EFFECT OF *IN OVO* INJECTION OF GLUCOSE ON EGG HATCHABILITY, CHICK HATCH-WEIGHT, PRODUCTIVITY AND CARCASS CHARACTERISTICS OF INDIGENOUS POTCHEFSTROOM KOEKOEK CHICKENS

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DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Animal Production), has not previously been submitted by me for a degree at this or any other university; that it is my work in design and execution, and that all material contained herein has been duly acknowledged.

Signature…… Date…….

Letsoalo Tshegofatso Maapeya Caroline

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DEDICATION

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ABSTRACT

Three experiments were conducted to determine the effect of *in ovo* glucose injection on egg hatchability, chick hatch-weight, productivity and carcass characteristics of indigenous Potchefstroom koekoek chickens. A complete randomized design was used in all the three parts of the study (from incubation, 1-49 days old unsexed chickens and 50-91 days old female chickens). On day 18 of incubation the developing eggs were subjected to the following treatments: 0^- (no glucose or water injected), 0^+ (only water injected), 5, 10, 15 or 20 mg of glucose per egg. Each treatment had three replications and there were 20 eggs per replicate. A quadratic model was used to determine *in ovo* glucose injection levels for optimal egg hatchability, chick hatch-weight and chick to egg weight ratio of Potchefstroom koekoek chickens. *In ovo* glucose injection improved ($P<0.05$) egg hatchability, chick hatch-weight and chick to egg weight ratio of the chickens. Egg hatchability, chick hatch-weight and chick to egg weight ratio Potchefstroom koekoek chickens were optimized at different injection levels of 4.50, 10.43 and 12.00 mg of glucose per egg, respectively.

Unsexed day-old chicks from the first experiment (according to their initial treatments and replicates) were used in a complete randomized design having six treatments, replicated three times, and having ten birds per replicate. Glucose injection levels increased ($P<0.05$) feed intake, growth rate, feed conversion ratio, live weight, metabolisable energy and nitrogen retention of female Potchefstroom koekoek chickens aged 1 to 49 days. However, growth rate, live weight, metabolisable energy intake and nitrogen retention of the chickens were optimized at glucose injection levels of 3.92, 4.36, 10.67 and 13.50 mg per egg, respectively.

Female chickens from the second part of the study (according to their initial treatments and replicates) were used in a complete randomized design having six treatments, replicated three times, and having five birds per replicate. *In ovo* glucose injection levels improved ($P<0.05$) on feed intake, growth rate, feed conversion ratio, live weight, metabolisable energy and nitrogen retention of female Potchefstroom koekoek chickens aged 50 to 91 days. However, only feed conversion ratio and metabolisable energy intake of the chickens were optimized at glucose injection levels of 12.15 and 5.57 mg per egg, respectively. Injection level also improved ($P<0.05$) carcass, breast,

drumstick, thigh, wing, gizzard and liver weights of female Potchefstroom koekoek chickens aged 13 weeks. *In ovo* glucose injection increased (P<0.05) breast meat tenderness, juiciness and flavour of female Potchefstroom koekoek chickens aged 91 days. However, breast tenderness, juiciness and flavour of female Potchefstroom koekoek chickens were optimized injection levels of 13.50, 19.25 and 10.83 mg of glucose per egg, respectively. No chicken deaths were observed.

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CHAPTER 1

INTRODUCTION

1.1 Background

In the developing countries the poultry industry can be divided into two sub-sectors, which are the commercial and traditional sub-sectors (Gueye, 1998). Exotic breeds of layer and broiler chickens with high egg and meat production, respectively, make up most of the commercial sub-sector (John, 1995). The indigenous chicken breeds such as the Potchefstroom koekoek chickens make up most of the traditional sub-sector. This is a sub-sector that is important for the livelihood of most rural households as it is found in rural areas (Sonaiya, 2001). Indigenous chickens provide meat and eggs of high quality (Miao *et al*., 2005). The indigenous chickens produce few eggs and have low body weights (Ebangi and Ibe, 1994; Safalaoh, 2001). Thus, indigenous chickens are less productive (King'ori *et al.,* 2003; Tadelle *et al.,* 2003). Therefore, improved nutritional management is necessary to assist in achieving optimal performance in terms of egg hatchability, chick hatch-weight, growth rate, feed conversion ratio, live weight, etc.

