigeria, Adeniyi and Oguntunji (2011) indicated that sales are one of the major reasons for engaging in rural chicken production. Marketing of indigenous chicken products is one of the few opportunities for poor households to generate income. The sale of chicken products mainly happens informally (Muchadeyi *et al*., 2004; Moges and Dessie, 2010) and when there is an emergency in the household.

Children and women were mainly involved in marketing of live chickens and eggs (Dinka *et al*., 2010; Natukunda *et al*., 2011). Akililu *et al*. (2007) reported that female-headed households in Ethiopia realized smaller poultry sales than in male-headed households. The quantity of poultry which is sold largely depends on the number of birds and the immediate need of cash. The rural indigenous chickens are sold at a higher price than broilers (Mtileni *et al.*, 2013). The reason could be because of the multiple uses of the indigenous chickens including for cultural and ritual purposes as well as food (FAO, 2010). In general, the price depends on the breed of the birds, sex of birds and in some cases age of the birds. Cocks are generally more expensive than hens. Chickens that are used for traditional rituals are also sold at a higher price. The price of chickens also varies among the months of the year. The price of chickens also increases during the holidays (Moges and Dessie, 2010). In Ethiopia, Dana *et al*. (2010) also reported that the market price of chickens is primarily dictated by weight, but farmers rated growth (males) and number of eggs as important factors after weight.

The sale of eggs from indigenous chickens varies from country to country. In Mozambique, for example, eggs are rarely sold. In Ethiopia, the scenario is different, about 70 % of people who own chickens traded in eggs (Moges and Dessie, 2010).

The channel for selling chickens depends on the production and the distance between the point of production and the market system. In remote rural areas, there are more sales between neighbors or the eventual consumer on the main road. Figure 2.1 shows the marketing channel of chickens in Mozambique. Most chicken owners sell their birds directly to consumers along major roads.

2.2.2 Household consumption

Meat and eggs are the main source of animal proteins for poor households (Mtileni *et al*., 2009; Dana *et al.*, 2010). Regarding chicken consumption, the best parts of the chicken carcass are consumed by the male head of the family. Traditionally, children used to eat the less desirable parts such as the neck, head, intestines, wings and shanks while women used to eat what was left by their husbands, or only the gravy without any meat. Nowadays there are fewer restrictions on the chicken portion or part eaten by some household members. Notwithstanding, the wings are still served to children, the legs and liver to women and the gizzard to the father.

Farmers usually produce indigenous chickens with minimum use of inorganic supplements and chemicals indicating that they have the potential to produce organic eggs and meat (Mtileni *et al*., 2013). Thus, indigenous chickens could be an alternative for consumers who prefer low fat and antibiotic or hormone-free white meat (Groom, 1990). Kingori *et al*. (2010) reported that consumer preference for indigenous chicken meat is attributed to the characteristic leaness, flavour, and the presumed organic nature of the product.

2.2.3 Socio-cultural roles

The birds are also given as gifts, sacrificed to ancestors and spirits (FAO, 2010) or according to their phenotype they are consumed as part of a ritual in traditional ceremonies (Dana *et al.*, 2010). Usually, the normal feathered and naked-neck strains are used for home consumption and quick income (household bank) while the frizzle-feathered chickens are required for the traditional ceremonies and social affairs. In certain southern parts of Mozambique,

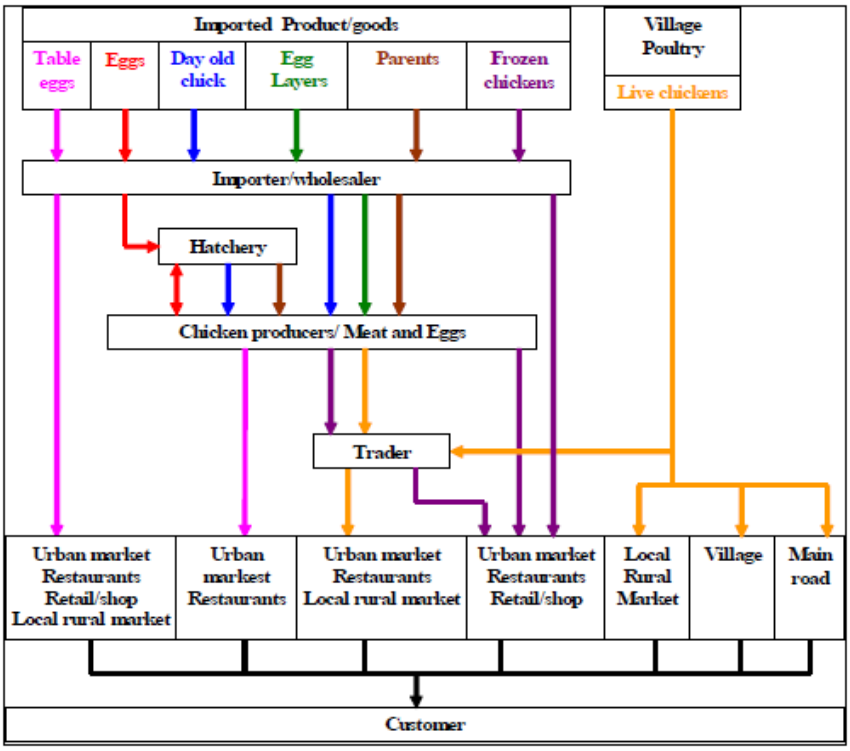

Figure 2.1. Marketing channels of chickens in Mozambique

Source: Anjos (2007)

when babies are born, the elderly put the baby and chicken on the lap to choose the baby's name. If a name is suggested and the chicken keeps quiet then the name is the right one. But if the chicken runs away, another name needs to be selected. On the other hand, people who come to visit the new mother offer a chicken to be raised, not consumed. One more role is the use of this chicken by traditional healers to treat diseases and solve some social problems.

When grandchildren visit their maternal grandmother they run to catch the chicken and they eat what they want. In addition, the grandson has the right to eat the back bones as a symbol of love and continuity of the family, because the back is equivalent to the pelvic region in the human body, considered an important part of the sexual act. In Limpopo National Park Support Zone in Gaza Province, Mozambique, data from Bagnol (2010) indicated that people rarely eat chickens and then only when they have visitors or during festive seasons such as Christmas and New Year.

2.2.4 Self-esteem

In the household, chicken is also seen as an important factors in the development of self-esteem of each member of the family in particular the children's own chickens. If the flock size increases, this means that this family member is lucky and considered in the community as a person who will have a successful future.

2.3 Local availability of chickens

Approximately 80 % of the rural households in Africa keep chickens, particularly the indigenous genotypes (Goromela *et al*., 2006). In Kenya for example, there are 22 million indigenous chichens and they are kept by 90% of rural households in small flocks of up to 30 birds (Kingori *et al*., 2010). The level of production varies with climate, economic, cultural, and social conditions of the households (Muchadeyi *et al*., 2007). Despite the high mortality, the productition rate is relatively high. Dessie and Ogle (2001) in Ghana reported that the egg production per clutch ranged from 15 to 20, with an average of 3.5 clutches per year. Hatchability ranges from 50 to 84% (Msami, 2007; Moges *et al*., 2010).

2.3.1 Adaptation to harsh environmental conditions

Keeping of indigenous chickens by rural communities in Africa has been practised for many generations (Guéye, 1998). Adaptation to the production environment was the most important attribute of the indigenous chickens (Dana *et al*., 2010). The higher adaptability of indigenous chickens to harsh environmental conditions is clearly demonstrated by their presence in remote

areas. Generally, chickens are not housed but live loosely and are exposed to rain and wind. Under the multi stress environment of rural areas they perform relatively well as dual purpose birds, they provide eggs and meat for most households living in rural areas. The chickens are better adapted to living on the ground, where they find most of their natural diet, consisting of worms, insects, seeds, and plants, while their four toed-feet are designed for scratching (Monges *et al*., 2010). Chickens can survive on little or no inputs and adjust to fluctuations in feed availability (Ajayi, 2010). They easily manipulate their ways away from the predators, when compared with commercial chickens, which are not able to escape when attacked by predators. The indigenous chickens are good scavengers, possess good maternal behavior and are relatively tolerant to disease when compared with the commercial breeds. They are able to live together as a flock in a small space, and roosters are polygamous and able to "fertilize" a large number of hens.

2.3.2 Local availability of feed resources

The locally available feed resource is the feed available in the rural households that chickens can consume. The possible feed resources include household waste (kitchen waste and household leftover) and material from the environment which include insects, crop products and by products, fruit plants, wild trees, and grass. This constitutes the main feed input for village chickens (Roberts and Gunaratne, 1992).

The household waste depends on the eating habits of the residents of the household. In Mozambique for example, beyond the maize and vegetables, the use of groundnuts and coconut milk in the diet is very common. The discarded material from groundnuts and coconut milk are used for feeding chickens. When the women in the households grind maize, rice or peanut using a traditional pounder pestle, chickens scratch among the debris that fall to the ground. During the preparation of vegetables or beans the damaged parts or parts with fungal contamination or that are not good for cooking are thrown to the ground and is thus available to the chickens.

Unfortunately, data concerning the feed resource base and the impact of aflatoxin in village chickens are limited. Indigenous chickens feed on what they find in the environment around the houses, such as cereal grains, kitchen wastes, food scraps, green forages, farm products, fruit, and insects. The chickens consume what they find on the ground, often without regard to the hygienic or sanitary condition of the products. Indigenous chickens consume what they can scavenge or are supplementated with such as agricultural foods. Thus, exposure to aflatoxin can occur through either scavenged products or supplemental agricultural food. In the tropics, fungal contamination of kitchen leftovers is widespread (Okoli *et al*., 2006; Muhammad *et al*.,

2010). Bryden (2012) stated that fungi are everywhere and formation of mycotoxins can occur in all agricultural commodities under appropriate field or storage conditions throughout the animal feed supply chain.

The most common method used to determine if birds have consumed aflatoxin-contaminated grains is to analyze crop contents (Deanna, 1990). Several problems are, however, associated with this method. First, crop samples often provide only small quantities of grain to test. Second, because aflatoxins are often highly concentrated in small portions of a load of grain (Deanna, 1999), birds that have been chronically exposed to aflatoxins may not have contaminated grain in their crops at the time of sampling. There is a large variation in the aflatoxin concentration of samples from the lot, and determination of the true aflatoxin concentration in the lot is difficult (Davis *et al*., 1980). This problem could be avoided if aflatoxin consumption could be detected in tissue samples. Herzallah (2013) indicated that AF can be detected in eggs, muscles (legs, breast), and organs (liver, kidney and gizzard). However, the liver is considered the better tissue to use for testing (Deanna, 1999).

A recent approach that has been used in the identification of consumed species is by characterization of DNA present in gut or faecal samples. Stanley *et al*. (2012) showed differences in the microbiota population between low and high performing birds. The conventional sources of energy and protein are expensive. Therefore, there is a need to evaluate alternative energy and protein sources, which can fully or partially substitute for conventional feed for poultry production. Energy sources are usually available in abundance, whereas protein sources are more limiting.

2.4 Potential protein sources for chickens

In the poultry industry, soybeans are the largest source of protein. However, several countries do not have enough quantities to supply the feed industry. On the other hand, rural famers do not have the financial capacity to buy feed for their birds. Thus, it becomes important to look for alternative low-cost products. Crop products such as cowpea, pigeon pea, and novel ingredients such as worms, maggots, could be a good feed alternative for the village chickens.

2.4.1 Cowpea (*Vigna unguiculata* L. Walp.)

Cowpea (*Vigna unguiculata*) is a grain legume produced in tropical and subtropical regions and is a protein source (Tshovhote *et al.*, 2003). Cowpea is an annual legume, commonly referred to as southern pea, black-eyed pea, frijoles or nhemba in Mozambique. Cowpea is one of the most

important sources of protein, carbohydrates, and vitamins in the diet of many populations especially in developing countries (Udensi, 2007). Cowpeas could, however, be also used as a feed for chickens.

The protein content of cowpeas has been reported to ranges from 22 to 26 % (Tshovhote *et al.*, 2003; El-jasser, 2011; Anjos *et al*., 2012). However, the presence of antinutritional factors (trypsin inhibitor, phytic acid and tannis) commonly found in legumes is a major factor limiting its use as a food (Liener, 1976) or chicken feed (Anjos *et al.*, 2012). Fortunately, there are several methods available to reduce or eliminate the anti-nutritional factors, including heat treatment, cooking (boiling), autoclaving, pressure cooking, microwave treatment, extrusion cooking, toasting, soaking, and germination (Akand and Fabiyi, 2010; Anjos *et al.*, 2012).

Several studies on cowpea as feed for chickens have been conducted. Eljack *et al.* (2010) using different levels (0, 100, 200 and 300 g/kg) of raw cowpea in broiler chicks diets observed no significant differences in total and weekly weight gain (g/bird), but it tended to be higher for birds fed the control diet. After six weeks, the body weight gain and feed conversion rate were significantly improved for birds fed all levels of cowpea compared to the control diet.

Chakam *et al*. (2010) assessed the effect of the incorporation level (150, 200, 250 and 300 g/kg) of cooked cowpea on the production performance of broilers in grower-finisher diets. The results showed that there was no significant difference among treatment groups for total feed consumption. Weight gain was significantly lower for birds fed the diet containing 300 g/kg cooked cowpeas and highest for those fed 200 g/kg cowpeas, and there was no significant difference between the birds fed 200 g/kg cowpea when compared with the control birds.

2.4.2 Pigeon pea *(Cajanus cajan*)

The following range of values was obtained for dry matter (95.9 to 96.3 %), crude protein (21 to 21 %), crude fat (4 to 6 %), crude fibre (7.2 to 7.5 %) and ash (3.8 to 4.0 %) respectively, for the raw and roasted seeds of pigeon pea (Akande *et al*., 2010). Amaefule and Obioha (2005) reported that 10% raw or processed (toasted for 30 minutes, boiled for 30 minutes, or soaked in water for 24 hours) pigeon pea can be used in pullet diets without any adverse effects.

The performance of chickens fed pigeon pea at varying levels of dietary inclusion was evaluated. Amaefule *et al*. (2006) reported that pullets fed 20 % boiled pigeon pea meal had a higher daily protein intake and live weight at point of lay. Boiled pigeon pea seeds as a replacement for soybean meal in the diets of broiler chickens was also evaluated by Igene *et al*.

(2011). The results indicated that pigeon pea meal can replace soybean meal protein up to 50% level in the finisher diet and supported growth or total weight gain without any significant depression. However, the dressed weight percentage was lowest in the chickens fed the diet containing 50% pigeon pea meal compared to those fed the control diet.

The effect of roasted pigeon pea seed on chicken performance was also evaluated. Amaefule *et al.* (2011) found that chicks fed 30 or 40 % raw pigeon pea had significantly lower live weight, BWG, protein efficiency ratio and higher feed conversion ratio than those fed the control diet. Amaefule *et al.* (2011) also found that the diet with 30% raw pigeon pea needed to be supplemented with methionine and at 40% raw pigeon pea should be supplemented with both lysine and methionine to improve growth performance of the chicks. These results may be an indication that methionine may have nbeen used to donate sulphur needed for detoxification of ANFs present in the raw pigeon pea diets. Ani and Okeke (2011) indicated that feed intake, weight gain and efficiency of feed utilization declined at the 32.5 % level of roasted pigeon pea inclusion in the diet of chicks. However, 27 % roasted pigeon pea may be incorporated into broiler finisher diets without any deleterious effect on growth performance of broiler birds.

2.5 Challenges to village chicken production

An extensive production system is the dominant practice in village chicken production. High chick loss in the first two months of life is one of the major constraints to poultry production. Farmers in the village reported that disease and predators were the main causes of chick loss. Newcastle disease (commonly known as “Muzungo” in Mozambique) was identified as a major and economically important health constraint, responsible for high mortality. Similar problems were reported in Botswana (Mushi *et al.,* 2006) and in South Africa (Nyoni and Masika, 2012).

In developing countries where Newcastle Disease is endemic, outbreaks regularly result in high mortalities, and in countries where it is not endemic, sporadic outbreaks make vaccination advisable. The implementation of an effective Newcastle disease control program has resulted in increased chicken numbers, increased household purchasing power, increased home consumption of chicken products, and increased decision-making power for women (Copland and Alders, 2005).

External parasites were also mentioned as important constraints and they contribute to poor hatchability, because the hens leave the nest because of parasites. To minimize the problem, the rural families spread ash in the nest. *Ascaridia galli*, *Heterakis gallinarum* and *Syngamus*

trachea (Anjos, 2005) and antibodies to NCD, infectious bursal disease, and infectious bronchitis were also detected in some birds in Zimbabwe (Mushi *et al*., 2006).

Among challenges faced by chickens farmers the most critical were disease problems, feed and water shortages, and inadequate extension service (Mutibvu *et al.*, 2012). Mapiye *et al.* (2008) highlighted shortage of feed, poor health and housing management as the major constraints. Mlambo *et al.* (2011) also showed that village chicken production was mainly affected by feed supply and disease outbreaks.

2.5.1 Seasonality of feed availability

The amount and availability of feedstuffs per bird of the SFRB are dependent on the location, season, farming systems, biomass of the village flocks, and climate (Dessie and Ogle, 2000; Mavale, 2001). In most environments, the SFRB will not provide adequate amounts of nutrients to support the needs for growth or egg production (Dessie and Ogle, 1992; Mwalusanya *et al.*, 2002; Rashid *et al*., 2005).

The physical and chemical assessment of the crop contents is a good indicator to estimate the nutritional value of the scavenged feed. Several studies were conducted to assess the physical and nutrient composition of crop contents of village chickens. The chemical composition of feed eaten by village chickens did not provide enough nutrients to support the needs for growth or egg production (Tadelle and Ogle, 2000; Rashid *et al*., 2005; Momoh *et al*., 2010) and varied with season, climate, location and age of birds (Mtambo, 1999; Tadelle and Ogle, 2000; Mwalusanya *et al.*, 2002; Rashid *et al.*, 2004; Minh *et al*., 2006).

In Tanzania, the most important scavengeable feed resources in the dry season were cereal grains and their by-products, oil seeds and oil seed cakes, and in the wet season were forage leaves, flowers, seeds, garden vegetables, insects, and worms (Goromela *et al*., 2008). In Bangladesh, Rashid *et al*. (2004) reported that crude protein, calcium and phosphorus contents were significantly higher in layer feed compared to grower feed, but other nutrients did not vary significantly and supplementing with protein and energy increased egg production and body weight (Biswas *et al.*, 2005; Okitoi *et al.*, 2009).

The physical composition (proportion of various components) of the crop contents of the village chicken is estimated by the visual observation and differences between countries and within countries can be also observed. In Mozambique, Anjos (2005) observed that the feed ingredients were composed of household waste (maize meal, bones, egg shell, cassava peel, and

yam peel), fruit (maize flour, sorghum, millet) grain and beans (maize, sorghum, millet, cowpea, bambara groundnut). In Bangladesh, Rashid *et al*. (2004) found feed ingredients consisted of household waste (cooked rice, vegetable trimmings and stump, egg shell, stomach and scale of fish) and grain (paddy, rice, and broken rice, maize). In conclusion, the scavenged feed is highly dependent on the surrounding environment.

The amount and availability of the material from the environment available to chickens depend on the location, season, farming systems, biomass of the village flocks, and climate (Dessie and Ogle, 2000; Mavale, 2001; Momoh *et al.*, 2010). During the wet season there are abundant flies, earthworms, locusts, snails, and grass, while in the dry season the most abundant feed resources (FRB) are cereals and cereal by products, roots and tubers, and leaves of trees and shrubs. The amount of kitchen wastes appear to be small in the rainy season indicating that in this period there is less food available in the most rural households compared with the dry period (Goromela *et al.*, 2006).

Sonaya (2012) predicted that climate change will obviously have an impact on the future availability of the feed resources. To cope with this, efforts should be directed to identifying new feed resources and feeding techniques in order to mitigate the negative impacts of climate change on indigenous chicken production systems.

2.5.2 Low quality of feeds and anti nutritional factors

Feed quality is critical to attaining gains from overall improved feed conversion, and improved animal health from ensuring specific nutrients is available to the chicken. In addition, contamination of feed ingredients with fungi during storage if not monitored regularly and corrected, can not only diminish the quality of the feed but can cause animal death.

Plants actively respond to challenges by insect herbivores with rapid induction of biochemical defenses. These defenses can include a combination of secondary metabolites and protein, which may act as toxins, antifeedants (any substance that inhibits normal feeding behavior) or antinutrients (Major and Constabel, 2008). Before the threat to existence by insects, deoxyribonucleic acid (DNA) embeds in the plant seed anti-nutritional factors and other substances as tools to help it survive the riskiest stage of its life cycle. These “tools” can be either acutely toxic (such as lectins, cyanogenic glycosides, non-protein aminoacids), unpalatable (saponins, tannis, non-protein aminoacids, bitter alkaloids), anti-nutritive (non-protein aminoacids, cyanogenic glycosides, isoflavones, alkaloids), or digestion reducers through protease inhibitors such as lectins or oligosaccharides (Enneking and Wink, 2000).

Anti-nutritional factors may also be formed during heat/alkaline processing of protein products, yielding Maillard compounds such as sulphur amino acids, D-amino acids, and lysinoalanine (Gillan *et al*., 2005).

2.5.2.1 Protease inhibitors

Protease inhibitors are proteins of wide distribution in the plant kingdom, and common constituents of legume seeds, with toxicological or anti-nutritional properties. Plants legumes such as soybeans (*Glycine max*), cowpeas (*Vigna unguiculata*) and fava beans (*Vicia faba*), tend to contain higher levels of protease inhibitors. Protease inhibitors alter the normal regulatory process of exocrine pancreatic secretion by blocking the function of digestive enzymes (proteases) in animals leading to malnutrition and other disturbances (Enneking and Wink, 2000).

The protease inhibitors present in soybeans have been extensively studied and are usually used as models for all other plant protease inhibitors. The protease inhibitors found in soybean has been grouped into two main groups, with the first acting mainly against trypsin (Kunitz inhibitor) and the second, (Bowman-Birk inhibitor) specifically against trypsin and chymotrypsin (Leeson and Summers, 2001). Trypsin inhibitor concentration is expressed by trypsin inhibitor units (TIU) per mg dry matter (DM) and can be classified into four groups such as very low activity (2-4 TIU mg^{-1} DM), low activity (4-7 TIU mg^{-1} DM), medium activity (7-10 TIU mg^{-1} DM) and fairly high activity (10-13 TIU mg^{-1} DM) (Mikić *et al.*, 2009). The major negative effect caused by trypsin inhibitor is not the impairment of protein digestion but an excessive secretion of exocrine pancreatic enzymes (Leeson and Summers, 2001). Figure 2.2 shows the mode of action of anti-trypsin factors and nutritional consequences of these factors. Cholecystokinin mediates a number of physiological processes including digestion and is released by the cells located in the mucosal epithelium of the small intestine, stimulated by

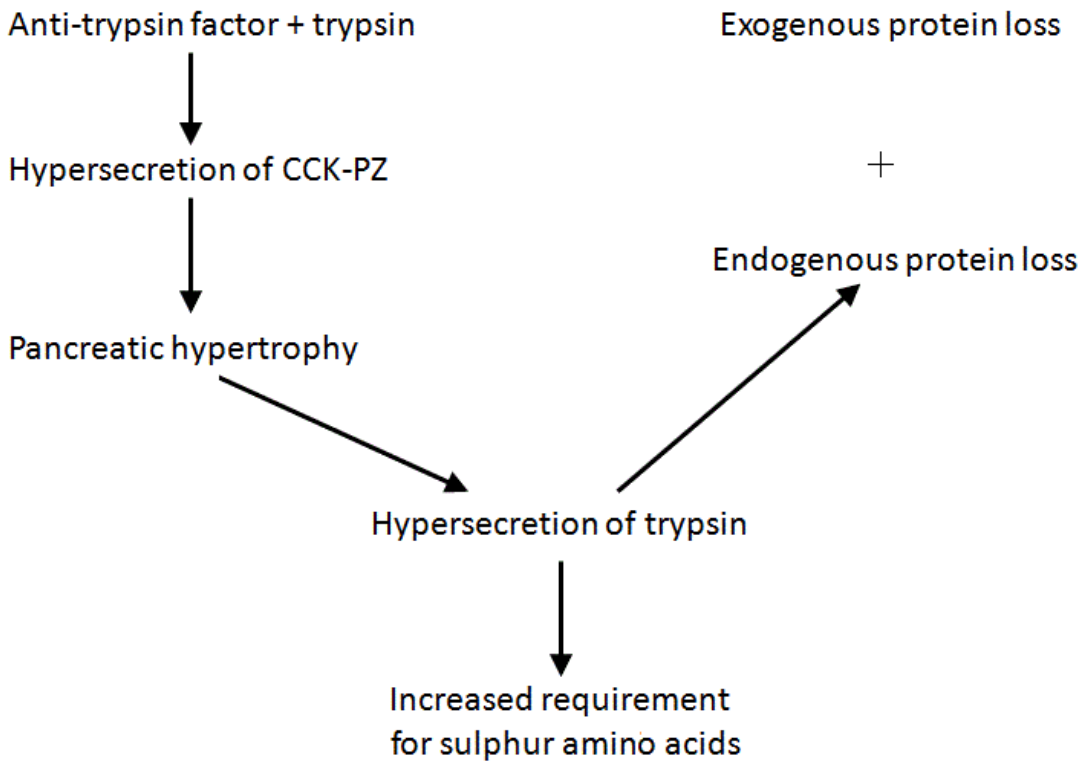

Figure 2.2. Mode of action of anti-trypsin factors and nutritional consequences

Source: Larbier and Leclercg (1994).

monitor peptide released by pancreatic cells. When protein digestion is complete, the monitor peptide is destroyed by trypsin and cholecystokinin. However, in the presence of trypsin inhibitors in the diet the monitor peptide is not destroyed, and the pancreas is continuously stimulated by cholecystokinin. Excessive stimulation of the pancreas causes hypertrophy and hyperplasia.

The protease inhibitors are thermo labile thus their activity is easily counteracted by heating. The degree of reduction or elimination of the proteases inhibitors depends upon the temperature and time of exposure to the heat, as well as the particle size and moisture content of the feed ingredient (Leeson and Summers, 2001). If heating fails to completely destroy the anti-nutritional factors, the result is a negative impact on animal performance. On the other hand, excessive heating reduces the availability of lysine (via the Maillard reaction) and possibly, to a lesser extent, other amino acids. The Maillard reaction, result in the formation of linkage that are resistant to hydrolysis by endogenous digestive enzymes. If the reaction proceeds far enough, the amino acids may become so firmly bound that they are not recovered by acid hydrolysis of the protein (Leeson and Summers, 2001). Urease activity is frequently used as a "marker" to indirectly reflect the presence of anti-nutritional factors in soybeans (American Oil Chemists Society, 1980).

2.5.2.2 Polyphenolic compounds

Polyphenolic compounds are commonly found in both edible and non edible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kahkonen *et al*., 1999). The most relevant in chicken nutrition includes free phenolic acids, polymeric phenols (tannins), and microconstituent phenolics such as gossypol and sapine.

Tannins are polymerised compounds with the greatest anti-nutritional activity and are abundant in sorghum, beans with coloured pericarps, and the hull of rapessed. They are able to precipitate protein present in the raw material or as digestive enzymes (Larbier and Leclercq, 1994). Tannins depress chicken growth performance and feed utilization through forming complexes with proteins and carbohydrates or inhibition of digestive enzymes.

2.6 Contamination of chicken feed resources

The quality and safety of food are factors that determine the market value of the products. Contamination with fungi have been detected in different countries and mostly on agricultural products such as cereals, wheat, rice, maize, cassava, peanuts and sunflower. Many of these fungi produce toxic secondary metabolites known as mycotoxins which are associated with

certain disorders in animals and humans (D'Mello and Macdonald, 1997). Animal feed ingredients and compounded feeds, due to the presence of nutrients and moisture contents, can support the multiplication of fungi at all stages in the food chain, such as production, harvesting, handling, processing, and storage (Manafi *et al*., 2009). Devegowda and Murthy (2005) reported that mycotoxins are one of the factors that may contribute to the reduction of chicken production and product quality. Bryden (2012) also reported that the major problem associated with mycotoxin contaminated animal feed is not only the acute disease but also the metabolic disturbances resulting in poor animal performance.

The most commonly encountered mycotoxins in feedstuffs and foods are the aflatoxins, zearalenone, deoxynivalenol (vomitoxin), and the fumonisins (Richard *et al*., 1993). Mycotoxins in humans were first reported in the Second World War when a Russian soldier showed symptoms of dermal necrosis, hemorrhage, and destruction of bone marrow after having consumed grains contaminated with fungi (CAST, 2003).

2.6.1 Aflatoxins

Aflatoxins (AF) are mycotoxins produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parasiticus* fungi. The fungi responsible are ubiquitous and can affect many dietary staples such as rice, maize, cassava, nuts, peanuts, chilies, and spices (Fufa and Urga, 2010; Adebesin *et al.*, 2001; Kaaya and Eboku, 2010; Hell and Mutegi, 2011). Aflatoxin contamination is influenced by high humidity, high temperatures, insect and rodent activity, and inadequate drying of the crops. Contamination occurs frequently in countries of Africa (Kaaya and Eboku, 2010; Hell and Mutegi, 2011). This contamination can occur at any stage of food production from pre-harvest to storage.

2.6.1.1 Aflatoxin metabolism

Six groups of aflatoxins have been identified based on their fluorescent properties under ultraviolet light, and their chromatographic mobility (Singh, 1995). Aflatoxin B_1 and B_2 produce a blue fluorescence while G_1 and G_2 produce a green fluorescence under ultraviolet light (Figure 2.3). The other two metabolic products of aflatoxins are M_1 and M_2 which occur in milk of lactating mammals that have consumed AF contaminated feed (Murphy *et al*., 2006).

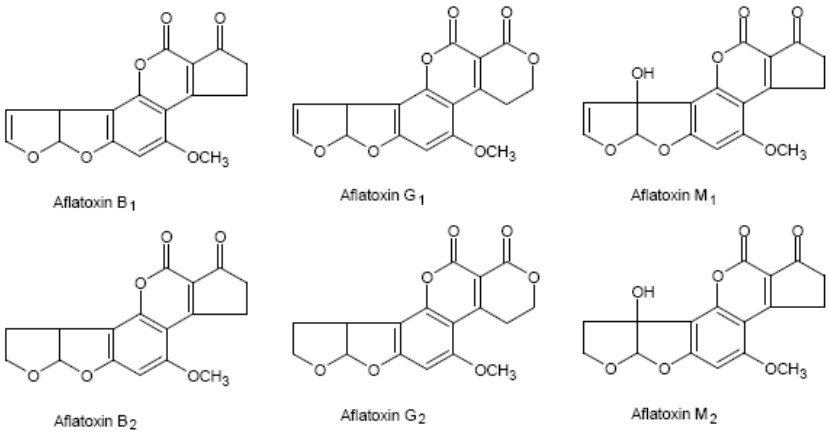

Figure 2.3. Chemical structure of the different aflatoxins

Source: Grace (2013)

The effects of aflatoxins on animals depend on various factors such as genetics, physiological phase, nutritional status, and environmental factors. Fetuses are very susceptible to even low levels of aflatoxin, and young and fast-growing animals are more affected than adults (Grace, 2013).

Aflatoxin B_1 (AFB_1), the most toxic of the AFs, is a potent liver carcinogen, causing hepatocellular carcinoma (HCC) in humans and a variety of animal species (Wu, 2013). Chronic exposure to aflatoxins has also been reported to cause stunted growth (Leroy, 2013).

Aflatoxin B_1 is metabolized by the liver through the cytochrome P450 enzyme system to the major carcinogenic metabolite AFB_1 – 8, 9- epoxide (AFBO) or to the less mutagenic hydroxylated metabolites AFM_1, Q_1, or P_1 (Murphy *et al*., 2006).

There are several pathways that AFBO can take: 1) it can be conjugated to glutathione and excreted; 2) it can be hydrolyzed to AFB1-8, 9-dihydrodiol which can be further metabolized to AFB_1-dialcohol which can then be excreted; or 3), it can readily bind with the N7 position of guanine in DNA forming AFB-DNA adducts, that are responsible for the mutagenic and carcinogenic effects of aflatoxin. Figure 2.4 shows the biotransformation pathways for aflatoxin B_1.

Aflatoxin (AF) causes cell membrane damage through increased lipid peroxidation in laboratory animals (Souza *et al*., 1999; Rastogi *et al*., 2001). Galvano *et al*., (2001) reported that, antioxidant substances such as selenium and some vitamins (A, C and E) can protect against mycotoxin-induced damage by their potential capacity to actas superoxide anion scavengers.

2.6.1.2 Aflatoxins in chickens

Aflatoxicoses have caused economic losses to the poultry industry, affecting ducklings, chickens, and turkeys. The effects in animals vary with dose, length of exposure, species, breed, and diet or nutritional status with younger animals more susceptible than older ones to the toxic effects of AF (CAST, 2003; Quezada *et al*., 2000). Aflatoxicosis in chickens is characterized by reduced feed consumption, reduced body weight gain and poor feed utilization. Bryden *et al.* (1979) reported that chickens fed greater than 1mg AF/kg diet had reduced body weight gain and feed consumption, and poor feed conversion efficiency.

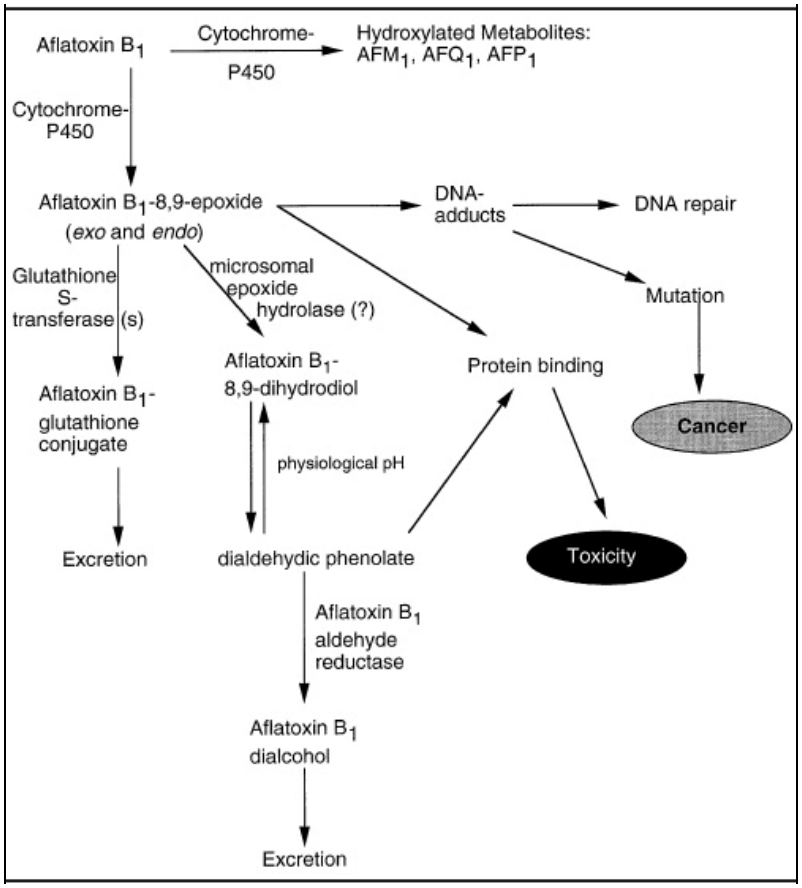

Figure 2.4. Aflatoxin pathways

Source: Bammler *et al*. (2000)

Similar results were found by Huff *et al*. (1986) and Santurio *et al.* (1999) who observed that chickens fed 2.5 mg/kg and 3 mg/kg of dietary aflatoxin had lower weight gains than controls. Quezada *et al*. (2000) also observed that body weight gain of chickens fed 2 mg AF/kg diet showed a marked decrease, compared with those fed an aflatoxin–free diet.

