Effect of dietary *Moringa oleifera* leaf meal on egg fertility and sperm quality of Potchefstroom koekoek indigenous chicken

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DECLARATION

I, the undersigned, hereby declare that this research dissertation submitted to the North West University for the Degree of Masters of Science in Agriculture in Animal Science is the result of my own work and has not been presented elsewhere for a higher degree. All sources of information have been acknowledged by references.

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GENERAL ABSTRACT

The current study was conducted to evaluate the effect of *Moringa oleifera* leaf meal (MOLM) inclusion in layer rations on growth performance, egg laying performance, egg quality parameters and hatchability performance of Potchefstroom koekoek (PK) hens. In addition, the study also evaluated the effect of MOLM on growth performance, semen and sperm quality parameters of Potchefstroom koekoek cockerels. Forty-eight PK hens at age twenty-two weeks were randomly allocated to two dietary treatments: MOLM0 (control) = diet with no MOLM inclusion; MOLM70 = diet with 70g/kg of MOLM inclusion. Each treatment was replicated four times with each pen holding six hens as the experimental unit. Non-fertile eggs were collected for nine weeks. When hens reached thirty-one weeks of age, eight cocks were introduced to the study and randomly allocated to a mating ratio of 6 hens: 1 cock. Fertile eggs were collected for 14 days to measure hatchability performance. In addition, thirty-two Potchefstroom koekoek cockerels were assigned to two dietary treatments: MOLM0 (control) and MOLM70, for the duration of twenty weeks. Each experimental unit (a pen) contained 4 birds and was replicated 4 times. Feed intake, weight gain, feed conversion ratio (FCR), Semen motility parameters (curvilinear velocity (VCL), average pathway velocity (VAP), straight line velocity (VSL) straightness, wobble and linearity), progression parameters (PM, NPM & static), pH, concentration, velocity, volume and morphology were evaluated. There were no significant ($P>0.05$) effects on feed conversion ratio, average weight gain and egg laying performance for all treatments. However, diet significantly ($p<0.05$) affected feed intake. Furthermore, there was no significant difference ($P>0.05$) noticed in terms of egg weight, egg content, egg shell thickness and all internal egg parameters apart from albumen length. However, diet significantly affected ($P<0.05$) egg length, diameter, shape index, shell weight and shell percentage. Dietary treatment MOLM70 improved the hatchability performance of Potchefstroom koekoek. The two way interaction significantly affected semen volume. Diet

MOLM70 resulted in higher sperm velocity (rapid), semen concentration, semen pH, motility (VCL, VSL AND VAP) and progressive motility than control (MOLM0). It was concluded that MOLM70 could be acceptable as sustainable feed resource in laying hen diets and it is potential useful in increasing the fertility of male Potchefstroom koekoek chicken.

Keywords: *Moringa oleifera* leaf meal, growth performance, egg production, egg quality, hatchability, semen and sperm quality

DEDICATION

This work is dedicated to my family, which has been tremendously supportive financially, morally and socially throughout my study. My parents Mr W.J Tutubalang and Mrs M.M Tutubalang, my siblings Rebaone, Tholwana and Botlhale not forgetting my cousins Sam Sekgalo and Tshepiso Mokoena, thank you for inspiring me throughout my education. All this is for you. Above all, I thank God almighty.

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CONTENTS

List of tables

List of figures

List of abbreviations

BPSE	Beltsvile Poultry Semen Extender
CP	Crude Protein
FCE	Feed conversion efficiency
MOLM	*Moringa Oleifera* leaf Meal
PUAF	Polyunsaturated Fatty Acids
NWU	North-West University
WHO	World Health Organization
%	percentage
CRD	completely randomised design
EC	egg content
ESA	egg surface area
EV	egg volume
EW	egg weight
FI	feed intake
G/Kg	grams/kilograms
GLM	general linear model
HDEP	hen day egg production
HU	hugh unit

Kg	kilogram
SAS	statistical analysis system
SEM	standard error of means
SI	shell index
SP	shell percentage
ST	shell thickness
SW	shell weight
VCL	cuevillinear velocity
VAP	average path velocity
VSL	straight-line velocity
LIN	linearity

CHAPTER 1

1. GENERAL INTRODUCTION

1.1 Background

Poultry is now by far the largest livestock species internationally (FAO, 2004), accounting for more than 30% of all animal protein consumption (Permin & Pedersen, 2000). The contribution of local poultry production to the nutritional and economic status of rural households is well recognized (Norris & Ng'ambi, 2006). Indigenous chickens have been a product of their environment and have survived under harsh conditions for many generations (Umesiobi, 2000; Fourie *et al.*, 2004). Yet, there has been a drop in the number of indigenous chickens, mainly due to their poor productive and reproductive performance (Larbi *et al.*, 2013). The productivity of indigenous chickens can be increased through improved management, especially targeting nutrition through supplementation (Ndegwa *et al.*, 1996). The dietary protein requirement for laying birds has been estimated at 140-180 g/kg for light and medium sized exotic birds (NRC, 1984; Harms *et al.*, 1966). Fertility and hatchability are usually the major parameters of reproductive performance that are most sensitive to genetic and environmental influences (Stromberg, 1975).

The use of indigenous leaf meal as feed source such as *Moringa oleifera* has been proven to have beneficial effect on production and performance of poultry (Nouman *et al.*, 2014). *Moringa oleifera* leaf meal (MOLM) is best known for its high leaf protein (27%) content, adequate amino acid profile, high levels of vitamins A and E, and low level of anti-nutritional compounds (Yang *et al.*, 2006). These compounds are known to have beneficial effect on spermatogenesis. Dietary manipulation by fatty acids has been recommended as a method of improving cockerel semen quality, due to the strong relationship that exists between nutrition and overall flock fertility (Hudson & Wilson, 2003). Several studies showed that supplementation with alpha-linolenic acid significantly enhanced semen fertility (Zaniboni *et al.*, 2006; Wathes *et al.*, 2007; Al-Daraji *et al.*, 2010). Sebola *et al.* (2015) reported high differences in fatty acids composition of *Moringa oleifera* leaf meal while alpha-linolenic acid had the highest value. Dietary lipid or fatty acid sources have been thought to affect cockerel sperm composition and functionality in different ways (Bongalhardo *et al.*, 2009), even when deposited proportionately in the sperm (Kelso *et al.*, 1997; Cerolini *et al.*, 2003). Even though various studies have been done on the Moringa species there is little information regarding the utilisation of *Moringa oleifera* leaf meal on native chicken. Therefore the objective of this study is to evaluate the effect of *Moringa oleifera* leaf meal on the semen quality of cockerel, egg fertility and hatchability as well as egg quality traits of Potchefstroom koekoek chicken.

1.2 Problem statement

Nutrition plays an important role in the fertility of animals. Chickens that are reared by small scale farmers are susceptible to nutritional deficiency and other diseases, such that their sperm fertility, and quality is reduced (Sonaiya & Swan, 2007). Hence, it's very important to formulate diets that will not only meet production requirements but also improve the reproductive performances of the poultry. There is little information regarding the effect of *Moringa oleifera* leaf meal on reproductive parameters of indigenous chicken.

1.3 Justification

Despite the fact that fertility can be determined in the native chickens, it is very important to understand and improve the semen quality of the native cocks. The highest achievement of every producer in the poultry industry is to maintain breeder males capable of producing viable spermatozoa that can fertilize eggs which will hatch with minimum mortality. However, the numbers of hatched eggs is dependent on the quality and quantity of the spermatozoa, thereby determining the profitability of the production. The widespread claim of *M. oleifera*'s nutritional and medicinal properties on humans can be extended and further be investigated in chickens as a feed source that improves the quality of semen and egg fertility. Therefore, it is very important to understand and improve the semen quality of the native cocks. This study seeks to assess the effect of dietary MOLM on egg fertility and sperm quality of indigenous chicken.

1.4 Objectives

- To evaluate the effect of MOLM on growth and laying performance of Potchefstroom koekoek hens
- To evaluate the effect of MOLM on external and internal egg parameters and hatchability performance of Potchefstroom koekoek chicken.
- To evaluate the effect of MOLM on growth and semen parameters of Potchefstroom koekoek roosters.

1.5 Study questions

- Does MOLM have an effect on growth performance of Potchefstroom koekoek chicken?
- Does MOLM have an effect on egg hatchability of Potchefstroom koekoek chicken?
- Does MOLM have an effect on reproductive performances (semen quality and egg fertility) of Potchefstroom koekoek chicken?

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CHAPTER 2

2. LITERATURE REVIEW

2.1 Introduction

Indigenous chickens (*Gallus domesticus*) are also known as village, rural, scavenging, family, or traditional chickens, they have different names in local languages (Alders *et al.*, 2009). Indigenous chicken production is an essential agricultural activity of almost all rural communities across the country. The poultry production systems in South Africa can be characterized into three distinct systems: commercial production, semi-intensive production and household production (South African Poultry Association, 2006). The household production system is where indigenous chicken are likely to be found also it is characterized by low input and low output. Alders *et al.* (2009) mentioned that indigenous chicken production is for home consumption and savings for small expenses such as school fees, purchase of medicines and slaughter at important social gatherings also, they are raised extensively in relatively small flocks numbering between 1–50. They are an important source of protein in the form of meat and eggs. Through occasional sale of indigenous chicken eggs and/or live chickens, income is generated. Indigenous chickens are a source of poultry manure, are a helpful tool in poverty alleviation of the rural villages and they play important role in biological pest control (Kgwatalala, 2013).

In chicken fertility is the first and most important factor and reveals the reproductive ability of males and females expressed by their capability when mated together to produce chicks. When an egg fails to show any indication of developing embryo it is said to be infertile (Miazi *et al.*, 2012). A trait of economic importance in the chicken industry is hatchability because it has a strong effect on chick production (Wolc *et al.*, 2010). It is influenced by several factors such as turning of eggs, egg weight, egg size, storage, humidity, shell strength, and genetic factors within the chickens kept. Ultimate profitability on hens is dictated by the number of fertile eggs produced for hatching. Low fertility is considered to be mainly a problem in males, though both females and males contribute to decreased fertility. There are many causes of low fertility; however, feeding male chickens with plant sources that are enriched with lipids such as MOLM can improve fertility.

2.2 Nutritional requirement and availability of feed for indigenous chickens

Sonaiya (2003) defines indigenous chickens as improved or unimproved chicken raised intensively or semi- intensively in small numbers and they are raised under communal production system. They account for more than 80% of poultry production in rural areas (GuEye, 1998). In South Africa indigenous chicken are culturally, economically and nutritionally important (Alabi *et al.,* 2012). They are mainly kept for meat and eggs, and their tastes are favoured more than those of exotic chicken (Dessie & Ogle, 2001). Indigenous strains need fewer nutrients than the exotic ones (Badhaso, 2012). They have the ability to change feed protein and energy to human food. Indigenous chickens range freely in the house hold compound and they scavenge for feed by nature, with little or no feed supplementation (Pedersen, 2002; Naido, 2003). They pick earthworms, insects, green grass, kitchen wastes (Moreki, 2001) and obtain minerals from top soil (Aganga *et al*., 2000). According to Nhleko *et al*. (2003), Kingori *et al*. (2003) and Pedersen (2002) these chickens are the most adaptable household animals that can survive roosting in trees, sheltered in cages, with little space to rest at night, unsheltered outside or harsh environmental conditions. Indigenous chickens use very low labour, capital and space, which give opportunity to people with limited space of land to practice chicken production (Muchadeyi *et al*., 2004). A protein requirement of indigenous laying hens differ from 16 to 18% of the diet and is needed for growth, egg production, maintenance and feather growth.

2.3 Potchefstroom koekoek

The Potchefstroom Koekoek is a South African registered chicken strain locally developed during the 1950s at the Potchefstroom Agricultural College by the late Chris Marais (Fourie & Grobbelaar, 2003). This breed is a combination of the Black Australorp Bared Plymouth Rock and White Leghorn. It has a barred colour pattern hence the name Koekoek. The purpose of the breed was for the hens to lay brown shelled egg with deep yellow skin colour carcass. Nowadays the meat of this breed is preferred over that of the commercial broiler hybrids and the meat of this breed is also popular among local communities (Grobbelaar, 2008). Their colour pattern is carried on a sex-linked gene that is useful for identification and separation (Fourie & Grobbelaar, 2003). The strain is well known among rural farmers in South Africa and neighbouring countries for egg and meat production as well as their ability to hatch their own offspring (Grobbelaar, 2008).

2.4 Botanical background of *Moringa oleifera*.

Moringa is a tree with an open umbrella-shaped crown, ranging in height from 5–12 m, with a straight trunk (10–30 cm thick) and a corky, whitish bark (Schwarz. 2001). The plant has leaflets 1–2 cm in diameter and 1.5–2.5 cm in length depending on climate. The tree produces a tuberous tap root which explains its tolerance to drought conditions (F/FRED, 1992). Moringa is adaptable to a broad range of environmental conditions from hot, dry, humid and wet conditions. It is originally considered a tree of hot semi-arid regions (annual rainfall 250–1500 mm). Moringa, when found in well drained soils with plenty water, grows rapidly, reaching higher heights, but has poor tolerance to both sandy soils, heavier clay soils and water limited conditions. The tree can be established in slightly alkaline soils up to pH 9 as well as acidic soils as low as pH 4.5 (Shindano & Chitundu, 2008) and is well suited for a wide range of adverse environments that would not be suitable for other fruit, nut and tree crops. Moringa can be found in the wild or cultivated and sold as a supplement on the health market or added to drinks such as Moringa Zinga. In India and several parts of Africa, it is cultivated on a large range in nurseries or orchards. The leaves, seeds, flowers, pods (fruit), bark and roots are all seen as a vegetable and each part is uniquely harvested and utilized. For example, fresh leaves are picked, shade dried, ground to a powder, and then stored for later use as a food flavouring or additive (Shindano & Chitundu, 2008). Dried or fresh leaves are also used in foods such as soups and porridges (Lockett *et al.*, 2000), curry, gravy, and in noodles, rice or wheat. For maintaining a healthy livestock farmers have added the leaves to animal feed (Sarwatt *et al.*, 2002; Fahey, 2005; Sánchez *et al.*, 2006) while utilizing the manure and vegetable compost for crop growth (Fahey, 2005; Save Gaia International Foundation, 2005). The nutritive value of Moringa has been reported to be similar to that of soybeans and rapeseed meal (Soliva *et al.*, 2005). Under developed countries suffering from malnutrition, use powdered leaves to enhance the nourishment of children (WHO Readers Forum, 1999; Lockett *et al.*, 2000; McBurney *et al.*, 2004).

2.5 Effect of *moringa oleifera* on poultry.

Due to the rising interest in *Moringa oleifera* and fact that the leaves have a high protein content, there has been a lot of study on the potential of *Moringa oleifera* leaf meal as an alternative feed ingredient for poultry. Though, Abou-Elezz *et al* (2012) found out that the digestibility of diets containing *Moringa oleifera* leaf meal were lower than control diets, especially for crude protein, which was partly due to the fibre content, which also limits its energy value in poultry (Abou-Elezz *et al.*, 2012). Studies by Moreki & Gabanakgosi, (2014); Gadzirayi & Mupangwa, (2014) showed that the use of *Moringa oleifera* leaf meal in broilers results in decreased performance. Tesfaye *et al.*, (2013); Jiya *et al.*, (2014); Gakuya *et al.*, (2014) state that broiler performance always declines when MOLM is included at 20% or more in the diet. In layers, fresh *Moringa oleifera* leaves added *ad libitum* to commercial feed improved laying performance but results were negative when the quantity of commercial feed was reduced (Abou-Elezz *et al.*, 2012). Also Sebola *et al* (2015) reported that inclusion of MOLM improved the growth performance and carcass characteristics of three chicken strains with intake between 50 and 70 g/kg.

2.6 Factors affecting laying performance of indigenous chicken

2.6.1 Hatchability determining factors

Hatchability refers to the percentage of eggs hatched, and is affected by numerous factors such as fertility, egg quality, egg size (Neshiem & Card, 1972; Williamson & Payne, 1978; Mandlekar, 1981) handling of eggs and management conditions during incubation and hatching. Farooq *et al.* (2001) considered egg and shell weight as the two most important factors affecting hatchability, provided that management is not a limiting factor.

2.6.2 Storage effect on hatchability

Tiwari & Maeda (2005) reported that eggs stored with the small end up had higher hatchability as compared to the large end up. They attributed this to little water loss that could indirectly influence hatchability. Bauer *et al.* (1990); Wineland (2007) suggested that the egg cannot provide a good environment for the embryo to hatch if it is set with the small end up. During long storage of eggs the pH of the albumen is affected because of the loss of carbon dioxide (Dawes, 1975), carbon dioxide maintain embryonic viability and result in a decline in hatchability (Kirk *et al.*, 1980; Deeming, 2000; Heier & Jarp 2001). Reis *et al.* (1997) observed that eggs which are incubated on the day of lay they hatch heavier chicks than those stored for a long time. Small eggs tend to produce smaller chickens with a lower performance than chickens hatched from larger eggs (Among *et al.*, 1984; Farooq *et al.*, 2001). Although many studies have shown that the correlation between length of storage periods, pre-incubation egg weight, hatchlings weight and growth performance of different species of poultry are positively strong (Ayorinde *et al.*, 1994; Danczak & Majewska, 1999; McLoughlin & Gous, 1999; Farooq *et al.*, 2001; Heier & Jarp, 2001; Nahm, 2001).

2.6.3 Effect of egg size on hatchability

At the beginning the hen will start to lay small eggs and in few weeks will go to medium size and then to the large size egg. Even if egg size can be manipulated by using fat levels, protein and enzymes, some other factors such as age and body weight of the hen, yolk weight and nutrient intake can influence egg size. Asuquo & Okon (1993) studied the effects of age in egg size on fertility and hatchability of eggs. Asuquo & okon (1993); Kalita (1994); Wilson (1991), reported a higher hatchability for intermediate sized eggs compared to too small or too large eggs. Egg size has an effect on hatchability (Neshiem & Card, 1972; Williamson & Payne, 1978; Mandlekar, 1981). Mandlekar (1981) reported hatchability of large (51-56 g) and medium eggs (45-50 g) to be 88.2% and 84.8% respectively. Hatchability of 97% was reported for medium sized eggs (50g) which was the best hatchability (Abiola *et al.*, 2008). Yolk size increases with an increase of egg size (North & Bell, 1990). Good hatchability is achieved when egg weight ranges from 55-56 g (North & Bell, 1990). Senapati *et al.* (1996) reported positive correlation between egg weight and hatchability. Abiola, (1999) mentioned that a close correlation between egg weight and hatching weight in domestic chickens has also been documented. According to Du Plessis & Erasmus (1972), Ricklefs (1983), Tufft and Jensen (1991) the effect of the egg weight on body weight at market age has been found to be independent of the age of the breeders from which the eggs originated. Given the high correlation between egg weight and final body weight, the economic importance of egg weight is apparent (Wilson, 1991). Egg size has been widely studied in the context of life-history theory because it can be highly variable. Some studies have shown that egg size can affect both parental and offspring fitness. Williams (1994) studied the relationship between egg size and offspring quality in birds and came up with equivocal results. The author reported that egg size typically affects hatching mass more strongly than it affects hatching size in birds because the main effect of egg size lies in the mass of the residual yolk sac that the chick retains at hatching. Fertility and egg quality were found to influence hatchability and weight of the day-old chick (Farooq *et al.*, 2000).

2.6.4 Egg factors affecting Hatching performance

Normally fertile eggs have all the important nutrients for embryo development of the chick to hatch. Furthermore there are some chemical and physical conditions of the egg that may reduce or cause no hatchability. The contribution may be due to environmental or hen factors. Eggs become fertile around day four after the introduction of the cock. In the processes of embryo development and successful hatching physical characteristic of an egg play a mature role (Narushin & Romanov, 2002). The influential egg variables are: weight, porosity and shell thickness, shape index and the consistency of the contents. Incubation is more successful when eggs are pointed compared to the round ones, if the shell thickness is greater than average and the contents are firm. An increase in egg weight is caused by the shell thickness and firm interiors which are higher than average and increased egg weight leads to an increased hatchability performance. Hatchability and chick hatch-weight may be closely related to the weight of the eggs, since the principal impact of egg size lies in the mass of the lingering yolk sac that the chick retains at hatching (Abiola, 1999; Donald *et al.*, 2002; Rashid *et al.*, 2005; King'ori *et al.*, 2007).

2.6.5 Artificial incubation

Incubation is the act of bringing an egg to hatching. The modern incubator is a simulated artificial design that mimics the mother-hen's role of providing fertile eggs with optimum environmental conditions (temperature, egg turning and humidity) to stimulate embryonic development until hatching (French,1997). Hill (2001); Lourens *et al.* (2005) showed that environmental temperature is the most important factor in incubation efficiency. A constant incubation temperature of 37.8°C is the thermal homeostasis in the chick embryo and gives the best embryo development and hatchability (Wilson, 1991; Lourens *et al.*, 2007). A high constant temperature during incubation initially accelerates embryonic growth, utilization of nutrients and energy from the yolk and albumen reserves, but later decreases embryonic development as a result of limited metabolic processes by insufficient exchange of oxygen (Rahn *et al.*, 1974; Lourens *et al.*, 2005). Lourens *et al.* (2005) reported significant embryo mortality and lower hatchability in chicken eggs when they were subjected to an incubation temperature of 38.9°C. An increase in environmental temperature may cause metabolizable energy to be diverted from growth and development to functions involved in homoeothermy (Meijerhof & Albers, 1998). For successful hatching, incubation procedures are important. Egg turning during incubation is important for successful hatching and influences hatchability. Van schalkwyk *et al.* (2000), Yoshizaki & Saito (2003) reported that no turning of eggs during incubation results in low hatchability and delays hatch by a few days. The optimum turning frequency is 96 times per day (Wilson, 1991; Elibol & Brake, 2003). There is increased fertile hatchability with increasing turning angle from 20-45° from the vertical (Funk & Forward, 1953). Byerly & olsen (1936) reported that egg turning in the third week has little effect on hatchability and Card (1926) observed that eggs turned during the first 6 days hatched nearly as well as those turned throughout incubation. Some causes and problems associated with poor hatchability are early embryonic death, egg rots, broken yolk, dead-in-shell checks, prolonged pre-incubation storage, poor breeder nutrition, breeder age, contamination, incubator and the hatcher malfunctions (Deeming, 1995; van Schalkwyk *et al.*, 2000; Chabassi *et al.*, 2004, Hassan *et al.*, 2004; Ipek & Hassan, 2004; Malecki *et al.*, 2005).

2.6.6 Nutrient availability at hatching

After the chick emerges out of the egg, several changes must take place in order for it to effectively adjust to its new surroundings. One of the essential changes that must happen will be the shift away from the chick's sole reliance on the lipid rich yolk sac for supplements to carbohydrate rich exogenous food sources. According to Romanoff (1960) the newly hatched chick does retain some residual yolk after hatching, it was not sufficient to support the chick during an extended period of starvation post-hatching (Bigot *et al.*, 2003). The yolk provides protein and energy immediately after hatching, and acts as a supplement to exogenous feed (Sklan *et al.*, 2000). Lambson (1970) stated that in the recently hatched chick, the yolk sac was exhibited to be absorbed specifically into the circulation and transported via the yolk stalk into the small intestines. Yolk absorption will increase when chicks get access to feed; absorption takes place via the yolk stalk into the intestines. Geyram *et al.* (2001); Sklan (2001) reported In order to accommodate the chick's change in nutrient intake, rapid intestinal development also occurred during the immediate post-hatching period with dramatic increases in villi size and volume appearing in the small intestines during the first day after hatching. Bigot *et al.* (2003) observed growth of the intestine can occur even if the chicks were not fed, but it is not the same as growth that occurred in chicks with access to feed and the difference remained even after the delayed chicks were given feed. Intestinal absorption of exogenous carbohydrates and proteins has formerly been considerable to be low in newly hatched chicks and it increases with age (Sulistiyanto *et al.*, 1999). Noy & Sklan (1999) outlined that the beginning reduction in hydrophilic compounds is due to the presence of hydrophobic lipid compound from the yolk being present in the intestinal tract.

2.7 Embryonic development and hatchability.

Chick embryo tissues contain a high proportion of polyunsaturated fatty acids in the lipid fraction (Speake *et al.*, 1998) and therefore need antioxidant defence (Surai, 1999). Tissues of newly hatched chicks express a range of antioxidant defences including natural antioxidant (vitamin E, carotenoids, glutathione, ascorbic acid) and antioxidant enzymes (superoxide dismutase, glutathione peroxidise and catalase) as well as antioxidant enzyme cofactors (Se, Zn, Mn and Fe) (Surai *et al.*, 1999). Of these, vitamin E, carotenoids and metals, are delivered from the maternal diet via the egg and the others are synthesised in the tissues. It is well accepted that maternal diet composition is a major determinant of antioxidant system development during embryogenesis and in early postnatal development (Surai, 1999). Vitamin E (Surai & Speake, 1998) and carotenoids (Surai *et al.*, 2001) are transferred from feed into egg yolk and further to embryonic tissues. The antioxidant system of the newly hatched chick includes the fat-soluble antioxidants vitamins E and carotenoids (Surai *et al.*, 1996), water soluble antioxidants ascorbic acids (Surai *et al.*, 1996) and glutathione as well as antioxidant enzyme (Surai, 1999). Among them vitamin E is considered to play a central role in antioxidant protection during embryogenesis (Surai, 1999). Therefore, during egg incubation fat-soluble antioxidants are transferred to the developing embryonic tissues mainly during the last week of incubation (Surai *et al.*, 1996; Surai 1999).

2.8 Sperm quality

Nutrition has been shown to influence the quality of sperm in chickens (Hocking & Bernard, 1997). Chicken sperm are unique in their structure and chemical composition. Spermatozoa of chicken contain an extremely high proportion of long chain polyunsaturated fatty acids (PUFAs) that are needed to maintain integrity, flexibility and fluidity. However, PUFAs are susceptible to lipid per-oxidation by free radicals that can occur during stress, and the high concentration of PUFAs in chicken sperm must be protected in order to preserve sperms' integrity. Surai, (2002) stated that in recent years it has been shown that vitamin E is located in sperm and provides antioxidant protection, especially in stress conditions. An antioxidant such as vitamin E added at levels of 100 mg/kg to the diet can help to preserve PUFA concentrations in sperm and maintain fertility. Other antioxidants such as carotenes, water soluble antioxidants like ascorbic acids and certain minerals can also have similar beneficial effects. Antioxidants should not be overlooked in the diet as they play an important role in protecting and promoting sperm production and quality.

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CHAPTER 3

EFFECT OF *MORINGA OLEIFERA* LEAF MEAL ON GROWTH AND LAYING PERFORMANCE OF POTCHEFSTROOM KOEKOEK HENS

Abstract

A feeding trial was conducted to determine the growth and laying performance of Potchefstroom koekoek (PK) hens fed dietary *Moringa oleifera* leaf meal (MOLM) for a period of 9 weeks. A total of 48 laying hens at the age of 22 weeks were bought from one of the local farmers. Hens were randomly allocated to two dietary treatments: MOLM0 (control) = diet with no Moringa inclusion; MOLM70 = diet with 70g/kg of Moringa inclusion. Each treatment was replicated four times with each pen holding six hens as the experimental unit. There were no significant ($P>0.05$) effects on feed conversion ratio and average weight gain for all treatments. However, diet significantly ($p<0.05$) affected feed intake. Furthermore, there was no significant ($P>0.05$) difference on egg-laying performance. The findings indicate that MOLM can be added up to 7% of the ration without affecting growth performance and egg production.