The liver is the target organ of aflatoxin in chickens; however kidney, gizzard and spleen can also significantly increase in relative weight (Dwyer *et al.*, 1997; Miazzo *et al.,* 2005). Gross pathological lesions in birds fed AF included pallor, discoloration of liver, enlargement of liver and kidneys (Hussain *et al.*, 2008), and gall bladder distension (Tessari *et al.*, 2006). Kubena *et al.* (1989) similar to Smith and Hamilton (1970) indicated that chicks fed 5 mg AF/ kg diet had significant increases in the relative weights of liver, kidney, gizzard, and proventriculus. Enlargement of the liver and other organs could be the result of oedema and/or the increase in lipid content (Smith and Hamilton, 1970). These findings are in agreement with Bryden *et al.* (1979) who reported an increase in relative liver weight of chickens fed 1 mg AF/kg diet for more than 2 weeks.

Microscopic changes in the livers of chickens fed AF were also observed. Ortatatli *et al.* (2005) reported slight to moderate hydropic degeneration, small fatty vacuoles in hepatocytes in centrilobular, a small amount of bile-duct proliferation located in portal areas, periportal fibrosis located in portal areas, and periportal fibrosis in livers of chickens fed a diet containing 100 ppb AF. Ergün *et al*. (2006) indicated that histological changes in hepatocytes of chicks fed aflatoxin included increased lipid droplets, high glycogen content, and mild mononuclear cell infiltration in the portal area. The histopathologic changes observed in the liver of birds fed AFB_1 (1.0 mg/kg diet) are similar to those reported by Gowda *et al*. (2008). Gowda *et al.* (2008) also reported decreased antioxidant function in terms of level of peroxides, superoxide dismutase activity, and total antioxidant concentration in liver due to 1.0 mg/kg diet AFB_1. Similar results were found by Che *et al*. (2011) who reported that liver superoxide dismutase activity was reduced, and myeloperoxidase activity increased by the fungal contaminated diet.

Aflatoxin has been reported to have an effect on nutrient metabolism and activity of enzymes that are important for feed digestion and absorption of nutrients. C hicks fed 1.0 mg AFB_1/kg diet had significantly reduced serum total protein, albumin, cholesterol, and calcium levels (Gowda *et al.*, 2008). Rosa *et al.* (2001) fed a 5 mg AF/kg diet to chicks from day one to 22 days and observed that the level of serum total protein, albumin, and globulin decreased. Decreases in the concentration of total plasma proteins, albumin, and globulins have been proposed as indicators of the alteration in protein synthesis observed in aflatoxicosis (Solcan *et*

al., 2013). Pimpukdee *et al*. (2004) also reported that broiler chicks fed aflatoxin had reduced serum vitamin A levels. Yarru *et al*. (2009) concluded that chicks fed 2 mg AFB_1/kg diet had physiological responses associated with altered gene expression in livers of broiler chicks.

2.6.2 Effects of antinutritional factors in chickens

Chohan *et al*. (1993) reported that among chicks fed full fat soybean meal diets, those fed the heat treated soybeans had significantly higher 21 d body weight gain than those fed diets containing the raw soybean or trypsin inhibitor. This indicates that the level of Trypsin inhibitor present in soybean impaired growth.

Palliyeguru *et al*. (2007) showed that although increasing amounts of the non-toasted full-fat soya bean increased the feed intakes of the birds, there was a marked reduction in protein digestibility, weight gain, and feed conversion efficiency. In addition, there was a linear increase in sub clinical necrotic enteritis in the duodenum, jejunum, mid small intestine, and ileum with increasing concentrations of raw soy bean. Mikić *et al*. (2009) reported that trypsin inhibitor (TI) reduces feed intake by diminishing digestion and absorption. In addition, it has been reported that TI also induces pancreatic enzyme secretion, hyper secretion, and the fast stimulation of pancreatic growth, hypertrophy, and hyperplasia.

The adverse effects of soluble non-starch polysaccharides (NSP) on nutrient digestion and absorption are due to their ability to increase the viscosity of digesta, to modify the physiology of the gastrointestinal tract, and to change the ecosystem of the gut (Choct, 2002). Clinical manifestation of dietary NSP in poultry include decreased growth, increased feed conversion, sticky droppings, watery excreta, and pasty vents (Leeson and Summers, 2001)

The effects of replacement of maize with a low-tannin sorghum diets on chicken performance and intestinal mucosa integrity were evaluated. Torres *et al*. (2013) found that feed conversion and weight gain were impaired at day 42 in broilers fed the high-sorghum diet (100 % maize replacement) compared with those fed the control diet and those fed the 50 % inclusion level of the low-tannin sorghum. The intestinal mucosa was negatively affected by the diet containing 50 and 100 % the low-tannin sorghum. Tannins are also responsible for an astringent taste of the feed that induces a lower feed intake due to reduced palatability (Medugu *et al*., 2012).

2.7 Reducing the effects of anti-nutritional factors

To reduce the ANF and improve the nutritional quality of local feed a wide range of processing techniques, such as boiling, toasting, soaking, decortications, autoclaving, have been used, and more recently supplementation with enzymes have also been used (Amaefule and Nwagbara, 2004; Amaefule *et al*., 2006; Babiker *et al.*, 2006; Saeed and Khadiga, 2007, Udensi *et al.*, 2007). It is necessary to develop improved methods for processing feed ingredients so that their potential for use in poultry diets is completely achieved (Akande and Fabiyi, 2010).

2.7.1 Heating and extrusion

Chemical composition, amino-acid profile and anti nutritional factors of different heat treatment (extrusion, cooking-100°C-30 minute, toasting-100°C-30 minute) of soybeans for broiler diets were evaluated. Ari *et al*. (2012) found that Crude Fibre values were highest with toasting (28.3%) while cooking gave the lowest value (12.5 %). For Ether Extract, the highest value was obtained in cooked soybeans (19.5 %) while the lowest t (9.7 %) was obtained in extruded soybeans. Total ash percentages ranged from 4.3 to 4.5 %, while nitrogen free extractive percentage ranged from 13.75 to 26.31 %. Calcium and phosphorus values were highest (1.08% and 0.33 %) in roasted and extruded soybeans, respectively. Eith respect to amino acid profiles (g/100 g protein), methionine values were highest (1.0 and 1.1 %) in cooked and roasted and least in extruded soybeans (0.5 %). A reduction in Trypsin Inhibitor Activity (TIA) due to treatment, was observed) with cooking causing the greatest reduction (85 %), followed by extruded soybeans (61 %). Urease assay values ranged from 0.02 to 0.09 (ΔpH). Alajaji and El-Adawy (2006) also found that boiling and microwave cooking of chickpea caused a slight increase in total essential amino acids, but they were not influenced by autoclaving.

Consumption of raw velvet beans by chickens reduced body weight gain and increased the weight of pancreas, gizzard and proventriculus as well as lengths of the small and large intestines and ceaca. Most of these changes were partial or completely reversed by dry heating/roasting of the raw velvewt beans (Carew *et al*., 2003).

The effect of soybean heat treatment (autoclaving, roasting and microwaving) on the growth performance of broilers chickens was evaluated. The treatments included: T1 (control diet-raw soybean); T2 (autoclaved soyben meal; 121°C, 20 min); T3 (autoclaved soyben meal; 121°C, 30 min); T4 (roasted soyben meal; 120°C, 20 min); and T5 (microwaved 46°C, 540 watt, and 7 min). Tousi-Mojarrad *et al*. (2014) observed higher body weight gain and higher feed conversion rate in chickens fed heat processed soybeans compared with those fed raw soybeans.

2.7.2 Addition of enzymes

Alam *et al*. (2003) reported that body weight of broilers fed diets containing exogenous enzymes was higher than control, with Roxazyme-G best performing enzyme product, followed by Alquerzim and Feedzyme. Dressing yield of broilers fed supplemental enzymes was significantly increased compared to controls. This indicates that the anti-nutritive effects of NSP (containing in diet based on rice poli and soybean meal) on the performance of broilers were overcome by addition of enzymes.

The influence of enzyme supplementation on performance and digestibility of dry matter and protein in young broiler chicks was examined in a diet based on triticale. Pourreza *et al*. (2007) reported that supplemental enzymes significantly improved body weight, body weight gain, feed intake, and feed conversion ratio. The results suggested that the nutritional value of cereal grains such as triticale can be improved by supplemental enzymes (200 g/kg) and in particular xylanase.

2.7.3 Soaking and fermentation

Soaking the cereals in water or adding non-starch polysaccharides degrading enzymes decreases the anti-nutritive activity of the non-starch polysaccharides (NSP). Addition of antibiotics to diets also has been shown to increase the nutritive value of diets containing high levels of NSP suggesting that the action of these materials is, at least in part, mediated by the gut microflora (Annison and Choct, 1991).

Ibrahim *et al*. (2002) found that soaking cowpea for 16 h in a bicarbonate solution caused a remarkable reduction in anti nutritional factors. Cooking pregerminated cowpeas was most effective. Fermentation completely removed trypsin inhibitor, oligosaccharides and significantly reduced phytic acid. However, tannins noticeably increased. Ramakrishna *et al*. (2006) also reported that raw dry Indian bean had a high trypsin inhibitory activity which decreased by 51 % after a 12 h water soaking period and which further decreased to a level of 17 % at 32 h germination. Phytate phosphorus constituted nearly 56 % of total phosphorus in the raw seed and 34 % after germinating for 32 h. Polyphenols concentration was not reduced by soaking.

2.8 Combating contamination of poultry feeds

Avian species especially chicks, goslings, ducklings, and turkeys are susceptible to AFB_1 toxicity (Dalvi, 1986). Unfortunately, data concerning feed resource base and the impact of

aflatoxin in indigenous chickens are limited. Indigenous chickens feed on what they find in the environment around the houses, such as cereal grains, kitchen wastes, food scraps, green forages, farm products, fruit, and insects. The chickens consume what they find on the ground, often without regard for the sanitary conditions of the products. In Africa and other tropical countries, fungal contamination of kitchen leftovers is widespread (Okoli *et al*., 2006 and Muhammad *et al.*, 2010), which may contribute to contamination of indigenous chickens.

Bryden, (2012) stated that fungi are everywhere and formation of mycotoxins can occur in all agricultural commodities under appropriate field or storage conditions throughout the animal feed supply chain. Thus, it will not be wrong to say that feed resource base may be contaminated by fungi and that can be a problem for feed security (Bryden, 2012).

The best way to control mycotoxin formation is to prevent the growth of fungi in feed. Good conditions during harvest, transportation, and storage of the feed are important in preventing the growth of fungi (Daghir, 2008).

Aflatoxin is a recurrent problem in many parts of the world, affecting millions of animals and a major effort is focused on how to neutralize the effects of the toxin. Physical, chemical, and biological methods have been used, with varying degrees of success, to neutralize aflatoxin (Park, 1993). Currently the utilization of non-nutritive adsorbents that bind the aflatoxins in the gut appears to be an effective strategy to reduce toxic effects of aflatoxin.

2.8.1 Adsorbents

Adsorbents are compounds that are not absorbed in the gastrointestinal tract and have the ability to bind physically with mycotoxins, preventing their absorption. It is important to note that the adsorbents are not selective, so they may bind other components of the diet (Leeson *et al*., 1995). Phillips (1999) reported that aflatoxin binding agents should be rigorously tested, paying particular attention to their effectiveness and safety in aflatoxin-sensitive animals and their potential for interactions with critical nutrients.

Activated charcoal, hydrated sodium calcium aluminosilicates, bentonites and diatomaceous earth have been evaluated as adsorbents to ameliorate the toxic effects of AF in chickens. The occurrence of zeolites, bentonites, and diatomite deposits in Mozambique, represent a very valuable material with a wide range of uses, the importance of which is currently increasing (Cílek, 1989).

2.8.1.1 Activated charcoal

Activated charcoal is the residue from the destructive distillation of vegetable origin organic matter, and is porous, low in ash content and high in surface area (Leeson *et al.*, 1995). A study of the toxicity of aflatoxin B_1 in broiler chicks and its reduction by activated charcoal was carried out by Jindal *et al*. (1994). The results showed that adding 200 ppm activated charcoal to a 0.5 ppm aflatoxin B_1 diet reduced the negative effect of AFB_1 on body weights and feed intake. Teleb *et al*. (2004) also reported that addition of 0.5 % activated charcoal to a diet contaminated with 30 μg/kg AFB_1 reduced mortality and improved body weight gain and efficiency of feed utilization. In contrast to these results, Denli and Okan, (2006) found that the addition of 2.5 g/kg activated charcoal to a diet containing 80 μg AFB_1/kg diet did not ameliorate the toxic effect of aflatoxin on chicken performance. Kubena *et al*. (1990) reported that adding 0.5% activated charcoal to the diet did not appear to have protective properties against the effects of 7.5 mg AFB_1/kg of diet.

Khadem *et al*. (2012) evaluated the effects of yeast, zeolite and activated charcoal, alone or in combination, in aflatoxin B_1 contaminated diets on the performance, biochemical traits, and organ weights of broilers by. The results showed that the combination of yeast plus charcoal and zeolite plus charcoal improved feed intake, body weight gain and feed conversion ratio. Furthermore, the final live body weight of the chickens fed the combination of yeast plus zeolite and charcoal diet was higher compared to those of other treatment groups.

2.8.1.2 Hydrated sodium calcium aluminosilicate (HSCAS)

Hydrated sodium calcium aluminosilicate (HSCAS), a sorbent compound obtained from natural zeolite, was demonstrated to sorb aflatoxin with a high affinity (Ramos and Hernández, 1997). Philips *et al*. (1988) reported that HSCAS when added to the diet of chickens at a level of 0.5 % significantly decreased the growth inhibitory effect of 7.5 mg AFB_1 /kg of feed. The friable and pale appearance of livers from the chicks fed 7.5 mg AFB_1 /kg of diet were not observed in chicks fed HSCAS plus7.5 mg AFB_1 /kg of diet. These results indicated that the HSCAS was effective in ameliorating the negative effects of AFB_1.

The efficacy of a hydrated sodium calcium aluminosilicate (0.5 % HSCAS) added to diets containing 3.5 mg/kg aflatoxin and 5.0 mg/kg diacetoxyscirpenol alone and in combination were evaluated. Kubena *et al*. (1993) reported that body weight gain, efficiency of feed utilization, mortality, and oral lesion scores were not significantly influenced by HSCAS in the absence of toxins, indicating that the HSCAS is inert. Kubena *et al*. (1993) also reported that

the reduction in body weight gain caused by 3.5 mg AF/kg of diet was diminished by the addition of 0.5 % HSCAS to the diet. The combination of diacetoxyscirpenol and AF caused a larger reduction in body weight gains of chicks compared with those fed AF alone at all time periods. However the addition of HSCAS to the diet containing a combination of AF and diacetoxyscirpenol diminished the adverse effects on body weight gains. Chung *et al*. (1990) reported that 0.5 or 1 % dietary HSCAS does not impair manganese, vitamin A or riboflavin utilization, but that zinc utilization was reduced slightly as a result of HSCAS ingestion.

The liver is the main organ involved in the detoxification of AFB_1, and also where most residues accumulate. Neeff *et al*. (2013) studied the efficacy of the HSCAS to reduce residual concentrations of AFB_1 metabolites in the liver and kidney of broilers fed AFB_1. The results indicated that the HSCAS was effective in reducing aflatoxin residues in liver and kidney of chicks fed 2.5 mg AFB_1/kg of diet from hatch to day 21. However, the histopathology data indicated that the HSCAS did not prevent or reduce the severity of lesions associated with aflatoxicosis.

2.8.1.3 Bentonite

Bentonite clay carries a uniquely strong negative ionic charge which causes it to "magnetically" attract any substance with a positive ionic charge such as bacteria, toxins, and metals (Hedayati *et al*. 2014). Magandane (2013) reported that Mozambique has a bentonite mine, in Maputo province, Boane District. The bentonite has been mined since 1967 in this place, but its economic and social significance for the country is negligible. The products are supplied to a number of customers throughout South Africa, Zimbabwe and Mozambique but only 25 % is utilized in local industry, mainly for drilling construction and small foundry industries (MIMOC, 2011). Bentonites are efficient at sequestering aflatoxin; they decrease the bioavailability of the toxin in the gastrointestinal tracts of birds when they are incorporated in the diet (Magnoli *et al.*, 2007).

Santurio *et al.* (1999) reported that the addition of 5.0 g sodium bentonite /kg of feed into chicken diet containing 3 mg AF/kg feed improved body weights at 42 d of age by 31.3 %, increased food intake by 23.8 % and improved productive efficiency by 40.1 %. In addition, liver, heart, pancreas and crop weights and biochemical variables were not affected by dietary sodium bentonite. Chickens fed sodium bentonite alone had similar performance to those feed the control diet containing no aflatoxin.

Pasha *et al*. (2008) also reported that chickens fed sodium bentonite at a level of 0.5 % had significantly higher body weight compared with those fed the control diet. Kermashani *et al*. (2009) observed that chickens fed AFB_1 showed sign of aggression, pale colored combs and skin, fluffy feathers, and retarded growth rate at week 3 and later. However, chickens fed sodium bentonite (0.5 or 1 %) alone or and sodium bentonite plus AFB_1 had a normal appearance.

Rosa *et al.* (2001) observed that natural sodium bentonite (0.3 %) from southern Argentina was effective on reducing the bioavailability and the toxic effects of AF (5mg AFB_1/kg diet) in growing broilers chicks from 30 to 52 day of age. No differences on body weight gain were observed between birds fed the control diet and those fed AF plus sodium bentonite, suggesting that sodium bentonite ameliorated the negative effects of AF. Indresh *et al.* (2013) also reported that the addition of 0.75 or 1.0 % bentonite into an AF (0.5 ppm) diet prevented the reduction in body weight, feed intake, and poor feed conversion ratio of the birds fed the AF contaminated diet.

Exposure of chickens to aflatoxin leads to reduce the immunity. Manafi (2012) reported that addition of high grade sodium bentonite to diets containing AF significantly improved antibody titers against ND and IBD vaccine compared to the AF control diet. Indresh *et al.* (2013) observed that serum antibody titres against (ND) and (IBD) vaccination that were significantly depressed in chicks fed AF diet were restored with the inclusion of 1 % bentonite in the AF diet.

Even though all bentonites samples contained predominantly smectites their aflatoxin adsorption capacities differed substantially. Therefore, it is important to assess the properties of the clays to assure effectiveness before they are introduced in animal feed (Velazquez, 2011).

2.8.1.4 Diatomaceous earth

Diatomaceous earth (DE) is a natural occurring siliceous sedimentary mineral formed from microscopic skeletal remain of unicellular algae-like plants called diatoms. The typical chemical composition of oven-dried diatomaceous earth is 86 % silica, 5 % sodium, 3 % magnesium and 2 % iron oxide (Mordirsanei *et al.*, 2008).

The efficacy of DE to reduce the negative effects of AFB_1 in chickens was evaluated. Modirsane *et al*. (2008) reported that dietary inclusion of DE (30 g/kg^{-1}) alone did not affect body weight gain or serum biochemical values. The addition of DE (30 g/kg^{-1}) into the aflatoxin (1 mg/ kg^{-1})

diet, improved feed intake, body weight gain, and feed conversion ratio compared to birds fed AF alone. In contrast, Denli and Okay (2006) found that the addition of 2.5 g/kg diatomite to diets containing 0, 40 or 80 μg AFB_1/kg feed, failed to prevent the harmful effects of AFB_1. The contrasting results could be due to the different concentrations of AFB_1 usedor to the different source of clay used.

Diatomaceous earth has also been reported as having the ability to reduce the incidence of gastro-intestinal parasites. Bennett *et al.* (2011) observed that DE has the potential to control internal (*Capillaria spp, Eimeria spp.* and *Heterakis*) and external parasites (e.g. *Ornithonyssus sylviarum*).

2.8.2 Antioxidants

An antioxidant is a substance that inhibits oxidation or reactions promoted by oxygen, peroxides or free radicals. Effective antioxidants are free radical scavengers that stop radical chain reactions (Huang *et al*., 2005). Free radicals are produced during normal metabolism but can induce body damage if they are present in excessive concentrations (Habibi *et al.*, 2014).

According to Surai (2002), the antioxidant system in the cell is based on three major lines of defence. The first line is responsible for preventing free radical formation by removing precursors of free radicals or by inactivating catlaysts and consists of three antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase. The second line is based on chain-breaking antioxidants that prevent and restrict chain formation and propagation. The last line deals with damaged molecules in the cell as a result of free radical action and is responsible for the repair or removal of the damaged molecules.

Several synthetic and natural antioxidants have been used to ameliorate the toxic effects of mycotoxins. Vitamins (A, E, C) and provitamins (â- carotene, carotenoids), phenolic compounds, curcuminoids, and sulfur containing compounds (glutathione, methionine) are known to exhibit antioxidant action against ochratoxin (OTA) and AFB_1 (Gowda and Ledoux, 2008).

Denly *et al*. (2003) reported that supplementing an aflatoxin diet with vitamin A (15 000 IU/kg feed), increased the body weight of quails by 9.3 % compared with those fed aflatoxin alone. The concentration of liver function enzymes serum glutamic pyruvic transaminase (GPT), serum glutamic- oxaloacetic transaminase (GOT), and alkaline phosphatase (ALP) all increased

when AFB_1 was added to the diet, but partially decreased when vitamin A was added to the AF diet.

There are several herbs that have been shown to have antioxidant properties, including clove oil, turmeric, garlic, and onion (Gowda *et al.*, 2004) because of their ability to act as superoxide anion scavengers against mycotoxin-induced damage (Galvano *et al*., 2001). A study was conducted to assess the effects of ginger root herb and its extracted essential oil on growth performance, antioxidant status, and serum metabolites of broiler chickens under heat stress. Habibi *et al*. (2014) reported that in chickens fed 150 mg/kg ginger essential oil, the total superoxide dismutase (TSOD) activity in liver increased. Malondialdehyde concentrations in the liver also decreased in the groups fed ginger powder (7.5 g/kg) and essential oil compared to those fed the control diet. These data suggest that ginger powder and ginger essential oils may be suitable replacements for synthetic antioxidants in broiler diets.

Turmeric (*Curcuma longa*) is a rhizoma perenial plant in the ginger family (Kim *et al.*, 2013). Curcumin, derived from the rhizome *Curcuma longa*, is one of the primary ingredients in turmeric and curry powders that are used as spices and as a food coloring agent in Asian and Africa countries (Tayyem *et al.*, 2006; Epstein *et al*., 2010). The active ingredient of curcumim is diferuloylmethane, a hydrophobic polyphenol with a yellow colour, having anti-inflammatory, antioxidant, and anti-cancer properties (Epstein *et al.*, 2010).

The efficacy of turmeric alone or combined with adsorbents in reducing the toxic effects of aflatoxin has been evaluated. Gowda *et al*. (2008) reported that chicks fed turmeric powder (TUM) or hydrated sodium calcium (HSCAS; 0.5 %) alone had similar feed intake, body weight gain, and feed: gain when compared with chicks fed the control diet. The addition of 0.5 % TUM (74 mg/kg curcumins) to the AFB_1 (1 mg/kg diet) diet increased feed intake and significantly increased weight gain when compared with chicks fed AFB_1 alone. However, the addition of HSCAS alone or a combination of TUM plus HSCAS into the AFB_1 diet improved chick performance to control values. The level of lipid peroxides increased in livers of chicks fed AFB_1, but supplementation of the AFB_1 diet with TUM or HSCAS, significantly reduced lipid peroxide levels to control values, suggesting antioxidant and protective effects of TUM and HSCAS against AFB_1.

Gowda *et al*. (2009) reported that the addition of 74 and 222 mg/kg total curcuminoids from TUM to an AFB_1 diet significantly improved weight gain and feed efficiency. An increase in the relative liver weight of birds fed AFB_1 was significantly reduced with the addition of 74,

222 and 444 mg/kg total curcuminoids from TUM to the AFB_1 diet. The inclusion of 222 mg/kg total curcuminoids also reduced the adverse effects of AFB_1 on serum chemistry in terms of total protein, albumin and gamma-glutamyl transferase activity. The decreased antioxidant functions due to AFB_1 were also alleviated by the inclusion of 222 mg/kg total curcuminoids. Dietary supplementation of 222 mg/kg curcuminoids to a diet containing 1.0 mg AFB_1/kg diet provided the greatest amelioration of the toxic effects of aflatoxin and demonstrated the highest antioxidant activity.

The effect of aflatoxin on the expression of liver antioxidant genes were evaluated in broilers. Yarru *et al*. (2009) observed that the decreased expression of superoxide dismutase, glutathione S-transferase and epoxide hydrolase genes due to AFB_1 was ameliorated by turmeric powder containing curcuminoids (74 mg/kg). Increased expression of interleukin 6 (IL-6), cytochrome P450 1A1 and 2H1 genes due to AFB_1 was also alleviated by turmeric.

2.9 Summary

Chicken meat and egg are important source of animal protein for the majority of the African population. Chickens production can be divided into two groups which include commercial production and household production. The commercial production with large flocks is mainly for market while the poor smalholder producers keep small flocks for home consumption and for sale during emergencies. Chickens are an important source of protein, income, and play important socio-cultural roles.

Antinutritional factors and aflatoxins causes depression of growth performance, changes in weight of internal organs, and affects gut health. Aflatoxin also contributes to poor growth performance, changes on biochemistry parameters, and changes in the weight of internal organs. Both antinutrional factors and aflatoxins interfere with the availability and utilization of nutrients. Anti-nutritional factors can be reduced by soaking, roasting, microwaving, extrusion, germination, and supplementation with exogenous enzymes. The negative effect of aflatoxin can be also being reduced throught addition of adsorbents and antioxidants to diets contaminated with aflatoxin.

The amount of feed available for scavenging in relation to the carrying capacity of the land areas and flock dynamics across the different seasons and agro-ecological has not been documented. It is important to identify and assess alternative feeds for scavenging chickens. Farmer perceptions on the availability of feed resources and extent of utilization of non-conventional available feeds need to form the foundation of all technologies developed.

There are several locally available feed resources that chickens eat during the scavenging period but households also supplement the diet of the chicks with feed based mainly on maize and household waste or leftover. The nutritive value of the feed ingredients consumed by scavenging chickens also needs to be determined. There are inconsistencies in data on the appropriate processing methods to reduce the negative effects of antinuritional factors. Methods that are applicable to resource-poor communal farmers should be explored.

Contamination of feed ingredient with mycotoxins at all stages of production chain (production, harvesting, handling, processing and storage) may induce health problems and mortality. Therefore, products that can act as aflatoxin binders should be investigated. Finally, understanding of nutrient utilization by the village chickens will provide information on growth rate and feed conversion ratio.

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CHAPTER 3 - Farmer perceptions on the availability of feed resources, and extent of utilization of non-conventional available feeds

Abstract

The objective of the current study was to assess farmer perceptions on the availability and utilisation of non-conventional feed resources for chickens. A structured questionnaire was administered to 240 households in a mountainous locality (Namaacha district), a community near a dam resource (Boane district) and a community with a flat terrain (Marracuene district) in Maputo, Mozambique. Scavenging was the major source of nutrients for chickens. More than three out of four farmers supplemented their chickens with household refuse and cereal and cereal by-products, particularly during the dry season. Two out of five of the respondents experienced feed shortages during the dry season. Nearly 90 % were willing to use novel protein sources (e.g. houseflies, earthworms and snails) as chicken feed. The probability of farmers to house their chickens at night was highly influenced ($P<0.05$) by topography, feed availability and presence of predators. District, level of education, number of adults, and number of children highly influenced ($P<0.05$) a household's likelihood of using kitchen waste. District highly ($P<0.05$) influenced a household's likelihood of experiencing feed shortage. The level of importance of village chickens was influenced $(P<0.05)$ by gender and age. Nine out of 10 households had a huge interest in increasing the productivity of their chickens. Increasing chicken productivity requires developing sustainable feeding strategies for the chickens.

Keywords: Village chickens, feed resource base, non-conventional feed resources.

3.1 Introduction

Rural farmers own over 90 % of the national chicken flock, which is estimated to be around 25 million birds (International Rural Center, 2010). Village chickens play a vital role in many rural and peri-urban households, as a source of meat, eggs and income. They also mitigate the impacts of the acquired immune deficiency syndrome (AIDS) (Alders *et al.*, 2007; Moreki *et al.*, 2010). Despite the crucial role these chickens play, their contribution to the national gross domestic product is low. The high prevalence of Newcastle disease and feed shortages are the most commonly cited challenges that face village chicken producers (Woolcock *et al.*, 2004).

Village chickens get their feed largely through scavenging feed resources around the homesteads. The quantity and quality of the scavenging feed resource base (SFRB) is seasonal and, at times, may be inadequate to meet nutrient requirements for meat and egg production

(Woolcock *et al*., 2004; Momoh *et al*., 2010). Chickens are, thus fed rotten agricultural produce and kitchen left-overs. Thus, exposure to aflatoxins can easily occur. Fungal contamination of kitchen leftovers is widespread in tropical countries (Okoli *et al*., 2006; Muhammad *et al*., 2010). Mycotoxins can develop when the grain is still under field or storage conditions and throughout the animal feed supply chain (Bryden, 2012). The impact of aflatoxins on the health and performance of the chickens, and, consequently, household food security, is not fully understood.

To sustainably increase village chicken productivity and increase the contribution of chickens to household food security and the national economy (Mapiye *et al*., 2008), it is important to establish farmer perceptions on appropriate feeding strategies for village chickens. Utilisation of non-conventional feeds resources has the potential to facilitate the production of organic chicken products that fetch premier prices in identified niche markets. Therefore, the objective of the current study was to establish farmer perceptions on the availability and extent of utilization of available non-conventional feed resources among resource-poor farmers.

3.2 Materials and methods

3.2.1 Study sites

The survey was conducted in Namaacha, Boane and Marracuene districts of Maputo province. Naamacha district lies 80 km west of Maputo on the border with Swaziland. It is located in the Lebombo Mountains. The range of the mountains is relatively low with altitude between 450 and 800 m above sea level. The district has a total land area of 2.196 km^2 (INE, 2011). The climate is tropical humid. There are predominantly two two seasons: hot and high rainfall between October and April; and the fresh and dry, between April to September. The average annual rainfall is 751.1 mm and the average temperature is 21°C. Main sources of water are the Movene, Mabenga, and Calichane, Impaputo and Umbeluzi rivers as well as the Lebombo dam. Agriculture is the main economic activity in the district. The main horticultural crops grown are maize, groundnuts, beans (*Vigna unguiculata* and *Phaseolos vulgari*s), sweet potatoes, cassava, peanuts, maize, and fruits. The predominant livestock species are cattle, goats, sheep, chickens, ducks, and pigs. Michangulene lies at 26 17' 29" S and the 32' 11 and 23" E and at an altitude of 94 m. The annual temperature is approximately 28°C. The annual precipitation varies between 600 and 800 mm. The region is largely semi-arid.

Boane district (40 km from the city of Maputo) is located at 26°.02'S and 32°17'E. The district has a total land area of 820 km² and population about 102 457 residents, which corresponds to a population density of 124.9 habitants per km² (INE, 2011). The climate is sub- humid with the hot and wet seasons experienced between November and March, while the dry season occurs from April to October. The average annual rainfall is 752 mm and the annual average temperature is 23.7°C with a maximum relative annual humidity of 80.5 %. The water courses of Boane belong to the hydrographic basins of Umbeluzi, Matola and Tembe rivers. The southern area is covered by the rivers, benefits from irrigation and low humidity, being able to grow vegetables, bananas and citrus. The main crops grown are maize, cassava, beans, bananas, and citrus. Livestock such as cattle, sheep, and poultry are produced for household consumption and sale.

Marracuene is located 30 km north of Maputo. The climate of the district is rainy tropical savanna, influenced by the proximity of the sea. It is characterized by warm temperatures with an average annual value of more than 20°C with relative humidity ranging from 55 to 75 %. The rainfall is moderate, with an annual average of 500 mm. The rainy season lasts from October to April, with 60 % of the rainfall received between December and February. Agriculture is the economic base of the district. The main crops cultivated are rice, maize, cassava, sweet potato, and bananas. The predominant species of livestock are cattle, sheep, and poultry.

Namaacha district represented households in mountainous locations, while households interviewed in the Boane district were located around a dam that supplies water for both domestic and household use. Marracuene district has a generally flat terrain.

3.2.2 Sampling procedure

Data were collected using diagnostic surveys. For each district, three villages and 10% of households of each village were randomly selected. Pre-tested structured questionnaires were administered to a total of 240 households across the three districts. In each district, 80 individual households were selected randomly.

3.2.3 Data collection

To assess the farmer perceptions, participatory rural appraisal (PRA) and structured questionnaires were used. The questionnaire covered aspects of household demography, the

chicken breeds used, flock sizes, chicken management, and utilization of non-conventional feed resources. The interviews were conducted in the vernacular language.

To collect primary data of village chicken production, importance, and challenges to rearing village chickens, face-to-face interviews with nine key informants were conducted. The key informants were management from the International Rural Poultry Centre, personnel from the technical department in the National Extension Service, extension officials, district livestock technicians, and community leaders. Transect walks were conducted in the villages. Data were also collected through direct observations. Participatory mapping and seasonal calendars were used to gather data on challenges facing village chicken production.

Using groups of fairly homogenous farmers, in each district, resource maps showing the general conditions of the village and its environment including farming fields, grazing lands, dams, major roads, common market places, schools and other major community resources were prepared. Seasonal calendars were used to gather information on the availability of feed and problems that occur in each season. Data on commonly used non-conventional feed resources and their availability were collected from each group. Feed calendars were produced to illustrate the changes in the scavenging feed resource base.

Focus group discussions were conducted to assess the importance and availability of feed resources. Opportunities for village chicken production were assessed. The major challenges facing village chicken production in each community were ranked.

3.2.4 Statistical analyses

All data were analysed using SAS (2008). The PROC FREQ procedure was used to determine differences among districts, season, type of feed, dietary supplementation; frequency, availability of feed and feed shortages. The general linear models procedure was used to compare productivity of the chicken breeds in each village. An ordinal logistic regression (PROC LOGISTIC) was used to predict the odds of a household, regarding the level of importance of village chickens, to experience chicken feed shortages, to house chickens at night, to use kitchen waste and maize bran. The variables fitted in the logit model included district, age, gender, level of education, number of adults, number of children, feed, lack of market, prevalence of diseases and parasites and the level of predation. The model used was:

$\text{Ln } [P/1-P] = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_t X_t + \varepsilon$

Where:

P is the probability of level of importance of village chickens experiencing chicken feed shortages, housing chickens at night, feeding chickens with kitchen waste and maize bran;

[P/1−P] is the odds of (household saying village chickens are important; experiencing chicken feed shortages; housing chickens at night, feeding chickens with kitchen waste and maize bran);
β_0 is the intercept;
$\beta_1 \ldots \beta_t$ are the regression coefficients of predictors;
$X_1 \ldots X_t$ are the predictor variables;
ε is the random residual error.

3.3 Results

3.3.1 Household characteristics and flock composition

Seven out of ten households 70 % were female. The household characteristics are shown in Tables 3.1 and 3.2. Chicken flocks were composed of three main breeds, normal feathered, naked-neck and frizzle. The normal feathered chicken was the most popular with higher flock size, compared with naked-neck and frizzle.

The importance of village chickens is shown in Table 3.1. Farmers were asked whether the chickens are not important, important or highly important. None of the farmers regarded chickens as not important. Important means low ranking, while highly important means ranked number 1 amongst other species kept. Among the households interviewed, the majority of them said that chickens are very important. Another large group of households reported that chickens are important. In Namaacha and Marracuene districts, the majority of interviewed households reported that chickens are very important, while for the majority of households in Boane chickens were important. The main purpose for keeping village chickens is meat consumption followed by as a source of income. The results showed that 90.3 % (n = 177) of all respondents (n = 196) kept the normal feather breed chickens for home consumption. All breeds are not kept for egg production, but are mainly preserved for reproduction.

Table 3.1. Characteristics of household demography, importance of chickens, flock composition and chicken productivity in Maputo

	Namaacha	Boane	Marracuene
Household demography			
Proportion of females (%)	71.2	71.6	66.2
Number of children	2.1	2.5	2.4
Number of adults	3.34[ab]	3.9[a]	3.19[b]
Importance of village chickens			
Important	46.48	49.38	38.37
Higly important	53.52	48.15	59.30
Chicken flock composition			
Cocks	15	10	10
Hens	21	22	40
Growing chickens	50	15	20
Chicks	20	13	20
Chickens productivity			
Eggs per clutch	15	20	10
Average eggs per clutch	14.5[a]	13.1[b]	13.9[ab]
Number of clutches per year	3.0[a]	3.1[a]	3.2[a]
Eggs incubated per clutch	12.6[a]	12.1[a]	13.3[a]
Eggs hatched per clutch	10.7[a]	10.6[a]	11.4[a]
Chicks weaned per hen per clutch	7.7[a]	7.9[a]	8.6[a]

[abc] Values in the same row with different superscripts are different ($P<0.05$).

Table 3.2. Management of village chickens in Namaacha, Boane and Marracuene districts

	Namaacha	Boane	Marracuene

Common supplementary chicken feeds			
Kitchen waste (%)	14.3	27.4	30.0
Household leftover (%)	34.3	28.8	17.5
Crushed maize grain (%)	35.7	17.8	7.5
Maize bran (%)	5.7	17.8	33.8
Provide water to the chickens (%)	1.3	23.8	10.8
Household providing feeds to chickens			
All chickens together	80.0	74.3	64.9
Only chicks	0.0	2.7	2.7
Chicks and hens	20.0	23.0	32.4
Experiencing feed shortages (%)	15.6	25.7	15.6
Housing practices			
Housed during the night	67.1	40.5	48.2
Never housed	30.1	58.2	50.6

The key informants reported that, village chickens are an integral component of all households (resource-poor). Increased production has the potential to improve food security and assist in poverty alleviation in rural populations. The constraints to the improvement of village chickens are the frequent outbreaks of Newcastle disease and feed scarcity. In general, vaccination campaigns were highly successful. No support programs are, however, in place to address productivity issues of the chickens after the diseases has been controlled. Feed supplementation and housing should be considered.

The odds of farmers regarding village chickens as either important or very important (Table 3.3) were influenced by gender and age. District, level of education and the number of adults did not affect the relative importance of the chickens. Female farmers were 1.07 times more likely to consider chickens more highly important than male farmers. Adults farmers were 1.6 times more likely to consider chickens highly important than young farmers. For each unit increase in number of adults the odds of households considering village chickens as highly important increased by 1.3.

3.3.2 Chicken housing

About half of the interviewed households housed chickens during the night. Table 3.4 shows odds ratio estimates of households housing chickens at night. The probability of farmers housing chickens at night was influenced ($P<0.05$) by topography, availability of feed, and predators. Common predators were snakes, simba, dogs, and wild birds. The farmers in Boane district were 1.3 times more likely to house their chickens during the night than farmers in flat zones. Farmers in Namaacha were 2.4 times more likely to house their chickens during the night. Farmers with no feed constraints were highly likely ($P<0.05$) to house chickens at night than those facing feed shortages.

3.3.3 Feed sources and shortages

Table 3.5 shows the common scavenging feed resource base (SFRB) available in the three districts of Maputo. The major SFRB are insects and ants, followed by cereal and cereal by-product and household waste/leftovers (mostly during the dry season). During the wet season there was an abundant supply of flies, earthworms, and grass while in the dry season the most abundant SFRB are cereals and cereal by products, roots and tubers, fruits, insects, and leaves of trees and shrubs.

Table 3.3. Odds ratio estimate, lower (LCI) and upper confidence (UCI) of importance of chickens

Predictors	Odds ratio	Lower CI	Upper CI	Significance
Topography (flat versus dam[1])	1.20	0.41	1.49	NS
Topography (dam versus mountain)	2.10	0.47	1.80	NS
Age[2]	1.68	0.88	2.93	*
Gender (female versus male)	1.07	1.50	1.71	*
Education (no versus yes)	1.69	0.917	3.11	NS
Number of adults	1.13	0.993	1.30	NS

The higher the odds ratio the stronger the predictor of farmers saying village chickens are important CI: confidence interval; NS: not significant (*P>0.05*); * *P<0.05*

[1]Topography; dam = Boane distrist were there is a hydrographic basis of Umbeluzi (Dam on the little Lebombo Boane); mountain= Namaacha distrit; flat = Marracuene district a flat terrain (crossed by the river and by the sea side)

[2]adults >35 years versus youth ≤35 years

Table 3.4. Odds ratio estimate, lower (LCI) and upper confidence (UCI) of housing chickens at night

	Housing at night			
Predictors	Odds Ratio	Lower CI	Upper CI	Significance
Topography (flat versus dam[1])	1.33	1.67	2.61	*
Topography (dam versus mountain)	2.47	1.19	5.13	*
Age[2]	1.10	0.58	2.07	NS
Gender (female versus male)	1.69	0.88	3.24	NS
Education (no versus yes)	1.29	0.67	2.48	NS
Number of adults	1.13	0.74	1.03	NS
Number of children	1.02	0.88	1.18	NS
Feed (no versus yes)	1.47	1.83	2.63	*
Lack of market(no versus yes)	2.45	0.10	1.52	NS
Disease prevalence(no versus yes)	1.43	0.67	3.05	NS
External parasites (no versus yes)	1.03	0.45	2.37	NS
Predators (yes versus no)	1.17	1.65	2.11	*

The higher the odds ratio the stronger the predictor of farmers house chickens at night or not housing. CI: confidence interval; NS: *P>0.05*; * *P<0.05.*

[1]Topography; dam = Boane distrist were there is a hydrographic basis of Umbeluzi (Dam on the little Lebombo Boane); mountain= Namaacha distrit; flat = Marracuene district a flat terrain (crossed by the river and by the sea side)

[2]adults >35 years versus youth ≤35 years

Table 3.5. Common scavenging feed resource base available in Maputo

		Namaacha (%)		Boane (%)		Marracuene (%)	
SFRB	n	Dry	Wet	Dry	Wet	Dry	Wet
Household waste	186	41.86[b]	4.65	23.44[a]	0.00	31.65[ab]	0.00
Household leftover	186	17.1	29.3	18.0	13.1	32.1	1.3
Cereal/by-product	174	24.2	9.7	24.6	0.0	36.4	1.8
Roots and tubers	49	33.3	66.7	34.8	4.4	21.7	13.0
Tree leaves	37	42.7[b]	57.1	20.0[a]	20.0	35.0[ab]	5.0
Fruits	32	12.5	37.5	33.3	0.0	27.8	33.3
Insects	150	28.0	22.0	25.5	14.9	22.6	39.6
Locusts	77	29.2[b]	29.2	11.1[a]	18.3	23.1[b]	42.3
Earthworms	96	33.3	33.3	37.0	14.8	29.2	47.9
Snails	33	22.2	55.6	12.5	25.0	31.3	31.3
Ants	105	62.5[b]	0.0	25.0[a]	10.7	22.6[a]	13.2
Flies	52	6.3[b]	81.3	0.0[a]	22.2	14.8[b]	51.9
Aquatic plants	5	00.0	00.0	0.0	0.0	75.0	0.0
Grass	113	37.5[c]	54.2	6.3[b]	50.0	8.8[a]	14.0

[abc] Means in the same row with different superscripts are different (*P<0.05*).

The availability of household waste was higher ($P<0.05$) in the dry season compared with the wet season. In contrast, household leftovers did not show a significant difference between the two seasons. Chickens received feed supplement (cereals and by-products) which are rich in carbohydrates and energy. These sources are, however, unbalanced and are deficient in other nutrients, particulary protein to fill the needs of the chickens for maintenance and production.

Households are ready to expand their chicken enterprises. The households were also asked about the use of flies, earthworm and snails as sources of proteins for chickens.Nearly 9/10 households had no reservations for using non-conventional animal protein sources as chicken feed. Those few households who did not prefer using non-conventional protein sources felt that they are difficult to harvest, and that they may harbour diseases that could be transmitted to humans.

Even though scavenging was the major feeding system, the majority of the households interviewed responded that chickens scavenge around and they provided feed supplements with maize, crushed maize, maize bran, and kitchen waste. Farmers also reported that supplementary feed is given to chickens of different age groups together, and the rest of the farmers interviewed provide feed only to chicks and hens.

The staple food in the three districts is maize, rice, cassava, groundnut and coconut milk with vegetables, meat/fish or beans. The major supplementary feeds are shown in Table 3.1. Household leftovers are the major source of feed supplements, followed by kitchen waste and crushed maize grain and maize bran. It was observed that the debris from the traditional grinding of maize, rice or peanut is also popular with chickens.

The by-products coming from the processing of maize (maize bran) and rice (rice bran) are stored and use as supplementary feed for the chickens. Leaves of cassava, sweet potato and beans are used as vegetable mixed with coconut and groundnut milk. Cassava roots and sweet potato are eaten boiled, roasted or cooked with vegetables and groundnut. The groundnut and coconut residues from the milk preparations are discarded and given to the chickens. Most often, the kitchen scraps are constituted by part of the foods that are spoiled with mould or for example damaged beans that are not used in human consumption.

District, level of education, number of adults and number of children influenced ($P<0.05$) a household's likelihood of using kitchen waste (Table 3.6). Households in Boane were 4.1 times more like more likely to use kitchen waste than those in Namaacha. Educated farmers were 3.3

times more likely to use kitchen waste than uneducated farmers. When the number of adults increased, the households were more likely to use kitchen waste. For each unit increase in the number of children, the odds of using kitchen waste increased by 1.3.

Table 3.7 shows the odds of households experiencing feed shortages. Households in mountain zones were more likely to use maize bran for feeding chicken than those near dams (odds ratio of 8.26). Educated farmers were 3 times more likely to feed chickens with maize bran (odds ratio 3.01). Topography highly influenced a household's likelihood of experiencing feed shortage. Age, gender of head of household, and level of education had no influence on experiencing feed shortages ($P>0.05$). The majority of the households in the three districts provided water for the chickens. Households in Boane were, however, less likely to provide water than those in Namaacha and Marracune. Nearly 30 % of the households did not provide water to their chicks.

3.3.4 Predation

Farmers who faced a high challenge of predation were highly inclined ($P<0.05$) to house chickens at night than those without the challenge. Snakes and dogs were the most common predators of eggs followed by simba and mongoose. Half of the respondents revealed that wild birds are highly responsible for chick losses. Chick loss from snakes and simba was lower ($P<0.05$) in Namaacha compared with Boane and Marracuene. The biggest ($P<0.05$) predators for growing chickens were simba and snakes in Boane and Marracuene. Adult birds were less affected by snakes and simba. Dogs, however, were the major predators in Namaacha district.

3.3.5 Chicken productivity

The production and reproduction performance of village chickens are shown in Table 3.8. There were differences in production and reproductive performance among breeds. The eggs per clutch per normal feathered hen in Boane were significantly lower compared with hens in Namaacha district but similar to those in Marracuene district. Hatchability varied among the three breeds and among the districts. On average, the hatchability was 85.9, 82.3 and 91.4 % for normal-feathered, naked neck, and frizzled chickens,

Table 3.6. Odds ration estimate, lower (LCI) and upper confidence (UCI) of houssehould feeding chickens with kitchen waste and maize bran

Predictors	Kitchen waste				Maize bran			
	Odds Ratio	Lower CI	Upper CI	Significance level	Odds Ratio	Lower CI	Upper CI	Significance level
Topography (flat versus dam[1])	1.01	0.49	2.09	NS	2.17	0.98	4.81	NS
Topography (dam versus mountain)	4.14	0.20	0.91	*	8.26	0.06	0.90	*
Age[2]	1.11	0.44	1.80	NS	1.66	0.26	1.36	NS
Gender (female versus male)	1.27	0.38	1.60	NS	1.36	0.32	1.66	NS
Education (no versus yes)	3.33	1.28	1.70	*	3.01	0.28	1.53	NS
Number of adults	1.13	0.72	0.94	*	1.10	0.72	1.11	NS
Number of children	1.30	1.11	1.80	*	1.03	0.85	1.23	NS

The higher the odds ratio the stronger the predictor of farmers using kitchen waste and maize bran; CI: confidence interval. NS: not significant (*P>0.05*); * *P<0.05*

[1]Topography; dam = Boane distrist were there is a hydrographic basis of Umbeluzi (Dam on the little Lebombo Boane); mountain= Namaacha distrit; flat = Marracuene district a flat terrain (crossed by the river and by the sea side)

[2]adults >35 years versus youth ≤35 years.

Table 3.7. Odds ratio estimate, lower (LCI) and upper confidence (UCI) of households experiencing feed shortage

Predictors	Odds ratio	Lower CI	Upper CI	Significance
Topography (flat versus dam[1])	1.34	0.36	1.53	NS
Topography (dam versus mountain)	4.60	0.20	0.92	*
Age[2]	1.32	0.39	1.45	NS
Gender (female versus male)	1.28	0.64	2.54	NS
Level of education (no versus yes)	2.10	1.05	4.18	NS
Number of adults	1.14	0.75	1.01	NS
Number of children	1.08	0.92	1.26	NS

The higher the odds ratio the stronger the predictor of farmers experiencing feed shortage CI: confidence interval. NS: not significant (*P>0.05*); * *P<0.05*

[1]Topography; dam = Boane distrist were there is a hydrographic basis of Umbeluzi (Dam on the little Lebombo Boane); mountain= Namaacha distrit; flat = Marracuene district a flat terrain (crossed by the river and by the sea side)

[2]adults >35 years versus youth ≤35 years

Table 3.8. Productivity of village chicken breeds in Maputo districts

Variable	Breed	Namaacha	Boane	Maracuane
Eggs per clutch	Normal	14.5 ± 0.64[c]	13.1 ± 1.21[b]	13.9 ± 0.43[a]
	Nacked-neck	1.8 ± 1.00[a]	13.0 ± 1.80[b]	17.5 ± 2.5[b]
	Frizzle	4.4 ± 0.92[b]	10.5 ± 0.37[a]	12.8 ± 1.60[a]
Number of clutches	Normal	3.0 ± 0.15[a]	3.1 ± 0.12[a]	3.2 ± 2.30
	Nacked-neck	0.4 ± 0.24[b]	2.5a ± 0.57[a]	2.5 ± 2.30
	Frizzle	1.04 ± 0.25[b]	3.0 ± 0.47[ab]	3.7 ± 2.30
Eggs incubated per clutch	Normal	12.6 ± 0.97[a]	12.1 ± 0.43	13.3 ± 0.41
	Nacked-neck	1.7 ± 0.95[b]	13.0 ± 1.80	17.0 ± 2.30
	Frizzle	3.3 ± 0.91[b]	10.0 ± 1.37	12.8 ± 1.50
Eggs hatched per clutch	Normal	10.7 ± 0.54[a]	10.6 ± 0.41	11.4 ± 0.36
	Nacked-neck	1.4 ± 0.86[b]	11.5 ± 1.70	13.0 ± 2.80
	Frizzle	3.0 ± 0.79 [b]	9.4 ± 1.2	11.3 ± 1.60
Chicks weaned/hen/clutch	Normal	7.7 ± 0.42[a]	7.9 ± 0.49[a]	8.6 ± 0.46
	Nacked-neck	0.6 ± 0.65[c]	7.8 ± 1.90[b]	11.5 ± 2.6
	Frizzle	2.0 ± 0.59[b]	6.5 ± 1.40[a]	7.7 ± 2.10

[abc] Values in the same column with different superscripts are different (*P<0.05*).

respectively. The chicks weaned per hen were, on average, 74, 66.9 and 67.9 % for normal-feathered, naked neck, and frizzled chickens, respectively. The number of normal feathers chicks weaned per hens was higher in Marracuene compared with Boane and Namaacha, but in Boane was higher compared with Namaacha. Hatchability and chicks weaned per hen from eggs of frizzle hens was higher compared with naked-neck hens. The major challenges in poultry farming included outbreak of diseases, predators, theft, and shortage of feed.

3.4 Discussion

Women make significant contributions to the rural economy in all developing countries and in Sub-Saharan Africa represent 50 % of the agriculture labour force (FAO, 2011).The observation that 70 % of the heads of households were female could be because males were mostly at work. Most of the farming activities were, therefore, performed by women, including keeping village chickens. In the present study, most men are employed on private farms, in towns or they emigrated to Swaziland or South Africa.

Crop and livestock production contribute to food security and household income. These results agree with Muchadeyi *et al.* (2007) who reported that 18 % of the households ranked livestock as the major source of income and 71 % ranked crops as the main contributor.

Chicken production is based on local village chicken breeds. Moges *et al.* (2010) reported that in Bure district, Ethiopia, scavenging made up 83% of the local chicken ecotypes. Mlambo *et al.* (2011) also reported that a free range backyard production system was predominant (95 %) among the households. The flock sizes varied among the breeds. Mengesha *et al.* (2008), in Ethiopia, observed an average flock size of 5.6 per household. In Mozambique, Mabunda-Matola (2003) also reported that the majority of households in communal areas keep between 6 and 15 chickens. In the present study, the average flock size per household was 7.

The present study indicated that hen's produce on average 7 to 30 eggs per clutch with a maximum of 2 to 3 clutches/hen/year. As a result, the total number of eggs produced ranged from 14 to 90 eggs/year/ hen. Dessie and Ogle (2001) in Ghana reported that the average egg production per clutch was 15 to 20, with 3 to 4 clutches per year. Hatchability and rate of chick survival are the major determinant factors of productivity in chickens (Habte *et al*., 2013).
In the present study, hatchability varied among the breeds and location. The hatchability of the three village breeds (normal feathers, naked-neck and frizzle) averaged 86 %, with the frizzle breed having the highest hatching rate. In Ethiopia, Zewdu *et al.* (2013) reported an average egg hatchability of village chickens of 85 %. Islam *et al*. (2008), in Bangladesh, found different

hatchabilities among Fayoumi (78 %), White Leghorn (76 %) and Rhode Island Red (76 %) breeds. Habte *et al*. (2013), in Ethiopia, also observed that the hatchability of local chickens was higher (83 %) than commercial breeds (44 %) under village conditions. The highest hatchability was reported for Boane compared with Marracuene and Namaacha. The differences in hatchability could be related to differences in the availability of feed resources. Kingori (2011) argued that feed and water provided in close proximity to the hen will keep her in good condition and reduce embryo damage due the cooling of the eggs if she has to leave the nest to scavenge for feed.

The percentage of chicks weaned per hen per clutch average 70 %. Normal feathered hens had a higher rate in the number of chicks weaned. These results could indicate that hens with normal feathers are good mothers or their chicks are resilient to adverse factors in the environment compared with naked neck and frizzled birds. The fact that normal feather hens had more chicks weaned could explain the higher number of normal feather chickens per household.

The majority of the households house their chickens at night. Farmers with no feed constraints and those who had predator constraints are more likely to house chicks at night. Few farmers in Ethiopia and Zimbabwe prepared separate overnight houses for village chickens but the majority of village chicken owners kept birds in various night sheltering places including perches inside the house, on the floor covered by bamboo made materials, on ceilings of the house, and under ('medeb') locally constructed sitting places (Moges *et al.,* 2010; Muchadeyi *et al*., 2007).

The major SFRB are insects and ants, followed by cereal and cereal by-products and household waste/leftovers. During the wet season there are abundant flies, earthworms, locusts, snails, flies, and grass, whereas in the dry season the most abundant SFRB are cereals and cereal by products, roots and tubers, and leaves of trees and shrubs. Goromela *et al.* (2006) reported that, during the rainy season, there is an abundant supply of insects, worms, and green forages, while in the dry season, chicken diets are mostly composed of cereals grain and cereal by-products. There was significantly more household waste in the mountainous Namaacha district than in Boane. Abubakar *et al.* (2007) reported that, in Cameroon, there was more maize and food scraps. In Tunisia, Ben *et al*. (2013) reported that 90 % of the farmers provided supplementary feeding once per day to their chickens that included maize, barley, wheat, and household waste products.

The topography, level of education, number of adults and number of children influenced a household's likelihood of using kitchen waste. Households in Boane, where there is a huge dam, use more kitchen waste than farmers in the mountain. The main reason for the results could be due to agriculture activities that are more dynamic in a dam area than in the mountain. These findings conform to the shortage of feeds where farmers in the mountainous district were more likely to experience feed shortages than farmers in the dam area. The amount of kitchen wastes is small in the rainy season (Goromela *et al.,* 2006). In addition, the fact that female heads of households were more likely to use kitchen waste than males could be because women are more involved in household activities, including feeding chickens. Mlambo *et al.* (2011) reported that, in Zimbabwe, flock ownership was dominated by women and children. In Ethiopia, Tadele and Ongle (2001) found that the food leftovers were more or less constant throughout the year, but the portion of the feed resources from the environment and the grain supplement varied with seasons. The household leftovers were available in all seasons, but higher in the wet season compared with the dry season. Goromela *et al.* (2007) reported that the variation is in the amounts and in the flora and origin of species available as food for the rural household.

The effect of season and farming system on the chemical composition of crop contents and their physical components were evaluated by several authors (Rashid *et al*., 2004; Anjos, 2005; Momoh *et al*., 2010). Cereal grains, kitchen wastes, green forage and insects/worms were the main crop contents and their composition varied with season and age of the bird (Momoh *et al.*, 2010). For example, the green forage component of the crop contents was high during the late dry season, and was also high in laying and growing chickens (Momoh *et al*., 2010). Mwalusanya *et al.* (2002) intimated that the chemical composition of feeds consumed by scavenging chickens was below the nutritional requirements of all classes of birds.

Households offered chickens spoiled or moldy leftovers and kitchen waste. Makkar (2014) reported that it is important to provide ingredients of good quality and free from deleterious substances, such as mycotoxins and heavy metals. Aflatoxins are a concern to village chickens because of the frequency of contamination of feeds and the threat they pose to chicken health (Otim *et al.,* 2005). Van Rensburg *et al.* (1985) showed that prepared meal samples contained measurable amounts of aflatoxin and that aflatoxin B_1 constituted 89 % of the total aflatoxin contents. Apart from mycotoxins, anti nutritional factors in beans and cassava (Udensi *et al.,* 2007; Sarkiyayi and Agar, 2010), which are provided to chickens, can contribute to the poor performance of the chickens (Yarru *et al.,* 2013; Chen *et al.,* 2014). The effects of these compounds on chicken performance need to be determined.

The majority of households fed all classes of chickens together. Zewdu *et al.* (2013) reported that 84 % of the respondents were feeding their chicken flock as one group.

The main purpose of keeping village chickens is for household meat consumption and as a source of income. The results agree with earlier reports (Mengesha *et al.* 2008; Moges *et al.* 2010). In Ethiopia, Dana *et al*. (2010) reported that production of eggs for consumption was the principal function of chickens. In Kenya, Justus *et al.* (2013) found that village chickens are kept mainly for subsistence and commercial purposes. Chickens are also important for church contributions, celebrations, emergencies and other socio-cultural functions. Chickens are, therefore, important for food security and for improving the livelihoods of rural households (Guéye, 2000; Alders *et al.* 2010; Mlambo *et al.*, 2011). Households' in the current study expressed the desire to increase the number of chickens. The odds of farmers saying village chicken is important were influenced by gender and age. Male heads of households were likely to own other livestock species such as cattle and goats, while females mostly relied on chickens for their livelihoods.

The major constraints to village chicken production were high disease prevalence, predators, theft, and shortage of feed. In Ethiopia, Yitbare and Atalel (2013) observed that disease and predators were the main constraints. Newcastle Disease is a major challenge that hinders the expansion of village chicken production (Bell *et al*., 1990; Mavale, 2001; Adwar and Lukešová, 2008; Dinka *et al.*, 2010). Several studies also reported that a high prevalence of both ecto- and endoparasites in village chickens (Permin *et al.*, 1997; Mushi *et al.*, 2008; Nnadi and George, 2010) is one of the constraints to keeping village chickens. These were uncommon in the present study, most likely because the chickens could be resilient to these parasites or that the lack of housing reduces the build-up of these parasites in chickens. Predators were a major constraint to village chicken productivity. Several authors reported predators as a constraint to an increase in village chicken production (Mapiye and Sibanda, 2005; Dinka *et al.*, 2010).

3.5 Conclusions

Village chickens play an important role in supplying income, meat and eggs for communal households and they are valued by women. The scavenging feed resources (SFRB) is the dominant feed system with supplementary feed based on maize grain, agricultural by-products, kitchen waste, and household leftovers. The SFRB could be contaminated by aflatoxin and some contain anti-nutritional factors. Most feedstuffs are likely to be deficient in protein. The

availability of scavenging feed resources varies with season, topography, gender, age, and level of education of the household.

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CHAPTER 4 - Chemical composition, amino acid digestibility and true metabolisable energy of cowpeas as affected by roasting and extrusion processing treatments using the cecectomized rooster assay

(Submitted to Journal of Applied Poultry Research)

Abstract

Nutritional differences due to variety, source and effects of heat processing on cowpeas (*Vigna unguiculata*) were assessed. The precision-fed cecectomized rooster assay was used to determine amino acid digestibility and true metabolizable energy of cowpeas. Mozambican cowpea (nhemba) treatments included either raw or roasted, whereas those (black- eyed beans) sourced from the United States of America (USA) were raw, roasted or extruded. Crude protein (CP) content was higher for raw nhemba (228 g/kg) in comparison to raw black-eyed beans (207 g/kg). Raw nhemba had a higher crude protein and amino acid content (except tryptophan) than raw black-eyed bean. The level of trypsin inhibitors of nhemba was three times higher than black-eyed bean (6700 vs 2200 TIU/g). Both roasting and extrusion increased the CP content of cowpeas. Extruded and roasted black-eyed beans contained higher amino acid concentrations compared with raw black-eyed beans. Heat treatment had no effect on methionine, threonine, proline, alanine, valine or leucine. The amino acid content of nhemba was reduced by heat treatment. Additionally, heat treatment reduced the concentration of trypsin inhibitors to below 2000 TIU/g. Roasting had no effect on amino acid digestibility in black -eyed beans, but increased digestibility of nhemba by 3.4 %. True metabolizable energy (TME_n) was significantly increased (3535 versus 3164kcal/kg) by extrusion.

Key words: amino acid digestibility, black-eyed beans, cecectomized rooster assay, heat processing, nhemba, trypsin inhibitors, urease assay.

4.1 Introduction

Due to the high demand for soybean and maize grain for human consumption, there is a pressing need to replace conventional feed ingredients with locally available products (Emenalom *et al*., 2005; Kana *et al*., 2013). One of the most important considerations in diet formulation is the availability and nutritional value of amino acids (Gilani *et al*., 2005; Bryden

and Li, 2010; Kim *et al.*, 2011) which depends, to a large extent, on the nutrient composition and concentration of anti-nutritional factors in the feedstuffs.

Cowpea (*Vigna unguiculata*), a grain legume produced in tropical and subtropical regions, is a protein source (Tshovhote *et al.*, 2003) that is used to feed chickens and has a composition equivalent to lupins and field peas (Tshovhote *et al.*, 2003). One of the major challenges with cowpea utilization in poultry feeding is the presence of anti-nutritional factors (ANFs), particularly trypsin inhibitors, lectins, tannins, and phytic acid (Umoren, 1997; Leeson and Summers, 2001). Soaking, boiling, roasting, autoclaving, and dehulling are some of the techniques that have been shown to reduce the negative effects of anti-nutritional factors (Amaefule and Nwagbara, 2004; Medugu *et al*., 2012). Bryden and Li. (2010) pointed out that, although heat treatment reduces or eliminates the effects of ANFs, it also reduces the quality of protein. For example, lysine is sensitive to high temperatures and the low digestibility of lysine in cotton seed meal reflects heat processing of the meal. Effects of heat treatment on protein quality of nhemba and cowpeas are unknown. An understanding of the nutritional value of feed ingredients is essential for optimizing chicken production (Barneveld and Edwards, 2012) Tshovhote *et al*. (2003) showed that the concentrations of amino acids, chemical composition, digestibility, and nutritive value of cowpeas varied among cultivars. One common method to estimate true amino acid digestibility is the precision-fed cecectomized rooster assay (Fernandez and Parsons, 1996). The precision fed rooster assay is a rapid feeding test using roosters whose caeca have been surgically removed to accurately assess amino acid digestibility (Parsons, 1986). In addition to accuracy, many samples can be tested in a relatively short time with few birds and roosters can be used for several assays (Lemme and Bryden. 2004). The objective of the current study was to determine the effect of heat treatment on the digestibility of amino acids, and on the metabolizable energy values of nhemba and black-eyed beans using the precision-fed cecectomized rooster assay. This study was conducted to test the hypothesis that heat treatment will improve the digestibility of amino acids in chick diets.

4.2 Materials and methods

4.2.1 Feed ingredients

One cultivar of cowpeas (*Vigna unguiculata*) called nhemba and a single cultivar of black-eyed beans (*Vigna unguiculata*) were used in the current study Nhemba varieties were supplied by the National Agricultural Research Institute, Mozambique (IIAM) and the black-eyed beans were purchased in Missouri and extruded at Wenger Manufacturing, Inc. (Sabetha, KS, USA).

4.2.2 Processing of cowpeas

The raw cowpeas were first ground in a mill (Retsch cutting mill model SM 200, Haan, Germany) using a 2cm x 2cm screen, and then ground a second time in the same mill using a 1cm x 1cm screen to further reduce particle size. Both nhemba and black- eyed beans were roasted using a hotbox oven at 120°C for 30 min. The seeds were removed from the oven and cooled, then ground. In addition, another sample of raw black- eyed beans was ground to 1/32″ and exposed to heat steam. The preconditioned held it at about 88°C for 4 to 5 minutes, and then it also got 102°C in the head immediately prior to extrusion. After that the black eyed bean were extruded with temperatures ranging from 60-111°C at a pressure of 400 psi, then cooled to room temperature. The final product was little puffed disks- tasted cooked. The extruded pellets were then ground in a mill using a 2cm x 2cm screen. The feed ingredients used in the assay are shown in Table 4.1.

4.2.3 Laboratory analyses

The amino acid profiles of the cowpeas were determined using the methods described by AOAC (2006) and crude protein content was measured using the Kjeldahl method (AOAC, 2006). The total fat was determined by ether extraction (AOAC Official Method 920.39 (A), 2006). Fiber was determined using AOAC (2006) Official Method 978.10 (2006). Moisture was analyzed according to AOAC (2006) Official Method 934.01, 2006, in a vacuum oven. Ash was according to Official Method 942.05 (2006).

The nhemba cowpea cultivars were analyzed in duplicate for urease activity. Urease activity was used as a "marker" to indirectly reflect the presence of ANFs, mainly trypsin inhibitors. The assay was done as described by AOAC (1980) whereas the trypsin inhibitor assay was done according to AOCS (1997). For the urease assay, 0.2 g of sample was finely ground and put into a test tube (test sample) to which 10 ml of the buffered urea solution was added. A stopper was inserted and the contents were mixed by shaking gently without inverting the tube. The tube was then placed in a water bath at 30^{o}C, and start time of heating recorded.

Another 0.2 g of the finely ground sample was put into a test tube (blank sample) and 10 ml of a phosphate buffer solution added. A stopper was inserted and the tube was mixed gently without inverting the tube; the blank sample was also placed in the water bath at 30^{o}C. Approximately 5 min elapsed between preparation of the test and blank samples. During the 30 minutes of incubation, the tubes were taken out of the water bath, shaken once, and returned. After 30 minutes, tubes were removed from the water bath, and cooled slightly at room temperature.

Table 4.1. Description of protein sources/treatments used in cecectomized rooster assay

Treatment	Description
T1	Raw black eyed bean
T2	Roasted black eyed bean
T3	Raw nhemba
T4	Roasted nhemba
T5	Extruded black eyed bean

Approximately 5 ml of the supernatant (enough to cover the electrode of the pH meter) was then transferred into a beaker and the pH measured with a pH meter. The pH of the supernatant liquid was recorded 5 min after removal of the test tubes from the water bath. Differences between pH of the test samples and pH of the blank were calculated as an index of urease activity. The (relative) urease activity index was, thus, estimated as the difference in pH between the test sample and that of its respective blank.

4.2.4 Rooster assay

A precision-fed cecectomized rooster assay, as described by Sibbald (1979), was conducted. Twenty five 50-week old cecectomized Single Comb White Leghorn roosters were used at the University of Illinois, Urbana-Champaign, Illinois, USA. Cecectomy was performed under anesthesia when the birds were 25 weeks old, according to the procedure of Parsons (1985). All surgical and animal care procedures were conducted under a research protocol approved by the Institutional Animal Care and Use Committee, University of Illinois, Urbana. All the birds were housed individually in cages with raised wire floors. They were kept in an environmentally controlled room and subjected to a 16h light period daily.

Before the start of the experiment, the roosters were deprived of feed for 24 h and then crop – intubated with approximately 30 g of one of the treatment samples, with four individually caged roosters assigned to each treatment sample. After crop intubation, urine and faeces from each rooster were collected for 48 h on plastic trays placed under each cage. The excreta samples were freeze-dried, weighed, and ground to pass through a 0.25 mm screen.

Endogenous corrections for amino acids were made using four roosters that had been fasted (free access to water, but no feed) throughout the experiment period (48 h). To estimate amino acid digestibility of the cowpeas, the extruded black eyed bean was used as the positive control (Table 4.1).

4.2.5 Measurements

Each feed and faecal sample was analyzed to determine amino acid concentrations Standardized digestibility (SID) of amino acid (%) was calculated by using the method described by Sibbald (1979) calculated as:

$$SID\ (\%) = \frac{Amino\ acid\ intake - (ileal\ amino\ acid\ intake\ out\ flow - basal\ endogenous\ amino\ acid\ losses)}{Amino\ acid\ intake}\ X\ 100\ \%$$

The difference between apparent amino acid digestibility and standardized amino acid digestibility was that the latter provided a correction factor for apparent amino acid digestibility coefficient. Feed and excreta from cecectomized roosters were also analyzed for total nitrogen (AOAC, 2000).

Gross energy (GE) content was estimated using an adiabatic bomb calorimeter standardized with benzoic acid. True metabolisable energy (TME) corrected for nitrogen (TMEn) was also calculated by the method of Sibbald (1976).Corrections for endogenous energy and nitrogen were made by using excreta from roosters that had been fasted for 48 h. The ME was corrected to nitrogen equilibrium, assuming that nitrogen retained would produce additional urinary energy in the excreta amounting to 8.22 kcal/g of nitrogen. These latter values were also corrected for endogenous energy of non-nitrogenous origin, as measured by using the fasted birds to obtain TMEn. The TMEn values were calculated as:

$TMEn = (FE_f - (EE_f + 8.22\ N_f) + (EE_u + 8.22\ N_u))/FC$

where FE_f is the GE of the total feed consumed, EE_f and EE_u refer to the energy in the excreta collected from the fed and fasted birds, respectively, N_f and N_u refer to the amount of nitrogen (in g) retained by the fed and fasted birds, respectively, and FC is the amount of dry feed consumed (in g).

4.2.6 Statistical analyses

Data were analyzed using the General Linear Models procedure of SAS®, version, 9.3[28]. Separation of means was performed using Fisher's protected least significant difference. Significance level was determined at $P<0.05$.

The statistical model used was:

$Y_{ij} = \mu + T_{i +} \varepsilon_{ij,}$ where

Y_{ij} = observation on i^{th} dietary treatment (T) and j^{th} rooster

μ is the overall mean

T_i= fixed effect of treatment (1, 2, 3, 4 and 5)

ε_{ij} is random error.

4.3 Results

4.3.1 Chemical composition of the different cowpea varieties

Proximate analyses and ANF concentrations in the cowpeas are shown in Table 4.2. Crude protein content was higher for raw nhemba (228 g/kg) in comparison to raw black-eyed beans (207 g/kg). Heat treatments increased the CP content of nhemba (roasting) and black-eyed bean (both roasting and extrusion). The fat content in raw nhemba beans was 17% higher compared with raw black-eyed beans. Roasting nhemba or black-eyed beans resulted in higher concentration of crude fat content. In the present study, crude fiber content in black-eyed beans was reduced after roasting and extrusion processes.