3.1 Introductions

The cost of feeding chickens for ideal growth performance has become high. Therefore, developing countries like South Africa have seen a drop in the contribution of indigenous poultry to food security (Bhatti *et al.*, 1990). This is caused by their poor productive performance (Bhatti *et al.*, 1990) and an increase in commercially produced exotic poultry breeds for meat and eggs (Gueye, 2000). Indigenous chickens remain predominant in the rural areas regardless the introduction of exotic birds. Indigenous chicken breeds can be produced at a low cost and they are recognized to be products of their own environment. They withstand harsh environmental conditions and diseases more than the exotic birds. Formulating feed using cheaper local resources is important for sustainable production of indigenous chickens, because of high costs of poultry feed. Aganga *et al* (2003) reported that indigenous chickens have low output in terms of slow growth rates, low egg production, small egg size, and as well as poor hatchability. Inclusion of MOLM in the diet of indigenous chicken can improve egg laying rate and egg mass production (Kakengi *et al.*, 2007; Abou-Elezz *et al.*, 2011). Most studies that have investigated the use of MOLM in poultry diets have been carried out with broilers. Growth and laying performance of indigenous strains needs to be investigated extensively. Therefore, the purpose of this study was to investigate the effect of MOLM on growth and laying performance of Potchefstroom koekoek hens.

3.2 Material and methods

3.2.1 Study area

This study was conducted at the North-West University Experimental Farm (Molelwane), in the North West Province (25.8° S and 25.5° E). Temperatures range from 22 to 35 °C in summer (August–March), and long-term average annual rainfall ranges from 200–450 mm per annum. The average minimum and maximum temperatures during the winter months (May–July) are 2 and 20 °C, respectively.

3.2.2 Laying hens, diets and experimental design.

A total of 48 laying hens at the age of 22 weeks of age, were randomly assigned to two treatments with four replicates and six laying hens per pen in a completely randomised design and distributed in pens with dimensions of 60 x 50 x 75 cm. Dietary treatments included basal diet without MOLM (control) and a basal diet with 70gMOLM/kg (Table 3.1). Adaptation period was considered for one week and then feeding with experimental diets for nine (9) weeks. Feed and water were provided *ad libitum* during the experimental period under continuous lighting throughout the trial. The basal diet was formulated to be isonitrogenous and isoenergetic.

Table 3.1 Gross composition of *Moringa oleifera* leaf meal (MOLM)-based experimental diets (g/kg)

	Diet[1]	
Ingredients	MOLM0	MOLM70
MOLM	0	70.0
Yellow maize	670.6	647.1
Prime gluten 60	50.0	50.0
Full fat soya meal	70.0	70.0
Soya bean meal	85.3	58.2
Sunflower oilcake	80.0	80.0
Limestone powder	12.3	7.1
Potassium carbonate	1.2	0.9
Mono calcium phosphate	9.8	10.0
Salt	3.2	3.2
Soya oil	7.8	13.5
Premix	6.8	6.8
Lysine	2.7	2.7
Methionine	0.3	0.7
Total	1000	1000
Chemical analysis of diets on an 'as fed basis (MOLM) MOL (g/kg)		
Dry matter	896.0	851.0
Crude protein	189.0	189.0
Ether Extract	52.0	61.0
Ash	49.0	45.0
Acid detergent fibre	36.0	47.0
Neutral detergent fibre	96.0	106.0
Crude Fibre	36.0	34.0
Metabolisable energy (KCal/kg)	3157.6	3157.2
Lysine	9.7	9.7
Methionine	4.0	4.3

[1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM70 = broiler finisher diluted with 70g MOLM/kg.

3.2.3 Measured Parameters

3.2.3.1 Growth performance

Feed offered to the hens and refusals were weighed daily using an Adam 6010 model electronic balance (Adam, Gauteng Province, South Africa). Average weekly feed intake (AWFI) was calculated by subtracting refusals from feed offered to the hens. The hens were weighed at the beginning of the experiment and subsequently on a weekly basis. Average weekly body weight gain (AWG) was determined as interim weight (difference between the weight at end of previous week and average live weight at end of current week) divided by the feeding period in days as follows:

$$AWG = \frac{Average weight\ at\ end\ of\ the\ previous week\ -\ at\ the\ end\ of\ the\ current\ week}{Number of\ days\ between the\ two\ weight\ weight}$$

Weekly feed conversion ratio (FCR) was calculated by dividing feed consumption with body weight gain (AWG) per week as follows: $FCR = \frac{Feed\ consumed}{weight\ gained}$

3.2.2.1 Laying Performance

Eggs were collected two times a day from each pen at 08:00 and 17:00 hours. The sum of the two collections along with the number of birds alive on each day was recorded and summarized at the end of the period.

3.3 Statistically analysis

$Y_{ijk} = \mu + T_i + W_j + (T*W)_{ij} + \varepsilon_{ijk}$

Y_{ijk} = Observation (egg laying performance and correlation), μ = population mean, T_i = effect of diet, W_j = effect of weeks, $(T*W)_{ij}$ = interaction between diet and weeks and ε_{ijk}= random error term.

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

Y_i = observation (growth performance), μ = population mean, T_i = effect of diet and ε_{ij}= random error term.

Data on growth performance and laying performance were analysed using GLM procedure of SAS (2010) as a completely randomized design. Differences among treatment means were determined using the PDIFF option in the LSMEANS statement of GLM procedure of SAS (2010). Level of significance was set at P<0.05.

3.4 Results

Table 3.2 Average feed intake, growth rate and feed conversion ratio of Potchefstroom Koekoek females chicken fed *Moringa oleifera* leaf meal.

	Diet(g/kg)		
Parameters	MOLM0	MOLM70	S.E
Av feed intake (g)	95.30^{a}	81.09^{b}	1.64
Feed conversion ratio	1.97	2.87	2.03
Growth rate (g/d)	37.82	26.63	7.53

a,bMeans on the same row with different superscripts are significantly different (P < 0.05). 1Diet: MOLM0 = basal diet without MOLM inclusion; MOLM70 = basal diet diluted with 70g MOLM/kg. SE=Standard error

Average feed intake, growth rate and feed conversion ratio of Potchefstroom Koekoek hens fed MOLM are shown in Table 3.2. For growth rate and feed conversion ratio, there was no significant effect between the two diets. However, hens that were feeding on control diet were consuming more (P<0.05) feed than hens that were feeding on MOLM70.

The relationship between weeks and response in egg laying performance is presented in Figure 3.1. There were no significant dietary effects on egg laying performance of Potchefstroom Koekoek hens. In week 4 and 5, there was rapid increase in egg laying performance across both dietary treatments. Although, not significant, egg laying performance was lower in cockerels fed diet MOLM70 at weeks 1,2,3,4 and 13 compared to diet MOLM0.

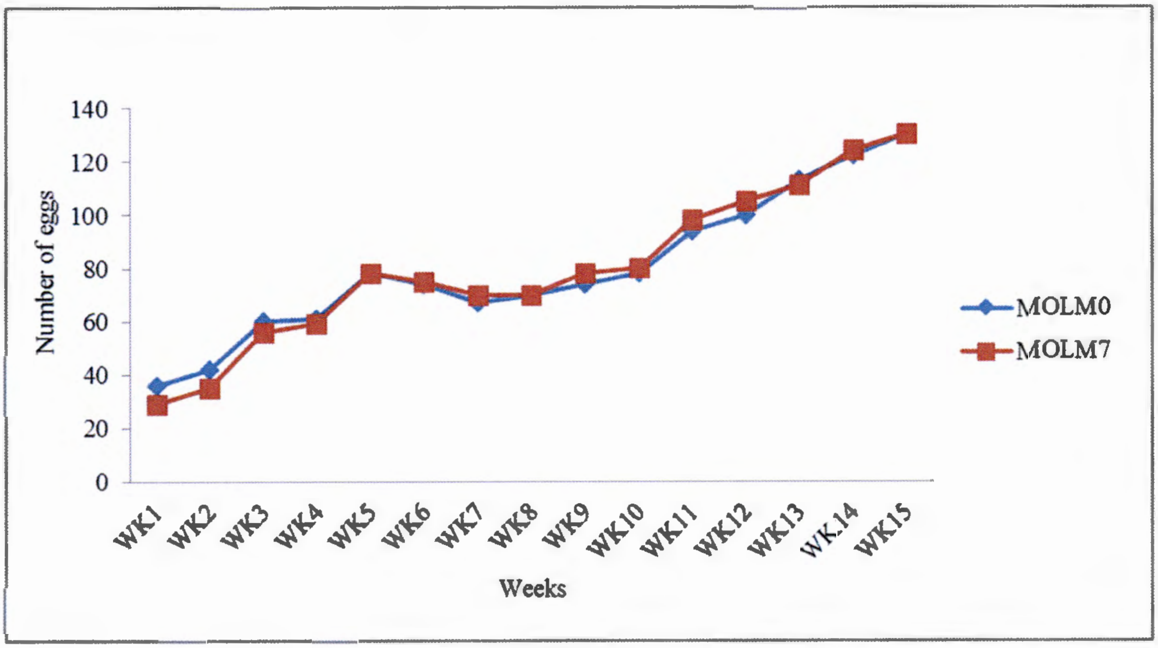

Figure 3.1 Effect of *Moringa oleifera* leaf meal (MOLM) on weekly egg production rate of Potchefstroom koekoek hens

Table 3.3 Correlation coefficient and probability of difference among weight gain, growth rate and egg laying Performance in weeks for Potcherfstroom koekoek hens.

		Weight gain	Growth rate	Egg laying rate	
	WG		0.34 (0.408)	0.25 (0.556)	
Week22	GR	0.72 (0.045)		0.79 (0.020)	Week30
	EL	0.65 (0.08)	0.08 (0.855)		
	WG		0.26 (0.532)	-0.08 (0.855)	
Week23	GR	0.99 (0.01)		-0.35 (0.398)	Week31
	EL	0.53 (0.177)	0.43 (0.282)		
	WG		-0.17 (0.686)	-0.21 (0.617)	
Week24	GR	0.54 (0.167)		-0.49 (0.213)	Week32
	EL	0.84 (0.001)	0.43 (0.294)		
	WG		-0.06 (0.893)	-0.23 (0.590)	
Week25	GR	-0.33 (0.418)		0.06 (0.893)	Week33
	EL	0.84 (0.009)	-0.46 (0.254)		
	WG		0.04 (0.928)	-0.18 (0.663)	
Week26	GR	-0.13 (0.758)		-0.55 (0.159)	Week34
	EL	0.69 (0.059)	-0.51 (0.196)		
	WG		0.44 (0.279)	-0.06 (0.889)	
Week27	GR	-0.003 (0.994)		-0.08 (0.845)	Week35
	EL	0.64 (0.085)	-0.41 (0.312)		
	WG		0.40 (0.330)	-0.22 (0.604)	
Week28	GR	-0.24 (0.559)		-0.56 (0.149)	Week36
	EL	0.24 (0.563)	0.04 (0.920)		
	WG		-0.11 (0.22)	0.55 (0.01)	
Week29	GR	-0.41 (0.313)		-0.33 (0.01)	Overall
	EL	0.06 (0.890)	-0.49 (0.215)		

Week 22 to 29 below the diagonal (within each parameters). Week 30 to overall above the diagonal (within each parameters).

The correlation between body weight gain and egg laying performance is shown in table 3.3. On overall performance, the correlation between body weight gained, growth rate and egg laying performance were negative. However the correlation between body weight gain and egg laying performance were moderately positive (r=0.55, prob=0.001). The overall correlation between weight gain and egg laying performance were positive (r = 0.55, prob= 0.001). The overall correlation between growth rate and egg laying performance were negative (r=-0.33, prob=0.001).

3.5 Discussions

3.5.1 Growth performance

The observation that the inclusion of MOLM in Potchefstroom koekoek hen diets had some effect on feed intake compares well with the published reports (Olugbemi *et al.*, 2010a; Gadzirayi *et al.*, 2012; Alebachew *et al.*, 2016). These workers found statistically significant differences in feed intake of laying hens when MOLM was included in the diet. However, the present study did not agree with the findings of Etalem *et al.* (2014) who noted that addition of MOLM to Dominant CZ layers at 10% had no effect on average feed intake. The difference in the present study's findings on feed intake to these reports could be due to strain and age differences in the hens used. The reduction of feed intake in the present study may be due to reduced palatability of the diet (Kakengi *et al.*, 2003) and one of the reasons can be increased bulkiness of diet. In addition, the hen's digestive system is simple and has a limited capacity to digest high fibrous ingredients efficiently. Also chickens lack the enzymes necessary for utilizing high fibrous ingredients (Son *et al.*, 2002; Esonu *et al.*, 2006; Ige *et al.*, 2006).

Similar to the findings of the current study, Olugbemi *et al.* (2010b); Etalem *et al.* (2014) observed that inclusion of MOLM on chicken diet did not affect FCR and growth rate. The lack of dietary treatment effect on FCR and growth rate suggest that 7% MOLM inclusion can act as a protein source in diets for indigenous chickens.

3.5.2 Laying performance

The observation that the inclusion of MOLM in PK diets had no effect on egg laying performance compares well with other researchers (Kwari *et al.*, 2011; Olabode & Okelola. 2014). These workers found no statistically significant differences in egg production when leaf meal was included in layer diets. In contrast, Kakengi *et al.* (2007), Abou-Elezz *et al.* (2011), and Lu *et al.* (2016) noted that egg laying performance decreased when MOLM was higher than 10% when included in layer diet. The authors further explained the decrease in egg production as caused by the bulkiness of the diet due to the 10% and above of MOLM. Therefore, the results in the current study suggest that MOLM can be included up to 7% in layer diets without major effects on egg laying performance of Potchefstroom koekoek hens.

3.6 Conclusions

Lack of dietary effect on body weight gained, feed conversion ratio and egg laying performance suggests that 7% inclusion of *Moringa oleifera* leaf meal was within the optimal range. The reduction in results obtained in the average feed intake of hens fed MOLM70 diet in this study could be improved by proper processing to detoxify anti-nutritional factors and reducing crude fibre before they can be integrated in diets of Potchefstroom koekoek hens.