Amino acid concentrations in the cowpeas are shown in Table 4.3. With the exception of tryptophan, amino acid concentrations were higher in the pooled nhemba cowpea samples in comparison to black-eyed beans. Nonetheless, amino acid N totalled 99 % of the N measured as CP in the nhemba compared with 93 % in black-eyed beans. The extruded and roasted black-eyed beans had higher amino acid values compared with raw black- eyed beans. With the exception of methionine, threonine, proline, alanine, valine and leucine, the amino acid contents of nhemba cowpeas were reduced by the heat treatment.

4.3.2 Effect of processing on anti-nutritional factors

The concentration of ANFs was higher in nhemba compared to the black-eyed beans. The trypsin inhibitor (TI) content in nhemba averaged 6700 TIU/g, whereas the black eyed cowpea contained 2200 TIU/g.

Urease indirectly reflects the presence of ANFs in soy products. Heat treatment reduced the urease activity from 0.33 in raw material to between 0.22 and 0.25 values in heat-processed cowpeas.

4.3.3 Amino acid digestibility

Amino acid digestibility coefficients among the five cowpea treatments are shown in Table 4.4. The amino acids in the raw black-eyed beans were more digestible compared with the amino acids in raw nhemba. Roasting did not improve ($P>0.05$) digestibility of black eyed bean, but increased ($P<0.05$) digestibility of proline, phenylalanine, leucine and arginine of nhemba cowpeas. The different results could be due to differences in cultivars, growing conditions,

Table 4.2. Chemical composition (as-is basis) and anti nutritional factors of nhemba and black-eyed beans

	Nhemba		Black-eyed beans		
	Raw	Roasted	Raw	Roasted	Extruded
Component(as is) g/kg					
Moisture	121.4	97.3	167.6	85.0	17.5
Crude protein	228.1	249.4	207.2	238.9	244.6
Crude fat	12.0	17.0	10.0	18.6	12.8
Crude fibre	49.7	49.5	37.1	25.6	22.7
Ash	35.3	33.0	33.5	31.5	46
Anti –nutritional factors					
Typsin inhibitor, TIU/g (average)	6700	<2000	2200	< 2000	< 2000
Phytic acid, %	9.2 -10.8	ND	9.2	ND	12.1
Urease assay, %	ND	ND	1.6	ND	3.0

ND: indicates none detected

Table 4.3. Amino acid composition (as-is basis) of nhemba and black- eyed beans

	Nhemba		Black eyed bean		
Amino acid content (as is g/kg)	Raw	Roasted	Raw	Roasted	Extruded
Methionine	3.4	3.5	3.0	3.3	3.6
Cystine	2.7	2.3	2.1	2.3	2.2
Methionine + cysteine	6.3	5.8	5.1	5.6	5.8
Lysine	16.6	14.0	14.3	15.1	15.4
Arginine	16.5	15.8	14.0	1 6.3	16.8
Tryptophan	2.3	1.9	2.6	2.6	3.0
Tyrosine	7.5	6.9	6.2	6.3	6.8
Threonine	8.7	8.8	7.2	8.2	8.6
Serine	10.2	9.6	8.2	9.2	9.2
Phenylalanine	13.6	13.6	11.4	12.5	13.4
Aspartic acid	26.5	26.1	22.4	26.1	26.6
Glutamic acid	37.8	37.5	33.6	35.8	40.0
Proline	9.3	9.7	9.0	9.2	10.8
Glycine	9.7	9.5	7.9	8.8	10.3
Alanine	10.2	10.6	8.9	9.6	10.8
Valine	11.7	12	10.4	11.4	12.9
Isoleucine	10.2	9.8	8.6	9.4	10.8
Leucine	18.1	18.2	15.7	16.8	19.1
Histidine	7.5	7.3	6.7	7.0	7.3
Hydroxyproline	0.4	0.4	0.0	0.4	0.6
Hydroxylysine	0.0	0.0	0.1	0.0	0.1
Taurine	0.6	0.8	0.0	0.8	0.7
Lanthionine	1.0	0.0	0.0	0.0	0.0
Ornithine	0.2	0.3	0.2	0.2	0.2
Total	226.4	218.6	192.5	211.3	229.2

Table 4.4. Effect of cowpea source and processing method on amino acid digestibility, using cecectomized rooster assay

Amino Acid Concentrations[3] (%)	Cowpea treatments[2]					P-value	SEM	CV %
	RNH	RONH	RBEB	ROBEB	EBEB			
ASP	0.77^{b}	0.79^{b}	0.87^{a}	0.86^{a}	0.88^{a}	0.0004	1.6188	3.88
THR	0.73^{b}	0.77^{b}	0.85^{a}	0.84^{a}	0.86^{a}	0.0003	1.7958	4.43
SER	0.72^{b}	0.76^{b}	0.86^{a}	0.86^{a}	0.88^{a}	0.0001	1.8338	4.49
GLU	0.80^{c}	0.83^{c}	0.89^{ab}	0.88^{b}	0.92^{a}	0.0001	1.3005	3.02
PRO	0.69^{c}	0.77^{b}	0.87^{a}	0.84^{a}	0.89^{a}	0.0001	1.9018	4.68
ALA	0.72^{d}	0.77^{cd}	0.83^{ab}	0.81^{bc}	0.88^{a}	0.0002	1.8118	4.54
CYS	0.66^{c}	0.69^{bc}	0.86^{a}	0.82^{ab}	0.79^{abc}	0.0361	4.6288	12.14
VAL	0.74^{c}	0.76^{c}	0.84^{b}	0.83^{b}	0.89^{a}	0.0001	1.5293	3.76
MET	0.75^{c}	0.79^{c}	0.87^{ab}	0.86^{b}	0.91^{a}	0.0001	1.3765	3.29
ILE	0.73^{c}	0.78^{c}	0.85^{b}	0.84^{b}	0.91^{a}	0.0001	1.4562	3.55
LEU	0.75^{d}	0.81^{c}	0.88^{b}	0.86^{b}	0.92^{a}	0.0001	1.3919	3.31
TYR	0.72^{c}	0.76^{c}	0.84^{ab}	0.83^{b}	0.88^{a}	0.0001	1.7965	4.46
PHE	0.77^{d}	0.82^{c}	0.88^{b}	0.87^{b}	0.93^{a}	0.0001	1.3759	3.23
LYS	0.77^{b}	0.72^{b}	0.85^{a}	0.83^{a}	0.85^{a}	0.0002	1.7210	4.28
HIS	0.77^{b}	0.76^{b}	0.85^{a}	0.84^{a}	0.86^{a}	0.0011	1.6995	4.17
ARG	0.74^{d}	0.83^{c}	0.90^{b}	0.90^{b}	0.94^{a}	0.0001	1.0808	2.50
TRP	0.99^{a}	101.1^{a}	0.96^{bc}	0.95^{c}	0.99^{ab}	0.0052	1.0273	2.09
Average	0.75	0.79	0.87	0.85	0.89			
TME_n[4]	2806^{d}	3347^{ab}	316^{bc}	3101^{c}	3535^{a}	0.0001	0.0798	5.00

[a-c] Values in the same row not sharing common superscript letters differ (*P<0.05*)

[1] Mean of 4 individually – caged roosters

[2] **Cowpea key**: RNH= Raw nhemba; RONH= Roasted nhemba; RBEB= Raw black eyed bean; ROBEB= Roasted black eyed bean; EBEB= Extruded black eyed bean

[3]**Aminoacid key**: ASP= Aspartic acid; THR = Threonine; SER= Serine GLU= Glutamic acid; PRO= Proline; ALA= Alanine; CYS= Cystine; VAL= Valine; MET= Methionine; ILE= Isoleucine; LEU= Leucine; TYR= Tyrosine; PHE =Phenylalanine; LYS= Lysine; HIS= Histidine; ARG= Arginine; TRP= Tryptophan

[4] **Energy digestibility key**: TME_n = True metabolisable energy

storage time, and size of the beans. Extrusion increased amino acid digestibility of black- eyed beans, specifically valine, isoleucine, leucine and arginine. Roasting had no effect on (*P>0.05*) the digestibility coefficients of aspartic acid, threonine, serine, proline, lysine and histidine in black-eyed beans. Digestibility coefficients were significantly increased (*P<0.05*) by 5.6, 4.3, 4.8, 5.4 and 4 % for valine, isoleucine, leucine, phenylalanine, and arginine, respectively, in extruded black-eyed beans.

4.3.4 Energy digestibility

True metabolisable energy (TME_n) was significantly increased (3535 versus 3164 kcal/kg) by extrusion in black-eyed beans when compared with raw black-eyed beans (Table 4.4). However, roasting reduced (*P<0.05)* the energy digestibility of black-eyed beans.

4.4 Discussion

Crude protein, amino acid profiles, and ANF concentrations in the nhemba and black eyed bean cowpeas were evaluated. Crude protein content was higher for raw nhemba (228 g/kg) in comparison to raw black eyed beans (207 g/kg). These data compare to reported protein concentrations in local Nigerian cowpea varieties Danborno and Kannanado of 221 and 198 g/kg, respectively (Owolabi *et al.*, 2012). Mamiro *et al*. (2003) observed 261 g CP/kg in Tanzanian cowpeas, while cowpeas grown in Saudi Arabia (Hussain and Basahy, 1998) contained 230 g/kg CP. These different results could be due to differences among cultivars or growing conditions (Mugendi *et al*., 2010).

Heat treatments increased the CP content of nhemba (roasting) and black-eyed bean (both roasting and extrusion). The fat content in raw nhemba beans was 17 % higher compared with raw black eyed beans. Roasting nhemba or black eyed beans resulted in higher concentration of crude fat content. In contrast to our results, Osman (2007) found that crude fat content was significantly reduced by roasting. Similar results to those found by Osman (2007) were reported by Balai (2014) who observed that decorticating plus roasting at 120°C for 30 minutes reduced the fat and fiber concentrations in cowpea. Mubarak (2005) found that cooking of mung bean seeds had no effect on the crude fiber content, in agreement with our roasted nhemba findings. In the present study, crude fiber content in black eyed beans was reduced after roasting and extrusion processes.

In the present study, similar to the tendency of protein concentrations, there were also variations between the cultivars (nhemba and black eyed bean) in amino acid concentrations. Tshovhote *et*

al. (2003) also found differences in amino acid concentrations among cowpeas; the concentrations of all amino acids except methionine and tyrosine were higher in the village cowpea cultivar than in the other two cultivars, Glenda and Agrinawa, in their study These differences could be due to the different cultivars, different techniques of production and/or conservation methods.

Cultivar influenced the concentration of the various ANFs in cowpeas, and should be considered when feeding animals. In our study the results showed that the concentrations of trypsin inhibitor and phytic acid were higher in nhemba cowpea than in black eyed beans. Similar results were found by Owolabi *et al*. (2012), who reported that the ANF composition in Nigerian cowpeas was higher in local varieties of cowpea compared to improved varieties. Phytic acid, however, was not significantly different between the local and improved varieties.

Several authors reported that processing of grain legumes could reduce the anti- nutritional factors (Hefnwy, 2011; Owolabi *et al.,* 2012). In the present study, the heat treatment (roasting or extrusion) of nhemba and black- eyed bean seeds reduced the level of trypsin inhibitor (TI) to levels below 2000 TIU/g. Similar results were reported by Udensi *et al*. (2007), who found that roasting cowpeas at 120°C for 30 minutes decreased the level of TI, phytic acid, and haemoglutinin. Nasara (2014) observed a reduction of trypsin inhibitor in decorticated roasted cowpea, whereas Umapathy and Erlwanger (2008) reported that trypsin inhibitors, lectins and tannins were reduced by autoclaving the raw cowpea under a pressure of 1 atm at 120°C for 15 min. Anuaonye *et al*. (2012) also found that extrusion of pigeon peas reduced anti nutritional factors such as trypsin inhibitors, phytic acid, and tannins.

Processing method and the bean cultivar could influence the amino acid digestibility. The results showed that amino acid concentration of cowpeas varies with heat treatments. Roasting did not improve digestibility of black eyed bean, but increased digestibility of proline, phenylalanine, leucine and arginine of nhemba cowpeas. The different results could be due to differences in cultivars, growing conditions, storage time, and size of the beans. Extrusion increased amino acid digestibility of black eyed beans, specifically valine, isoleucine, leucine and arginine. In contrast, Brenes *et al*. (2008) reported that methionine, cysteine and lysine concentrations were higher in raw chickpeas than extruded chick peas. Alajaji and El-Adaw (2006) also found that boiling and microwave cooking chickpea caused a slight increase in total essential amino acids, but they were not influenced by autoclaving. These results suggested that the amino acid availability from different peas may be affected by the specific heat processing method used.

In the current study, raw nhemba had higher true metabolisable energy values (2806 kcal/kg DM versus 2576 kcal/kg) than those reported by Tshovhote *et al*. (2003), but similar to those observed by Sarmiento-Franco *et al. (*2011) in chickens fed in Mexico. Compared with chicks fed raw nhemba, TME_n was increased in chicks fed the diet containing roasted nhemba (3347 versus 2806 kcal/kg). The improved TMEn values are in accordance with the results of Nell *et al.* (1992), who found a marked improvement in metabolisable energy values of autoclaved cowpeas, with values increasing from 2949 to 3102 kcal /kg DM.

There was evidence that the cowpeas contained anti-nutritional factors such as trypsin inhibitor and likely lectin (Anjos *et al*., 2012) which, being thermo-labile, can be removed by the heat treatment and, may consequently, change the energy digestibility of the untreated cowpeas. Nell *et al.* (1992) reported that autoclaving resulted in significant ($P<0.05$) improvements in digestible energy (DE) and true metabolisable energy (TME) of cowpeas when determined in pigs and poultry, respectively.

The decreased energy digestibility observed in roasted black eyed bean, compared to the improvement in nhemba could be due to differences in varieties, the levels of anti nutritional factors, or the roasting treatment itself.

4.5 Conclusions

Both heat treatments reduced trypsin inhibitors in cowpeas. Amino acid and energy digestibility values of raw nhemba cowpeas were significantly lower compared with the raw black eyed bean cowpeas. Extrusion increased amino acid digestibility of black-eyed beans, specifically valine, isoleucine, leucine, and arginine. Roasting increased amino acid digestibility and increased true metabolisable energy of nhemba cowpeas but was detrimental for black- eyed beans. Heat treatment could be used to improve the digestibility of amino acids in cowpeas for chicken diets in Mozambique and elsewhere.

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CHAPTER 5 - Effects of heat and enzyme treatment of cowpeas (*Vigna unguiculata*) and pigeon peas (*Cajanus cajan*) on performance and gut health of chickens

Abstract

An experiment was carried out to assess the effectiveness of roasting, extrusion, and addition of enzymes to cowpeas (*Vigna unguiculata*) and pigeon peas (*Cajanus cajan*) on the performance and gut health of chickens. A total of 180 day-old Ross male broilers were used. Birds were randomly assigned to 30 pens of six birds per pen in a completely randomized design. Diets included various combinations of raw or heat-treated Mozambique cowpeas (nhemba) or pigeon peas, with or without an added enzyme mix (proteinase, xylanase and phytase). Cowpeas and pigeon peas were substituted for soybean meal at 400 g/kg inclusion rate. Feed and water were provided *ad libitum*. Roasting cowpea or enzyme incorporation had no ($P>0.05$) effect on body weight gain and the cumulative performance index. Roasting pigeon pea improved ($P<0.05$) performance of chicks and no mortality was observed. Compared to birds fed the control diet, duodenal crypt depth was significantly lower ($P<0.05$) in birds fed raw cowpea, raw cowpea with enzymes, and roasted pigeon pea diets. Raw cowpeas with or without enzymes elicited an immune response in the duodenum that was absent in birds fed the control diet or roasted cowpeas. Partial replacement of corn and soybean meal with 40 % roasted pigeon peas was demonstrated to be feasible.

Keywords: cowpea; pigeon pea, cumulative performance index; gut health, processing.

5.1 Introduction

Chickens play an important role in providing meat and eggs, and income for rural households. In smallholder production systems, the major constraints to chicken productivity are the high prevalence of diseases and parasites, and the low availability of quality feed. Protein sources such as soybean, fishmeal, and bone meal are becoming scarce and expensive. The potential of increasing the use of locally available feeds, which can contribute to meeting nutritional requirements of the chickens, need to be explored.

Cowpea (*Vigna unguiculata*) and pigeon pea (C*ajanus cajan*) are alternative feed ingredients for chickens with a huge potential for use in smallholder chicken production systems. Cowpea is widely grown in arid and semi-arid environments. In Mozambique, for example, cowpea

represents 17 % of the total land area under grain legume production. It is, however, considered a woman's crop and has little commercial value. The good grain and the tender leaves are used as vegetables. The seeds are exploited for household consumption and also for feeding chickens. Pigeon pea is also produced in by resource poor farmers for household consumption. Feeding pigeon peas n to chickens is likely to enhance its value and can contribute to household food security.

Cowpea and pigeon pea contain anti-nutritional factors that bind nutrients and damage the gut lining (Tshovhote *et al.*, 2003; Mingbin *et al*., 2009). The main anti-nutritional factors are trypsin inhibitors, polyphenolic compounds, and phytic acid. The anti-nutritional factors also increase gut viscosity and thus decrease nutrient absorption, the latter being used for the growth of the intestinal microflora. Growth performance of the birds is, consequently, compromised. Chickens cannot fully utilize the fibre present in plant-based feed ingredients, so exogenous enzymes could be incorporated in their diets to enhance the breakdown of the non-starch polysaccharides (Ani *et al.*, 2012).

Growth performance and gut health can be used to assess the response of the chickens to diets. When feeding scavenging chickens, it was estimated that cowpea or pigeon pea can constitute up to 50 % of the diet. Although soaking, cooking, roasting, extrusion, and addition of enzymes can reduce the negative effects of the anti- nutritional factors, their effect on cowpea and pigeon pea needs to be investigated. Roasting is an appropriate and easy method to process these grain among resource-limited farmers because they can use the firewood for roasting the peas. Some farmers practice roasting, but no empirical studies are available on the effectiveness of the technology for smallholder farmers. The objective of this study was to compare the effect of roasted cowpea and pigeon pea supplemented with an enzyme mix on performance and gut health of chickens.

5.2 Materials and methods

5.2.1 Grain legumes

Three cultivars of nhemba (*Vigna unguiculata*) INIA 36, IT 16, and IT 18 that were pooled and pigeon pea (*Cajanus cajan*), were used in the current study. The grains were provided by the National Research Institute of Agriculture, Mozambique (IIAM).

5.2.2 Determination of urease activity of grains

Raw beans were first ground in a mill (Restch- saw mill; SM 2000; Serial: 127211118J) with a 2 cm x 2 cm screen, then ground again in a mill with a 1cm x 1cm screen to obtain a particle size of 1.5mm mesh. Raw beans were roasted using a hotbox oven at 120°C for 45 min. The seeds were then cooled before they were ground again through a 1.5 mm sieve. The beans were analyzed in duplicate for urease activity using a method described by the Association of Official Analytical Chemists (1980), to assess the reduction of trypsin inhibitors after heating.

A 0.2 g (± 0.001 g) of the finely ground sample was put into a test tube and 10 mL of the buffered urea solution was added to the tube and this was considerd the test sample. The tubes was then capped, mix/swirl gently (without inverting the tube) and placed in a water bath at 30^{o}C and the time the samples were placed into the water bath was recorded. A 0.2 g (± 0.001 g) of sample finely grounded was putted into a test tube and added 10 mL of the phosphate buffer solution (blank). After the tub was stopper, mix/swirl gently (without inverting the tube) and placed in water bath at 30^{o}C and noting the time put into the bath. Five minute intervals were allowed between the preparation of the test and blank samples.

The contents of both test and blank sample tubes were swirled every five minutes for 30 minutes in the water bath. After 30 minutes, the tubes were removed from the water bath let stand for a few minutes, then approximately 5 ml of the supernatant liquid were transferred into a beaker with the electrode of the pH meter immersed in the liquid. Approximately five minutes after removal from the water bath, the pH of the supernatant liquid was determined. The difference between pH of the test and blank samples were calculated as an index of urease activity.

5.2.3 Experimental design and birds

One hundred and eighty day-old male broiler (Ross 708) chicks were purchased from a commercial hatchery, weighed, wing banded, and assigned to stainless steel chick batteries. All pens had the same initial body weights (about 41 g/bird). A completely randomized design was used with five replicate pens of six chicks assigned to each of six dietary treatments. The chicks were allowed *ad libitum* access to feed and water from hatch to day 14. The temperature in the house ranged between 35 and 36.1 ^{o}C at the beginning of the experiment and between 30 and 31.1^{o}C towards the end of the experiment. The chicks were subjected to different light regimes: 24 hours of light (2 foot candles) from days 5 to 9, 20 hours of light (2 foot candles) and 4 hours of darkness from days 10 to 14. The health status of the birds was assessed at least twice daily

during the week days and once daily on weekends. All abnormalities and bird mortalities were recorded, including body weights of dead chicks and culls.

5.2.4 Diets

A maize-soybean meal-based basal diet (mash form) formulated to meet the nutritional requirements of chicks (1-14 days post-hatch), as recommended by the National Research Council (NRC, 1994), was used. Table 5.1 shows the ingredient composition of the basal diet. Acid insoluble ash (1 %) was added at the expense of corn as indigestible marker for digestibility measurements. The six dietary treatments used are shown in Table 5.1. For the diet containing a combination of enzymes that included a commercial phytase (Ronozyme), Cibenza DP100 (protease) and Cibenza CSM (xylanase, α-galactosidase and β-glucanas) was used. To make the enzyme premix, 60 g of Cibenza DP100 plus 60g of Cibenza CSM, and 60 g of Ronozyme phytase were mixed with 5.82 kg of ground maize. The nutritional composition of each diet is shown in Table 5.2.

5.2.5 Measurements

5.2.5.1 Gut morphology

At day 12, two birds per pen were injected with 10 mg/kg of bromodeoxyuridine (BRDU) as a marker for gut epithelial growth. Two days later, the same birds were injected with BRDU, before they were weighed and euthanized. Samples of duodenum (1 cm empty piece), midgut (1 cm empty piece), ileo-cecal junction with 1 cm of ileum attached and a cross section of mid cecal pouch were collected from each bird. The lumen was flushed gently with Notox and preserved in labeled bottles containing 10-20 × volume of Notox (samples were pooled by treatment). A 1-cm piece of duodenum was collected from one bird per pen, flushed with Notox, and fixed with 10-20× volume of Notox in a labeled bottle. The fixed tissues were processed and embedded in a paraffin wax. Tissue blocks were then sectioned to 5 micron thickness stained with haematoxylin and eosin (H&E) or immunostained with anti-BRDU or anti-IgA and examined using an Olympus light microscope. The H&E stained slides were used for examination of gut morphology and measurement of gut morphometry. The anti-BRDU stained slides were used for examination of cell proliferation. The ant-IgA stained slides were used for examination of immune response.

Table 5.1. Dietary treatments used in a broiler trial comparing the effects of heat treatment with or without added enzymes, using 400 g/kg replacement of soybean meal with either cowpeas (*Vigna unguiculata*) or pigeon peas (*Cajanus cajan*)

Ingredients g/kg	T1	T2	T3	T4	T5	T6
Maize-soy	593.4	351.4	351.4	379.3	351.4	379.3
Soybean	318.1	168.1	168.1	140.9	168.1	140.9
Cowpea	0.0	40.0	40.0	40.0	0.0	0.0
Pigeon	0.0	0.0	0.00	0.00	40.0	40.0
Soybean iol	10.0	33.3	33.3	10.0	33.3	10.0
Dicalcium	18.0	18.6	18.6	18.0	18.6	18.0
Limestone	11.1	11.4	11.4	11.8	11.4	11.8
Salt	3.6	3.5	3.5	3.5	3.5	3.5
$NaHCO_3$	2.6	2.8	2.8	2.7	2.8	2.7
Aliment	1.9	3.2	3.2	3.2	3.2	3.2
L-Lysine HCL 78%	1.3	1.3	1.3	1.7	1.3	1.7
Threonine	0.7	1.4	1.4	1.3	1.4	1.3
Tryptophan	0.0	0.0	0.0	0.0	0.0	0.0
Choline CL 60 %	0.8	1.9	1.9	2.0	1.9	2.0
Trace Mineral mix	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin mix	0.5	0.5	0. 5	0. 5	0. 5	0. 5
Mold guard	0.5	0. 5	0. 5	0. 5	0. 5	0. 5
Santoquin-mix6	0.1	0. 1	0. 1	0. 1	0. 1	0. 1
Sand	35.4	0.00	0.00	22.5	0.00	22.5
Total	1000	1000	1000	1000	1000	1000

T1: control; T2: raw cowpea; T3: roasted cowpea; T4: raw cowpeas with enzymes; T5: raw pigeon pea; T6: roasted pigeon pea. $NaHCO_3$: sodium bicarbonate; Min: organic trace mineral mix.

Table 5.2. Calculated nutritional composition of the diets

	Treatments					
Nutrient composition	T1	T2	T3	T4	T5	T6
ME (kcal/kg)	2900	2900	2900	2900	2900	2900
Crude protein (%)	20.4	19.6	19.6	20.1	19.6	20.1
Available phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45
Calcium (%)	0.92	0.92	0.92	0.92	0.92	0.92
Sodium (%)	0.22	0.22	0.22	0.22	0.22	0.22
Choline (ppm)	1600	1600	1600	1600	1600	1600
Digestible Lysine (%)	1.07	1.07	1.07	1.07	1.07	1.07
Digestible TSAA (%)	0.80	0.80	0.80	0.80	0.80	0.80
Digestible Threonine (%)	0.72	0.72	0.72	0.72	0.72	0.72
Digestible Tryptophan (%)	0.23	0.20	0.20	0.20	0.20	0.20

T1: control; T2: raw cowpea; T3: roasted cowpea; T4: raw cowpeas with enzymes; T5: raw pigeon pea; T6: roasted pigeon pea; Digestible TSAA= digestibile total sulfur amino acids.

The height and width of 5 randomly selected villi, the depth of 5 randomly selected crypts, and the thickness of mucosa tissue at 5 different locations per sample were measured using an Olympus light microscope with a 10× eyepiece lens and a 10× objective lens giving a magnification of 100X. The villi height, villi width, crypt depth, crypt depth/villi height (CV) ratio, and the thickness of the mucosal tissue (TMUC) were compared among treatments. Greater villi height and lower villi width indicate a greater available absorption area. A lower crypt depth and crypt depth/villi height ratio indicates less need of cell proliferation to maintain gut integrity. The mean of 5 villi heights, villi widths, crypt depths and TMUCs for each sample were used for statistical analyses.

5.2.5.2 Digesta viscosity

For viscosity and *Clostridium* measurements, birds from all treatments were used. Jejunum and ileums from two birds per pen were collected and their contents gently squeezed onto tared weigh boats and the digesta weights recorded. Digesta samples were centrifuged for 10 minutes at 2400 RPM. Following centrifugation, 0.5 ml supernatant was carefully removed and placed in a viscometer sample cup. After attaching the cup to the viscometer, 1 minute equilibration time was allowed for each sample. Viscosity measurements were performed using an LVDV-1' Viscometer (with cone/ plate attachment) at a constant temperature of 25°C at 100 RPM.

5.2.5.3 Culturing Clostridium perfringens

Tied-off ileums from two birds per pen were collected and refrigerated in saline for subsequent clostridial culture. Ileal digesta contents were placed into sterile 50 ml tubes, accurately weighed to within 0.1g, then diluted (w/w) with sterile phosphate buffered saline to 10× (0.1), 1000×(0.001) and 100000 × (0.00001) dilutions. One ml of each dilution was transferred into sterile petri dishes, and then 20 ml of molten SPS agar (BD manual SPS agar) tempered to 48°C was added to each petri dish. The plates were swirled to facilitate mixing and allowed to solidify. Once the plates solidified, they were placed into BD EZ anaerobe gas pack pouches to provide an anaerobic atmosphere, and then incubated at 37°C for 18 hours. Following incubation, all black colonies were counted.

5.2.5.4 Growth performance

The birds were weighed by pen on days 0, 7 and 14, and feed consumptiont was determined by emptying any feed from the feeder back into the pail for that pen, weighing the pail plus feed then subtracting the weight of the empty pail from the weight of the pail plus feed. Mortality was checked twice daily and weights of dead birds were used to adjust feed conversion. Body weight gain was estimated as the difference in body weight from two successive measurements.

The feed to gain ratio corrected for dead birds was estimated as: (feed consumed) / (incremental pen weight + incremental dead bird weight). The cumulative performance index (CPI) was calculated as:
((cumulative livability x ((body weight x 1000) / Day of study) x 10) / (cumulative feed to gain corrected for dead bird weight)).

5.2.5.5 Apparent ileal digestibility

At day 15, ileal digesta from all remaining birds, and excreta from all pens, were collected for digestibility measurements. Apparent digestibility was estimated using the following equation:
Apparent ileal digestibility coefficient %= 1 - ((Nutrient in excreta x Marker in excreta) / (Nutrient in diet x marker in diet)).

5.2.6 Statistical analyses

Data were analyzed by analysis of variance (ANOVA) procedures appropriate for a randomized complete block design using the General Linear Models procedure (SAS, 2008). When the ANOVA was significant, means were separated by Fisher's protected least significant difference (LSD) method.

5.3 Results

5.3.1 Growth performance

The effects of dietary treatments on growth performance of chicks are shown in Table 5.3. At day 7, body weight gain (BWG), and cumulative performance index (CPI) of chicks fed raw cowpea or raw pigeon pea were lower ($P<0.05$) than BWG of chicks fed the control diet. Roasted cowpea or roasted cowpea plus enzymes did not improve performance of chicks above that of chicks fed raw cowpea. BWG and CPI of chicks fed roasted cowpea were similar to those fed raw cowpea but significantly lower compared with those fed the control diet. Chicks fed roasted cowpea with enzymes had lower BWG and CPI compared with those fed raw or roasted cowpea. The chicks fed roasted pigeon pea had similar ($P>0.05$) BWG, FC and CPI compared with those fed the control diet. Mortality was higher ($P<0.05$) in the chicks fed raw

Table 5.3. Effects of nhemba and pigeon pea on 7 and 4 days growth performance of chicks fed diets containing enzymes and roasted beans

Day	Parameters (g)	T1	T2	T3	T4	T5	T6	P-value
7	BWT	0.141^{a}	0.116^{c}	0.124^{bc}	0.110 ^{c}	0.123^{bc}	0.131^{ab}	0.0009
	BWG	0.101^{a}	0.077^{bc}	0.084^{bc}	0.070^{c}	0.083^{bc}	0.090^{ab}	0.0007
	Fgdb	1.353^{c}	1.623^{ab}	1.534^{bc}	1.749^{a}	1.683^{ab}	1.421^{c}	0.0012
	CPI	148.78^{a}	101.24^{bc}	105.29^{bc}	82.56^{c}	92.75^{c}	132.37^{ab}	0.0023
	mortpct	0.000	3.334	12.500	10.000	12.500	0.000	
14	BWT	0.390^{a}	0.307 ^{b}	0.314 ^{b}	0.296^{b}	0.319^{b}	0.360^{a}	0.0001
	BWG	0.249^{a}	0.191^{b}	0.190^{b}	0.186^{b}	0.196^{b}	0.229^{a}	0.0001
	Fgdb	1.389^{b}	1.621 ^{b}	1.603^{b}	1.549^{b}	1.745^{a}	1.573^{b}	0.0001
	CPI	188.99^{a}	132.43^{b}	119.08^{b}	119.22^{b}	115.03^{b}	145.18 ^{b}	0.0003

^{abc} Values in the same row with different letters are significantly different ($P<0.05$)

Key: BWT = body weight; Body weight gain =BWG; Feed gain day bird = Fgdb; Cumulative performance index = CPI; mortpct= mortality

T1: control; T2: raw cowpea; T3: roasted cowpea; T4: raw cowpeas with enzymes; T5: raw pigeon pea; T6: roasted pigeon pea.

beans, roasted cowpea, roasted cowpea plus enzymes and raw pigeon pea compared with chicks fed the control diet or roasted pigeon pea. At day 14, the chicks fed raw cowpea, roasted cowpea, roasted cowpea plus enzymes or raw pigeon pea had lower ($P<0.05$) BWG and CPI, compared to chicks fed the control diet.

5.3.2 Digesta viscosity

The effect of heat and enzyme treatment of cowpea and pigeon pea on chicken digesta viscosity is shown in Figure 5.1. Jejunal digesta viscosity of chicks fed raw cowpea was significantly ($P<0.05$) lower compared with those fed raw pigeon pea. Compared with chicks fed the control diet, no significant ($P>0.05$) differences were observed in digesta viscosity of chicks fed other treatments.

5.3.3 Clostridium growth

The effect of heat and enzyme treatment of cowpea and pigeon pea on growth of *Clostridium perfringes* in chickens is shown in Figure 5.2. Chicks fed raw cowpea and raw cowpea plus enzymes had similar clostridial growth to chicks fed the control diet. The *Clostridium* colonies formed in chicks fed roasted cowpea were not different ($P>0.05$) from those fed raw pigeon pea. However, chicks fed roasted cowpea, raw pigeon pea and roasted pigeon pea had a higher number ($P<0.05$) of *clostridium* colonies compared to chicks fed the control diet.

5.3.4 Duodenum morphology

Table 5.4 shows the result of the effect of heat and enzyme treatment of cowpea and pigeon pea on chickens. The results showed no significant differences ($P>0.05$) in mucosal tissue thickness, villi length or villi width among birds fed dietary treatments. Chicks fed raw cowpea, raw cowpea plus enzymes and raw pigeon pea had lower ($P<0.05$) duodenum crypt depths compared with those fed the control diet. Chickens fed roasted cowpea and those fed roasted pigeon pea had similar ($P>0.05$) duodenum crypt depths compared with those fed the control diet.

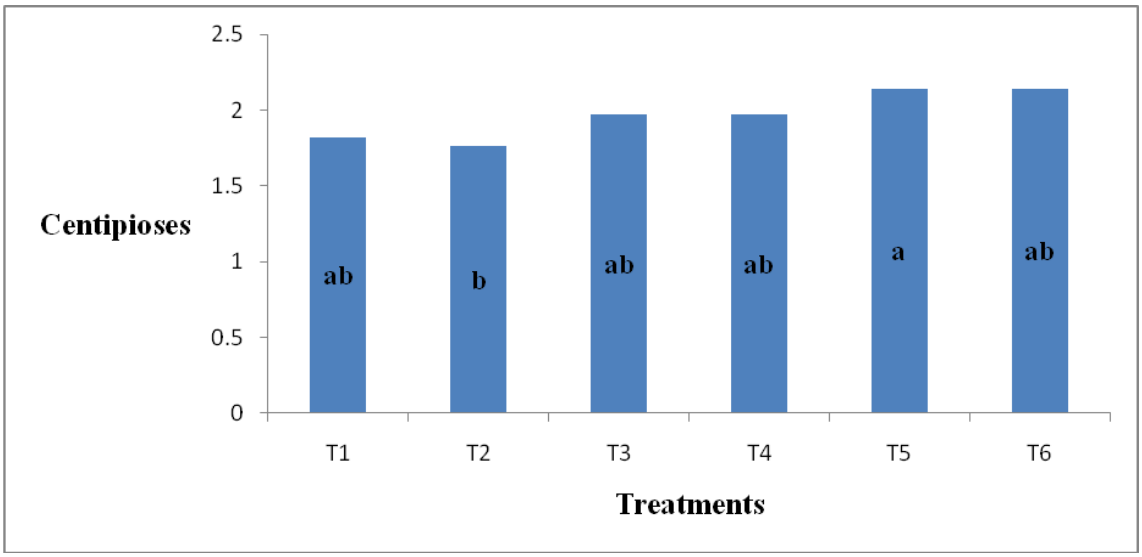

Figure 5.1. Effect of heat and enzyme treatment of cowpea and pigeon pea on ileal digesta viscosity of chicks

T1: control; T2: raw cowpea; T3: roasted cowpea; T4: raw cowpeas with enzymes; T5: raw pigeon pea; T6: roasted pigeon pea. Centipioses: cP

$^{ab}P<0.05$

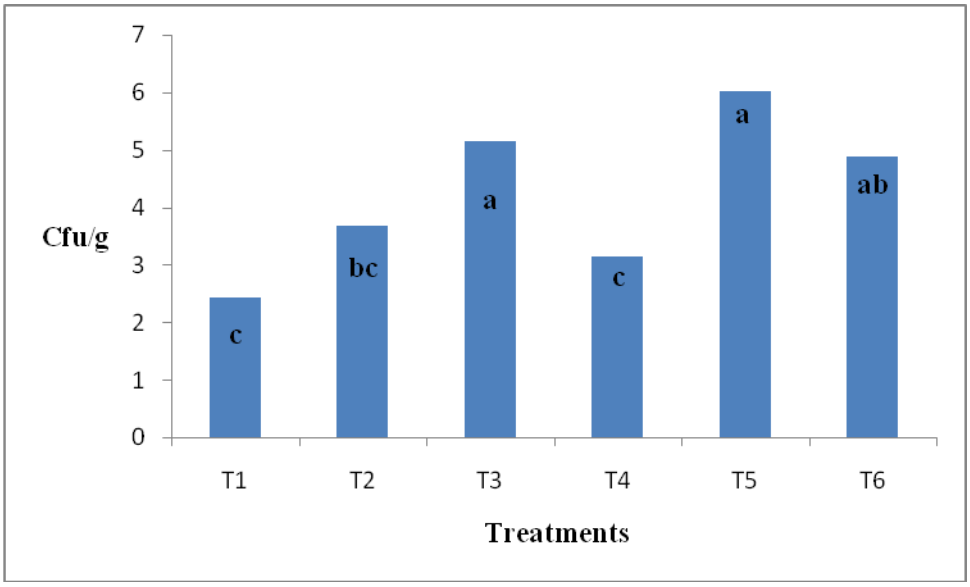

Figure 5.2. Clostridial growth in chicks fed cowpea and pigeon pea

The results are given as log Cfu/g (Colony-forming units per gram) of ileal content

T1: control; T2: raw cowpea; T3: roasted cowpea; T4: raw cowpeas with enzymes; T5: raw pigeon pea; T6: roasted pigeon pea; Cfu/g: colony-forming units per gram

$^{abc}P<0.05$

Table 5.4. Effects of dietary treatments on villus length and width, crypt depth, and mucosa tissue thickness of broilers

Parameter (μm)	T1	T2	T3	T4	T5	T6	P-value
TMUC	2424[a]	2044[a]	2118[a]	2314[a]	2283[a]	2517[a]	0.3880
VilLngth	2158[a]	1852[a]	1895[a]	2104[a]	2075[a]	2290[a]	0.4570
vilwdth	237[a]	189[a]	212[a]	224[a]	210[a]	194[a]	0.3651
crypt	267[a]	192[b]	223[ab]	210[b]	207[b]	227[ab]	0.1141
CV ratio	0.132[a]	0.104[a]	0.119[a]	0.101[a]	0.101[a]	0.100[a]	0.4752

[abc] Values in the same row with different letters are significantly different (*P<0.05*)

TMUC= mucosa tissue; VilLngth = villus length; vilwdth= villus widths

T1: control; T2: raw cowpea; T3: roasted cowpea; T4: raw cowpeas with enzymes; T5: raw pigeon pea; T6: roasted pigeon pea

5.3.5 Immune function

Raw cowpeas (with or without enzymes) elicited an immune response (Figure 5.3a) in the duodenum that was absent in the control diet and very much reduced by roasting (Figure 5.3b). The pigeon pea was not as immunogenic as the cowpeas. A high proliferation of immune cells in response to the antigens was seen in the raw cowpeas. Chicks fed raw pigeon peas had more proliferation in the core of the villus than those fed roasted cowpeas, as shown by the increased immunoglobulin A (IgA) staining in the duodenum of chicks fed raw pigeon peas compared to those fed roasted pigeon peas.

5.3.6 Ileal apparent digestibility

Table 5.5 shows the result of dietary treatments on DM digestibility. Apparent ileal dry matter digestibility of chicks was higher in chicks fed heat treated cowpea or raw cowpea with enzymes, compared with those fed raw cowpea or the control diet.The heat treatment of cowpeas improved the apparent digestibility compared with chicks fed the raw cowpea. In constast, no differences were observed in chicks fed raw or roasted pigeon pea. The apparent ileal nitrogen digestibility of chicks fed raw cowpea was higher compared with those fed the control diet. Chicks fed raw cowpea and raw cowpea with enzyme had similar apparent ileal nitrogen digestibility.

Roasted pigeon pea improved the apparent digestibility of nitrogen compared with those fed raw pigeon pea. The apparent ileal nitrogen digestibility of raw cowpea was lower than the raw pigeon pea.

5.4 Discussion

Body weight gain (BWG) and cumulative performance index (CPI) of the chicks fed 400 g/kg raw cowpea and pigeon pea beans were significantly lower than those fed the control diet. A similar result was found by Amaefule *et al.* (2011) when they found that chicks fed fed 30 % or 40 % raw pigeon pea had lower BWG, protein efficiency ratio and feed conversion ratio than those fed control diet.

Muamer *et al.* (2013) observed that cowpea inclusion at 10 % reduced body weight of chicks at two weeks of age when compared with controls. Anjos *et al.* (2012) and Abdelgani *et al.* (2013) also reported that chicks fed (20 %) and (15 %) raw cowpea had similar BWG to those fed the

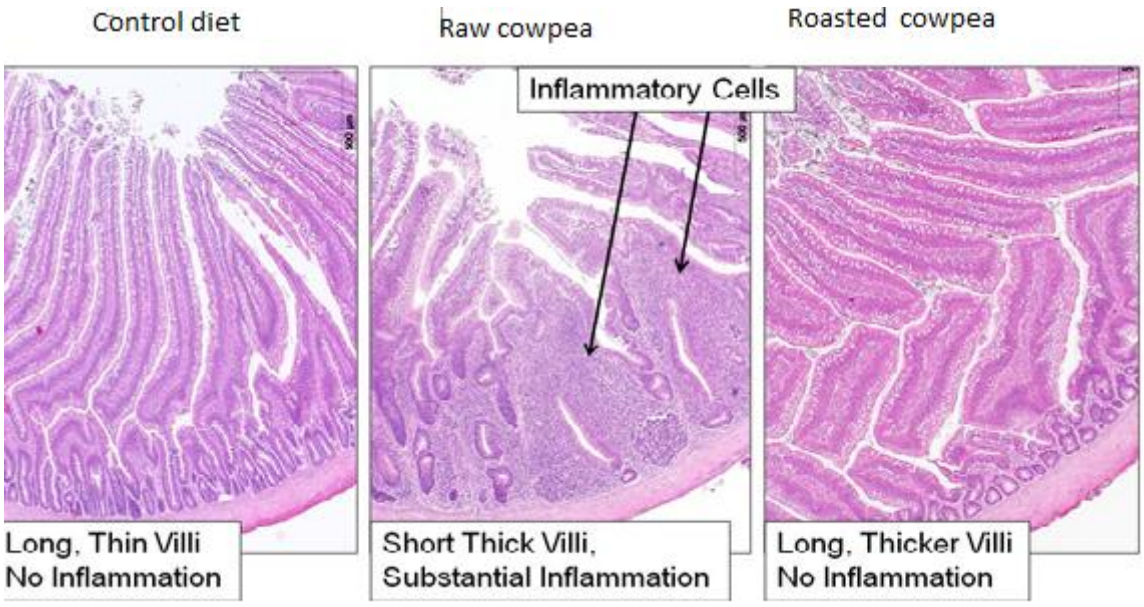

Figure 5.3a. Morphology of Duodenum from Corn Soy, Raw Cowpea and Roasted Cowpea Fed Birds (H&E, 40x)

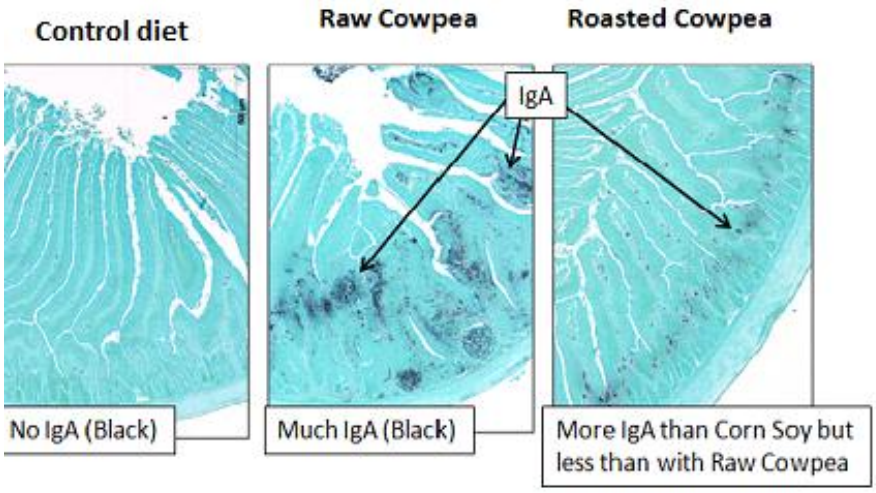

Figure 5.3b. Morphology of duodenum from corn soy, raw cowpea and roasted cowpea fed birds: 010015 (H&E, 40x); IgA: Immunoglobulin

Table 5.5. Values of apparent ileal dry matter and nitrogen digestibility in the diets containing enzyme and roasted beans

Treatments	Apparent Dry matter digestibility %	Apparent Nitrogen digestibility %
T1	0.73	0.21
T2	0.72	0.34
T3	0.91	0.73
T4	0.90	0.73
T5	0.84	0.63
T6	0.84	0.71

T1: control; T2: raw cowpea; T3: roasted cowpea; T4: raw cowpeas with enzymes; T5: raw pigeon pea; T6: roasted pigeon pea

control diet. On other hand, the low BWG observed in chicks fed raw cowpeas in the current study may be attributable to the concentration of anti-nutritional factors, such as trypsin inhibitors, present in the raw cowpeas, that have been shown to reduce growth performance (Tshorhote *et al.*, 2003; Belal *et al.*, 2011).

Cowpeas also contain galactose oligosaccharides, non-starch polysaccharides (NSP), which have anti-nutritive properties that may affect the performance of chickens (Leeson and Summers, 2001). Bedford (2000) reported that highly digestible diets are absorbed prior to the establishment of an environment favourable to bacterial growth. For poorly digestible diets, nutrients escape digestion and absorption and enter the mid-lower small intestine where it acts as a substrate and allows bacterial populations to grow. Consequently, there will be a great demand of energy and protein requirement from the diet which is ultimately taken at the expense of the host (Bedford, 2000).

The use of enzymes to improve growth performance of chickens has been shown by several authors (Bedford, 2000; Sundu *et al*., 2006; Mingbin *et al*., 2009). Adding a combination of xylanese, protease and phytase enzymes to raw cowpea diets did not overcome the negative effects of the raw cowpeas. These results are not in agreement with those reported by Belal *et al.* (2011) who found a positive effect of adding enzymes to raw cowpea with BWG being higher than those fed the control diet. Amaefule *et al.* (2011) also found that a diet containing 30% raw pigeon pea needed to be supplemented with methionine, and at 40 % raw pigeon pea the diet needed to be supplemented with both lysine and methionine to improve growth performance of the chicks. These results can be an indication that methionine could have provided sulphur needed for detoxification of ANFs contained in the raw pigeon pea diets.

The better performance of chicks fed roasted compared with raw pigeon pea, was reported earlier by Amaefule and Nwagbara (2004). In the present study, the BWG, FG, CPI and mortality of chicks fed 40 % roasted pigeon pea were similar to chicks fed the control diet. These results are not in agreement with Ani and Okeke (2011) who found that feed intake, weight gain and efficiency of feed utilization declined at the 32.5 % level of roasted pigeon pea inclusion in the diet of chicks. However, the inclusion of 27 % roasted pigeon pea into broiler finisher diets had no deleterious effect on growth performance of broiler chicks.

Maintenance or enhancement of gut health is essential for the welfare and productivity of chickens when antibiotics are not added to feed. In the present study, digesta viscosity was evaluated. The results showed that digesta viscosity was slightly higher in birds fed raw pigeon

pea and lower with those fed raw cowpea, but the difference among treatments were not significant when compared with those fed the control diet. Although not significant, the increased digesta viscosity observed in chicks fed raw pigeon pea, was in line with the depressed growth performance of the chicks. The poor growth performance could be partially due to the reduction in feed passage rate throughout the gastrointestinal tract (Yasa, 2003).

Enzymes added to poultry diets, especially diets rich in NSP reduced viscosity in the diet and digesta (Khattak *et al.*, 2006). In the present study, the digesta viscosity of the chicks was slightly improved by the addition of enzymes in the raw cowpea diet when compared with those fed the control diet. Roasting raw pigeon pea also reduced the digesta viscosity when compared with those fed raw pigeon pea, and the values were similar to those fed the control diet. These results suggest that processing or adding enzymes could reverse or prevent the negative effect of the raw beans.

Gut microflora affects the immune status of the bird through its influence on the intestinal wall, and the diet is perhaps the most important factor influencing gut microflora (Choct, 2009). In the present study, compared with chicks fed the control diet, *Clostridium perfringes* colonies were higher in chicks fed roasted cowpea and those fed the raw pigeon pea. The difference between chicks fed roasted cowpea and those fed raw pigeon pea could be due to the concentration of tannis in raw pigeon pea. Inhibitory effects of tannins from different sources have been demonstrated by Elizondo et al. (2010).

Mucosa tissue, villus lenght and width were not affected by dietary treatments. The poor performance of chicks fed raw cowpea, raw cowpea and enzymes, and raw pigeon pea were in line with the lower crypt depth when compared with those fed the control diet. Histological staining of the duodenum for IgA did support a benefit in roasting cowpea.

It is most likely that increased digesta viscosity and changes in gut size that induces significant changes in the performance of broiler chickens, especially when the birds are young (Yasar, 2003). In the present study, roasting pigeon pea improved apparent ileal nitrogen digestibility, and is consistent results observed in growth performance. In contrast, the higher apparent illeal nitrogen digestility observed in all cowpea treatments were not in line with growth performance, suggesting that, roasting was not enough to reduce the negative effect of the antinutrional factors in cowpea. The enzymes helped to improve apparent ileal nitrogen and dry matter digestibility of cowpeas. Similar results were reported by Iyayi (2013) who found that the ileal digestibility of phosphorus, crude protein, and amino acids was higher when a 30 %

roasted cowpea (50 ^{o}C) diet, was supplemented with 500 units of phytase enzyme. In addition, in the same study, Iyayi (2013) reported that phytase significantly increased feed intake and body weight of the chicks, which is in contrast to the results obtained in our study. The contrasting results between our study and that of Iyayi (2013) could be due to different levels of bean inclusion and different roasting methods.

5.5 Conclusions

Partial replacement of soybean and corn meal with 40 % roasted cowpea, or raw cowpeas supplemented with enzymes compromised bird performance. Roasting pigeon peas was an effective treatment for reducing the anti-nutritional factors in these beans. Partial replacement of corn and soybean meal with 40 % roasted pigeon peas was demonstrated to be feasible. Lack of gut inflammation in birds fed roasted, but not raw, cowpeas suggest that heat treatments were effective in at removing at least some of the ANF in local cowpeas.

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CHAPTER 6 - Effects of roasting, extrusion and enzymes on black-eyed beans (*Vigna unguiculata*) on growth performance and gut health of chickens

Abstract

An experiment was conducted to assess the effects of roasting, extruding and addition of enzymes to black-eyed beans (*Vigna unguiculata*) on growth performance and gut health of chickens. A total of 336 day-old Ross male broilers were used. Birds were randomly assigned to 42 pens of 8 birds per pen for 14 days in a completely randomized design. Diets included various combinations of raw or heat-treated black-eyed bean with or without added enzymes (proteinase, xylanase and phytase), with beans substituting for soybean meal at 400 g/kg inclusion. Feed and water were provided *ad libitum*. At day 7, feeding raw black-eyed beans reduced ($P<0.05$) body weight compared to chicks fed the control diet. Roasting or adding enzymes to the raw black-eyed beans did not improve body weight gain; however, extrusion of black-eyed beans increased ($P<0.05$) body weight gains to levels comparable to the control diet. By day 14, all diets performed similarly to those of the control diet.

Keywords: cowpeas; chick performance; gut health; processing, exogenous enzyme.

6.1 Introduction

Cowpea (*Vigna unguiculata*) is grown by resource-limited communal farmers in arid and semi-arid environments in the tropics. Cowpeas produce tender leaves and grain which are used for household consumption (Matikiti *et al.*, 2012). Cowpea is considered more tolerant to drought than soybean. One of the most remarkable features of cowpea is that it thrives in dry environments with available cultivars producing a crop with as little as 300 mm of rainfall (Gomez, 2004). It is referred to as the "hungry-season crop" since it is the first crop to be harvested before the cereal crops are ready (Etana *et al.*, 2013). Black-eyed beans have the disadvantage that it contains anti-nutritional factors (ANF's) such as trypsin inhibitors, lectins and tannins that reduce chicken performance. The anti-nutritional factors can also results in pathophysiological changes in chicken gut morphology leading to impaired absorption of nutrients.

The black-eyed bean (*Vigna unguiculata* subs. *unguiculata*) is an improved variety of cowpea. Black-eyed beans have gained considerable importance since being introduced in Zimbabwe as

a highly nutritious and relatively low input crop for smallholder farmers and has an added advantage of having a high market potential (Matikiti *et al*., 2012). Black-eyed beans respond well to improvements in management, producing higher yields, and have received much recognition on the international market compared to unimproved varieties. It is, however, prone to attack by insects. Furthermore, compared with unimproved varieties, black-eyed beans contain a lower concentration of anti-nutritional factors.

Roasting, extrusion, and incorporation of exogenous enzymes into diets have been reported to reduce the negative effects of these anti-nutritional factors in chickens. However, use of exogenous enzymes to improve the digestibility of alternative feeds for chickens has not been well researched. The effects of these enzymes depend on factors such as the age of the bird and type of the diet (Bedford, 2000; Hajati, 2009). The objective of the current study was, therefore, to determine the effects of extrusion and roasting of black-eyed beans (400 g/kg inclusion), and supplemention of black-eyed bean diets with exogenous enzymes on performance and gut health of chickens.

6.2 Materials and methods

6.2.1 Feed ingredients

Black- eyed beans (*Vigna unguiculata)* purchased in Missouri, USA extruded at Wenger Manufacturing, Inc. (Sabetha, Kansas, USA) and were used in the current study.

6.2.2 Processing of black-eyed beans

The raw black- eyed beans (RBEB) were first ground *(*Restch- saw mill; SM 2000; Serial: 127211118J) through a 2 cm x 2 cm screen, followed by a 1cm x 1cm screen to obtain a particle size of 1.5 mm mesh.The raw black-eyed beans were roasted (ROBEB) using a hotbox oven at 120°C for 45 min. The seeds were removed from the oven and cooled, then ground. A second batch of the raw black-eyed beans were also extruded at 120°C for 3 min, cooled, and ground through a 2 cm x 2 cm screen then ground again through a 1cm x 1cm screen to obtain a particle size of 1.5 mm mesh. In addition, a third batch of raw black- eyed bean were ground as described previously, and extruded at 111°C, then cooled to room temperature. The expanded, extruded pellets were then ground through a 2 cm x 2 cm screen.

6.2.3 Birds and housing

A total of 336 day-old male broilers (Ross 708) chicks were purchased from a commercial hatchery, weighed, wing banded, and assigned to stainless steel chick batteries. All pens had the same initial body weight (about 41 g/bird). A completely randomized design was used with seven replicate pens of eight chicks assigned to each of the six dietary treatments. The chicks were fed starter diets from day 0 to 14. Lighting comprised 24 hours of light (approximately 2 foot candles) from days 1 to 9, followed by 20 hours of light (approximately 2 foot candles) and 4 hours of darkness from Days 10 to 14. The temperature in the house ranged between 35 and 36.1^{o}C at the beginning of the experiment and between 30 and 31^{o}C towards the end of the experiment. The health status of the birds was assessed daily. All abnormalities were recorded, including body weights of dead chicks and culls.

6.2.4 Treatments and experimental design

Six dietary treatments were used as shown in Table 6.1. Tables 6.1 and 6.2 have shown the ingredient and nutritional composition of the diets, respectively. Treatment 1 was the control maize-soya meal diet, while treatment 2 was a diet containing the raw black-eyed beans (RBEB). Treatment 3 was a diet that contained roasted black-eyed beans (ROBEB) and Treatment 4 contained extruded black-eyed beans (EBEB). Treatment 5 was a diet that contained raw black-eyed beans supplemented with a mixture of enzymes. Treatment 6 was a diet that contained extruded black-eye beans supplemented with a mixture of enzymes. The enzyme mixture included a combination of a commercial phytase (Ronozyme), Cibenza DP100 (protease) and Cibenza CSM (xylanase, α-galactosidase and β-glucanas) were used. Sixty g of each commercial enzyme was mixed with 5.82 kg of ground maize to make a premix. A randomized complete design was used, and included 6 treatments with 7 replicate pens of 8 birds per pen. A maize-soybean meal-based basal diet (mash form) formulated to meet the nutritional requirements of chicks (1-14 d post-hatch), as recommended by the National Research Council (NRC, 1994), was used. The raw black- eyed beans, treated black- eyed beans and treated black- eyed beans plus enzymes were included at 400 g/kg inclusion.

6.2.5 Measurements

6.2.5.1 Gut morphology

At day 12, two birds per pen were injected with 10 mg/kg of bromodeoxyuridine (BRDU) as a marker for gut epithelial growth. Two days later, the same birds were again injected with

Table 6.1. Ingredient composition of experimental diet used in the study (g/kg)

	Treatments					
Ingredients	1	2	3	4	5	6
Maize	593.4	351.4	351.4	379.3	351.4	379.3
Soy bean	318.1	168.1	168.1	140.9	168.1	140.9
Raw BEB	0.0	400.0	0.0	0.0	400.0	0.0
Extruded beans	0.0	0.0	0.0	400.0	0.0	400.0
Roasted beans	0.0	0.0	400.0	0.0	0.0	0.0
Soy bean oil	10.0	33.3	33.3	10.0	33.3	10.0
Dicalcium - foods	18.0	18.6	18.6	18.0	18.6	18.0
Limestone	11.1	11.4	11.4	11.8	11.4	11.8
Salt	3.6	3.5	3.5	3.5	3.5	3.5
Sodium bicarbonate	2.6	2.8	2.8	2.7	2.8	2.7
Alimet	1.9	3.2	3.2	3.2	3.2	3.2
L-lysine hydrochloride 78	1.3	1.3	1.3	1.7	1.3	1.7
Threonine	0.7	1.4	1.4	1.3	1.4	1.3
Choline 60	0.8	1.9	1.9	2.0	1.9	2.0
Organic trace mineral mix	2.0	2.0	2.0	2.0	2.0	2.0
Foods vit mix	0.5	0.5	0.5	0.5	0.5	0.5
Mould guard	0.5	0.5	0.5	0.5	0.5	0.5
Santoquin-mix6	0.01	0.01	0.01	0.01	0.01	0.01
Sand	3.54	0.00	0.00	2.25	0.00	2.25
Total	100.0	100.0	100.0	100.0	100.0	100.0

Treatment 1: control maize-soya meal; Treatment 2: raw black- eyed beans (RBEB); Treatment 3: roasted black- eyed beans (ROBEB); Treatment 4: extruded black- eyed beans (EBEB); Treatments 5: raw black-eyed beans plus enzymes; Treatment 6: extruded black- eyed beans plus enzymes

Table 6.2. Calculated nutrient composition of the diets

Calculated nutrient composition	1	2	3	4	5	6
ME (kcal/kg)	2900	2900	2900	2900	2900	2900
Crude Protein (%)	20.44	19.66	19.66	20.12	19.66	20.12
Available phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45
Calcium (%)	0.92	0.92	0.92	0.92	0.92	0.92
Sodium (%)	0.22	0.22	0.22	0.22	0.22	0.22
Choline (ppm)	1600	1600	1600	1600	1600	1600
Digestible lysine (%)	1.07	1.07	1.07	1.07	1.07	1.07
Digestible sulphur amino acid (%)	0.80	0.80	0.80	0.80	0.80	0.80
Digestible threonine (%)	0.72	0.72	0.72	0.72	0.72	0.72
Digestible tryptophan (%)	0.23	0.20	0.20	0.20	0.20	0.20

Treatment 1: control maize-soya meal; Treatment 2: raw black- eyed beans (RBEB); Treatment 3: roasted black- eyed beans (ROBEB); Treatment 4: extruded black- eyed beans (EBEB); Treatments 5: raw black- eyed beans plus enzymes; Treatment 6: extruded black- eyed beans plus enzymes.

BRDU, before they were weighed and euthanized. Samples of duodenum (~ 1 cm), midgut (~ 1 cm), ileo-cecal junction with 1 cm of ileum attached, and a cross section of mid cecal pouch were collected from each bird. The lumen was flushed gently with Notox and preserved in labeled bottles containing 10-20 × volume of Notox (samples were pooled by treatment).

A 1-cm piece of duodenum was collected from one bird per pen, flushed with Notox and fixed with 10-20× volume of Notox in a labeled bottle. The fixed tissues were processed and embedded in paraffin wax. Tissue blocks were then sectioned to 5 microns thickness, stained with haematoxylin and eosin (H&E) or immunostained with anti-BRDU, and examined using an Olympus light microscope. The H&E stained slides were used for examination of gut morphology and measurement of gut morphometry. The anti-BRDU stained slides were used for examination of cell proliferation. The height and width of 5 randomly selected villi, the depth of 5 randomly selected crypts, and the thickness of mucosa tissue at 5 different locations per sample were measured using an Olympus light microscope with a 10× eyepiece lens with a 10× objective lens given a magnification of 100×. The villi height, villi width, crypt depth, crypt depth/villi height (CV) ratio and mucosa tissue thickness (TMUC) were compared among treatments. Greater villi height and lower villi width indicate a greater absorption area. Lower crypt depth and crypt depth/villi height ratio indicates less need of cell proliferation to maintain gut integrity. The mean of villi heights, villi widths, crypt depths and TMUCs for each sample were used for statistical analyses.

6.2.5.2 Growth performance

The birds were weighed by pen on days 0, 7 and 14, and feed consumption was determined. Mortality was checked twice daily and weights of dead birds were used to adjust for feed conversion. Body weight gain was estimated as: = (body weight at a weigh day) – (body weight at the previous weigh day). The feed: gain ratio corrected for dead birds was estimated as: (feed consumed) / (incremental pen weight + incremental dead bird weight).

The feed consumed was estimated as the difference between the total weight of feed offered and the residual for each week/pen divided by the number of birds per pen. The cumulative performance index (CPI) was calculated as: = ((Cumulative livability x ((body weight x 1000) /Day of study) x 10)/ (Cumulative feed: gain ratio corrected for dead bird weight)) were calculated.

6.2.6 Statistical analyses

Data were analyzed using the General Linear Models procedure of SAS® (SAS, Institute, Cary, NC, USA). When the null hypothesis was rejected, means were separated using Fisher's protected least significant difference method.

6.3 Results

6.3.1 Growth performance

The effects of dietary treatments at days 7 and 14 on body weight, body weight gain (BWG), feed: gain ratio and CPI, are shown in Table 6.3.

On Day 7, chicks fed raw black-eyed beans (RBEB) had lower *(P<0.05*) growth performance than those fed the control diet. Roasting raw black- eyed beans did not improve growth performance, but extrusion of raw black- eyed beans significantly improved body-weight gain (BWG) and feed:gain ratio, and the CPI of the chicks was similar(*P>0.05*) to those fed the control diet. Addition of enzymes to the diet containing raw- black-eyed beans (RBEB) did not improve BWG and CPI compared to chicks fed RBEB. Chicks fed extruded RBEB supplemented with enzymes had similar (*P>0.05*) BWG, FG and CPI to those fed the control diet.

Day 14, with the exception of chicks fed the extruded raw black- eyed beans, all other groups had similar BWG to those fed the control diet. The chicks fed extruded BEB had lower (*P<0.05*) BWG and higher (*P<0.05*) F: G ratio than those fed the control diet.

6.3.2 Gut health

6.3.2.1 Gut morphology

The measurements of villus (length and width), crypt depth, and mucosa tissue of broiler duodenum are shown in Table 6.4. Measurement of mucosa tissue and the villi width of chicks from all treatments revealed results similar (*P>0.05*) to those of the chicks fed the control diet.
The villi length of the chicks fed treatments 2, 3, 4 and 5 were similar to those fed the control diet. In contrast, the chicks fed treatment 6 had lower (*P<0.05*) villus length compared to those fed treatment 2 RBEB and those fed on the control diet.

Table 6.3. The effects of dietary treatments on body weight, body weight gain (BWG), feed: gain ration and cumulative performance index (g)

		Treatment						
Day	Parameter	1	2	3	4	5	6	P-value
7	Body weight	0.15^{a}	0.13^{b}	0.12^{b}	0.14^{a}	0.12^{b}	0.14^{a}	0.0001
	BWG	0.11^{a}	0.09^{b}	0.08^{b}	0.10^{a}	0.08^{b}	0.10^{a}	0.0001
	F:G	1.24^{c}	1.40^{a}	1.42^{a}	1.26^{c}	1.45^{a}	1.30^{c}	0.0019
	CPI	0.132^{a}	0.121^{c}	0.117^{c}	0.127^{a}	0.115^{c}	0.131^{a}	0.0001
14	Body weight	0.36^{a}	0.33^{bc}	0.33^{c}	0.32^{c}	0.32^{c}	0.35^{ab}	0.0090
	BWG	0.21^{a}	0.20^{a}	0.20^{a}	0.18^{b}	0.20^{a}	0.21^{a}	0.0199
	F:G	1.46^{bc}	1.53^{ab}	1.53^{ab}	1.58^{a}	1.49^{abc}	1.43^{c}	0.01221
	CPI	187^{a}	144^{c}	137^{c}	156^{bc}	136^{c}	169^{ab}	0.0003

^{abc} Values in the same row with different letters are significantly different ($P<0.05$)

Treatment 1: control maize-soya meal; Treatment 2: raw black-eyed beans (RBEB); Treatment 3: roasted black-eyed beans (ROBEB); Treatment 4: extruded black-eyed beans (EBEB); Treatments 5: raw black-eyed beans plus enzymes; Treatment 6: extruded black-eyed beans plus enzymes

BWG = body weight gain; F: G = Feed to gain ratio; CPI = cumulative performance index

Table 6.4. Villi length and villi width, cript and mucosa tissue on broiler duodenum (µm)

Parameter (µm)	1	2	3	4	5	6	P-value
TMUC	2393[a]	2164[a]	2115[a]	2065[a]	2421[a]	1971[a]	0.1829
VilLngth	2167[ab]	1938[ab]	1900[ab]	1849[ab]	2224[a]	1785[c]	0.1613
vilwdth	201[a]	221[a]	187[a]	192[a]	202[a]	187[a]	0.3528
crypt	226[a]	226[a]	215[a]	216[a]	198[a]	186[a]	0.3826
cvratio	0.105[ab]	0.117[a]	0.113[ab]	0.119[a]	0.089[b]	0.104[ab]	0.1781

[abc] Values in the same row with different letters are significantly different *(P<0.05*)

Treatment 1: control maize-soya meal; Treatment 2: raw black-eyed beans (RBEB); Treatment 3: roasted black-eyed beans (ROBEB); Treatment 4: extruded black-eyed beans (EBEB); Treatments 5: raw black-eyed beans plus enzymes; Treatment 6: extruded black-eyed beans plus enzymes

TMUC= mucosa tissue; VilLngth = villus length; vilwdth= villus width

6.4 Discussion

Black-eyed beans are potential sources of energy and protein for chickens. Its use is, however, still limited due to the effect of anti nutritional factors present in the beans. Anti-nutritional factors adversely affect the digestibility of protein, as well as the bioavailability of amino acids, and the protein quality of feed (Gilani *et al.*, 2012).

On Day 7, feeding raw black-eyed beans significantly reduced BWG when compared to chicks fed the control diet. These findings agree with Arija *et al*. (2006) who reported a significant reduction in growth performance and an increase in relative weights of the pancreas, liver, and jejunum in chickens fed a diet containing 300 g/kg raw kidney bean. The anti-nutritional factors in the bean could have interfered with digestion and, therefore, reduced feed consumption (Marzo *et al.*, 2002).

The efficacy of heat treatment in reducing anti nutritional factors in beans has been reported earlier (e.g. Abdon *et al*., 2013; Balail, 2014). In the present study, roasting raw black-eyed beans did not improve body weight gain when compared with the RBEB. These findings are in contrast with Emiola *et al.* (2007) who reported that feed intake and BWG of birds fed a control diet and diet containing 50% heat-treated (roasting or boiling) kidney bean meals were similar and significantly higher than those fed raw or dehulled meals. The difference in the findings could be due to the different type of bean used (black-eyed beans versus kidney beans), as well as processing temperature employed (120^{o}C for 45 minutes versus 120^{o}C for 30 minutes). Abdon *et al*. (2013) reported that roasted cowpeas contained lower crude protein, ether extract, crude fibre, ash and metabolizable energy than soaked peas, a finding which may help to explain the poor performance of birds in the current study.

Extrusion has been shown to be effective in reducing trypsin, chymotrypsin inhibitors and haemagglutinating activities in peas (Alonso *et al.*, 1998). In the present study, extrusion significantly improved BWG, feed: gain ratio and the cumulative performance index. This beneficial effect could be due to an inactivation of the anti-nutritional factors in the beans and an increase in the digestibility of nutrients.

The exposure of feed material to high temperatures for short times (extrusion) has the favourable effect of high rates of destruction of micro-organisms and destruction of heat-labile anti nutrients. High shear forces may also denature protein and disrupt the food matrix, thereby improving the digestibility of nutrients (Arija *et al.*, 2006).

Feed enzymes can make an impact on gastrointestinal microbial ecology by reducing undigested substrates and anti-nutritive factors (Kiarie *et al.,* 2013). In the current study, addition of enzymes to a diet containing raw black-eyed beans did not improve performance as observed in chicks fed raw black-eyed beans. The poor performance could be related to the age of the chicks. The responses to enzyme supplementation depend on the age of the bird, which is apparently related to both the type of gut microflora present and the physiology of the chicken (Hajati *et al.,* 2009).

Iyayi *et al*. (2013) also reported that phytase supplementation of the cowpea- diets significantly improved the growth performance of the birds, as result of increased feed intake and feed efficiency. Chicks fed the extruded black-eyed beans plus enzymes performed similarly to those fed the control diet. These results suggest that although the combination of extrusion plus enzymes was beneficial for the growth performance of chicks, there was no significant influence of enzyme addition to extruded peas, indicating that extrusion alone is sufficient to create the same level of benefit.

At 14 days of age, chicks fed raw black-eyed beans performed similarly to those fed the control diet. These results suggest that older birds can probably tolerate the levels of anti-nutritional factors better than birds that are seven days old. This poor performance of 7-day-old chicks compared to 14-day-old chicks could be attributed to three key factors. First, the age of the chicks; second, it could also be due to low feed intake as a consequence of the bitter taste of the beans; and finally, it may be that after seven days, the birds became used to the bitter taste, ate more, and consequently increased their feed intake and BWG. On the other hand, the anti-nutritional factors present in black –eyed beans may decrease nutrient digestibility and absorbability due to increased digesta viscosity of the gut content. It is most likely that increased digesta viscosity and changes in gut size can induce significant changes in the performance of broiler chickens, especially when the birds were young (Yasar, 2003). With the exception of chicks fed extruded black-eyed beans, all other groups had similar BWG to those fed the control diet.

On day 14, gut measurements (of the villus, crypt and mucosa tissue) of all groups of chicks were not different to those fed the control diet. These findings are consistent with the growth performance of the chicks at day 14, which suggests the posited relationship between gut health and good growth performance. There is a paucity of research on the effect of cowpea on gut morphology. The gut morphology parameters were similar to broilers fed 20% cooked cowpeas

(Iheukwumere *et al.*, 2008), but differed significantly from broilers fed 20 % raw and 20 % toasted soybean seed meal. Makinde *et al*. (1996) reported that pigs fed 100 % cowpea had greater crypt depth than pigs fed soybean meal during the first 7 d post weaning.