3.7 Reference

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CHAPTER 4

EGG PARAMETERS AND HATCHABILITY PERFORMANCE OF POTCHEFSTROOM KOEKOEK CHICKEN IF FED *MORINGA OLEIFERA* LEAF MEAL

Abstract

The objective of the current study was to investigate the effect of *Moringa oleifera* leaf meal (MOLM) on parameters and hatchability performance of Potchefstroom koekoek hens. A total of 56 Potchefstroom koekoek chickens (48 hens and 8 cocks) were randomly allocated to a mating ratio of 6:1 and 2 dietary regimens: A control (commercial layer diet); and the treatment of MOLM (70gMOLM/kg inclusion). Each treatment was replicated 4 times with each pen holding 7 chickens as the experimental unit. Chickens were offered dietary treatment for 4 weeks before collecting fertile eggs. Fertile eggs were collected for 14 days. Internal and external egg quality parameters measured in the current study were affected by dietary inclusion of *Moringa oleifera* leaf meal and some of the quality parameters were improved when MOLM was included in the diet. There was no significant ($P>0.05$) difference in yolk height, albumen height, egg weight and egg content in all weeks. However, hens fed MOLM diets had higher ($P<0.05$) albumen diameter at week 1; albumen length at week 1,4 and 7; albumen weight at week 1; albumen index at week 5; yolk index at week 3; haugh unit at week 4. Generally, there was no significant difference ($P>0.05$) noticed in terms of egg weight, egg content, egg shell thickness and all internal egg parameters apart from Albumen length. However, diet significantly affected ($P<0.05$) egg length, diameter, shape index, shell weight and shell percentage. Dietary treatment with MOLM70 experienced positive effects on

hatchability of Potchefstroom koekoek. The findings from the current study can be helpful in improving the external egg quality, internal egg quality and hatchability performance of Potchefstroom koekoek hens.

4.1 Introductions

Egg quality is important for both consumers and poultry breeders. Egg quality measurement involves internal and external qualities (Song *et al.*, 2000). External egg characteristics include egg weight, shell weight, egg width, egg length and egg shape index, these traits are important for consumer acceptability (Wolanski *et al.*, 2007). Internal characteristics include albumen index, yolk index and Haugh unit, and they are significant for the egg production industry (Song *et al.*, 2000). Thick albumen is the primary interior quality trait, believed to be an important factor for egg freshness (Toussant & Latshaw, 1999). Egg quality is greatly influenced by storage condition, hen physiology, production system and environmental conditions of the rearing system (Scott & Silversides, 2000; Jin *et al.*, 2011).

Poultry diets are formulated from a mixture of ingredients, including cereal grains, cereal by-products, fats, plant derived protein sources, vitamin and mineral supplements, crystalline amino acids and feed additives. The aforementioned ingredients affect the quality of the egg laid by birds and embryo development. Embryo development is based on the chicken egg nutrients, different sizes of eggs (Tumova *et al.*, 2007, Tumova *et al.*, 2009), different mineral content in serum and egg shell (Tumova *et al.*, 2014). However, Peebles *et al* (2001) and Tona *et al* (2010) further reported that embryo development is influenced by strain and hen age. It has been well documented that the enrichment of chicken eggs with n-3 PAFA could be reached by dietary supplementation with plant sources that are rich in n-3 PAFA such as *leucaena* leaf meal, cassava leaf meal and *Moringa oleifera* leaf meal (Cherian & Sim, 1991: Jiang *et al.*, 1991: Crespo & Eseve-Garcia, 2002).

Feeding diets with *Moringa oleifera* leaf meal that contain n-3 PAFA could improve egg quality and embryo development. It can, therefore, be postulated that *Moringa oleifera* leaf meal inclusion in diets may affect the quality of egg and embryo development. Currently, there is little information on the effects of *Moringa oleifera* leaf meal inclusion in indigenous chicken diets on egg quality. The present study was, therefore, conducted to investigate the effect of *Moringa oleifera* leaf meal in Potchefstroom Koekoek diets on egg quality and hatchability.

4.2 Material and methods

4.2.1 Study site, management of chickens, diet formulation and experimental design

The study site, management of chickens, diet formulation and experimental design were as described in details under section 3.2.1 to 4.2.2 respectively.

4.2.2 Experimental procedure

Hens from experiment 1 (one) at the age of 31 weeks and eight cockerels at the age of 30 weeks were used. They were housed in eight deep litter pens, each with six hens and one cockerel. They were offered diets shown in table 4.1 for 4 weeks before collecting fertile eggs. The eggs were collected for 14 days. Candling was done on the 7th and 18th day of incubation and the eggs were transferred into a hatchery on the 18th day. Harvesting of chicks was on the 21st day. Eggs were placed in an incubator with the broad end pointing upwards. The temperature and humidity was set to 37.5 °C and 82.5%, respectively for incubation and 37.0 °C and 85% for hatching. Non-fertile eggs collected from experiment 1 were used to do the physical and internal parameters. A 0.01 g sensitivity level electronic balance (RADWAG) was used to weigh the eggs.

4.2.3 Egg collation

Hens at the age of 23 weeks were used in this study. Non fertile eggs were collected for the period of 8 weeks before mating.

4.2.4 Determination of Egg Quality Analysis

Egg weight was recorded. Then the eggs were broken out in a flat plate. The heights of the albumen and yolk were measured with a tooth pick and mm ruler. Length and diameters of albumen, and diameters of yolk were measured with a calliper. Shells of broken eggs were dried in air. The weight of dried egg shells was measured and shell thicknesses of eggs were measured with a micrometer. Calculations were performed according to the following formulae:

$$Eggshapeindex = (\frac{egg\ width}{egg\ length}) \times 100$$

$$shell\ ratio = \left(\frac{sheell\ weight}{egg\ weight}\right) \times 100$$

$$yolk\ ratio = \left(\frac{yolk\ weight}{egg\ weight}\right) \times 100$$

$$Albumen\ ratio = \left(\frac{albumen\ weight}{egg\ weight}\right) \times 100$$

$$Yolk\ index = \frac{yolk\ height}{yolk\ diameter}) \times 100$$

$$Albumen\ index = \left[\frac{albumen\ height}{(albumen\ length\ + albumen\ diameter\ \div 2)}\right] \times 100$$

$$haugh\ unit\ (HU) = 100\ \times \log(albumen\ height + 7.57 - 1.7\ \times egg\ weight\ ^{0.37})$$

4.2.5 Determination of Hatchability

Hatchability: the percentage fertility rate, hatching yield and hatchability was calculated using the following formulas given by Sahin *et al.*, (2009):

$$Fertility = \frac{Number\ of fertile\ eggs}{number\ of eggs\ set} \times 100$$

$$Hatching\ yield = \frac{Number\ of chicks\ hatched}{number\ of eggs\ set} \times 100$$

$$Hatchability\ of\ fertile\ eggs = \frac{numberof chicks\ hatched}{number\ of\ fertile\ eggs} \times 100$$

4.3 Statistically analysis

$Y_{ijk} = \mu + T_i + W_j + (T*W)_{ij} + \varepsilon_{ijk}$

Y_{ijk} = Observation (external and internal egg quality), μ = population mean, T_i = effect of diet, W_j = effect of weeks, $(T*W)_{ij}$ = interaction between diet and weeks and ε_{ijk}= random error term.

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

Y_{ij} = observation (hatchability performance), μ = population mean, T_i = effect of diet and ε_{ij}= random error term.

Data on external and internal egg quality and hatchability performance were analysed using GLM procedure of SAS (2010) as a completely randomized design. Differences among treatment means were determined using the PDIFF option in the LSMEANS statement of GLM procedure of SAS (2010).

4.4 Results

4.4.1 External Egg Parameters of Potchefstroom Koekoek Hens.

The effect of *Moringa Oleifera* leave meal on the external egg characteristics of Potchefstroom koekoek hens is shown in Table 4.1. Diet had effect (P<0.005) on external parameters of the egg. There was no interaction (P>0.05) that existed between feed type and number of weeks in egg weight and egg content. In week 4 and 6, hens fed on *Moringa Oleifera* leave meal laid eggs with higher (P<0.05) egg length value than those fed the control diet but no significant (P>0.05) was observed for the other weeks. In week 1, however, hens fed diet MOLM70 produced eggs with lower (P> 0.05) diameter compared to those fed the control diets while chicken fed diet MOLM0 laid eggs that have higher egg shape index until week 4. Higher

(P<0.05) egg shell weight was observed for hens fed dietary MOLM70 at week 1, 2, 3 5, and 7, but no significant effect was recorded in the other weeks. Chicken fed diet MOLM70 produced eggs with a thicker (P<0.05) shell at week 7 than those fed dietary MOLM0, in contrast, at week 8 chicken fed dietary MOLM0 had thicker shell compared to those fed dietary MOLM70. Shell percentage was improved in eggs of chicken fed diet MOLM0 from week 1 to 6 while no significant effect was observed in the last two weeks.

On overall performance there was no significant difference (P>0.05) noticed in terms of egg weight, egg content and egg shell thickness. However, diet significantly affected (P<0.05) egg length, egg diameter, egg shape index, egg shell weight and shell percentage. Inclusion of dietary MOLM70 improved egg length, egg shape index, egg shell weight and shell percentage. However, in terms of egg diameter, dietary MOLM0 was higher (P<0.05) when compared to hens fed MOLM70. Hens that consumed dietary MOLM70 had higher (P>0.05) egg weight than hens fed dietary MOLM0.

Table 4.1 Effect of *Moringa Oleifera* leave meal on the external egg parameters of Potchefstroom koekoek hens.

Parameters	Diet	1	2	3	4	5	6	7	8	overall
EW (g)	C	46.95±0.62	46.50±0.77	46.86±0.57	47.02±0.49	44.64±0.53	44.91±0.55	44.44±0.63	46.86±0.85	45.65±0.22
	M	47.30±0.72	46.22±0.91	45.59±0.87	45.93±0.80	44.71±0.97	45.49±0.98	46.60±2.41	46.44±1.60	45.76±0.20
EL (mm)	C	51.76±0.38	51.63±0.50	52.25±0.22	52.36±0.25^b	51.45±0.28	51.54±0.32^b	51.29±0.35	52.97±0.43	51.81±0.12^b
	M	51.93±0.44	53.02±0.59	52.71±0.34	53.37±0.42^a	52.35±0.52	52.90±0.57^a	53.03±1.36	53.70±0.82	52.76±0.20^a
ED (mm)	C	40.61±0.50^a	40.26±0.27	40.15±0.21	40.47±0.18	39.83±0.22	39.78±0.17	39.69±0.27	40.22±0.51	40.06± 0.09^a
	M	38.42±0.58^b	39.45±0.32	39.69±0.32	39.82±0.30	39.62±0.40	39.87±0.30	39.88±1.04	39.59±0.97	39.48±0.15^b
ESI (%)	C	78.53±0.97^a	78.55±1.10^a	76.85±0.41^a	77.42±0.54^a	77.53±0.47	77.37±0.49	77.56±0.66	76.05±1.06	77.31±0.28^a
	M	74.06±1.13^b	74.42±1.30^b	75.28±0.62^b	74.64±0.89^b	75.78±0.87	75.44±0.88	75.20±2.55	73.72±2.00	74.87±0.45^b
ESW (g)	C	4.45±0.06^b	4.35±0.08^b	4.21±0.07^b	4.44±0.06	4.20±0.06^b	4.29±0.07	4.31±0.07^b	4.60±0.12	4.32±0.03^b
	M	4.96±0.07^a	4.77±0.09^a	4.56±0.10^a	4.57±0.11	4.52±0.12^a	4.50±0.12	4.93±0.28^a	4.31±0.23	4.65±0.04^a
EST (mm)	C	0.49±0.02	0.67±0.02	0.67±0.01	2.00±1.06	1.67±0.83	0.76±0.01	0.78±0.00^b	0.79±0.01^a	1.06±0.21
	M	0.47±0.02	0.66±0.03	0.68±0.02	0.75±1.75	0.76±1.53	0.77±0.01	0.83±0.02^a	0.74±0.02^b	0.68±0.34
EC (%)	C	40.64±0.62	40.22±0.70	40.21±0.53	41.28±0.44	39.14±0.47	39.53±0.49	38.95±0.56	40.92±0.75	39.95±0.20
	M	40.50±0.72	39.40±0.84	39.47±0.80	40.06±0.72	38.87±0.87	39.74±0.88	40.44±2.15	40.84±1.41	39.82±0.32
SP (%)	C	13.51±0.29^b	13.55±0.18^b	12.37±0.16^b	12.21±0.10^b	12.31±0.11^b	11.98±0.11^b	12.38±0.11	12.67±0.18	12.49±0.06^b
	M	14.43±0.34^a	14.83±0.21^a	13.46±0.25^a	12.76±0.16^a	13.08±0.20^a	12.66±0.20^a	13.23±0.44	12.07±0.34	13.53±0.10^a

^{a,b} Means (within each parameter) on the same column with different superscripts are significantly different ($P < 0.05$). MOLM0=diet containing 0% MOLM; MOLM70 = basal diet diluted with 70g MOLM/kg; SE=Standard error; EW= egg weight; EL= Egg Length; ED=Egg Diameter; ESI=Egg Shape Index; ESW=Egg Shell Weight EST=Egg Shell Thickness EC=Egg Content SP=Shell Percentage.

4.4.2 Internal egg parameters of Potchefstroom koekoek hens.

Yolk height and albumen height were not different (P>0.05) amongst diets in all weeks (Table 4.2). Yolk weight was higher (P<0.05) for hens fed dietary MOLM0 on the 2^{nd} week, but no difference on other weeks. Yolk diameter was lower (P<0.05) for hens fed dietary MOLM70 for week 2 and 5 than other weeks. Yolk index for hens fed dietary MOLM70 was greater than those from diet MOLM0 on the 3^{rd} week. Yolk ratio was not significant (P>0.05) at weeks 3 to 8. Dietary MOLM70 resulted in lower yolk ratio in week 1 and 2 as compared to eggs from control diet (MOLM0). At week 1, 4, and 7 albumen length was higher (P<0.05) for hens fed dietary MOLM70; however, there was no significant difference for other weeks. Albumen diameter was significantly influenced by MOLM70 during week 1 than eggs from diet MOLM0, except at week 8 when eggs from diet MOLM0 had significantly higher albumen diameter (AD). Hens fed dietary MOLM70 at week 1 had heavier albumen weight (AW) but there was no significant difference from week 2 to 8. There was a statistical difference between the diets at week 5 regarding albumen index. Haugh unit parameter had no significant effect at all weeks, except at week 4 where eggs from MOLM70 had the lowest value. On overall performance, dietary treatment did not affect any parameter except for albumen length that was higher for hens fed dietary MOLM70 when compared to those that received dietary MOLM0.

Table 4.2 Effect of *Moringa Oleifera* leave meal on the internal egg parameters of Potchefstroom koekoek hens.