6.5 Conclusions

It is concluded that at day 7, roasted and raw black-eyed beans supplemented with enzymes were not beneficial for improving the growth performance of the chicks. Extrusion significantly improved body weight gain, feed: gain ratio and cumulative performance index of the chickens. At day 14 chicks fed extruded black-eyed beans plus enzymes had similar BWG and F: G ratio to those fed the control diet.

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CHAPTER 7 - Efficacy of Mozambican bentonite and diatomaceous earth in ameliorating the toxic effects of aflatoxins in broiler chicks

(Submitted to World Mycotoxin Journal)

Abstract

The effectiveness of diatomaceous earth (DE) and bentonite clay (BC) in ameliorating the toxic effects of aflatoxin B_1 (AFB_1) in chicks was assessed. A total of 150 day-old male broiler chicks were assigned to six dietary treatments, with 5 replicate pens of five chicks per treatment. Dietary treatments were: 1) a positive control basal diet (BD) containing no AFB_1, no BC, or no DE; 2) BD + 0.50% BC but with no AFB_1or DE; 3) BD + 0.50% DE but with no AF or BC; 4) BD + 2 mg AFB_1/kg diet but with no BC or DE; 5) BD + 0.50% BC + 2 mg AFB_1/kg diet but with no DE; and 6) BD + 0.50% DE + 2 mg AFB_1/kg diet but with no BC. Compared with controls, feed intake (FI) and body weight gain (BWG) were depressed ($P<0.05$) in chicks fed AFB_1, with greater reduction in FI and BWG observed in birds fed the AFB_1 plus 0.50% DE diet. Feed conversion ratio was similar. Chicks fed AFB_1 alone had increased ($P<0.05$) relative liver weights compared to all other dietary treatments except for chicks fed AFB_{1+}DE. Chicks fed AFB_1 alone and those fed AFB_1+DE had heavier ($P<0.05$) relative kidney weights compared to chicks fed other treatments. Compared with the control chicks, livers from birds fed dietary AFB_1 and AFB_1 plus DE were similar. Liver lesion score of chicks fed AFB_1 plus BC was lower ($P<0.05$) than that of chicks fed AFB_1 but higher ($P<0.05$) than that of control chicks. Compared to controls, serum concentrations of glucose, albumin, total protein globulin, and calcium were decreased ($P<0.05$) in chicks fed AFB_1 alone, whereas serum concentrations of AST and GGT were increased ($P<0.05$). It can be concluded that BC was partially effective in reducing the toxic effects of AFB_1 whereas DE was not effective in reducing the severity of lesions caused by AFB_1.

Key words: aflatoxin, absorbents, broilers.

7.1 Introduction

Mycotoxins are toxic secondary metabolites produced by fungi that can be present on poultry feeds and are major threats to poultry growth performance and health (Shareef, 2009). The two main genera of mycotoxin producing fungi which impact poultry production are *Aspergillus* and *Fusarium*. *Aspergillus* is a tropical and semi-tropical fungus which produces aflatoxins and ochratoxin A. Aflatoxins contaminate food and animal feeds and cause serious health challenges and livestock production losses (Rensburg *et al.*, 2006). Contamination of feeds with

mycotoxins may induce livestock morbidity and mortality. Diets containing aflatoxins could induce pathological lesions in the liver, and impair hepatic antioxidant functions (Yang *et al.*, 2012).

In many smallholder chicken production systems, kitchen leftovers are a major source of nutrients (Mtileni *et al*., 2012). Fungal contamination of kitchen leftovers is also widespread in tropical countries (Okoli *et al*., 2006; Muhammad *et al.*, 2010). Therefore, it is important to identify and characterize products that can bind mycotoxins. Several authors (e.g. Rosa *et al.*, 2001; Mordisanei *et al.*, 2008; Franciscato, 2006) reported that diatomaceous earth and bentonite clays have the potential to reduce the toxic effects of aflatoxin B_1 in chickens. In Mozambique, deposits of diatomaceous earth and bentonite clays exist but their potential as a feed additive has not been assessed. Therefore, the objective of the current study was to determine the efficacy of Mozambican bentonite clay (BC) and diatomaceous earth (DE) to reduce the toxic effects of aflatoxin in chickens.

7.2 Material and methods

7.2.1 Experimental design and birds

One hundred and fifty day-old male broiler (Ross 308) chicks were purchased from a commercial hatchery, weighed, wing banded, and assigned to stainless steel chick batteries. All pens had the same initial body weights (about 41 g/bird). A completely randomized design was used with five replicate pens of five chicks assigned to each of six dietary treatments. The chicks were maintained on a 24h continuous lighting schedule and allowed *ad libitum* access to feed and water from hatch to day 21. The temperature of the room ranged between 35 and 36.1 ºC at the beginning of the experiment and between 30 and 31.1ºC towards the end of the experiment. The Animal Care and Use Protocol was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee. The birds were inspected daily for any health related problems.

7.2.2 Diets

A maize-soybean meal-based basal diet (mash form) formulated to meet the nutritional requirements of young broilers (1-21d post-hatch), as recommended by the National Research Council (NRC, 1994), was used. Table 7.1 shows the ingredient and chemical composition of

Table 7.1. Composition and nutrient value of basal diet (as-is)

Ingredients	Composition (%)
Corn	52.94
Soybean meal	37.92
Soybean oil	4.48
Dicalcium phosphate	1.72
Limestone	1.25
Salt	0.46
DL-methionine	0.19
Vitamin/mineral mix[2]	0.25
Sand	0.80
Total	100
Nutrient composition (calculated)[3]	
Crude protein (%)	23.00
Metabolizable Energy (Kcal/kg)	3100
Lysine (%)	1.26
Methionine (%)	0.53
Methionine + Cysteine (%)	0.90
Threonine (%)	0.86
Tryptophan (%)	0.31
Calcium (%)	1.00
Phosphorus (% Av.)	0.45

[2]Supplied per kilogram of feed: manganese, 100 mg;zinc, 100 mg; iron, 50 mg; copper, 11.25 mg; iodine, 1.5 mg; selenium, 0.15 mg; vitamin A, 7,700 IU; vitamin D_3, 2,750 ICU; vitamin E, 16.5 IU; vitamin B_{12}, 11 µg; vitamin K, 0.83 mg; riboflavin, 6.6 mg; thiamin, 1.1 mg; pantothenic acid, 6.6 mg; niacin, 27.5 mg; pyridoxine, 1.37 mg; folic acid, 0.69 mg; biotin, 33 µg; choline, 385 mg

the basal diet. AflatoxinB_1 (AFB_1) from ground *Aspergillus parasiticus* strain NRRL 2999 culture material (700 mg/kg culture material) was incorporated into the basal diet to achieve the required dietary AFB_1 concentration of 2 mg/kg diet.

Dietary treatments were: 1) a positive control basal diet (BD) containing no AF, no BC, and no DE; 2) BD + 0.50% BC but with no AF or DE; 3) BD + 0.50% DE but with no AF or BC; 4) BD + 2 mg AFB_1/kg diet but with no BC or DE; 5) BD + 0.50% BC + 2 mg AFB_1/kg diet but with no DE; and 6) BD + 0.50% DE + 2 mg AFB_1/kg diet but with no BC. The composition of the basal diet is given in Table 7.1. Dietary AF (B_1, B_2, G_1, and G_2) concentrations were determined. Briefly, feed samples were extracted with acetonitrile and water (86:14), and an aliquot of the extract was passed through a puriTox TC-M 160 cleanup column and suitably diluted with water before analysis using HPLC with cobra cell post column derivatization with fluorescence detection at 365 nm excitation and 440nm emission. Before the start of the experiment, all diets were screened according to the method described by Rottinghaus *et al.* (1982) for the presence of citrinin, T-2 toxin, vomitoxin, zearalenone, fumonisis and ochratoxin A.

7.2.3 Measurements

Chicks were weighed by pen on day 0, 7, 14 and 21 of the experiment. Feed was also weighed weekly per pen and feed conversion ratio was calculated. Mortalities were recorded as they occurred and the dead birds were necropsied. On day 21, three birds per pen were anaesthetized with carbon dioxide and blood samples collected via cardiac puncture for the determination of serum chemistries. Liver weight of each of the three birds was recorded and a piece of liver tissue (2 to 3 g) was collected and rinsed with ice-cold phosphate buffered saline (pH 7.4) containing 0.16 mg/ml heparin to prevent blood clot formation. Liver samples were also harvested from six birds per treatment, and fixed in 10% neutral buffered formalin for gross and histopathology evaluation. Fixed liver tissues were trimmed, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin for microscopic examination. Liver lesions were scored using a scoring system of 1 to 4 (1= liver unremarkable; 2= mild aflatoxin lesions; 3= moderate aflatoxin lesions; and 4= severe aflatoxin lesions).

Blood was centrifuged at 1400xg at 8ºC for 30 min (Sorval, RC 3 B plus) and the serum was preserved at -20ºC pending biochemical analyses. Serum samples were analyzed for total proteins, albumin, globulin, glucose, uric acid, gamma glutamyltransferase (EC 2.3.2.2), aspartate aminotransferase, and calcium using an auto analyzer (Kodak Ekatachem Analyzer, Eastman Kodak Co, Rochester, NY).

The shank colour of all surviving birds was determined using the DSM colour fan for broilers. Right tibiae were collected from three birds per pen for determination of tibia ash content. Tibiae were stripped of adhering tissue, dried at 100ºC, and fat was extracted with a mixture of ether and methanol (90 and 10%, respectively). Fat-extracted tibiae were than dried at 100 ºC for 24 h and ashed in a muffle furnace at 600ºC overnight.

7.2.4 Statistical analyses

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., 2011). Except for histology where chick was the experimental unit, pen was considered the experimental unit for all other response variables. An arcsine transformation was applied to percent mortality data before statistical analyses were performed. All statements of significance are based on the 0.05 level of probability.

7.3 Results

7.3.1 Growth performance

The effects of BC and DE on 21 day growth performance of chicks fed AFB_1 are shown in Table 7.2. Body weight gain (BWG), feed intake (FI) and feed to gain (FC) of chicks fed BC or DE were not different (*P>0.05*) from those of control chicks. In contrast, chicks fed the 2.0 mg AFB_1/kg diet alone had significantly depressed (*P<0.05*) BWG and FI when compared to control chicks. Although not statistically significant, the addition of 0.50 % BC to the AFB_1 diet tended to improve FI (6 %) and BWG (9 %) when compared with chicks fed only AFB_1. Feed conversion ratio was not different among treatments.

7.3.2 Liver and kidney weights

Effects of dietary AFB_1 and adsorbents on relative organ weights are shown in Table 7.3. Relative liver and kidney weights of chicks fed adsorbents alone were not different (*P>0.05)* from those of control chicks. Compared to control chicks, relative liver and kidney weights were significantly increased by dietary AFB_1. In chicks fed AFB_1 plus BC, the relative liver weight was 19 % higher than that of controls, but were lower (*P<0.05*) than that of chicks fed AFB_1 alone. Relative liver weight of chicks fed AFB_1 plus DE was greater (*P<0.05*) than that of control chicks but similar to those of chicks fed AFB_1 only. The relative kidney weight of chicks fed AFB_1 plus BC was higher (*P<0.05*) than that of control chicks but lower (*P<0.05)*

Table 7.2. Effects of bentonites and diatomaceous earth on 21 day growth performance and mortality of chicks fed containing 2 mg AFB_1/kg diet

Treatments[2]		Variables		
		Body weight gain (g)	Feed intake (g)	Feed:Gain (g:g)
BD		876[a]	1166[a]	1.33
BD + BC		864[a]	1149[a]	1.33
BD + DE		865[a]	1145[a]	1.32
BD + AF		777[bc]	1037[bc]	1.33
BD + AF+BC		847[ab]	1108[ab]	1.30
BD + AF +DE		734[c]	968[c]	1.32
ANOVA	S.E.M.:	26	34	0.016
	P-value:	0.003	0.002	0.862
Adsorbent source				
None		826[ab]	1101[ab]	1.33
Bentonite		856[a]	1129[a]	1.31
Diatomaceous earth		780[b]	1057[b]	1.32
Aflatoxin level				
0		869[a]	1153[a]	1.32
2 ppm		786[b]	1038[b]	1.32
Main factors		P-value		
Adsorbent		0.1218	0.1280	0.6089
Aflatoxin		0.0007	0.0004	0.5669
Adsorbent xAflatoxin		0.0978	0.1575	0.7051

[1]Data are means of five replicate pens of 5 chicks each for 21 days

[2]Treatments were the addition of $AFB_1$and adsorbents to the basal diet (BD).

[a-c]Values within columns with no common superscripts are different ($P<0.05$)

AFB_1= Aflatoxin B_1 from culture material; BD = Basal diet; BC = Bentonite clay; DE = Diatomaceous earth

Table 7.3. Effects of bentonite and diatomaceous earth on relative organ weight and liver lesions scores of chicks fed diets containg 2 mg AFB_1/kg diet[1]

Treatments[2]		Variables		
		Relative liver weight (%)[2]	Relative kidney weight (%)[2]	Liver histological lesion score[3]
BD		2.77[c]	0.78[c]	0.00[c]
BD + BC		2.82[c]	0.82[c]	0.00[c]
BD + DE		2.98[c]	0.82[c]	0.00[c]
BD + AF		3.98[a]	1.52[a]	3.50[a]
BD + AF+BC		3.29[bc]	1.16[b]	1.66[b]
BD + AF +DE		3.63[ab]	1.51[a]	3.66[a]
ANOVA	S.E.M.:	0.19	0.09	0.23
	P-value:	0.0008	< 0001	< 0001
Adsorbent source				
None		3.37[a]	1.15[a]	1.75[a]
Bentonite		3.06[a]	0.98[a]	0.83[b]
Diatomaceous earth		3.37[a]	1.16[a]	1.83[a]
Aflatoxin level				
0		2.86[b]	0.80[b]	0.0[b]
2 ppm		3.63[a]	1.39[a]	2.9[a]
Main factors			P-value	
Adsorbent		0.2429	0.1172	< .0001
Aflatoxin		< .0001	< .0001	< .0001
Adsorbent x Aflatoxin		0.1530	0.0902	< .0001

[1]Values are means of five replicate pens of 3 chicks each. [2]Treatments were the addition of $AFB_1$and adsorbents to the basal diet (BD). [abc] Values within columns with no common superscripts are different (*P<0.05*)

AFB_1 = Aflatoxin B_1 from culture material; BD = basal diet; BC = Bentonite clay; DE = diatomaceous earth

Liver lesions were scored using a scoring system of 0 to 4 (0= liver unremarkable; 1= mild aflatoxin lesions; 2= moderate aflatoxin lesions; 3= remarkable and 4= severe aflatoxin lesions).

than that of chicks fed only AFB_1. The relative kidney weight of chicks fed AFB_1 plus DE was higher (*P<0.05*) than that of control chicks but was similar to that of chicks fed the AFB_1 contaminated diet.

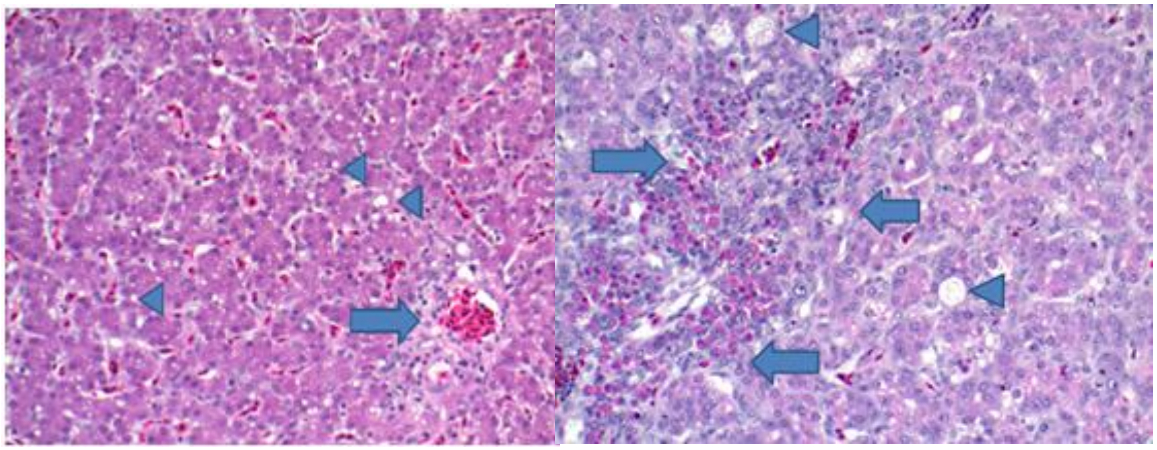
The liver lesion scores (Table 7.3) were analyzed based on grading of the severity of lesions with a value of 0 = no significant lesions and a value of 4 = severe lesions present. Mean liver lesion score of chicks fed the control diet and chicks fed adsorbents alone was 0.0. Chicks fed 2 mg/kg AFB_1 had a higher (3.5) mean score (*P<0.05)* compared with control chicks (0.00). Addition of DE to the AFB_1 diet did not reduce the severity of the liver lesions caused by AFB_1 (3.66 versus 3.50), but the addition of BC significantly (*P<0.05*) reduced the lesion scores caused by AFB_1 alone (1.66 versus 3.50).

7.3.3 Histopathology of the liver

Compared with the control chicks, birds fed dietary AFB_1 (Figure 7.1a and 7.1b) had multiple infiltrations of heterophils, lymphocytes, and macrophages. The portal tract areas were infiltrated by heterophils, and mild fibrosis and bile duct proliferation were observed in the portal tract area. Mild to marked hepatocellular vacuolation and swollen hepatocytes were present. Many of the hepatocellular nuclei were vesicular and pleomorphic. Small numbers of hepatocytes in the portal areas had mitotic figures.

Chicks fed AFB_1 plus BC (Figure 7.2a and 7.2b) had similar changes to those found in the group fed AFB_1 only, but the lesions were less severe with a few scattered infiltrations of lymphocytes and macrophages observed. Chicks fed AFB_1 plus DE (Figure 7.3) showed similar histological findings as observed in chicks fed only AFB_1 (Figure 7.1a).

Tables 7.4 and 7.5 show the effects of dietary treatments on serum chemistries. There were no significant differences in serum glucose, albumin, total protein and globulin in chicks fed adsorbents when compared to control chicks. Compared to controls, the addition of AFB_1 decreased (*P<0.05*) serum glucose, albumin, total protein and globulin. Chicks fed AFB_1 plus BC had significantly (*P<0.05*) increased serum albumin (30%), total protein (26%) and globulin (21%) when compared with chicks fed AFB_1 alone. No significant differences were observed in birds fed DE plus AFB_1 when compared with the AFB_1 dietary treatment. Serum concentrations of calcium, aspartate amino transferase (AST) and gamma glutamyltransferase (GGT) in chicks fed adsorbents were similar to controls and different (*P<0.05*) from those fed the AFB_1 diet.

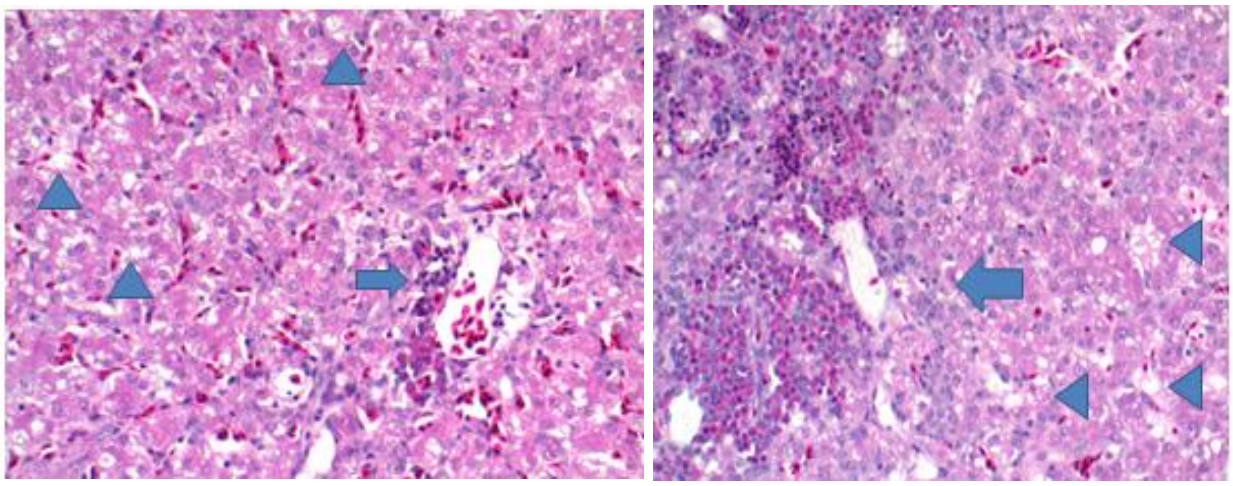


(a) (b)

Liver lesions from chicks fed treatment BD: Portal tract (arrow) and hepatocytes are within normal limits. Small single vacuoles are visible in a few hepatocytes (arrowheads). **(HE 20X obj)**

Liver lesions from chicks fed treatment BD+AF: Portal tract (arrow) is infiltrated by large number of inflammatory cells (heterophils and macrophages). Many of hepatocytes are vacuolated (arrowheads). **(HE 20X obj)**

Figure 7.1. Liver lesions from chicks fed (a) treatment BD and (b) treatment BD+AF (HE 20X obj)

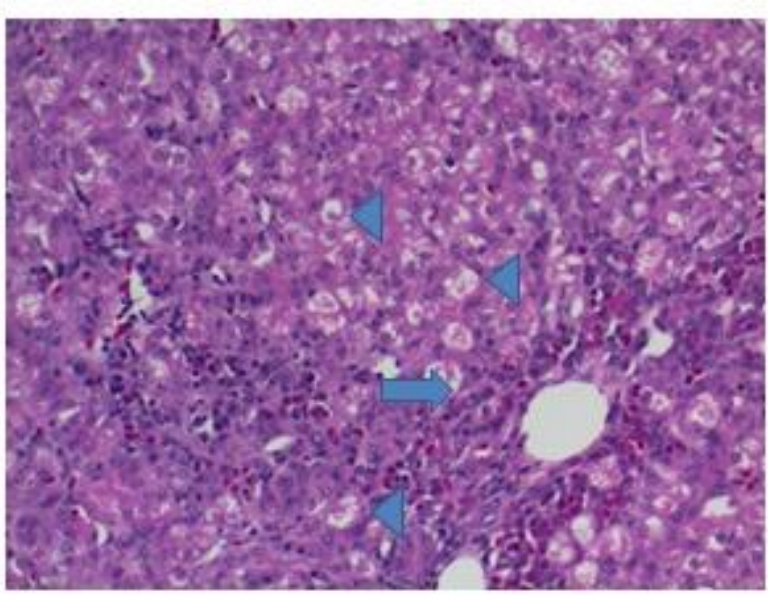


(a) (b)

Liver lesion from chicks fed treatment BD+AF+BC: The changes are mild in this liver. The portal tracts (arrow) are infiltrated by only small numbers of inflammatory cells (heterophils and macro-phages).The hepatocytes are mildly vacuolated (arrowheads). **(HE 20X obj).**

Liver lesions from chicks fed treatment BD+AF b): Portal tract (arrow) is infiltrated by large number of inflammatory cells ((heterophils and macrophages). Many of hepatocytes are vacuolated (arrowheads). **(HE 20X obj).**

Figure 7.2. Liver lesion from chicks fed (a) treatment BD+AF+BC and (b) treatment BD+AF+ BC (HE 20X obj)



Liver from Treatment BD+AF+DE: The portal tract (arrow) is infiltrated by large numbers of inflammatory cells (heterophils and macrophages). Many of the hepatocytes are vacuolated (arrowheads). **(HE 20X obj)**

Figure 7.3. Liver from Treatment BD+AF+DE

Table 7.4. Effects of bentonite and diatomaceous earth on glucose, total protein, and albumin and globulin levels in chicks fed diets containing 2 mg AFB_1/kg diet[1]

Treatments[2]		Serum variables			
		Glucose (mg/dL)	Albumin (g/dL)	Total protein (g/dL)	Globulin (g/dL)
BD		291[a]	0.99[a]	2.70[a]	1.75[a]
BD + BC		278 [a]	0.95 [a]	2.64[a]	1.69[ab]
BD + DE		292[a]	1.00 [a]	2.65[a]	1.65[ab]
BD + AF		221[b]	0.47[c]	1.67[c]	1.21[c]
BD + AF+BC		259[ab]	0.61[b]	2.02[b]	1.47[b]
BD + AF +DE		224[b]	0.36[c]	1.35[c]	0.99[c]
ANOVA	S.E.M.:	18	0.03	0.11	0.08
	P-value:	0.0238	<.0001	<.0001	<.0001
Adsorbent source					
None		256[a]	0.730[ab]	2.20[ab]	1.47[ab]
Bentonite		269[a]	0.780[a]	2.23[a]	1.58[a]
Diatomaceous earth		258[a]	0.680[b]	1.99[b]	1.31[b]
Aflatoxin level					
0		287[a]	0.98[a]	2.68[a]	1.70[a]
2 pmm		235[b]	0.48[b]	1.68[b]	1.22[b]
Main factors		P-value			
Adsorbent		75	0.044	0.022	0.021
Aflatoxin		0.0015	< 0.0001	< 0.0001	< 0.0001
Adsorbent x Aflatoxin		0.31	0.0025	0.0216	0.057

[1]Values are means of five replicate pens of 3 chicks each.

[2]Treatments were the addition of $AFB_1$and adsorbents to the basal diet (BD);

[a-c]Values within columns with no common superscripts are significantly different (*P<0.05*);

AFB_1 = Aflatoxin B_1 from culture material; BD = basal diet. ; BC = Bentonite clay; DE = Diatomaceous earth

Table 7.5. Effects of bentonite and diatomaceous earth on serum calcium, AST, GGT and uric acid concentrations in chicks fed diets containing 2 mg AFB_1/kg diet[1]

Treatments[2]		Serum variables			
		Calcium (mg/dL)	AST (IU/L)	GGT (U/L)	UA (mg/dL)
BD		10.45^{a}	199^{b}	12.33^{b}	7.87
BD + BC		10.29^{a}	190^{b}	12.13^{b}	8.18
BD + DE		10.22^{a}	202^{b}	12.87^{ab}	8.08
BD + AF		9.59^{bc}	313^{a}	15.53^{a}	6.65
BD + AF+BC		9.77^{b}	226^{b}	11.67^{b}	8.15
BD + AF +DE		9.26^{c}	323^{a}	11.33^{b}	5.50
ANOVA	S.E.M.:	0.132	26.588	0.953	0.962
	P-value:	<.0001	0.0026	0.0575	0.2957
Adsorbent source					
None		10.02^{a}	256^{a}	13.93^{a}	7.26
Bentonite		10.03^{a}	208^{a}	11.90^{b}	8.16
Diatomaceous earth		9.73^{b}	262^{a}	12.10^{ab}	6.79
Aflatoxin level					
0		10.32^{a}	197^{b}	12.44^{b}	8.04^{a}
2 pmm		9.54^{b}	287^{a}	12.84^{a}	6.77^{b}
Main factors		P-value			
Adsorbent		0.057	0.102	0.081	0.363
Aflatoxin		<0.0001	0.0004	0.612	0.117
Adsorbent x Aflatoxin		0.261	0.226	0.050	0.427

[1]Values are means of five replicate pens of 3 chicks each.

[2]Treatments were the addition of $AFB_1$and adsorbents to the basal diet (BD).

[a-c]Means within columns with no common superscripts are significantly different (*P<0.05*); AFB_1 = Aflatoxin B_1 from culture material; BD = basal diet; BC = Bentonite clay; DE = Diatomaceous earth; AST = Aspartate aminotransferase; GGT = Gamma-glutamyltransferase; UA = Uric acid

Chicks fed AFB_1 plus BC had lower (*P<0.05*) AST and GGT concentrations (28 and 25%, respectively) when compared to chicks fed AFB_1 alone. In chicks fed AFB_1 plus DE, there were no significant differences observed in calcium and AST, but the GGT concentration was significantly (*P<0.05*) reduced (27%) when compared to chicks fed the AFB_1 diet. There were no significant differences among the treatment groups for uric acid (UA).

7.3.4 Bone ash and shank colour

Table 7.6 shows that the shank colour of chicks fed adsorbents was similar to that of birds fed the control diet. Shank colour of chicks fed the AFB_1 diet was lighter (*P<0.05*) compared to chicks fed the control diet. The addition of BC appeared to offer protection as evidenced by shank colour that was intermediate between those of control and AFB_1 chicks. However, DE did not appear to offer any protection against the change in shank colour. There were no differences among treatments for any of the bone measurements (Table 7.6).

7.4 Discussion

Aflatoxins in poultry feed may cause health problems and subsequent economic losses (Ledoux and Rottinghaus, 2007). Therefore, it is important to find ways to minimize their harmful effects. Several researchers have studied the efficacy of non-nutritive adsorbents in reducing or preventing the negative effects of AFB_1 when chickens were fed diets contaminated with AFB_1 (Kubena *et al.*, 1990; Harvey *et al.*, 1993; Bailey *et al.*, 1998). In the present study, the efficacy of BC and DE in reducing the effects of AFB_1 was assessed in broiler chicks.

Chick performance was not negatively affected by the addition of BC or DE when compared to the control basal diet, suggesting that the adsorbents did not negatively affect the nutritional value of the diet, indicating that the concentration (0.50 % in total diet) used was safe for the chicks. These results are consistent with data reported by Miazzo *et al*. (2005) and Saifaeikatouli *et al*. (2010) in which 0.3 % and 1.5 % sodium bentonite, respectively resulted in similar performance to that of control chicks. In the present study Aflatoxin B_1 reduced (*P<0.05*) feed intake (FI) and body weight gain (BWG). Similar results were found by Yarru *et al*. (2013) and Xen *et al*. (2014) who fed 2 mg AFB_1 /kg feed to broiler chickens.

In the present study, it was hypothesized that when diets containing AF are supplemented with adsorbents that act as AFB_1 binding agents, chick performance should improve. Inclusion of bentonite in the AFB_1 diet improved BWG (9 %) and FI (6.8 %) to values close to that of control

Table 7.6. Effects of bentonite and diatomaceous earth on tibia variables and shank color values of chicks fed diet containing 2 mg AFB_1/kg diet[1]

Treatments[2]		Variables			
		Bone weight (g)	Ash weight (g)	Ash (%)	Shank colour
BD		2.06	1.07	2.14	3.93[a]
BD + BC		1.99	1.03	2.08	3.73[a]
BD + DE		2.06	1.04	1.91	2.86[ab]
BD + AF		1.99	1.01	1.20	2.20[b]
BD + AF+BC		2.14	1.09	1.05	3.26[ab]
BD + AF +DE		1.86	0.95	0.97	2.66[ab]
ANOVA	S.E.M.:	0.079	0.149	0.513	0.442
	P-value:	0.284	0.353	0.379	0.091
Adsorbent source					
None					3.06
Bentonite					3.50
Diatomaceous earth					2.76
Aflatoxin level					
0					3.51
2 pmm					2.71
Main factors			P-value		
Adsorbent					0.2873
Aflatoxin					0.0407
Adsorbent x Aflatoxin					0.2122

[1]Data are means of 5 replicate pens of 5 chicks each for shank color and 5 replicate pens of 3 chicks each for bone measurements

[2]Treatments were the addition of AF to the basal diet (BD)

[a-c]Means within columns with no common superscripts are significantly different (*P<0.05*)

AF = Aflatoxin B_1 from culture material; BD = basal diet. ; BC = Bentonite clay; DE = diatomaceous earth

chicks. Lopes *et al.* (2006) reported that the addition of bentonite to the AFB_1 diet improved BWG by 9.5 % but this was still lower than the performance of control birds. In contrast, Pasha *et al*. (2007) observed that chicks fed 100 µg/kg aflatoxin had suppressed body weight and feed consumption, and poor FCR values, which were significantly improved with the addition of 0.5% sodium bentonite to the aflatoxin-contaminated diet. These results agreed with our finding in which the BWG was improved by adding bentonite into the AFB_1 diet. The results of Lopes *et al.* (2006), appears to be consistent with and not different from our results.

It is also possible that the bentonite from Pakistan had higher non-selective binding properties, and, therefore, might have bound vitamins, minerals, and amino acids (Pash *et al.*, 2007). Some bentonites have been proven to be efficient at sequestering AFB_1. They decreased the bioavailability of the toxin in the gastrointestinal tracts of birds when they are incorporated in the diet (Magnoli *et al.*, 2008). The binding capacity of theses adsorbents varied with the rheological source and even among batches of a given source. In addition to reducing performance, AFB_1 causes mortality of the birds (Cravens *et al.*, 2013). In the current study, compared with the AF diet alone where we saw 12 % mortality, the addition of bentonite or diatomite to the AFB_1 diet prevented chick mortality, suggesting apparent protection against this deleterious effect caused by AFB_1.

Gross pathological lesions in birds fed the AFB_1 treatment included pallor, discolouration of the liver, enlargement of the liver and kidneys (Hussain *et al.*, 2008), and gall bladder distension (Tessari *et al.*, 2006). The liver is the target organ of AFB_1 in broilers; however, kidney, gizzard and spleen can also increase in relative weight (Dwyer *et al.*, 1997; Miazzo *et al*, 2005). The observed increase in relative liver and kidney weights in chicks fed AFB_1 alone is consistent with previous reports (Phillips *et al.*, 1988; Ledoux *et al.*, 1998; Neeff *et al.*, 2013). Addition of bentonite to the AFB_1 diet significantly reduced the increase in relative liver and kidney weight observed in chicks fed AFB_1 alone. Similar results were reported by Pash *et al.* (2007) who fed 0.5% bentonite and 0.1mg/ kg aflatoxin diet in broilers chickens. Rosa *et al.*(2001) have shown that the increase in relative liver and kidney weights of chicks fed the AFB_1iet could be reduced by bentonite addition (0.3%) to the AFB_1 (5mg/kg diet) diet, and Magnoli *et al,* (2011) reported that sodium bentonite was a good candidate for preventing aflatoxicosis. Addition of DE to the AFB_1 diet did not reduce the severity of the liver lesions caused by AFB_1, as the mean liver score of groups treated with AF alone was actually lower (3.50) than that of chicks fed the combination of AFB_1 and diatomite (3.66). These findings are in contrast to Denli *et al*. (2009) who reported that consumption of 0.5 % of AflaDetox (a natural product obtained from

diatomaceous earth) reduced the incidence and severity of liver lesions produced by 1mg/kg aflatoxin diet.

Adding DE or BC to the basal diet resulted in similar levels of glucose, albumin, protein, and globulin as in the controls suggesting that the two adsorbents did not negatively affect the nutritional value of the diet. Addition of AFB_1 to the basal diet reduced these serum metabolites. Decreases in the concentration of total plasma proteins, albumin, and globulins are indicators of the alteration in protein synthesis observed in aflatoxicosis (Solcan *et al.,* 2013). Rosa *et al*. (2001) and Zhao *et al.* (2010) reported that AFB_1 reduced total protein, albumin, and globulin concentrations.

Quezada *et al*. (2000) suggested that the changes occurring in protein concentration in hepatic and renal tissue are probably related to structural damage of liver and kidney as a consequence of AF exposure, reinforcing the suggestion that the liver and the kidney are primary target organs for AF with consequent deleterious effects on the metabolic activities and secretory capacity of these organs. The reduction in serum glucose could be due to the reduced feed intake or to a reduction in the activity of enzymes involved in carbohydrate utilization or both (Zhao *et al.,* 2010). Compared with chicks fed the AFB_1 diet alone, the addition of bentonite to the AFB_1 diet increased the level of total protein, albumin and globulin in serum of chicks; however the level was lower when compared with control chicks. Rosa *et al.* (2001) showed that serum biochemical changes could be ameliorated by bentonite addition (0.3 %) to an AF (5 mg/kg diet) diet in growing broilers fed dietary treatments from 30 to 52 d of age.