		Weeks								
Parameters	Diet	1	2	3	4	5	6	7	8	overall
YW (g)	C	18.52±0.45	19.00±0.37^a	17.25±0.24	16.20±0.21	15.71±0.22	15.63±0.22	15.41±0.23	15.84±0.31	16.48±0.11
	M	17.41±0.52	17.52±0.44^b	16.71±0.36	15.73±0.35	14.93±0.40	15.58±0.39	14.36±0.88	15.90±0.59	16.32±0.18
YH (mm)	C	16.85±0.29	16.95±0.19	17.38±0.14	17.70±0.13	17.49±0.14	16.53±0.22	17.37±0.15	17.92±0.24	17.24±0.07
	M	16.68±0.33	16.90±0.23	17.85±0.21	17.55±0.21	17.61±0.26	16.61±0.39	16.25±0.58	18.00±0.45	17.21±0.11
YD (mm)	C	39.36±0.52	39.09±0.31^a	38.58±0.30	38.98±0.26	37.40±0.37^a	37.65±0.24	37.28±0.25	36.71±0.38	38.08±0.12
	M	38.76±0.61	38.06±0.36^b	37.83±0.46	38.02±0.42	35.85±0.68^b	37.97±0.43	36.90±0.98	36.21±0.72	37.69±0.20
YI	C	0.43±0.01	0.44±0.01	0.45±0.01^b	0.45±0.00	0.47±0.01	0.44±0.01	0.47±0.01	0.49±0.01	0.456±0.01
	M	0.43±0.01	0.44±0.01	0.47±0.01^a	0.46±0.01	0.490.01	0.44±0.01	0.44±0.02	0.50±0.02	0.46±0.01
YR (%)	C	0.39±0.01^a	0.41±0.01^a	0.38±0.01	0.35±0.00	0.35±0.00	0.35±0.00	0.35±0.01	0.34±0.01	0.36±0.01
	M	0.37±0.01^b	0.38±0.01^b	0.38±0.01	0.34±0.01	0.33±0.01	0.35±0.01	0.31±0.02	0.35±0.01	0.36±0.01
AL (mm)	C	72.11±1.46^b	72.61±1.94	74.27±1.29	75.58±1.14^b	73.82±1.14	78.92±1.36	74.13±1.17^b	74.01±2.43	74.80±0.51^b
	M	78.32±1.70^a	74.94±2.31	74.99±1.96	80.14±1.88^a	74.13±2.10	81.59±2.45	83.50±4.50^a	75.75±4.59	77.28±0.83^a
AD (mm)	C	56.43±1.15^b	57.62±1.20	57.56±0.92	60.74±0.87	58.94±0.92	63.78±1.08	59.66±0.99	61.28±1.42^a	59.75±0.64
	M	60.39±1.34^a	58.19±1.42	57.82±1.39	61.10±1.43	58.69±1.70	66.97±1.94	63.72±3.79	54.61±2.69^b	60.19±0.64
AH (mm)	C	5.03±0.17	4.44±0.14	4.63±0.10	4.82±0.10	4.72±0.11	4.41±0.11	5.05±0.16	5.36±0.22	4.75±0.05
	M	4.84±0.20	4.38±0.16	4.85±0.15	4.45±0.17	4.96±0.21	4.39±0.20	4.75±0.60	5.57±0.41	4.69±0.78
AW (g)	C	21.30±0.41^b	21.65±0.49	22.35±0.47	23.91±0.39	22.33±0.39	22.82±0.43	22.48±0.50	24.20±0.68	22.61±0.17
	M	22.59±0.48^a	21.66±0.59	23.00±0.71	23.50±0.64	22.68±0.73	23.55±0.77	24.67±1.92	24.04±1.28	22.91±0.27
AI	C	7.89±0.31	7.11±0.32	7.13±0.22	7.22±0.23	7.32±0.25^b	6.42±0.22	7.72±0.30	8.20±0.49	7.25±0.10
	M	7.11±0.36	6.70±0.38	7.51±0.33	6.45±0.38	7.72±0.46^a	6.00±0.39	6.86±1.14	8.81±0.93	7.01±0.16
HU	C	73.83±1.15	69.00±1.29	71.23±0.85	72.08±1.29^a	72.16±0.94	69.64±1.00	74.58±1.09	76.19±1.69	71.97±0.41
	M	72.18±1.34	68.56±1.53	72.99±1.29	66.92±2.13^b	74.16±1.74	69.28±1.74	70.53±4.18	77.92±3.20	71.10±0.67

^{a,b}Means (within each parameter) on the same column with different superscripts are significantly different ($P < 0.05$). MOLO0=diet containing 0% MOLM: MOLM70 = basal diet diluted with 70g MOLM/kg.. SE=Standard error YW= yolk weight YH=yolk height YR= yolk ratio YD= yolk diameter YI= yolk index AW= albumen weight AH=albumen height AD= albumen diameter AL= albumen length AI= albumen index HU= haugh unit

4.4.3 *External Egg Parameters for fertile eggs.*

Table 4.3 showed the effect of MOLM on fertile egg physical parameters (weight, length, width, and shape index) of Potchefstroom koekoek. Diet had significant effects on fertile egg physical parameters (weight, length, width, and shape index) of Potchefstroom koekoek. Egg length was significantly high on egg from hens fed dietary MOLM70 while shape index of the eggs from hen fed diet MOLM70 was significantly low ($P<0.05$). However, egg weight and egg diameter did not differ ($P>0.05$)

Table 4.3 Effect of *Moringa oleifera* leaf meal on fertile egg physical parameters (weight, length, width, and shape index) of Potchefstroom koekoek.

	Diet		
Parameters	MOLM0	MOLM70	SE
Egg weight (g)	50.05	50.74	0.40
Egg length (mm)	53.78^{b}	54.54^{a}	0.19
Egg diameter (mm)	41.02	41.03	0.14
Egg Shape Index (%)	76.33^{a}	75.30^{b}	0.30

^{a,b}Means on the same raw with different superscripts are significantly different ($P < 0.05$). MOLM0 =diet containing 0% MOLM: MOLM70 = basal diet diluted with 70g MOLM/kg.. SE=Standard error.

4.4.4 *Hatchability.*

Figure 4.1 shows the effect of feeding MOLM on hatchability. The results revealed that, eggs from those hens fed dietary MOLM70 experienced the highest hatchability of eggs but eggs from hens that were fed the control (MOLM0) experienced the lowest hatchability of eggs. Figure 4.2 show the Relationship between different diets and responses in fertility rate. Out of 120 eggs that were incubated for MOLM0, 91 eggs were fertile, while for MOLM70 out of 120 eggs 93 were fertile. Eggs from chickens that were fed dietary MOLM70 experienced the highest (P<0.05) hatchability of fertile eggs. However, eggs from hens that were fed MOLM0 diet had the lowest (P<0.05) hatchability of fertile eggs (Figure 4.3).

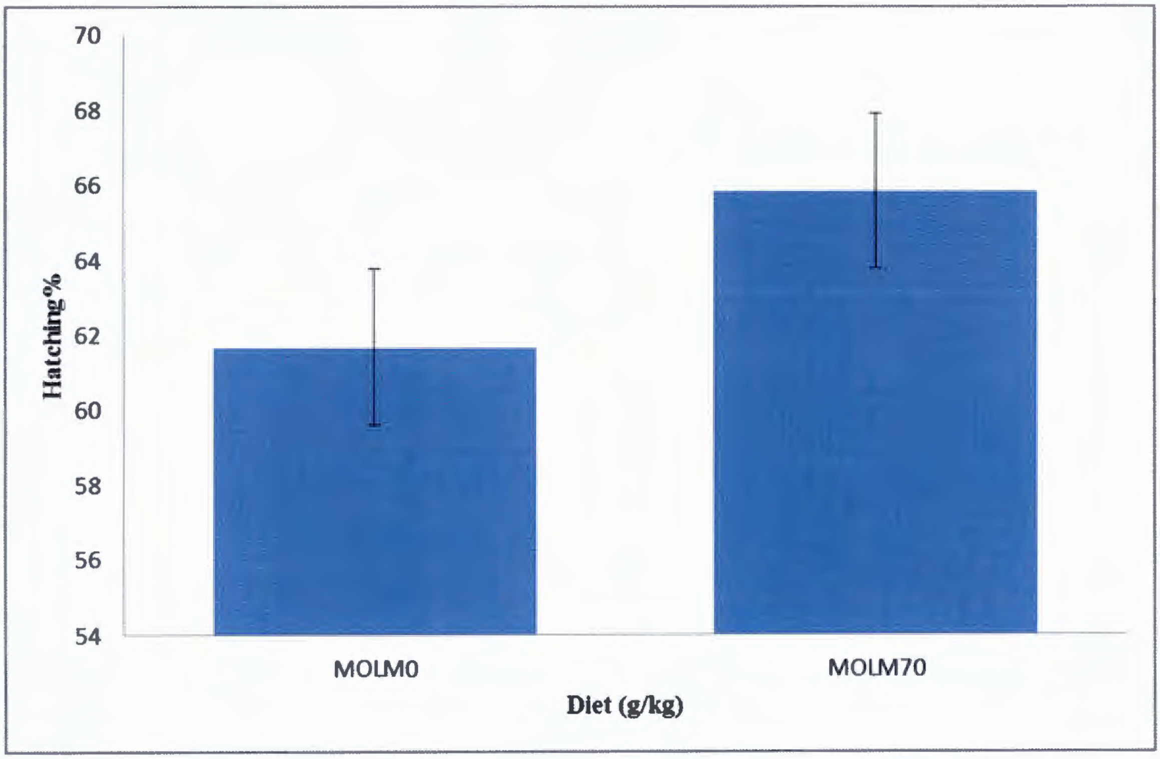

Figure 4.1 Relationship between different diets and responses in egg hatchability. MOLM0= diet containing 0% MOLM; MOLM70 = basal diet diluted with 70g MOLM/kg.

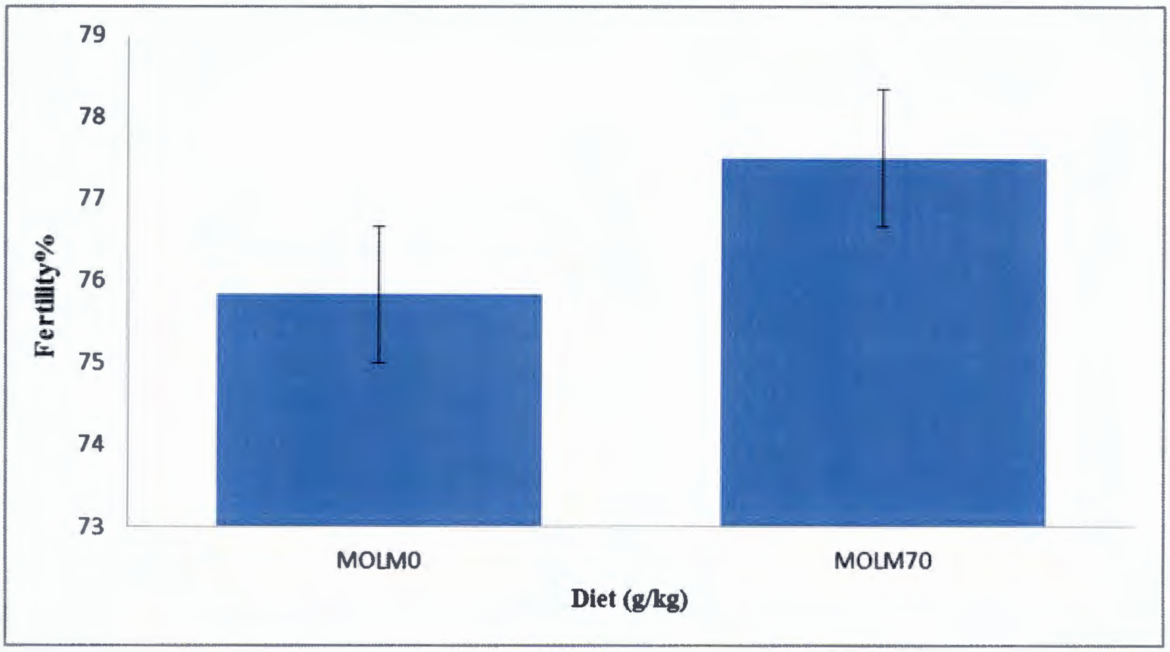

Figure 4.2: Relationship between different diets and responses in egg fertility of Potchestroom koekoek

MOLM0= diet containing 0% MOLM; MOLM70 = basal diet diluted with 70g MOLM/kg.

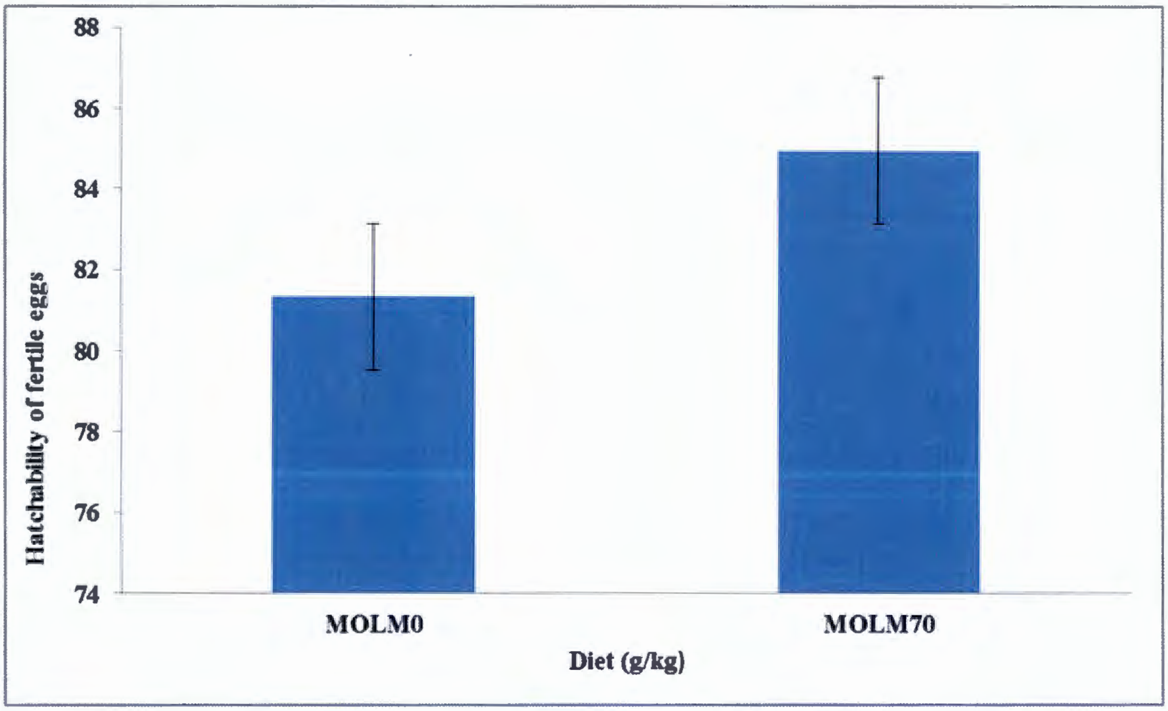

Figure 4.3: Relationship between different diets and responses in hatching of fertile eggs.

MOLM0= diet containing 0% MOLM; MOLM70 = basal diet diluted with 70g MOLM/kg.

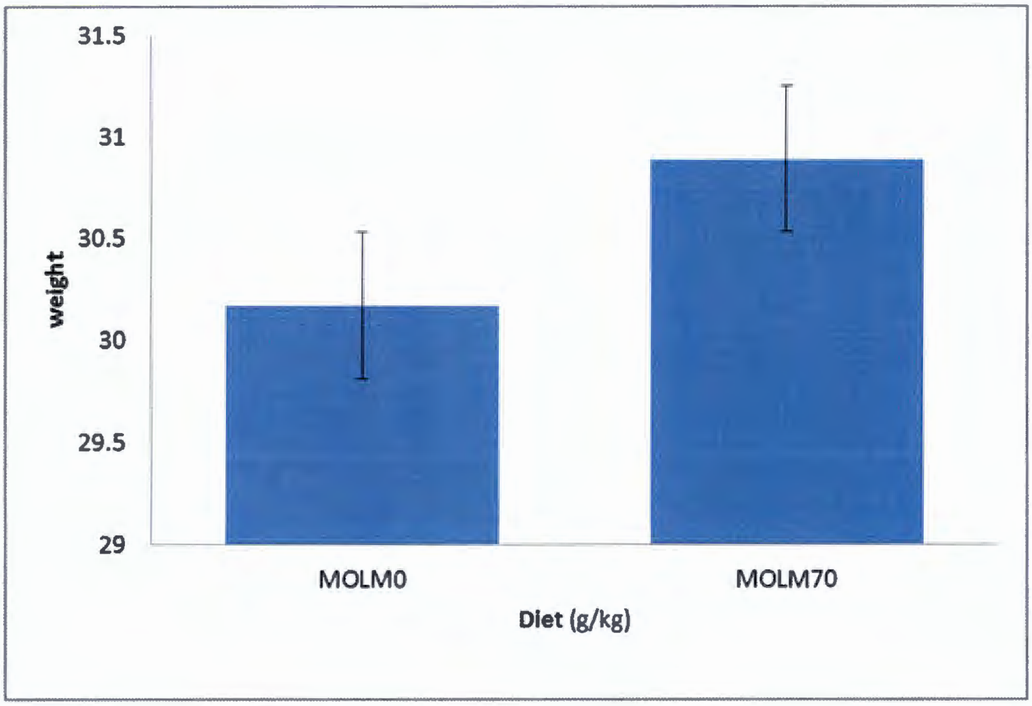

Figure 4.5: Relationship between different diets and responses in weight at day old.

MOLM0= diet containing 0% MOLM; MOLM70 = basal diet diluted with 70g MOLM/kg.

4.5 Discussion

4.5.1 External Egg Parameters of Potchefstroom Koekoek Hens.

Egg shell weight in the current study was lower than the shell weight reported by Ebenebe *at al.* (2013) for Isa brown breed of layers fed various levels of *Moringa oleifera* leaf meal. The reason may be that Isa brown breed are mainly bred for egg production. shell weight generated in this study contradict the work of Moreki et al. (2011) who reported that shell weight increased with age of the hen. The minimum shell thickness of 0.35 mm was established as an indicator for quality eggs (ISA, 2009). The minimum egg shell thickness recorded in the present study was higher than the minimum shell thickness suggested by ISA (2009). This implies that inclusion of dietary MOLM70 resulted in an improved egg quality in terms of shell thickness. Wubalem *et al.* (2016) reported that a hen cannot sustain shell quality for long without enough levels of calcium, phosphorus and vitamin D in the diet and other micronutrients function in calcium transport and bone matrix turnover. This supports the current study, due to the presence of these nutrients in *Moringa oleifera* leaf meal, the supplement contributes to the quality of egg shell (Hy-line, 2013). In addition, Lack of dietary treatment effect on egg weight and egg content showed that inclusion of MOLM70 was within the optimal range and therefore did not cause any significant change in egg weight and its contents. Similarly, Bhatnagar *et al.* (1996) reported a non-significant effect on egg weight at 0%, 5% and 10% inclusion levels of dietary *Leuceana* leaf meal.

Egg length and diameter were used to calculate egg shape index (SI) in this study. Eggs exist in different shapes and can be distinguished using egg shape index. Egg shape index less than 72 indicate sharp eggs, from 72 to 76 standard eggs and more than 76 round eggs respectively (Altuntaş and Şekeroğlu, 2008). In the present study, the shape of eggs of Potchefstroom koekoek that were fed MOLM0 diet was found to be round. However, the shape of those fed dietary treatment MOLM70 was found to be standard (normal). Standard eggs fit well to the trays and make less transportation loss. Egg shape index of hens fed dietary treatment MOLM70 diet were lower compared to the 77.7 reported by Tesfaye *et al.* (2014) for diet containing 5% of MOLM, reason could be the age of hens used in this study. The egg shape index recorded in this study was in contrast with the findings of Ebenebe *et al.* (2013) who reported a non-significant result in egg shape index. The results suggest that MOLM can improve eggs for consumer acceptability.

4.5.2 Internal egg parameters of Potchefstroom koekoek hens.

Yolk and albumen height were not affected in this study. However, Ebenebe *et al.* (2013) reported that MOLM had an effect on yolk and albumen height. Albumen weight of 27.9 g was reported by Tasfaye *et al.* (2015) for diet containing 5 % MOLM was higher than the results obtained in the present study. Increase in egg weight is related to increase in albumen weight in this study. This is in line with the findings of Alebachew *at al.* (2016).

The Haugh Unit (HU) is considered to be the best indicator of albumen quality and it is calculated from egg weight and inner thick albumen height. Generally, the higher the Haugh unit value the better the egg quality. Haugh unit greater than 72 is classified as grade AA, 60 to 72 haugh unit is classified as grade A and haugh unit less than 60 is classified as grade B. Zhao *et al.* (2010) noted that eggs are considered to be fresh when HU is 60 or above, and HU of less than 60 is considered inferior egg quality. HU of both dietary treatments were higher than the minimum value suggested by Zhao *et al.* (2010). Inclusion of MOLM to layer diet did not have any effect on HU. In addition, other researchers observed that adding *Moringa oleifera* leaf meal to layer diets resulted in better HU within the AA ranking (Alebachew *et al.*, 2016).

Isikwenu *et al.* (1999) noted the yolk index and haugh unit are best indicators of internal egg quality. A decrease in yolk index is related to losing moisture from the egg. Yolk index value reported by Garba *et al.* (2010) was similar to the values reported in the present study but lower than the values obtained by Alebachew *et al.* (2016). Oluyemi and Robert (2000) reported that egg yolk index should be between 0.30–0.50 for good quality egg yolk. The yolk index for all dietary treatment was within the recommended range. The overall non-significant results for internal egg parameters suggests that inclusion of MOLM was within the optimal range and therefore, did not cause any significant change in properties of the diet. As such, the results are in contrast with the finding of Ebenebe *et al.* (2013) who reported that inclusion of MOLM at 7.5 % resulted in poor egg quality indices and lower productivity. *Hatchability.*

Most sensitive parameters to genetic and environmental influences are hatchability and fertility (Stromberg, 1987). Stahl *et al.* (1986); Peebles and Brakes (1987) stated that factors affecting hatchability and fertility include bird strain, plane of nutrition, condition and length of storage of eggs, egg quality and mating ratio. Fertility, hatchability and hatchability of fertile eggs was actually improved for hens fed dietary MOLM70 as compared with the control (MOLM0) diet. Moyo *et al.* (2011) stated that MOLM contains higher levels of Zinc and Vitamin E, Park *et al.* (2004) and Mahmood and Al-Daraji (2011) reported that Zinc and vitamin E can play a benefitial role in hatchability of eggs. Zinc helps with the protection of genetic material structure or DNA chromatin in the sperm nucleus, an important structure for successful fertility. Durmus *et al.* (2004) noted increased hatchability with increasing Zinc in the diets of Brown parent stock layers.

Organic selenium supplementation of layers diets improved the environment of the sperm storage tubules in the hen's oviduct, increasing the interval of time the sperms can be stored, allowing the sperms to live longer, and increasing the number of sperm holes in the yolk layer (Agate *et al.*, 2000; Davtyan *et al.*, 2006; Petrosyan *et al.*, 2006; Hanafy *et al.*, 2009). Osman *et al.* (2010) reported that, supplementation of plant leaves containing selenium increased fertility and hatchability percentage. Narushin and Romanov (2002) stated that the physical characteristics of eggs such as the shape index, weight, length and width play a significant role in the embryo development and successful hatching. This might be the reasons for improved fertility and hatchability of hens fed dietary treatment with MOLM70 in the present study. Hatchability of fertile eggs noted in this study was higher than 77 % reported by Tesfaye *et al.* (2014) for dominant CZ layer hens fed diet containing 5 % MOLM but simmilar to 84 % noted by Molekwa and Umesiobi (2009) for Potchefstroom koekoek artificially inseminated with semen from high performing cocks. Fertility rate and hatchability of the present study was similar to the findings of Wubalem *et al.* (2016) who reported that inclusion of dietary *Moringa oleifera* leaf meal at different levels improved egg fertility and hatchability.

Chick weight at day-old was shown to be positively affected by the inclusion of dietary MOLM70. Improved day old weight in the current study agree with the finding of Etalem *et al.* (2014) who noted higher chick weight in hens fed 5 % MOLM. Coon *et al.* (2006) observed that chick weight can be improved by additional protein in the hen's diet. *Moringa oleifera* leaf meal is known to be a good source of protein (Alebachew *at al.*, 2016).

Conclusions

The findings from the study confirm the use and efficiency of MOLM for egg quality development in Potchefstroom koekoek hens. From the results, it can be concluded that the inclusion of dietary MOLM in the diet can have beneficial effect on hatchability performance in Potchefstroom koekoek hens.

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Chapter 5

EFFECT OF *MORINGA OLEIFERA* LEAF MEAL ON GROWTH PERFORMANCE, SEMEN PARAMETERS OF POTCHEFSTROOM KOEKOEK COCKERELS.

Abstract

The aim of the study was to investigate the effect of dietary *Moringa oleifera* leaf meal (MOLM) on the semen and sperm quality of Potcheftroom koekoek cockerels. Thirty two Potchefstroom koekoek cockerels were assigned to different diets: MOLM0 (control) = diet with no Moringa inclusion; MOLM70 = diet with 70 g/kg of MOLM inclusion, for the duration of 20 weeks. The experimental unit was a pen that contains 4 birds, replicated 4 times. Semen parameters (curvilinear velocity (VCL), average pathway velocity (VAP), straight line velocity (VSL) straightness, wobble and linearity), progression parameters (PM, NPM & static), pH, concentration, velocity, volume and morphology were evaluated. The two way interaction significantly affected semen volume. The dietary MOLM70 resulted in higher sperm velocity (rapid), semen concentration, semen pH, motility (VCL, VSL AND VAP) and progressive motility than the control (MOLM0). It can be concluded that MOLM70 is nutritionally enriched to enhance both reproductive and productive performance of Potchefstroom koekoek chickens.

5.1 Introductions

Semen quality is a measure of the capacity of semen to achieve fertilization which is essential in the production of hatching eggs and can be heavily influenced by nutrition (Peters *et al.*, 2008). One of the most important characteristics that determines fertility in the roosters is the quality of semen. However, it is important to distinguish the semen parameters in terms of sperm concentration, semen volume, semen pH, sperm motility and morphology. Tyler & Bekker, (2012) stated that increasing protein in the male diet has been demonstrated to reduce sperm production and there is a strong negative correlation that exists between spermatozoa concentration and dietary protein levels. The semen quality characteristics evaluation of poultry gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters *et al.*, 2004). The semen of the domestic chicken according to Hafez (1978) varies from a thick suspension to a watery fluid with a relative high density. The differences in volumes and sperm concentration of the indigenous chicken semen depends largely on the relative contribution of the various reproductive glands, the number of spermatozoa that could be obtained from a strain and the extent to which the genetic potentials can be exploited (Hafez, 1978). A cockerel's fertility is based upon the capability to perform a successful mating and the quality of semen produced. However, limited research has been done on the effect of leaf meal on the semen quality of indigenous roosters. Therefore the aim of the study was to evaluate the effect of *Moringa oliefera* leaf meal on the semen and sperm quality of Potchefstroom koekoek cockerels.

5.2 Material and methods

5.2.1 Experimental procedure

Thirty two (32) roosters of 20 weeks of age were used as the semen donors and was randomly assign to two dietary treatments (MOLM0 g/kg and MOLM70 g/kg). Each treatment was replicated 4 times with 4 cockerel birds per replicate. The birds were kept in individual cages measuring 60 x 50 x 75 cm. Roosters were provided with *Moringa oleifera* leaf meal (MOLM0 g/kg and MOLM70g/kg) and water *ad libitum*. From each treatment semen was collected and pooled three times a week by the dorso-abdominal massage method (Burrows and Quinn, 1937), six semen collections were performed for every group.

5.2.2 Measured Parameters

5.2.2.1 Growth performance

Growth performance was as described in details under section 3.5.1.