The degree of damage in the liver can be assessed by specific enzyme tests. Aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) activities provide a sensitive and specific measure of hepatic function or injury (Boone *et al.,* 2005) and are sensitive serological indicators of liver and kidney toxicity. The increased activity of AST (57 %) and GGT (26 %) in chicks fed the AFB_1 diet confirmed that there was major damage done to the livers, as previously suggested by the higher relative liver weights, liver discolouration, and the appearance of vacuoulated hepatocytes and an increased incidence of inflammatory cells observed in hepatic cells during histopathologic evaluation. Similar to the current study, Tedesco *et al*. (2004) and Neeff *et al*. (2013) also observed increased serum activity of AST and GGT when they fed AFB_1 at 0.8 mg/ kg and 2.5 mg/kg, respectively. In the current study, addition of bentonite to the AFB_1 diet significantly decreased the levels of AST and GGT, which were increased by AFB_1. The activities of AST and GGT observed in chicks fed the AFB_1 plus BC diet were similar to the AST and GGT concentrations of chicks fed the control

diet, suggesting that bentonite ameliorated the effects of AFB_1 on these serum enzymes. Similar results were reported by Santurio *et al*. (1999), who observed that the addition of bentonite to an AFB_1 diet reduced the AST and GGT concentrations to levels similar to those observed in the control diet without AFB_1. Addition of DE to the AFB_1 diet did not improve the concentrations of glucose, albumin, proteins, or globulin, which were negatively impacted by AFB_1. Modirsanei *et al.* (2008) also found that the addition of DE to an AFB_1 diet did not ameliorate the decreases in glucose or total protein, but observed a small increase in albumin concentration. Denli *et al.* (2009) fed birds a diet containing 1mg AFB_1/kg diet, and 5 g DE/kg diet (0.50%) and observed a significant increase in total proteins compared to the AFB_1 diet alone. These increases brought total protein levels up to levels similar to those found in the control diet. In the present study, addition of DE to the AFB_1 diet resulted in AST levels similar to the AFB_1 diet alone, suggesting that DE did not ameliorate the negative effects of AFB_1 on AST levels. In contrast, adding DE to the AFB_1 diet improved levels of GGT back to levels associated with the control diet. These results are not consistent with the results from Modirsanei *et al.* (2008) or Denli *et al.* (2009) who reported that there were no significant changes in those blood parameters.

In the present study, we have shown that the inclusion of AFB_1 into a control diet reduced blood calcium levels in chicks by 8 %. Vitamin D_3 is required for the normal absorption and metabolism of calcium and phosphorus (McDonald *et al.*, 2002). Bird (1978), Nassar *et al.* (1985) and Glahn *et al*. (1991) demonstrated a significant interaction between AFB_1 and vitamin D_3 metabolism. Even when the diet was adequate in vitamin D_3, aflatoxin reduced the calcium level in the serum by 20 % (Hamilton *et al.*, 1973). In addition, Glahn *et al. (*1991) reported that the kidney damage caused by AF might alter Ca and P metabolism in broilers. The probable mechanism by which AFB_1 affects calcium metabolism may well be the result of liver and kidney damage caused by AFB_1 leading to a reduction on the hydroxylation of vitamin D in the liver to 25 (OH) vitamin D, and on the subsequent second hydroxylation of 25 (OH) vitamin D_3 in the kidney to the active metabolite 1, 25 $(OH)_2$ vitamin D_3 (Nassar *et al*, 1985). Glahn (1991) also reported that plasma 25-(OH) hydroxy-vitamin D and 1, 25-$(OH)_2$ vitamin D (the most biologically active form of Vitamin D) levels decreased when birds were fed 2 mg AF/kg diet. The low levels of 1, 25- $(OH)_2$vitamin D in the serum could lead to a reduction of serum parathyroid hormone, causing the observed decrease in blood calcium levels. In our study, adding bentonite into the AFB_1 diet improved blood calcium levels, but they were still much lower than control values. These results are corroborated by findings from Santurio *et al.* (1999) whereby blood calcium levels were not increased by the addition of bentonite to AFB_1 diet. In the present study, BC and DE appear not to be effective in preventing the decrease in serum

calcium caused by AFB_1. This is reflected in almost equal serum Ca values of those birds fed AFB_1 plus DE and AFB_1 plus BC compared to those fed AFB_1 alone.

Bone is highly complex in structure, the dry matter consisting of approximately 460 g mineral matter/kg with calcium and phosphorus being the most abundant minerals (McDonald *et al.*, 2002). The status of the bone is often utilized as a way to determine mineral and vitamin D adequacy in poultry diets by assessing the dietary effects on bone ash or by determining the levels of Ca and P in the bone ash (Onyango *et al.*, 2003). Feeding 2 mg aflatoxin (AF)/kg diet from 1 to 21 days of age alone did not significantly affect the bone parameters with tibia ash being similar among treatments. The lack of differences among treatments agrees with Safaeikatouli *et al.* (2012). In contrast, tibia ash levels of chicks consuming AF (2 mg total aflatoxin (AF)/kg diet) (for 21 dayswere found to be significantly lower compared to control as indicated by Basmacioglu *et al*., (2005) who fed the same concentration of AF in broilers chicks. Similar to our study, sodium bentonite did not adversely affect tibia mineral concentrations of chicks fed nutrient-deficient diets (Southern *et al.*, 1994), which suggests that there was no interaction between bentonite and Vit. D_3 with respect to the mineral content of the tibias.

Aflatoxin can cause poor pigmentation in birds, presumably by interfering with the absorption, transport, and deposition of carotenoids (Tyczkowsi and Hamilton, 1987). In the present study, the shank colour was reduced by feeding 2 mg AF/kg diet. Significant improvements in hank colour were achieved by the inclusion of bentonite and diatomaceous earth, which suggests that the adsorbents were able to reduce the effects of aflatoxin on pigmentation in the birds.

7.5 Conclusions

Toxicity of aflatoxin was expressed as a significant depression in weight gain, feed intake, and increased mortality. The inclusion of adsorbents in chick diets at a level of 0.5% diet did not have any negative effects on weight gain, feed intake, or chick mortality suggesting that the adsorbents did not negatively affect dietary nutrients at this dietary inclusion level. Diatomaceous earth was not effective in ameliorating the toxic effects induced by 2 mg/kg AFB_1. However, bentonite clay (0.5% diet), though it did not completely protect chicks against aflatoxicosis, was more effective than the DE in reducing the adverse effect of AF in chicks. These results suggest that locally available BC in Mozambique can be used to reduce the toxic effects of aflatoxin that may be present in poultry feeds.

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CHAPTER 8 - Efficacy of adsorbents (bentonite and diatomaceous earth) and turmeric (*Curcuma longa*) to ameliorate the toxic effects of aflatoxin in chicks

(Submitted to British Poultry Science journal)

Abstract

A study was conducted to determine the efficacy of bentonite clay (BC), diatomaceous earth (DE) and turmeric powder (TUM) in ameliorating the toxic effects of aflatoxin B_1 (AFB_1).

Two hundred fifty Ross-308 day-old male broiler chicks were assigned to 10 dietary treatments (5 replicates of 5 chicks) from hatch to d-21. Dietary treatments were: basal diet; basal diet plus AFB_1 (2 mg) or BC (0.75%), or DE (0.75%), or TUM (200 ppm curcuminoids) and different combinations of AFB_1, BC, DE and TUM. Feed intake (FI), body weight gain (BWG) and feed gain (FG) of the birds fed BC or DE separately, were not different from control birds. Birds fed TUM only had similar FI and FG but lower BWG than control chicks. Aflatoxin B_1 reduced ($P<0.05$) FI, BWG, and serum concentrations of glucose, albumin, total protein, and calcium, but increased ($P<0.05$) FG and relative liver and kidney weights. Chicks fed the combination of AFB_1 and BC had similar FI and FG to control chicks. Chicks fed the combination of DE and AFB_1 had lower ($P<0.05$) FI (23.1%) and BWG (28.6%) compared with control chicks. Chicks fed the combination of TUM and AFB_1) also decreased FI (26.2 %) and BWG (31%) compared with control chicks. Chicks fed the combination of AFB_1, BC and TUM consumed significantly ($P<0.05$) higher amounts of feed compared with chicks fed only AF, but gained ($P<0.05$) less when compared with control diet chicks. Chicks fed the combination of AFB_1, DE, and TUM diet had poorer growth performance than those fed AFB_1 alone. None of the combination diets reduced ($P>0.05$) the severity of liver lesions.

Key words: aflatoxin, bentonite, diatomaceous earth, turmeric, broilers.

8.1 Introduction

Aflatoxins (AF) are a group of heterocyclic metabolites produced by storage fungi of the genus *Aspergillus*, particularly *A. flavus* and *A.parasiticus.* Even though 18 different aflatoxins have been identified, only aflatoxin B_1, B_2, G_1 and G_2 have been detected as natural contaminants of feed and feedstuffs (Leeson *et al.*, 1995).Contamination of feed ingredients with mycotoxins during production, harvesting, handling, processing and storage may induce sanitary disturbance and mortality. Fungal contamination of kitchen leftovers is also widespread in tropical countries (Okoli *et al*., 2006) and they can cause serious health problems in poultry, and

may result in serious economic losses (Ledoux and Rottinghaus, 2007).The adverse effects of AF on chicken performance are both dose and time dependent (Leeson *et al.*, 1995). Aflatoxin B_1 (AFB_1) is the most biologically active form of AF and causes poor performance, liver lesions, and immunosupresssion in poultry (Ledoux *et al*., 1999; Rosa *et al.*, 2001; Magnoli *et al.*, 2011). Different approaches, including physical, chemical, and biological treatments of contaminated feeds and feedstuffs, have been employed to detoxify AF (Smith *et al.*, 1994; Phillips *et al*., 1988; Harper *et al.*, 2010). In previous work, bentonites (Magnoli *et al*, 2011) diatomite, (Modirsanei *et al.*, 2008) and activated charcoal (Hesham *et al*., 2004) showed an ability to bind AFB_1 both *in vitro* and *in vivo*. All adsorbents are, however, not equally effective and some are more effective at higher concentrations (Neeff *et al.*, 2013). The most recent dietary approach to prevent mycotoxicoses in poultry is the combined use of antioxidants and adsorbents (Surai, 2002). Herbal components like turmeric (*Curcuma longa*), garlic (*Allium sativum*) and green algae (*Spirulinaplantesis*) counteract mycotoxins, improve growth performance, and also act as good antioxidants (Sawarkar *et al*., 2012, Elagib *et al*., 2013). The objectives of the current study were to assess the efficacy of two adsorbents and turmeric powder (TMP) singly or in combination to ameliorate aflatoxicosis in broiler chicks, and to determine the effect of TMP inclusion and adsorbents in poultry diets on chick performance.

8.2 Material and methods

8.2.1 Experimental design, birds and diets

Two hundred and fifty Ross 308 day-old male broiler chicks were purchased from a commercial hatchery, weighed, wing banded, and assigned to stainless steel chick batteries so that initial body weights (41 g/bird) were equal among treatments. A completely randomized design was used with five replicate pens of five chicks assigned to each of 10 dietary treatments. The chicks were maintained on a 24 h continuous light schedule and allowed *ad libitum* access to feed and water from hatch to day 21. The temperature of the room ranged from 35 to 36.1^{o}C at the beginning of the experiment to between 30 and 31.1^{o}C towards the end of the experiment. The protocol of the experiment was approved by the University of Missouri-Columbia Animal Care and Use Committee. Mortality was recorded as it occurred, and the birds were inspected daily for any health related problems.

8.2.2 Diets

A maize-soybean meal-based basal diet (Table 8.1), in mash form formulated to meet the nutritional requirements of young broilers (1-21d post-hatch) as recommended by the National Research Council (NRC, 1994), was used. Aflatoxin (700 mg/kg culture material) from ground

Aspergillus parasiticus strain NRRL 2999 culture material was incorporated into the basal diet to achieve the required dietary aflatoxin B_1 concentration of 2 mg/kg diet. Commercially available food grade turmeric powder (*Curcuma longa*), containing 24,700 mg/kg of curcuminoids was used as a source of antioxidants. Turmeric powder (0.81 %) was incorporated into the basal diet to produce dietary treatments containing 200 mg/kg curcuminoids. Dietary treatments included: 1) a positive control basal diet (BD) containing no aflatoxin (AFB_1), no bentonite (BC), no diatomaceous(DE) or no turmeric (TUM); 2) BD + 0.75 % BC, but with no AF, DE or TUM; 3) BD + 0.75 % DE but with no AFB_1, BC or TUM; 4) BD+ TUM but with no BC, DE or AFB_1; 5) BD + 2 mg AFB_1/kg diet but with no BC, DE or TUM; 6) BD + 0.75 % BC + 2 mg AFB_1/kg diet but with no DE or TUM; 7) BD + 0.75 % DE + 2 mg AFB_1/kg diet but with no BC or TUM; 8) BD+ 2 mg AFB_1/kg diet + TUM but with no BC or DE; 9) BD+ 2 mg AFB_1/kg diet + 0.75 % BC + TUM, but with no DE; 10) BD+ 2 mg AFB_1/kg diet + 0.75 % DE + TUM, but with no BC. Dietary AF (B_1, B_2, G_1, and G_2) concentrations were confirmed by analyses. In brief, feed samples were extracted with acetonitrile and water (86:14), and an aliquot of the extract was passed through a puriTox TC-M 160 cleanup column and suitably diluted with water before analysis using HPLC with cobra cell post column derivatization with fluorescence detection at 365 nm excitation and 440nm emissions. All diets were screened by the method of Rottinghaus *et al*. (1982) for the presence of citrinin, T-2 toxin, vomitoxin, zearalenone, fumonisins and ochratoxin A, before the start of the experiment and were below detection limits for these mycotoxins. Total curcuminoid content, including curcumin, bisdemethoxycurcumin, and demethoxycurcumin, were determined by the method described by Gowda *et al*. (2008).

8.2.3 Measurements

Chicks were weighed by pen at the beginning (day 1), day 7 and at day 21 of the experiment. Feed was weighed per pen at day 21, and feed conversion ratio was calculated from average feed consumption per pen (g) divided by average body weight gain per pen (g). Mortalities were recorded as they occurred and the dead birds were necropsied. On day 21, three birds per pen were anaesthetized with carbon dioxide and blood samples collected via cardiac puncture for determination of serum chemistries. Livers and kidneys were removed from the same three birds, and weighed.

Table 8.1. Composition and nutrient value of the basal diet (g/kg as-is)

Ingredient	g/kg
Corn	506.4
Soybean Meal	383.2
Soybean oil	52.4
Dicalcium	17.2
Limestone	12.4
Salt	4.6
Methionine	1.9
Vitamin Mix[2]	2.5
Sand	19.4
TOTAL	1000.0
Calculated nutrient composition[3]	
Crude Protein (g/kg)	230.0
Metabolizable Energy (MJ/kg)	12.9
Lysine (%)	12.7
Methionine (%)	5.3
Methionine + Cysteine (%)	9.0
Threonine (%)	8.6
Tryptophan	3.1
Calcium (%)	10.0
Phosphorus (% Av.)	4.5

[2]Supplied per kilogram of feed: manganese, 100 mg; zinc, 100 mg; iron, 50 mg; copper, 11.25 mg; iodine, 1.5 mg; selenium, 0.15 mg; vitamin A, 2.31 mg ; vitamin D_3, 68.75 µg ; vitamin E, 16.5 mg; vitamin B_{12}, 11 µg; vitamin K, 0.83 mg; riboflavin, 6.6 mg; thiamin, 1.1 mg; pantothenic acid, 6.6 mg; niacin, 27.5 mg; pyridoxine, 1.37 mg; folic acid, 0.69 mg; biotin, 33 µg; choline, 385 mg.

The liver weight of each bird was recorded and a piece of liver tissue (2 to 3 g) was collected and rinsed with ice-cold phosphate buffered saline (pH 7.4) containing 0.16 mg/ml heparin to prevent blood clot formation. Liver samples were also harvested from 6 birds per treatment, and fixed in 10 % neutral buffered formalin for gross and histopathology evaluation. Fixed liver tissues were trimmed, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin for microscopic examination. Liver lesions were scored using a score system of 1 to 4 (1= liver unremarkable; 2= mild aflatoxin lesions; 3= moderate aflatoxin lesions; and 4= severe aflatoxin lesions.

Blood was centrifuged at 1400g at 8^{o}C for 30 min (Sorval, RC 3 B plus) and the serum was collected and preserved at -20^{o}C until submitted for biochemical analyses. Serum samples were analyzed for total proteins, albumin, globulin, glucose, uric acid, γ-glutamyltransferase (EC 2.3.2.2), aspartate aminotransferase, and calcium using an auto analyzer (Kodak Ekatachem Analyzer, Eastman Kodak Co, Rochester, NY).

The shank colour of all surviving birds was determined using the DSM colour fan for broilers. Right tibiae were collected from three birds per pen for determination of tibia ash. Tibiae were stripped of adhering tissue, dried at 100^{o}C, and fat was extracted with a mixture of ether and methanol (90 and 10 %, respectively). Fat-extracted tibiae were than dried at 100^{o}C for 24 h and ashed in a muffle furnace at 600^{o}C overnight.

8.2.4 Statistical analyses

Data on growth performance, liver and kidney weights, gross pathology, histopathology (liver), serum chemistry, tibia ash and shank colour were analyzed using the GLM procedure of SAS software (SAS Institute Inc., 2011). Treatment means were compared by the Tukey's test and statistical significance was accepted based on the 0.05 level of probability. Data on mortality and liver lesion scores were analyzed using the Chisquare test Proc FEQ in SAS.

8.3 Results

8.3.1 Diet analyses, growth performance and mortality

Dietary AFB_1 concentrations were confirmed by HPLC analyses, and a screen of the basal diet for citrinin, T-2 toxin, vomitoxin, zearalenone, fumomnisins, and ochratoxin A indicated that concentrations of these mycotoxins were below detection limits. The effects of dietary treatments on 21 day feed intake (FI), body weight gain (BWG) feed conversion ratio (F: G), and mortality are shown in Table 8.2. Feed intake (FI), BWG, feed: gain ratio (FG) and

mortality of birds fed bentonite clay (BC) or diatomaceous earth (DE) alone were not different (*P>0.05*) from those of control birds. Compared with controls, chicks fed TUM (200 mg of total curcuminoids/kg of diet) alone had similar FI, FG and mortality, but significantly lower BWG. Chicks fed 2 mg AFB_1/kg diet had lower (*P<0.05*) FI, BWG and significantly higher (6 %) FG when compared to controls.

The inclusion of BC into the AFB_1 (2 mg/kg diet) diet significantly improved FI and BWG, but did not affect FG or mortality, when compared with chicks fed only AFB_1. Addition of DE to the AF diet was not effective (*P>0.05*) in reducing or preventing the negative effects of AF on performance variables such as FI, BWG, and FG. Compared with control chicks, chicks fed AFB_1 plus TUM, had significantly *(P<0.05*) lower FI, BWG, and significantly (*P<0.05*) higher FG, and higher mortality (20 % versus 0.0 %).

Compared to control chicks, chicks fed a combination of BC, TUM and AFB_1 had lower (*P<0.05*) FI and BWG. The addition of BC and TUM into the AFB_1 diet significantly (*P<0.05*) improved FI but not BWG when compared with chicks fed the AFB_1 diet.

Compared to control chicks, chicks fed a combination of DE, TUM and AF had lower (*P<0.05*) FI and BWG, and poorer (*P<0.05*) FG. The supplementation of the AFB_1 diet with a combination of DE and TUM did not prevent the decrease in FI and BWG, and the increase in FG observed in birds fed AFB_1 alone.

The inclusion of BC into the AFB_1 (2 mg/kg diet) diet significantly improved FI and BWG, but did not affect FG or mortality, when compared with chicks fed only AFB_1. Addition of DE to AF diet was not effective (*P>0.05*) in reducing or preventing the negative effects of AF on performance variables such as FI, BWG, and FG. Compared with chicks fed AFB_1, chicks fed AFB_1 plus TUM, had significantly *(P<0.05*) lower FI, and significantly (*P<0.05*) higher mortality (20 versus 4.0 %). Chicks fed AFB_1 plus TUM also had lower (*P*<0.05) FI and BWG, poorer (*P<0.05*) FG, and higher (*P<0.05*) mortality (20 versus 0 %) than chicks fed the control diet.

Compared to control chicks, chicks fed a combination of BC, TUM and AFB_1 had lower (*P<0.05*) FI and BWG. The addition of BC and TUM into the AFB_1 diet significantly (*P<0.05*) improved FI but not BWG when compared with chicks fed the AFB_1 diet. Compared to control chicks, chicks fed a combination of DE, TUM and AFB_1 had lower (*P<0.05*) FI and BWG, and poorer (*P*<0.05) FG. The supplementation of the AFB_1 diet with a combination of DE and TUM

Table 8.2. Effects of bentonite clay, diatomaceous earth and turmeric alone or in combination on 21 day growth performance and mortality of chicks fed diets containing 2 mg AFB_1/kg diet[1]

Treatments[2]		Variables			
		Feed intake (g)	Body weight gain (g)	Feed: Gain (g:g)	Mortality %[3]
BD		1197^{ab}	896^{a}	1.34^{e}	0.0^{b}
BD + BC		1206^{a}	893^{ab}	1.35^{de}	0.0^{b}
BD + DE		1218^{a}	913^{a}	1.33^{e}	0.0^{b}
BD + TUM		1108^{bc}	811^{bc}	1.37^{cde}	0.0^{b}
BD + AF		988^{d}	697^{d}	1.42^{bcd}	4.00^{b}
BD+ AF+BC		1149^{abc}	832^{abc}	1.38^{bcde}	0.0^{b}
BD + AF+DE		920^{de}	639^{d}	1.45^{ab}	8.00^{ab}
BD + AF +TUM		883^{e}	618^{d}	1.43^{abc}	20.0^{a}
BD + AF+BC+TUM		1088^{c}	786^{d}	1.39^{bcde}	0.0^{b}
BD +AF+ DE+ TUM		935^{ed}	624^{c}	1.50^{a}	4.0^{b}
ANOVA	S.E.M.:	32	29	0.025	4.1
	P-value:	< .0001	<.0001	0.0008	0.045

[1]Values are means of five replicate pens of 5 chicks each

[2]Treatments were the addition of AF and adsorbents to the basal diet (BD)

[a-c] Values within columns with no common superscripts are significantly different (*P<0.05*)

AF = Aflatoxin $B_1$from culture material; BD = basal diet; BC = 0.75 % Bentonite clay; DE = 0.75 % diatomaceous earth; TUM = 0.81 % turmeric that supplied 200 mg/kg curcuminoids

did not prevent the decrease in FI and BWG, and the increase in FG observed in birds fed AFB_1 alone. In fact, the addition of DE and TUM to the AFB_1 diet caused a significant decrease in BWG and poorer FG when compared with birds fed AFB_1 alone.

8.3.2 Liver and kidney weights

The effects of BC, DE and TUM on relative liver and kidney weights of chicks fed dietary treatments are summarized in Table 8.3. Addition of BC, DE and TUM alone to the BD did not affect relative liver and kidney weights when compared with liver of chicks fed the control diet

The addition of AFB_1 to the basal diet increased ($P<0.05$) the relative weight of the livers (27 %; Figure 8.1b) and kidneys (54.3 %; Figure 8.2b) compared to that of chicks fed the control diet (Figures 8.1a and 8.2a). Compared with chicks fed the AFB_1 only diet (Figure 8.2b), the addition of BC to the AFB_1 diet significantly reduced (16.8 %) the relative kidney weight (1.04 versus 1.25 %). Compared with chicks fed the AFB_1 only diet, inclusion of DE in the AFB_1 diet significantly reduced (15.2 %) the relative kidney weight (1.06 versus 1.25 %). Compared with chicks fed AFB_1 alone, the addition of TUM into the AFB_1 diet was not effective in preventing or reducing the increase in relative liver or kidney weight. Addition of a combination of both BC and TUM to the AFB_1 diet prevented the increase in relative liver and reduced the increase in kidney weights caused by AFB_1. In contrast, the addition of a combination of DE and TUM to the AFB_1 diet was not effective in reducing or preventing the increase in the weight of the liver caused by AFB_1 and actually increased the weight of the kidney compared to chicks fed AFB_1.

8.3.3 Histopathology of the liver

The effects of dietary treatments on liver histological scores are shown in Table 8.3. The liver histological scores of chicks fed BC, DE or TUM were not different ($P>0.05$) from scores of control chicks. Neither adsorbent nor combinations of adsorbent and TUM were effective ($P>0.05$) in reducing the lesion scores observed in chicks fed AFB_1. Sections of liver from chicks fed AFB_1 alone presented with mild fibrosis and bile duct proliferation in the portal tract area with swollen hepatocytes and marked hepatocellular vacuolation.

Table 8.3. Effects of bentonite clay, diatomaceous earth and turmeric on relative liver and kidney weigth and liver histological score[3] chicks fed diet containing 2 mg AFB_1/kg diet

Treatment[1]		Variables		
		Relative liver weight (%)[1]	Relative kidney weight (%)[1]	Liver histological score[2,3]
BD		3.2^{de}	0.81^{e}	0.00^{c}
BD + BC		3.36^{de}	0.80^{e}	0.00^{c}
BD + DE		3.22^{e}	0.79^{e}	0.00^{c}
BD+ TUM		3.55^{cde}	0.89^{de}	0.00^{c}
BD +AF		4.06^{ab}	1.25^{b}	2.17^{a}
BD + AF+BC		3.68^{bcd}	1.04^{c}	1.50^{b}
BD + AF+DE		3.61^{bcde}	1.06^{c}	2.0^{ab}
BD +AF+ TUM		3.91^{abc}	1.21^{b}	1.67^{b}
BD +AF+ BC+ TUM		3.57^{cde}	0.99^{cd}	2.0^{ab}
BD +AF+ DE+ TUM		4.15^{a}	1.43^{a}	1.67^{b}
ANOVA	S.E.M.:	0.159	0.048	
	P-value:	0.0011	<.0001	<.0001

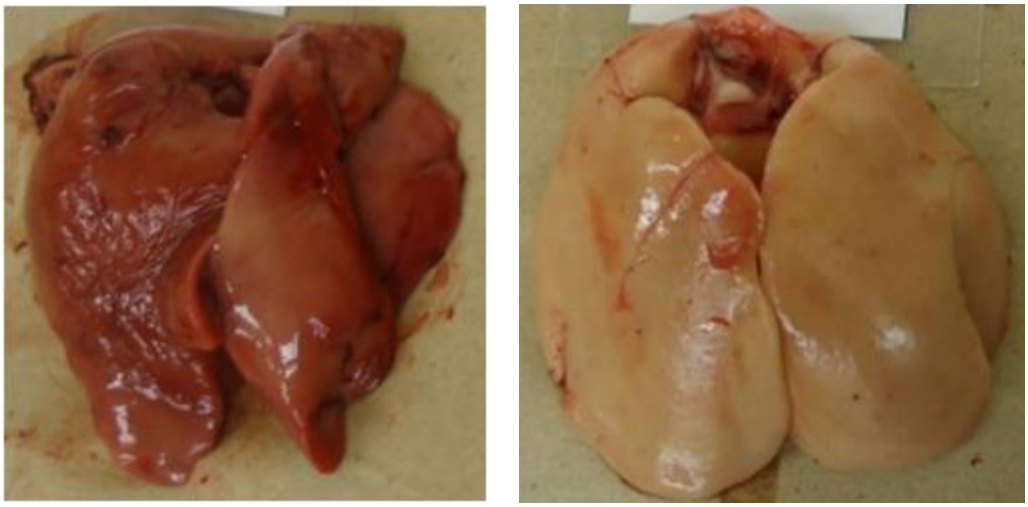
[1]Data are means of five replicate pens of 3 chicks each

[2]Data are means of 6 chicks per treatment

[3] Statistical analyses was performed using the Chisquare test PROC FREQ (SAS)

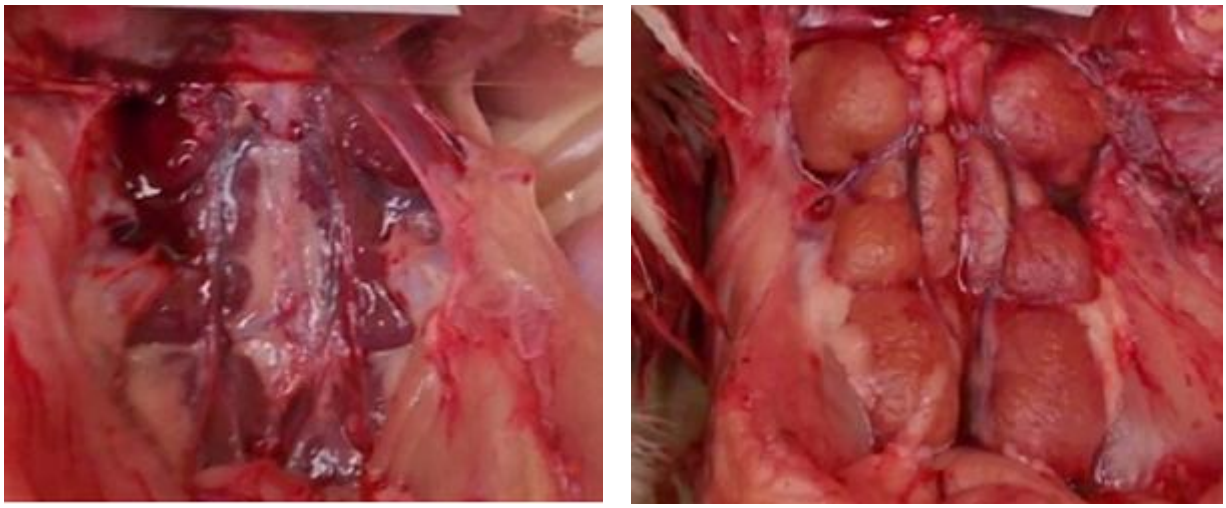
[a-e]Values within columns with no common superscripts are significantly different (*P<0.05*)

AF = Aflatoxin Culture Material; BD = basal diet; BC= Bentonite clay; DE= Diatomaceous earth; TUM: Turmeric



(a) (b)

Figure 8.1. Liver from basal diet (a) and from basal diet plus AFB_1 (b)



(a) (b)

Figure 8.2. Kindney from basal diet (a) and from basal diet plus AFB_1 (b)

8.3.4 Serum chemistry

The effects of dietary treatments on serum concentrations of glucose, albumin, globulin and total proteins are summarized in Table 8.4. Chicks fed BC or DE alone had similar (*P>0.05*) serum concentrations of glucose, albumin, and total protein and globulin values to those of control chicks. Chicks fed TUM alone had elevated levels (*P<0.05*) of glucose, but serum concentrations of albumin, total protein and globulin were similar to that of control chicks. Compared with the controls, the chicks fed the AFB_1 only diet had lower (*P<0.05*) serum concentrations of glucose, albumin, total protein, and globulin. Addition of BC into the AFB_1 diet increased the serum values above those of AFB_1 chicks, with serum concentrations of glucose, globulin, and total protein not different (*P>0.05*) from that of controls.

The serum concentrations of glucose, albumin, total protein and globulin in chicks fed AFB_1 plus DE was similar to those of chicks fed AFB_1 alone. Serum concentrations of glucose, albumin, globulin and total proteins of chicks fed the combination of AF and TUM were similar (*P>0.05*) to those of chicks fed AFB_1alone. Compared to controls, chicks fed the combination of AFB_1, BC and TUM had lower (*P<0.05*) serum concentrations of albumin and total protein but higher concentrations of serum albumin and total protein when compared to chicks fed AFB_1. Compared to controls, chicks fed the diet containing AFB_1, DE and TUM had lower (*P<0.05*) serum concentrations of albumin, total protein and globulin but similar (*P>0.05*) serum concentrations of glucose, albumin, globulin, and total proteins to those of birds fed AFB_1 only.

Effects of dietary treatments on serum concentrations of calcium, aspartate aminotransferase (AST), γ-glutamyltransferase (GGT) and uric acid (UA) are summarized in Table 8.5. Levels of calcium, AST, GGT and UA of chicks fed BC, DE or TUM alone were not different (*P>0.05*) from that of control chicks. Chicks fed the AFB_1 diet had lower (*P<0.05*) serum Ca but similar (*P>0.05*) levels of AST (202 versus 196 IU/L), GGT (15.27 versus 14.33 U/L), and UA (5.19 versus 6.25 mg/dL) when compared with chicks fed the control diet. Chicks fed the AFB_1 diet supplemented with BC, DE, or TUM had lower (*P<0.05*) serum calcium concentrations compared to controls, but similar (*P>0.05*) serum CA, AST, GGT and UA to that of chicks fed the AFB_1 only diet. Chicks fed the AFB_1 diet supplemented with combinations of BC and TUM or DE and TUM had lower (*P<0.05*) concentrations of serum Ca compared to controls, but similar (*P>0.05*) concentrations of AST, GGT, and UA to that of chicks fed AFB_1 only.

Table 8.4. Effects of bentonite clay, diatomaceous earth and turmeric on serum biochemical value of chicks fed diets containing 2 mg AFB_1/kg diet[1]

Treatment[2]		Serum Variables			
		Glucose (mg/dL)	Albumin (g/dL)	Total Protein (g/dL)	Globulin (g/dL)
BD		262^{bcd}	1.20^{a}	2.92^{a}	1.72^{ab}
BD + BC		299^{ab}	1.08^{ab}	2.60^{ab}	1.57^{ab}
BD + DE		285^{abc}	1.13^{a}	2.83^{a}	1.69^{ab}
BD+ TUM		312^{a}	1.11^{a}	2.93^{a}	1.77^{a}
BD +AF		210^{e}	0.61^{gf}	1.89^{ed}	1.31^{dc}
BD + AF+BC		248^{cde}	0.96^{bc}	2.63^{ab}	1.67^{ab}
BD + AF+DE		217^{de}	0.71^{ef}	2.19^{cd}	1.53^{bc}
BD +AF+ TUM		208^{e}	0.64^{efg}	2.13^{cde}	1.49^{bc}
BD +AF+ BC+ TUM		232^{de}	0.89^{dc}	2.45^{bc}	1.61^{ab}
BD +AF+ DE+ TUM		232^{de}	0.52^{g}	1.83^{e}	1.13^{d}
ANOVA	S.E.M.:	17.54	0.048	0.120	0.081
	P-value:	0.0004	<.0.001	<.0001	< .0001

[1]Data are means of five replicate pens of 5 chicks each

[2]Treatments were the addition of AF and adsorbents to the basal diet (BD)

[a-c] Values within columns with no common superscripts are significantly different ($P<0.05$)

AF = Aflatoxin from culture material; BD = basal diet; BC= Bentonite clay; DE= Diatomaceous earth; TUM = Turmeric

Table 8.5. Effects of bentonite clay, diatomaceous earth and turmeric on serum biochemical value of chicks fed diet containing 2 mg AFB_1/kg diet[1]

Treatments[2]		Serum Variables			
		Calcium (mg/dL)	AST (IU/L)	GGT (U/L)	UA (mg/dL)
BD		11.32^{a}	196^{ab}	14.33^{ab}	6.25^{ab}
BD + BC		11.13^{a}	175^{b}	14.20^{ab}	6.22^{ab}
BD + DE		11.35^{a}	161^{b}	13.93^{ab}	6.533^{ab}
BD+ TUM		10.81^{ab}	164^{b}	14.33^{ab}	7.61^{a}
BD +AF		9.75^{cd}	202^{ab}	15.27^{a}	5.19^{b}
BD + AF+BC		10.41^{bc}	186^{b}	13.67^{ab}	6.57^{ab}
BD + AF+DE		10.11^{cd}	187^{b}	13.60^{ab}	4.57^{b}
BD +AF+ TUM		9.59^{d}	206^{ab}	14.20^{ab}	4.91^{b}
BD +AF+ BC+ TUM		9.93^{cd}	195^{b}	13.67^{ab}	5.57^{ab}
BD +AF+ DE+ TUM		9.50^{d}	260^{a}	13.20^{ab}	5.91^{ab}
ANOVA	S.E.M.:	0.234	21.3	1.222	0.774
	P-value:	<.0001	0.223	0.821	0.308

[1]Data are means of five replicate pens of 5 chicks each;

[2]Treatments were the addition of AF and adsorbents to the basal diet (BD);

[a-c] Means within columns with no common superscripts are significantly different ($P<0.05$);

AF = Aflatoxin from culture material; BD = basal diet. ; BC= Bentonite clay; DE= Diatomaceous earth; TUM = TurmericAST – Alanine aminotransferase; GGT - Gamma-glutamyl transferase; UA – Uric acid

8.3.5 Tibia ash and shank colour

Table 8.6 contains the results of tibia variables and shank colour of chicks fed dietary treatments. There were no significant differences (*P>0.05*) in tibia weight, tibia ash weight, tibia ash percent or shank color of chicks fed BC or DE alone when compared with those fed the control diet.

Chicks fed TUM alone had lower (*P<0.05;* 1.95 versus 2.26 g) tibia weight and tibia ash weight but similar (*P>0.05*) percent ash and shank color to that of chicks fed the control diet. The AFB_1 diet decreased (*P<0.05*) tibia weight and tibia ash weight but did not affect (*P>0.05*) percent ash or shank colour when compared with the chicks fed the control diet. Compared with controls, chicks fed the AFB_1 plus BC diet had similar (*P>0.05*) tibia weight (2.11versus 2.26 g), tibia ash weight (1.09 versus 1.20 g), tibia percent ash (51versus 53 %) and shank colour (2.60 versus 2.95). In contrast, chicks fed the combination of DE and AFB_1 or TUM and AFB_1 had significantly (*P<0.05*) lower tibia weight, tibia ash weight, and shank color when compared to controls. The addition of BC and TUM to the AFB_1 diet improved tibia variables and shank color values comparable to chicks fed the control diet. In contrast, the addition of DE and TUM to the AFB_1 diet decreased (*P<0.05*) tibia weight, tibia ash weight, percent tibia ash and shank colour when compared with chicks fed the control diet. Chicks fed the combination of DE, TUM and AFB_1 also had lower (*P<0.05*) tibia weight and tibia ash weight than birds fed AFB_1 alone.

8.4 Discussion

Growth of the birds fed BC or DE alone was not different from those of control birds, indicating that the adsorbents were inert and nontoxic. Similar observations were made by Magnoli *et al*. (2011) and Denli *et al*. (2009) who reported that FI and BWG of chicks fed 0.3 % BC (Magnoli *et al*., 2011) or 0.5 % DE (Denli *et al.*, 2009) did not differ from control chicks.

Birds fed TUM (200 mg/kg curcuminoids) alone had similar FI but significantly lower BWG when compared with those fed the control diet. The reduction in BWG observed in this study is in contrast to a previous report by Al-Sultan (2003) who reported that chicks fed a diet containing 0.5 % TUM had higher (16.8 %) BWG when compared with the control group. Yarru *et al*. (2009) and Gowda *et al.* (2009) reported that chicks fed TUM (0.5 %), containing 74 mg/kg of total curcuminoids were not negatively affected, as these birds performed as well as those fed a control diet. Emadi and Kermanshahi (2007) also observed that in all periods FI,

Table 8.6. Effects of bentonite clay, diatomaceous earth and turmeric on tibia variables and shank color of chicks fed diets containing 2 mg AFB_1/kg diet[1]

Treatments[2]		Tibia variables and shank colour			
		Bone weight (g)	Ash weight (g)	Ash (g/kg)	Shank colour
BD		2.26[a]	1.20[a]	530[abc]	2.95[abc]
BD + BC		2.20[ab]	1.18[a]	540[a]	2.85[abcd]
BD + DE		2.24[a]	1.19[a]	530[ab]	3.25[ab]
BD + TUM		1.95[bc]	1.03[b]	530[abc]	3.45[a]
BD + AF		1.96[b]	1.01[bc]	520[cde]	2.37[cdef]
BD + AF +BC		2.11[ab]	1.09[ab]	510[bcd]	2.60[bcdef]
BD + AF +DE		1.70[cd]	0.86[cd]	510[e]	2.25[def]
BD + AF + TUM		1.68[d]	0.87[cd]	520[cde]	2.10[ef]
BD + AF +BC + TUM		2.07[ab]	1.09[ab]	530[abc]	2.75[bcde]
BD + AF +DE + TUM		1.67[d]	0.85[d]	510[de]	2.05[f]
ANOVA	S.E.M.:	0.079	0.044	4.19	0.245
	P-value:	< 0.0001	0.0001	0.0007	0.0016

[1]Data are means of five replicate pens of 3 chicks each

[2]Treatments were the addition of AF and adsorbents to the basal diet (BD)

[a-c] Means within columns with no common superscripts are significantly different (*P<0.05*)

AF = Aflatoxin from culture material; BD = basal diet. ; BC= Bentonite clay; DE= Diatomaceous earth TUM = Turmeric

Shank colour was determined using a DSM Yolk colour fan with scores of 1 (lightest) to 15 (darkest colour)

BWG and FG were not significantly different among the treatments, when they fed from 0 to 0.75 % turmeric rhizome powder. Al-Kassie *et al.* (2011) reported that a mixture of cumin (*Cuminumcyminum*) and turmeric (*Curcuma longa*) at dietary levels of 0.75 % and 1 % respectively enhanced the overall performance of broiler chicks. Abbass *et al.* (2010) studied the comparative efficacy of turmeric crude powder and salinomycin sodium on the occurrence of coccidiosis and growth performance of broilers and observed that, the body weight gain of the groups fed rations supplemented with salinomycin sodium (coccidiostat) and 3% turmeric powder (2280 and 2293 g, respectively) were higher than that of the infected group (1955g).

Studies on the effect of turmeric on performance of broiler have yielded inconsistent results. Turmeric has been shown by several authors to improve bird performance but other authors report that the inclusion of turmeric does not alter the performance of the birds. Turmeric contains essential oils (Li *et al.*, 2011) that have active components which possess antimicrobial, antifungal and antioxidant activities (Gowda *et al*., 2008, Abbass *et al.*, 2010 and AL-Kassie *et al.*, 2011), which can help to maximize the utilization of nutrients, contributing to the improvement in performance of broiler chickens. Li *et al.* (2011) reported large variations in composition of essential oils of turmeric rhizomes according to varieties and geographical locations. These differences could explain the inconsistent effects of turmeric on growth performance.

The negative effects of aflatoxin (AFB_1) in broiler chickens include decreased BWG, poor efficiency of feed utilization, liver damage, poor performance, and changes in immune responses (Ledoux *et al*., 1999; Verma *et al*., 2002). In the present study, decreased growth performance of chicks fed AFB_1 alone agrees with previous reports (Bailey *et al*., 2006; Yarru *et al.*, 2009). Addition of BC into AFB_1 diet prevented the growth-depressing effects of AFB_1. Similar results were reported by Rosa *et al*. (2001) using 30-d-old broilers fed 5 mg total AFB_1/kg diet plus 0.3 % sodium bentonite (SB) for three weeks. In contrast, the addition of DE into the AFB_1 diet did not prevent the reduction in growth performance caused by AFB_1.These results are consistent with a previous report indicating that DE failed to prevent losses in growth performance of chicks fed AFB_1 (Denli and Okan, 2006).

In the present study, TUM addition to the AFB_1 diet reduced feed intake and increased mortality when compared with chicks fed theAFB_1diet. These results are in contrast to Gowda *et al*. (2009) who reported that the addition of 74 and 222 mg/kg curcuminoids to a diet containing 1 mg/kg AFB_1 improved weight gain and feed conversion efficiency compared to birds fed AFB_1 alone. Similar results were also reported by Yarru *et al*. (2009) who also

observed an improvement in body weight gain of birds fed 1 mg/kg AFB_1 and supplemented with 74 mg/kg curcuminoids when compared to chicks fed AFB_1 alone. In the current study, chicks fed the diet containing 200 mg/kg TUM alone showed poor growth performance which was aggravated when adding AFB_1 to the diet (811g versus 639g, respectively). The contrasting results could be attributed to the different concentrations of AFB_1 used (2 mg vs. 1 mg/kg) or to differences in the composition of the TUM. Li *et al* (2011) reported that the concentration of the active components of TUM (curcuminoids and essential oils) depends on variety, geographic locations, sources, and cultivation conditions. This is further affected by the extraction and storage methods used.

With the exception of an increase in feed intake, the supplementation of the AF diet with a combination of BC + TUM did not reduce the toxic effects of AF, indicating that the combination was not effective in ameliorating the adverse effects produced by the AFB_1 diet. Supplementation of the AF diet with the combination of DE plus TUM caused a significant reduction in BWG when compared with chicks fed AFB_1 alone, suggesting that there was a negative interaction between DE and TUM.

The supplementation of the AFB_1 diet with a combination of both BC and TUM did not yield further benefits in terms of feed intake and BWG as compared with BC or TUM alone, suggesting a negative interaction between BC and TUM. The current results are in contrast with Gowda *et al*. (2008) who showed that the addition of TUM (74 mg/kg) and a hydrated sodium calcium aluminosilicate (HSCAS; 0.5 %) to an AFB_1 diet (1.0 mg/kg) significantly improved FI and BWG comparable to that of the control chicks, indicating a beneficial effect of TUM. Gowda *et al*. (2009) also reported that increasing the level of TUM to 444 mg/kg resulted in poor performance of chicks and attributed the decrease in growth performance to the pro-oxidant action of curcuminoids at the higher concentration. This hypothesis could partially explain differences observed in the present study when compared with Gowda *et al*. (2008) where they used a lower level of TUM.

Neither BC, DE nor TUM alone had any significant effect on relative liver and kidney weight of chicks. Previous reports showed no negative effect of BC, DE or TUM on the relative weight of these organs (Santurio *et al*., 1999; Denli *et al*., 2009; Gowda *et al.,* 2009; Yarru *et al*., 2009). Studies have suggested that AFB_1 negatively affects relative organ weights in broiler chicks (Kubena *et al*., 1990; Gowda *et al.,* 2009). In the present study, enlargement and discoloration of livers and kidneys, along with mild fibrosis and bile duct proliferation, and marked hepatocellular vacuolation, were observed in chicks fed AFB_1. These results agree with

earlier reports (Gowda *et al*., 2008; 2009; Yarru et al., 2009; Neeff *et al*., 2013) in which increased relative liver weights were observed in chicks fed 1 to 2.5 mg AFB_1/kg diet. This finding is in contrast to Modersanei *et al*. (2008) who indicated that the relative weights of liver, spleen, bursa of Fabricius and pancreas were not affected by feeding 1.0 mg/kg diet to 21 days of age. The difference in results could be due to the lower concentration of AFB_1 used by Modersanei *et al*. (2008).

The present study indicates that BC decreased the increase in kidney weights caused by AFB_1. The bentonite used in the current study was a crude product. According to Thieu and Pettersson (2008), improvements in processing and purification of bentonite are needed to enhance the surface area, which would probably result in better adsorptive capacity. Although our results did not show a significant reduction in the relative liver weight, the numerical decrease in relative liver weight (9.4 %) and the significant decrease in relative kidney weight (16.8 %) agrees with Rosa *et al.* (2001) who observed an 18.3 % reduction in relative liver weight and 27 % reduction in relative kidney weights of chicks fed a diet containing 5 mg AFB_1/kg diet and supplemented with 0.3 % BC. The addition of DE to the diet containing AFB_1 numerically reduced liver weight (11.1 %) and significantly reduced relative kidney weight by 15.2 %, indicating that DE partially ameliorated the toxic effect of AFB_1. Denli and Okan (2006) reported that compared to chicks fed 80 μg AFB_1/kg feed addition of 2.5 g/kg DE to the AF diet did not diminish the increase in liver weight. Contrary to our results and the study of Denli and Okan (2006), Denli *et al*. (2009) reported that the addition of 1, 2, and 5 g/kg of AflaDetox (a natural product obtained from diatomaceous earth) to AFB_1 contaminated diets ameliorated the effects on the relative weights of liver, resulting in values not significantly different from the control. These contrasting results are possibly due to the differences in the cationic composition of the compounds or physicochemical differences among the adsorbents used in the studies mentioned above (Denli *et al.*, 2009).

Liver weights of chicks fed the combination of TUM and AFB_1 were not different from relative liver weights of chicks fed AFB_1 alone, suggesting no positive effect on reducing the increase in relative liver weights. This result is not consistent with early reports on the effects of TUM on relative liver weight. Gowda *et al*. (2008) and Yarru *et al.* (2009) reported that liver weights of chicks fed diet containing AFB_1 (1.0 mg/kg) and supplemented with turmeric powder containing 74 mg/kg curcuminoids, was comparable with those of chicks fed the control diet.

The reduction in relative liver and kidney weights observed in chicks fed the AFB_1 diet with a combination of TUM and BC indicated that the combination significantly ameliorated the effects of AFB_1 on relative weight of liver and kidney observed in birds fed AFB_1 alone. Addition of the combination of DE and TUM to the AFB_1 diet was not effective in ameliorating the effect of AFB_1 on relative organ weights. In fact, relative liver weight of chicks fed the DE+TUM combination were not different from that of chicks fed AFB_1 alone, and relative kidney weight was higher than that of chicks fed AFB_1alone.These results suggest that the curcumin present in TUM and known to protect the liver against AFB_1 (Gowda and Ledoux, 2008) was not effective when combined with DE. However, our observations are in agreement with Gowda *et al*. (2008) who observed that the dietary inclusion of TUM and HSCAS into an AFB_1 diet did not result in any further benefits as compared with either TUM or HSCAS alone.

Feeding chicks a diet containing BC, DE or TUM alone did not negatively affect serum concentrations of glucose, total protein (TP) albumin, globulin, gamma glutamyltransferase (GGT) aspartate aminotransferase (AST), uric acid (UA) or calcium(Ca). Similar findings on serum variables were reported earlier (Magnoli *et al*., 2011; Modirsanei *et al*., 2008; Gowda *et al.*, 2008), indicating that the adsorbents and TUM were non-toxic. Gowda *et al.* (2008) and Neeff *et al.* (2013) surmised that the reduction in serum levels of TP, albumin, globulin, γ-glutamyltransferase, aspartate aminotransferase, and calcium are indicative of the negative effect of AFB_1 on hepatic and renal tissues.

The addition of BC to the AFB_1 diet raised serum concentrations of TP and globulin to levels comparable to those of chicks fed the control diet. In contrast, Rosa *et al.* (2001) reported that the addition of 0.3 % sodium bentonite to a diet containing 5 mg AFB_1/kg diet did not raise serum concentrations of TP and albumin to levels comparable to that of control chicks.

The changes in serum variables observed in chicks fed AFB_1 plus DE does not agree with some results reported by Modirsanei *et al*. (2008) who observed increased serum albumin, ALT and serum lactatedehydrogenase (LDH) activity, when they fed DE (30 mg kg^{-1}) and AFB_1 (1.0 mg kg^{-1}) to broiler chicks during experimental aflatoxicosis.The contrasting results could be related to differences in sources of DE (Iran versus Mozambique) or AFB_1 concentration (1 mg/kg vs. 2 mg/kg) used by Modirsanei *et al.* (2008).

Supplementation of 222 mg/kg TUM to an AFB1 diet significantly improved the serum values of total protein, albumin, globulin, γ-glutamyltransferase and aspartate aminotransferase compared with chickens fed AFB_1 alone or those fed AFB_1 plus 74 or 444 mg/kg TUM (Gowda

et al., 2009). In the present study, adding 200 mg/kg TUM to the AFB_1 diet did not improve the serum values of total protein, albumin, globulin, γ-glutamyltransferase and aspartate aminotransferase compared with chickens fed AFB_1 alone. The small difference noted between results using 200 mg/kg TUM versus Gowda *et al*. (2009), using 222 mg/kg TUM could be attributed to a lack of standardization, source and/or chemical composition of TUM.

Addition of both TUM and BC to the AFB_1 diet increased the level of total protein, albumin and globulin, but only globulin was increased to the level of chicks fed the control diet. In contrast, the addition of both TUM and DE to the AFB_1diet did not overcome the depression in serum proteins. No similar studies on chicks fed AFB_1 diet combined with BC plus TUM or DE plus TUM seem to be available for comparison with our result.

The inclusion of BC or DE individually to the control diet had no adverse effects on the bone ash. In contrast, TUM reduced the blood parameters. Khaneda *et al*. (2013) reported that the addition of bentonite (1 %) to a control diet had no significant effect on tibia ash. Safaeikatouli *et al*. (2012) reported that, although, not significant the addition of bentonite (3 %) increased blood (mg/dl^{-1}) calcium and phosphorous levels by 10 and 14 % respectively, tibia calcium and phosphorous levels by 8 and 1.4 %, respectively, and tibia ash by 7 %. These results suggest that supplementing silicate minerals to diets increased the degree of mineralization and development of the bone.

The addition of AFB_1 had a negative effect by reducing the value of the tibia variables. The absorbent BC ameliorated the effects of AFB_1 whereas DE did not. The addition of TUM also did not demonstrate an ameliorating effect. The combination of BC and TUM was partially effective in reversing the effect of AFB_1 on bone ash, ash weight and ash, whereas the combination of DE plus TUM did not. No similar studies on chicks seem to be available for comparison of results.

8.5 Conclusions

The addition of BC, DE or TUM to the basal diet caused no adverse effects on the performance and organ weights of the birds. Aflatoxin B_1caused marked deleterious effects on growth performance, increased relative organ weights, caused histopathogical changes in the liver, and alterations in serum chemistry values. The addition of the absorbent BC into the AFB_1 diet ameliorated the negative effects of AFB_1, whereas the addition of DE to the AFB_1 diet did not ameliorate any of the negative responses caused by AFB_1. The addition of TUM alone into the AFB_1diet also did not demonstrate an ameliorating effect. The addition of the combination of

DE and TUM to the AFB_1 diet was not as effective in reducing the effect of AFB_1 on BWG as the combination of BC and TUM. When BC and DE were fed in combination with TUM, the results showed a reduction in the comparative individual effectiveness of BC and DE in their ameliorating effect on BWG.

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CHAPTER 9 – General Discussion and Conclusions

9.1 General Discussion

Throughout the world, chickens are produced in many diverse mixed farming systems. Chickens are grown as a source of meat and eggs. Village chickens play a valuable role within households. They reduce risk, contribute significantly to alleviating poverty, secure household food supply, and promote gender equality. Women and children own most of the chickens and are directly responsible for their management. Despite their immense importance to rural livelihoods, village chicken productivity is low. Contributing factors include huge variability in management practices, limited investment in village chicken production, high prevalence of diseases and parasites, shortage of feeds, and lack of adequate protein consumption.

The study showed that the normal feathered, naked-neck and frizzled strains are the most common strains kept in the areas of Mozambique investigated. Hens produce between 14 and 90 eggs per year from 2 to 3 clutches/year. The percentage of chicks weaned per hen per clutch averaged 70 %. Normal feathered hens had a higher rate of chicks weaned, suggesting that they are either good mothers or the chicks are more resilient to adverse factors in the environment compared with naked-neck and frizzled birds. Most households housed the birds at night, especially in households with no feed constraints, and those who had predator constraints.

In general, the scavenging feed resource base (SFRB) constituted most of the feed input for village chickens. During the rainy season, there is an abundant supply of insects, worms, and green forages, while in the dry season, chicken diets are mostly composed of a higher supply of cereals grain and cereal by-products, as was reported earlier (Goromela *et al.*, 2006). There was significantly more household food waste fed in the mountainous Namaacha district than in Boane. The quantity and quality of feed varied not only with season of the year, but also the location, climate, with common agricultural activities contributing to the differences.

Use of non-conventional protein sources as chicken feed is limited by the presence of anti-nutritional factors. In Chapter 4, it was demonstrated that the concentration of trypsin inhibitors and phytic acid were higher in the local Mozambican nhemba cowpea than in black-eyed beans. The concentration of anti-nutritional factors also varied with cultivars, as was reported earlier (Owolabi *et al.*, 2012).

Roasting or extrusion of nhemba or black-eyed beans reduced the level of trypsin inhibitor to levels below 2000 TIU/g. In addition, amino acid concentrations varied with heat treatments. Roasting did not improve amino acid digestibility of black-eyed beans, but increased digestibility of nhemba cowpeas. The extruded black eyed bean cowpeas had higher amino acid values compared with raw black eyed bean cowpeas. The hypotheses that the nutritive value and amino acid digestibility of cowpeas vary with different cultivars, and that heat treatment reduces the anti-nutritional factors and improves digestibility of amino acids was, thus, not rejected. The results further indicated that the method of processing and the variety influence the nutritive value of feed ingredients. The decreased energy digestibility observed in roasted black-eyed beans, compared to the improvement in nhemba could be due to differences in varieties, the levels of anti-nutritional factors, or the roasting itself (Alajaji and El-Adawy, 2006; Brenes *et al.*, 2008).

The hypothesis that the effect of heat treatment and enzyme supplementation of cowpeas and pigeon peas on growth performance and gut health of chickens will differ was tested in Chapter 5. Roasting cowpea or enzyme incorporation had no effect on body weight gain and the cumulative performance index. Roasting pigeon pea improved apparent ileal nitrogen digestibility, consistent with the effect observed on growth performance. In contrast, the higher apparent ileal nitrogen digestibility observed in all cowpea treatments did not conform with effects on growth performance, suggesting that roasting was inadequate to reduce the negative effect of the anti-nutritional factors in cowpeas. The poor performance of chicks fed raw cowpea, raw cowpea and enzymes and raw pigeon pea were in line with the lower crypt depth when compared with those fed the control diet. Histological staining of duodenum for immunoglobulin A did suggest there are benefits for roasting this bean. Roasting or adding enzymes to the raw black-eyed bean did not increase body weight gain further. Extrusion of cowpeas, however, increased body weight gain to levels comparable to the control diet.

In the chapter 6 an experiment was conducted to assess the effects of roasting, extruding and addition of enzymes to black- eyed beans (*Vigna unguiculata*) on growth performance and gut health of chickens. The results shown that at day 7 roasting or adding enzymes to the raw black-eyed beans did not improve body weight gain; however, extrusion of black-eyed beans increased ($P<0.05$) body weight gains to levels comparable to the control diet. By day 14, all diets performed similarly to those of the control diet.

Feed can be contaminated with mycotoxins that compromise the health and growth performance of birds. In Chapter 7, the hypotheses that the toxic effects of aflatoxin B_1 (AFB_1) in chickens

will be ameliorated by the addition of 0.50% bentonite (BC) or 0.50% diatomaceous earth (DE) to diets containing 2 mg AFB_1/kg diet was not fully confirmed. Bentonite from Mozambique may be useful in preventing and/or reducing the toxic effects of aflatoxins, whereas DE was not effective. In Chapter 8, the hypothesis that the combination of BC plus DE and turmeric (TUM) will reduce the toxic effects of aflatoxins was not proved.

9.2 Conclusions

Village chickens play an important role in supplying income, meat and eggs for communal households and they are valued by women. The major constraints to village chicken production are high disease prevalence, predators, theft, and shortage of feed. Cowpea and pigeon pea can be used as non-conventional protein sources after heat processing.

Aflatoxin B_1 caused marked deleterious effects on growth performance, increased in relative organ weight, caused histopathogical liver changes, and alterations in serum chemistry values. The addition of BC, DE or TUM to the basal diet caused no adverse effects on the performance and organ weights of the birds. The addition of bentonite into the AFB_1 diet ameliorated the negative effects of AFB_1, whereas the addition of DE to the AFB_1 diet did not ameliorate any of the negative responses caused by AFB_1. The addition of the combination of BC and TUM to the AFB_1 diet was more effective in reducing the effects of AFB_1 on BWG than the combination of DE and TUM.

9.3 General Recommendations

9.3.1 Recommendations

Availability of protein-rich feeds is a major constraint to village chicken productivity. Predators are one of the causes of chicken loss, so farmer households should be encouraged to house the chickens, in particular the young chicks. Nutrients that are not optimally supplied by SFRB, ideally, should be provided as supplementary feeds. Peas should be roasting by the household farmers before providing it to chickens. Bentonite can be recommended for reducing the toxic effects of AF.

9.3.2 Further research

Chicken meat and egg are important source of animal protein for the majority of resource-poor farmers. Understanding of nutrient utilization by the village chickens will provide information on improving productivity and profitability of village chicken enterprises.

The effects of these anti-nutritional factors and aflatoxins in village chickens need to be understood. Further characterization of the antinutritional factors of the local beans might provide new insights of how to overcome the anti-nutritional factors in cowpeas. Combination of different treatments such as heat, enzymes and other methods of extraction of the compounds responsible for the performance lost deserves further attention. Cheap and appropriate technologies of processing the peas should also be developed.

Appendix 1. Questionnaire

Household Baseline Survey on Village chicken production in rural areas of Mozambique

Questionnaire N° ……

Date: _____ Village_____ Ward_____

1. Name of person being interviewed _____
   Age_____ Gender _____

2. Education, completed years of schooling: illiterate_____6st year of schooling_____ more them 6nd year.

3. Number of adult household members_____Number of children below 12 years_____

4. Indicate your major sources of income

Source of income	**Tick**	**Rank**
Crop production		
Livestock		
Agricultural labour		
Migration		
Monthly salaried jobs		
Business		
Handicraft		
Others (specify)		

5. Flock composition

Breeds	**Flock size (quantity)**				**Purpose (0 = NO, 1 = YES)**			
	Cock	hens	growers	chicks	Meat	Egg	Cash	Other
Normal feathered								
Naked-neck								
Frizzle								
Exotic layers								
Exotic broilers								

6. What are the reasons for having lower numbers? (Tick and rank 1-6 most important)

	Tick	**Rank**
higher demand/Higher sale rate		
lower of chicks per brood		
Higher mortality		
Long period inter brood		
Higher price		

a) How important are the indigenous chickens you? Not important_____important_____Very important_____?

b) Would you want to increase the number of indigenous chickens? Yes_____No_____

c) Give reasons for increasing the number of indigenous chickens? (Tick and rank 1-6 most important)

	Tick	**Rank**
Easyness of breeding		
Need of use for immediate expences		
School fees		

Increased the indigenous chickens trade		
Need of buying sheep and goat		
Consuption		
I don’t eat beef		

7. Chicken productivity

	Normal feathered	**Naked-neck**	**Frizzle**
Eggs per clutch			
Number of clutches per year			
Eggs incubated per clutch			
Eggs hatched per clutch			
Chicks weaned per hen per clutch			

8. What are the major challenges to chicken rearing? (Tick and rank 1-6 most important)

Challenges	**Tick**	**Rank**
Feed shortage		
Lack of acess to market		
High prevalence of disease		
High prevalence of external parasites		
Housing		
Predators		

9. Chicken housing practices (Tick):1) Housed permanently _____2) Housed during night_____3) never housed_____

10. If your birds are housed, please describe the housing type (Tick): 1) Simple construction with on-farm materials_____2) Improved chicken house_____

11. If your birds are NOT housed, give a reason:

a) Not enough money to build _____ Not necessary_____birds do well without_____ Other (specify)_____

b) Why do you think that they do well without house?

12. Identify the common Scavenging Feed Resource Base (SFRB) available

Type of feed	Dry season	Wet season
Household cooking waste		
Household leftover		
Cereal and cereal by-product		
Roots and tubers		
Leaves of trees and shrubs		
Fruits		
Insects		
Locusts		
Earthworms		
Snails		
Ants		
Flies		
Aquatic plants		
Grass		

13. How do you feed your chickens (Tick)? I provide feed to my chickens_____ Chickens scavenge only_____Chickens scavenge and I feed them_____

14. If you provide feed to your chickens, (Tick): Are you feed all chickens together?_____are you feed only chicks?_____ You feed chicks and hens?_____

15. What have been your major sources of feed for chickens in the past 12 months (Tick)? 1= Kitchen waste_____; 2= Complete ration_____; 3= Home made ration_____; 4= Crushed grain___; 5= Whole grain____; 6= Other (specify)

16. What do you think are the main constraints in chicken feeding? 1=Lack of feeds_____; 2= Cost of feed____; 3= shortage_____; 4= Lack of water_____; 5= Other (specify)_____

17. Have you experienced any shortage of feed for chickens in the past 12 months? 0 = No_____; 1 = Yes, some___; 2 = Yes, significant_____

18. Do you have any reasons for not preferring using the following ingredients as chicken feed?

Protein source	Yes	No
Flies		
Earthworm		
Snails		

a) If yes, what are the reasons?

……

19. Do you provide water to the chicken?

NO_____Why?_____ YES_____

20. Chicken losses (Tick and rank 1-3 most important)

	Tick	Rank
Predators		
Thieves		
Disease		

21. What are the most common predators?

	Snake	Simba	Dog	Birds	Others
Eggs					
Chicks					
Growers					
Adults					

22. How many birds did you lose in the last month?

23. Name the most important reason for chick losses in period. (*use codes :* 1= disease; 2= Predator; 3= Thieft; 4= Accident; 5 = Unknown *(multiple answers possible*)

a) Less than 1 month_____b) 1- 6 months_____c) Beyond 6 months_____

24. How many chickens did you sell June 20012 to May 2013 and what was the price?

	Cock		**Hens**		**Growers**	
	Quantity	Price	Quantity	Price	Quantity	Price
Normal feathered						
Naked-neck						
Frizzle						

25. Where do you sell most of the chicken products in the village? Home Gate_____Local market_____neighboring villages_____

26. Which types of meat do you prefer to eat? (*Use code below*)

Meat Type	**Choice**	**Why**
Pig		
Goat		
Chicken		
Beef		
Guinea fowl		
Duck		
Mice		
Termites		
Game meat		
a. Choice: 1=1st choice; 2=2nd choice; 3=3rd choice. b. Why : 1= price, 2= availability, 3= taste,4= other (specify)		

27. How many chickens do you think your household should have? _____. Why? _____

Appendix 2. Ethics approval - University of Illinois

Institutional Animal Care and Use Committee

University of Illinois

TO: Carl Parsons

FROM: Phil Solter

DATE: Friday, October 24, 2014

SUBJECT: Approval of Animal Use Protocol

Your animal use protocol submission entitled, "Digestibility of Nutrients in Feedstuffs for Poultry," was approved by the Institutional Animal Care and Use Committee (IACUC) on Friday, October 24, 2014. The IACUC approval number for this protocol is 14244.

Please note that changes in the protocol, animal numbers, or personnel must receive approval by the IACUC.

This approval is valid for a three-year period, which expires on Tuesday, October 24, 2017. If work will continue beyond the expiration date, a new protocol will need to be submitted and approved by the IACUC prior to Tuesday, October 24, 2017. Additionally, federal regulations and campus policy require annual administrative review of protocols. You will receive notification from the IACUC prior to the deadlines for these reviews as well as for the protocol expiration.

If you have any questions, please do not hesitate to contact the IACUC staff.

Sincerely,

Phillip Solter, Phd
Chair, IACUC
University of Illinois at
Urbana-Champaign

Appendix 3. Ethics approval - Missouri University

Ledoux, David R.

From: Allen, Jennifer Ashley
Sent: Wednesday, June 04, 2014 3:48 PM
To: Ledoux, David R.
Cc: Jennings, Cyndi L.; Kraus, Gail L.; Linville, Michael L.
Subject: ACUC Protocol #8140 Approved
Attachments: 8140.pdf

Dear Dr. Ledoux,

This memo serves as notification that your above-referenced Animal Care and Use Protocol or Amendment has been approved by the MU ACUC. Attached is your complete protocol. This e-mail is also being sent to your department administrator/facility supervisor by cc: to this message.

We wish to make every effort possible to avoid delays in the release of your grant funds! ACQA is required to verify that animal procedures in funded grants are described in your approved animal protocol before grant money can be released. Avoid delays by amending your protocol at any time before the grant award is made. If you submitted your protocol by TOPAZ you may amend it by following the instructions located here: https://research.missouri.edu/acqa_secure/forms/topaz_amendments.pdf.

Thank you for your continued cooperation with the MU ACUC. If you have any questions about your protocol or the ACUC review process, please don't hesitate to contact me at the ACQA office.

Jen Allen
Coordinator, Animal Care and Use Committee
Animal Care Quality Assurance Office
50 McReynolds Hall
573-882-4300
allenjenn@missouri.edu

Protocol Detail Report

Printed By: Allen, Jen A.
6/4/2014 3:47:09 PM

Protocol Information

Reference # 8140
Protocol # 8140
Protocol Type: Original
PI: Ledoux, David R.
Submittal Date: 5/20/2014
Author: Ledoux, David R.
Status: Approved
Inactive Date:
Approval Date: 6/4/2014
Effective Date: 6/4/2014
Renewal Date: 6/4/2015
Next Review Date: 6/4/2015
Expiration Date: 6/4/2017

Basic Information 1

* Save Often 1.1

Make sure you click on the "black disk" icon above to save your work as you answer questions. If you receive an error, the program will only be able to restore your work to the last SAVED VERSION.

Include Attachments 1.2

Attachments: Summon at MU References.docx

Please include ALL relevant attachments to this protocol here. To add the attachment click on the "paperclip" icon in the top right of this question header.

Animal Care and Use Committee Policies 1.3

For more information about the standard policies of the MU Animal Care and Use Committee please visit the ACQA website.

Protocol Number 1.4

Protocol Number (filled in by ACUC)

Reference Number 1.5

Reference Number (automatically assigned)
8140

Protocol Detail Report

Printed By: Allen, Jen A.
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1.6

Principal Investigator

Ledoux, David R. ledouxd@missouri.edu

1.7

Created By

Ledoux, David R. ledouxd@missouri.edu

1.8

Department

Click on the drop down list to select the department.

Animal Science

1.9

Title

Protocol Title
Evaluatiuon of the efficacy of feed additives to prevent or ameliorate the effects of toxins present in poultry feed

1.10

Triennial Re-Write

Is this protocol a re-write of an ACUC protocol that was previously approved at the University of Missouri?

- [x] Yes
- [ ] No

1.10.1

Yes

1.10.1.1

Historical Protocol Number

What ACUC number was this protocol previously approved under?
7182

1.10.2

No

2

Species Section

2.1

Species

Please click on the green plus sign to select the species.

2.1.1

Turkey #1

2.1.1.1

Strain/Stock/Breed

Please click on the green plus sign to select the strain.

ANY

UNIVERSITY *of* MISSOURI

OFFICE OF RESEARCH

July 24, 2014

David R. Ledoux, Ph.D.
Animal Science
112A ASRC

Dear Dr. Ledoux:

The MU Institutional Biosafety Committee (IBC) has reviewed your renewal protocol application, **"Evaluation of the efficacy of experimental adsorbents to prevent or ameliorate the toxic effects of mycotoxins present in poultry rations"**. They have **approved** your protocol at **BSL2\ABSL1** containment and physical conditions or lower (RG2 agent). This approval is valid for three years from July 2014.

Access to the laboratory must be restricted when biohazardous agents are present and "A sign incorporating the universal biohazardous symbol must be posted at the entrance to the laboratory." Please refer to the following regulatory compliance guidelines.

NOTE: The Select Agent Program limits T-2 exemption to less than 1000 mg of the toxin. Any amount equal to or over 1000 mgs will require enrollment into the select agent program.

The current guidelines for exempt quantities require you to maintain document regarding storage, shipping or use of this regulated toxin. This includes current inventory, date of usage and amounts and records of purchases. Also you are required to maintain justification (research protocol approval) and notify the Biosafety team if any changes affect location or quantities of toxin (e.g., moved from locked storage or transfer of toxin to colleagues or collaborators, etc.)

The "toxin due diligence" provision requires a person transferring toxins in amounts which would otherwise be excluded from the provisions to: (1) use due diligence to assure that the recipient has a legitimate need to handle or use such toxins; and (2) report to Federal Select Agent Program if they detect a known or suspected violation of Federal law or become aware of suspicious activity elated to the toxin.

205 Jesse Hall Columbia, MO 65211-1150 Phone: 573-882-9500 Fax: 573-884-8371