5.2.2.2 Semen parameters

Parameters such as sperm motility, concentration and viability were determined according to methods of Anderson (2001) and Lukaszewicz *et al.* (2008). Sperm motility was analysed on a warm microscope slide (x400magnification), by assessing a 10µL semen and Beltsvile Poultry Semen Extender (BPSE) mixture (1:10) and classifying 100 sperm cells as either motile or non-motile. Sperm were counted with the aid of haemocytometer (dilution at 1:200 in distilled water) from which the sperm concentration was then determined. Two hundred (2 x 100) individual sperm cells were viewed per slide for morphology assessment using the eosin-nigrosin stain method. The sperm output for each cockerel was calculated as the product of semen volume (mL) and sperm concentration (10^9/mL) (Anderson, 2001; Cerolini *et al.*, 2006).

5.3 Statistically analysis

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Y_{ij} = observation (growth performance), μ = population mean, T_i = effect of diet and ε_{ij}= random error term.

Data on growth performance was analysed using GLM procedure of SAS (2010) as a completely randomized design. Differences among treatment means were determined using the PDIFF option in the LSMEANS statement of GLM procedure of SAS (2010). Level of significance was set at $P<0.05$.

5.4 Results

5.3.1 Growth performance

There were no significant dietary effects on average weekly feed intake of Potchefstroom Koekoek cockerels (Figure 5.1). Although not significant, feed intake was numerically lower in cockerels fed dietary MOLM0 in all the weeks except for weeks 1 and 8 compared to those that received dietary MOLM70. Feed intake decreased at weeks 2, 7 and 11 in cockerels fed MOLM0 and for cockerels fed MOLM70 feed intake decreased in week 8. The effect of diet on the average weekly weight gain of PK cockerels is illustrated in Figure 5.2. Diet had no effect on weight gain of PK cockerels. However, there was no indication of weight loss in this study. The effect of diet on the average weekly FCR of PK cockerels is illustrated in Figure 5.2. Diet had no effect on FCR of PK cockerels. However, a linear decrease in FCR was observed throughout the experiment for cockerels fed all diets.

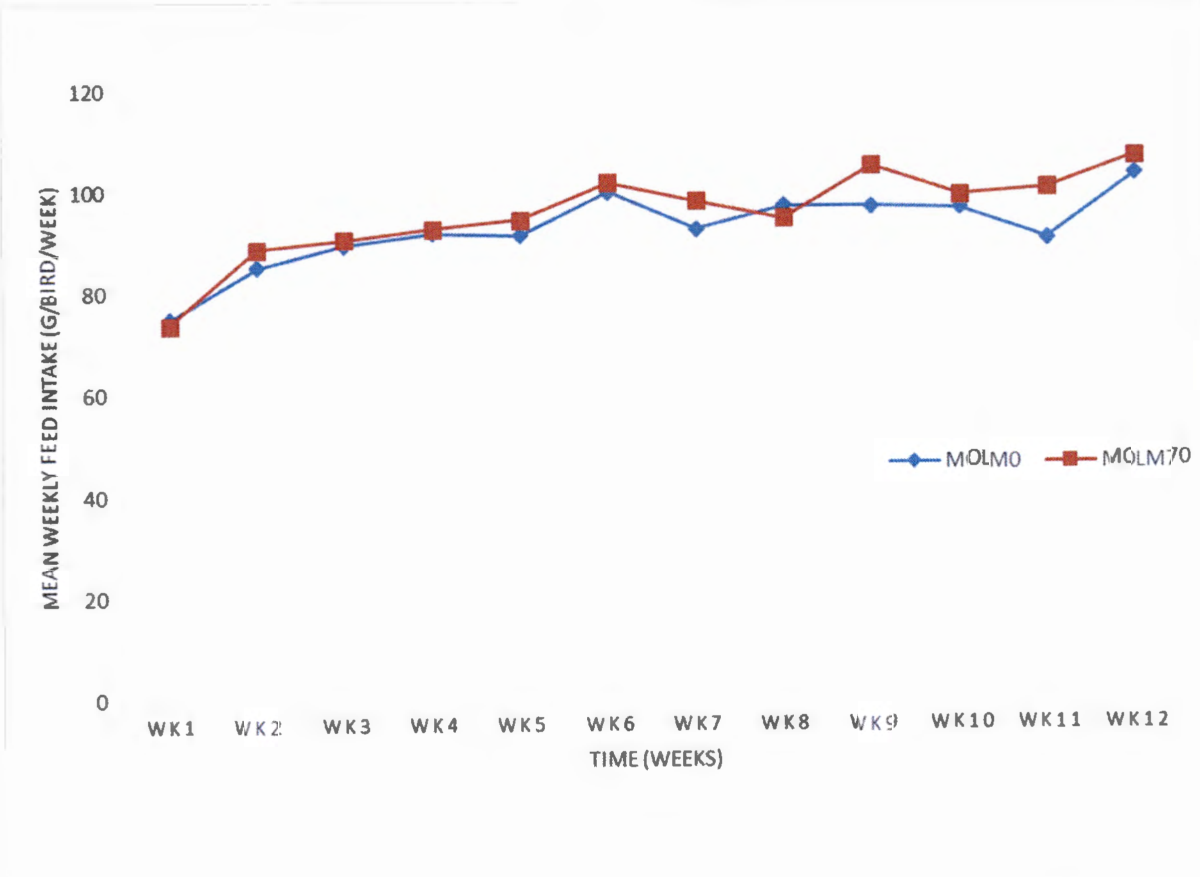


Figure 5.1 Mean weekly feed intake of Potchefstroom koekoek cockerels fed diets containing *Moringa oleifera* leaf meal.

[1]Diet: MOLM0 = basal diet without MOLM inclusion; MOLM70 = basal diet diluted with 70g MOLM/kg.

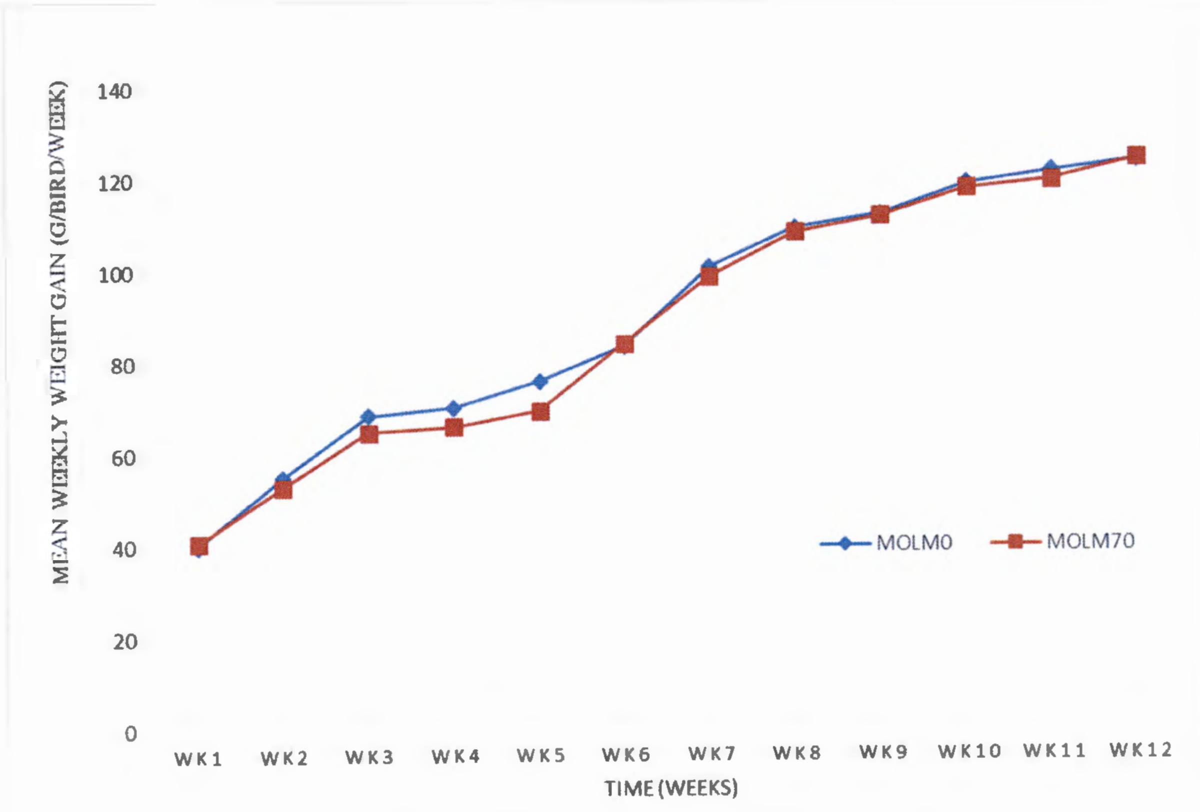


Figure 5.2 Mean weekly weight gain of Potchefstroom koekoek cockerels fed diets containing *Moringa oleifera* leaf meal

[1]Diet: MOLM0 = basal diet without MOLM inclusion; MOLM70 = basal diet diluted with 70g MOLM/kg.

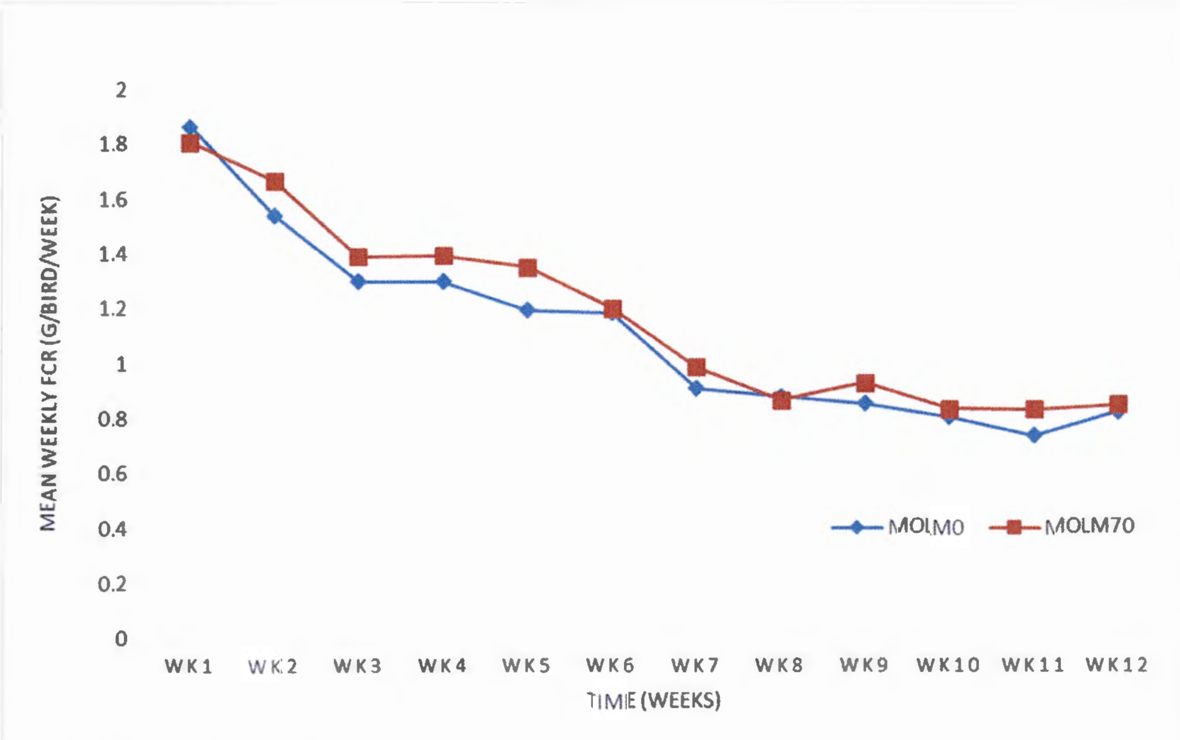


Figure 5.3 Mean weekly feed conversion ratio of Potchefstroom koekoek cockerels fed diets containing *Moringa oleifera* leaf meal.

[1]Diet: MOLM0 = basal diet without MOLM inclusion; MOLM70 = basal diet diluted with 70g MOLM/kg.

5.3.2 Semen parameters

The effect of diet and day and their interaction on semen parameters is presented in table 5.1. Dietary treatment had an effect on semen PH, but diet did not affect semen volume, sperm concentration and morphology (live, dead, mid and tail). Day had an effect on semen volume and morphology (mid and tail). However day did not affect sperm concentration, pH and number of dead and live sperm in morphology. The two way interaction (diet*day) significantly affected semen volume but did not affect other semen characteristics (sperm concentration, pH and morphology).

Table 5.1 Analysis of dietary treatment, day and the interaction (X) between treatment and day effect on semen quality

		Interaction		
Semen Characteristics		Treatment effect	Day effect	Treatment x Day effect
Semen volume (ml)		NS	**	**
Sperm concentration (10^8 cells ml $^{-1}$)		NS	NS	NS
pH		**	NS	NS
Morphology	Live	NS	NS	NS
	Dead	NS	NS	NS
	Mid	NS	**	NS
	Tail	NS	**	NS

^{Ab} means within same row with different superscript differ (P<0.05).

The effect of *Moringa olefera* leaf meal on semen parameters and morphology percentage is presented in table 5.2. There was no significant difference (P>0.05) noticed with regards to sperm concentration, semen volume, medium and slow velocity %, linearly, straightness, wobbles and morphology. Inclusion of dietary MOLM70 resulted in high (P<0.05) semen pH, progressive motility, VCL, VSL and VAP.

The effect of dietary *Moringa oleifera* leaf meal on the semen volume of Potchefstroom Koekoek cockerels is illustrated in Figure 5.4. Diet had no effect (P>0.05) on semen volume of Potchefstroom Koekoek cockerels. However, semen volume was higher in cockerels fed dietary MOML70 at all days except for day two where cockerels fed dietary MOLM0 had a higher semen volume.

Table 5.2. The semen characteristics of Potchefstroom Koekoek sampled for biochemical semen ingredients evaluation (mean ± SEM)

	Parameters	Diet (g/kg)	
		MOLM0	MOLM70
	Concentration(10^8 cells ml^{-1})	677.50 ± 0.041	719.19 ± 0.041
Semen	pH	6.940 ± 0.120^b	7.370 ± 0.120^a
characteristics	Volume (ml)	0.39 ± 37.415	0.45 ± 37.415
	Total motility		
Progression	PM	39.38 ± 2.419^b	57.73 ± 2.419^a
(%)	NPM	48.59 ± 1.279^a	39.89 ± 1.279^b
	Static	12.57 ± 2.156^a	2.38 ± 2.156^b
	Rapid	81.27 ±2.497^b	89.06 ± 2.497^a
Velocity (%)	Medium	55.84 ± 0.736	56.42 ± 0.736
	Slow	32.78 ± 0.743	32.86 ± 0.743
	VCL (µm/s)	86.70 ± 8.007^b	106.64 ± 7.124^a
	VSL (µm/s)	34.42 ± 3.370^b	45.54 ± 2.999^a
Motility	VAP (µm/s)	51.57 ± 4.987^b	66.19 ± 4.437^a
	Linearity (%)	39.93 ± 1.164	42.70 ± 1.035
	Straightness (%)	67.08 ± 1.057	68.83 ± 0.940
	Wobble (%)	59.29 ± 0.969	61.89 ± 0.863
	Live	80.08 ± 1.413	80.78 ± 1.413
	Tail	4.67 ± 0.411	3.67 ± 0.411
Morphology	Dead	12.86 ± 1.177	13.47 ± 1.177
	Mid	2.17 ± 0.270	1.67 ± 0.270

^{Ab} means within same row with different superscript differ (P<0.05). VCL=cuevillinear velocity; VAP= average path velocity; VSL straight-line velocity; LIN= linearity.

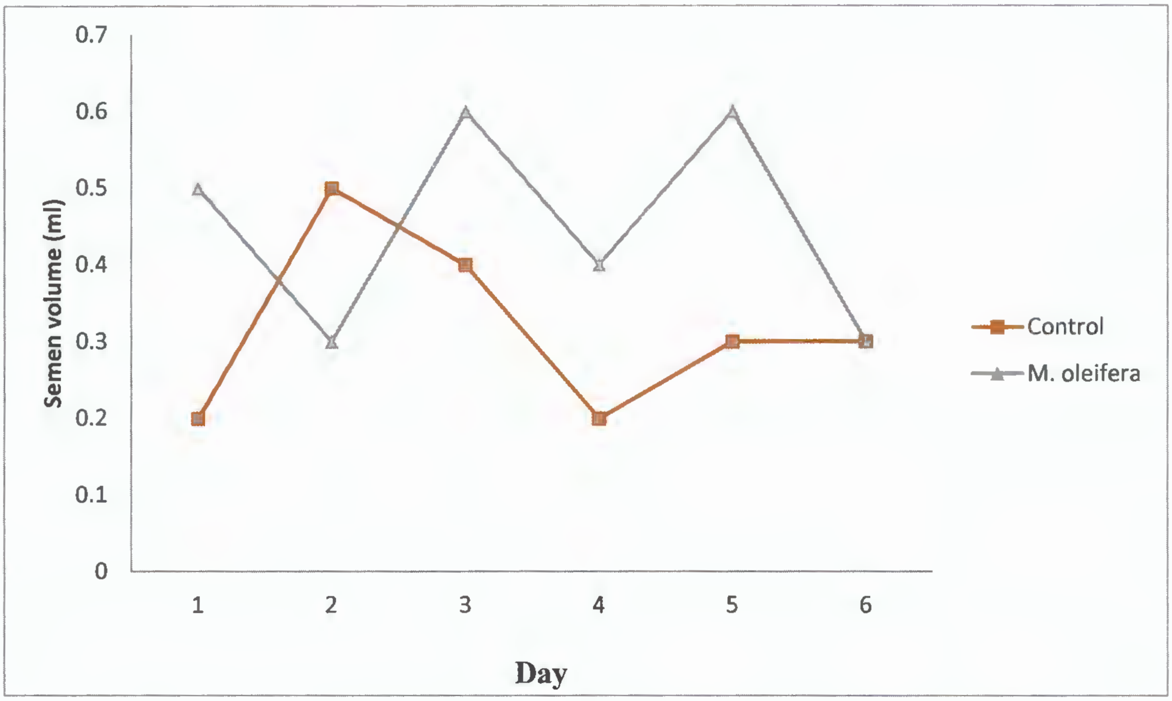
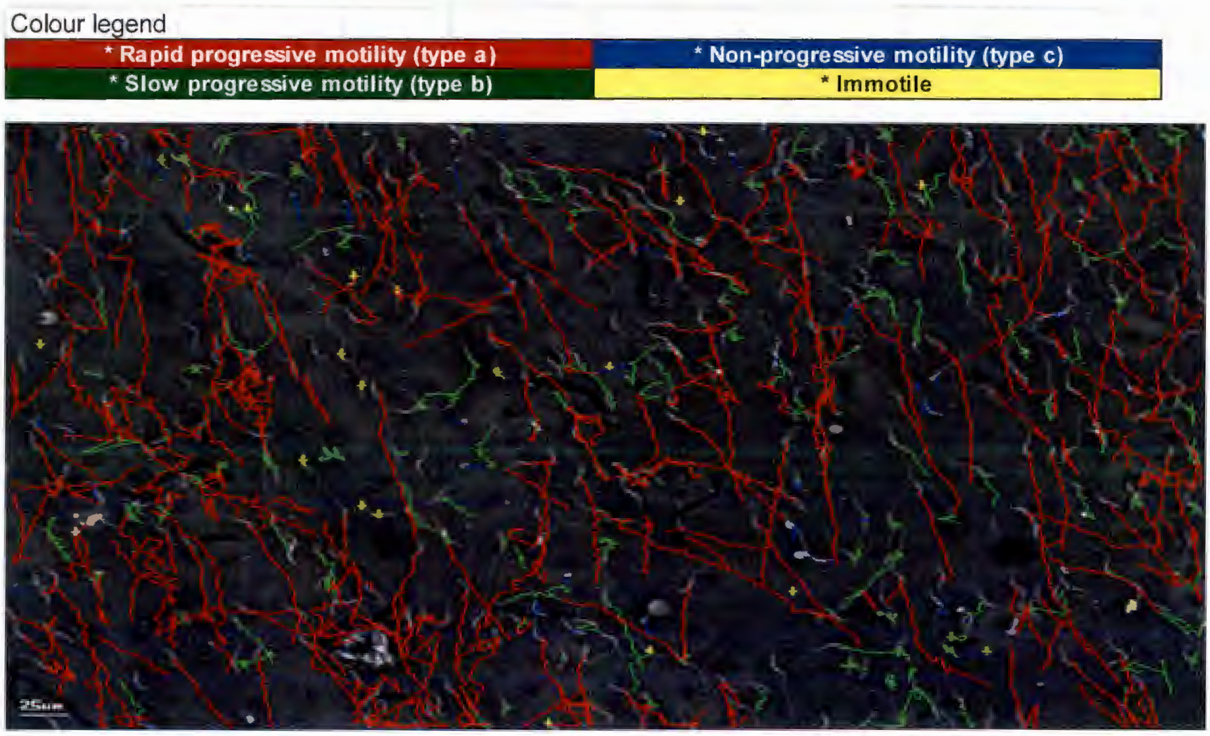


Figure 5.4 Effect of collection period (day interval) on semen volume of chickens fed *moringa oleifera* leaf meal.



Output of Sperm class Analyser

5.5 Discussions

5.5.1 Growth performance

The observation that the inclusion of MOLM in PK cockerels' diets had no effects on feed intake, weight gain and FCR contrast with those reported for broilers, when MOLM was included in the diets (Safa & Tazi, 2014). The differences observed might be caused by the breed of the chicken used. The general increase in body weights of PK cockerels indicated that the treatment had no adverse effect on growth and body weight of the cockerels. A good indicator of semen volume and semen concentration in some cockerel breeds is body weight (Yusuf, 2014). Generally, Adeyemo *et al.* (2007) mentioned that poultry breeds with heavier body weight have been found to have larger testes and produce more sperm cell during spermatogenesis and thus resulting in a higher semen concentration. These results imply that 7 % MOLM can safely be included in diets for indigenous chicken.

5.4.2 Semen parameters

Bearden *et al.* (2004) mentioned that low sperm concentration has been associated with low fertility. Therefore, the higher sperm concentrations recorded in MOLM70 suggests that testicular development and proper hormone balance in roosters were triggered by the inclusion of MOLM. This suggestion is supported by the findings of Saalu *et al.* (2011) who reported that rats fed MOLM showed high sperm production and normal seminiferous epithelium. Non-significant results of sperm concentration in this study is in conformity with the report of Olubowale *et al.* (2014) who recorded a non-significant sperm concentration when dietary lipid sources were included in the diet of Hy-line silver-Brown cockerels. However, the study was disagreeing with the findings of some authors who reported a significant effect of diet on sperm concentration Cerolini *et al.* (2006); Yusuf (2014).

The pH result obtain in birds fed MOLM70 was within the range obtained by Peters *et al.* (2008), Gebriel *et al.* (2009) and Orunmuyi *et al.* (2012). Improved mean pH (7.37) in roosters offered MOLM70 in this study improved sperm velocity by increasing the pH to alkaline. Ashizawa and Wishart (1987) and Ashizawa *et al.* (1997) stated that the percentage of motile sperm and sperm velocity were increased to alkaline pH (pH scale) in domestic roosters. Lower pH value will affect the live spermatozoa due to the lower production of lactic acid and process metabolism spermatozoa.

The significant difference reported for progressive motility in the present study highlight the positive effect of MOLM, in enhancing sperm motility by providing the substrate (ATP) needed for motility. Increased progressive motility from roosters fed MOLM70 may be attributed to Vitamin E and Selenium found in MOLM (Fuglie, 2001; Sebola *et al.*, 2017). Young *et al.*, (1986); Hansen & Deguchi, (1996) stated that dietary selenium causes an increase in sperm concentration, sperm motility and sperm capacity in farm animals, including poultry species.

The current study is in agreement with the findings reported by Fatoba *et al.* (2013) who noted significant higher sperm motility in albino rats treated with different levels of Moringa root extract. Similarly Macheba *et al.* (2013) recorded significant increase in sperm motility of tom (male turkey) fed plant root extracts. Plant material like MOLM contains substantial amount of beneficial anti-oxidants, phytochemicals, minerals and vitamins which are known to increase growth and stimulate reproduction in animals including poultry species (Nwangwa *et al.*, 2007; Machebe *et al.*, 2011).

An indicator of some disorders in spermatogenesis is sperm morphology. MOLM contain essential antioxidant and phenolic compounds that help in protecting the testis against morphologic, spermatogenic and oxidative chances brought about by toxic materials and certain anti-neoplastic agents (Siddhuraju and Barker, 2003; Saalu *et al.*, 2011). However in the present study, dietary treatment had no significant effect on sperm morphology. But it was not the case with the report of Yusef (2014) who noted a significant effect on sperm morphology of turkey fed dietary *Moringa oleifera* and *Gongronema latifolium* leaf meal. Fataba *et al.* (2013); Yusuf (2014); reported a significant effect of *Moringa oleifera* leaf meal on semen volume. However it was not the case in this study. In agreement with the present findings, Castellini *et al.* (2004) noted a non-significant effect of α-Linolic acid on semen volume of rabbit bucks.

5.6 Conclusions

In conclusion, findings from the present study indicate that, it is possible to improve the semen parametes of Potchefstroom koekoek through inclusion of MOLM. However this study can also be helpful in developing and propagating economically viable Potchefstroom koekoek flocks, making Potchefstroom koekoek production more profitable and conserve semen in cryo-gene bank for use in the future.

5.7 Reference

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Chapter 6

General discussion and conclusions

6.1 general discussion

The research was carried out to determine the effect of *Moringa oleifera* leaf meal (MOLM) on growth, laying performance, external and internal egg parameters and hatchability performance of Potchefstroom koekoek (PK) hens. Growth performance, semen and sperm quality parameters of PK cockerels fed dietary MOLM were also determined. It was observed in the results that there were no significant differences on feed conversion ratio, growth rate and egg laying performance across all treatments. Interestingly, hens fed MOLM70 had lower feed intake compared to hens on dietary MOLM0. This may be due to the increased bulkiness of the diet that resulted in reduced palatability of the diet. The results therefore suggest that inclusion of MOLM at 7 % may not cause any barriers to the protein and calcium digestion dynamics and utilisation by hens. Therefore, *Moringa oleifera* leaf meal can be included at 7 % without major effect on growth and laying performance of PK hens. Diets had no effect on all internal egg parameters apart from albumen length, where hens fed MOLM70 had higher albumen length compared to those that received the control (MOLM0) diet. The results on external egg parameters also showed a lack of significant effect of diet on egg weight, egg shell thickness and egg content. Inclusion of MOLM70 improved egg length, egg shape index, egg shell weight and shell percentage. However, in terms of egg diameter, hens fed dietary MOLM0 was higher than those under dietary MOLM70. Hens fed dietary MOLM70 had higher egg weight than hens on MOLM0 with no statistical difference between MOLM0 and MOLM70. There was no significant effect on feed intake, weight gain and FCR of PK cockerels. Also, there was no significant difference noticed in terms of sperm concentration, semen volume, medium and slow velocity %, linearly, straightness, wobbles and morphology. Inclusion of MOLM70 resulted in high semen pH, progressive motility, VCL, VSL and VAP. The results suggest that testicular development and proper hormone balance in roosters were triggered by the inclusion of MOLM. Overall, *Moringa oleifera* leaf meal was shown to have potential benefits if included in chicken diets. Collectively, the findings from the present study can be helpful in designing low-cost feed formulations that will be beneficial to small scale farmers and also to consumers with regards to improved growth performance, egg fertility and semen quality of indigenous chicken farming systems in the future.

5.2 Recommendations

- ✓ Farmers may consider including 7% of *Moringa oleifera* leaf meal on diet because it will improve the quality of poultry cockerel semen which could result to higher fertility and hatchability.
- ✓ Cost benefits analysis.
- ✓ The effect of dietary levels of MOLM inclusion on day old chicks can be researched till they reach sexual maturity age to have a comprehensive understanding of how MOLM is utilised.
- ✓ The effect of MOLM inclusion and temperature on semen parameters of Potchefstroom koekoek can be researched.