1.2 Problem statement

Indigenous chickens have low egg hatchability, low chick hatch-weight, low growth rate and high mortality rate (Tadelle and Ogle, 2000). Chicken embryos depend on nutrients within the egg. These nutrients are needed for metabolic processes of the growing embryo during incubation (Foye *et al.,* 2006). The embryos are very sensitive to energy deficiency during their development. Energy deficiency results in weak hatchlings or embryonic deaths (Shafey *et al.,* 2012). There is some evidence that *in ovo* feeding in broiler chicken production has a positive effect on chick development and productivity (Coles *et al.*, 2003). *In ovo* injection of glucose provides energy in broiler chicken embryos and, thus, improves egg hatchability, chick hatch-weight and performance of the chickens (Salmanzadeh, 2012). However, such information is not extensive and conclusive. In fact, such information was not found for indigenous chickens.

1.3 Motivation of the study

This study generated information on the effect of *in ovo* glucose injection levels for optimal hatchability, chick hatch-weight, feed intake, digestibility, growth rate, feed conversion ratio, metabolisable energy, live weight, mortality and carcass

characteristics of Potchefstroom koekoek chickens. Such information will help in devising feeding strategies for improving productivity of Potchefstroom koekoek chickens. Improvement in productivity of Potchefstroom koekoek chickens will be beneficial to poultry farmers.

1.4 Aim and objectives

The aim of this study was to determine optimal responses in feed intake, digestibility, growth rate, mortality and carcass characteristics of Potchefstroom Koekoek chickens to *in ovo* injection of glucose.

The objectives of the study were to determine:

i. the effect of *in ovo* injection of glucose on egg hatchability, chick hatch-weight and productivity of Potchestroom koekoek chickens.
ii. *in ovo* glucose injection levels for optimal responses in egg hatchability, chick hatch-weight and productivity of Potchefstroom koekoek chickens.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Chick embryos are dependent on the nutrients within the egg, these nutrients provide the developing embryo with energy and building blocks required for the metabolic needs during incubation (Foye *et al.,* 2006). Many nutrients have important structural, physiological and immunological roles in avian embryogenesis and growth performance. *In ovo* injection of nutrients may help overcome any constraint of inadequate egg nutrition during incubation (Selim *et al.,* 2012). To overcome some physiological limitations and also to improve the intestinal functionality and nutritional status of hatchlings, *in ovo* feeding was developed. This is a method of inserting nutrient solutions into the embryonic amniotic fluid in poultry (Uni and Ferket, 2003). This method makes use of the knowledge that neonatal birds naturally consume the amniotic fluids towards the hatching period (Romanoff and Romanoff, 1967). Thus, addition of a nutrient solution to the embryonic amniotic fluid delivers essential nutrients into the embryo. There are many potential nutrient supplements which can be included in the *in ovo* feeding solution.

2.2 Glucose requirement in chick embryos

Glucose is a simple sugar (monosaccharide) and an important carbohydrate in biology. It is used by cells as the primary source of energy (Clark and Sokoloff, 1999). It is, also, used in the manufacture of a number of products. The whole carbohydrate of an egg does not exceed 1%. Despite the small amount of carbohydrate supply, glucose is the most important source of energy needed for growth of the embryo (Starck and Rickelefs, 1998). The glucose naturally available in eggs may not be sufficient to meet the immediate metabolic demands of the embryo. However, glucose is the predominant energy source during the first week of embryonic growth (Moran, 2007). Whilst fatty acids of yolk lipids are the predominant energy sources during the second half of incubation (Noble and Cocchi, 1990; Sato *et al.*, 2006), a large mobilization of yolk nutrients into the embryo occurs during the peri-hatch period (Vieira and Moran, 1999) and is utilized during the hatching process (Chotinsky *et al*., 2001). Embryos prefer to use glucose rather than fatty acids for energy production because oxygen availability is limited before hatching and with the same amount of oxygen consumption, glucose oxidation provides more energy than lipid catabolism (Pearce and Brown, 1971). Glucose is used by cells as the primary source of energy

for the embryo (Clark and Sokoloff, 1999). However, at this stage of incubation, glycogen is utilized in greater amount in order to meet the high energy demand of the hatching process, and consequently glycogen reserves in the embryo are significantly depleted by the end of incubation (Shafey *et al*., 2012). The chicken embryo is very sensitive to a deficiency of energy during the hatching process. This deficiency can result in weak hatchlings and more severe conditions can result in embryonic deaths (Chotinsky *et al*., 2001). Energy supply is, therefore, a limiting factor for a successful exit from the egg. Therefore, improving the glycogen reserves in the embryo will provide the critical energy needed for hatching. Thus, many attempts have been made to improve the glycogen status of the embryos at the end of the incubation period and at hatching by employing *in ovo* feeding (Uni *et al.*, 2005). Introduction of external carbohydrates, as readily available energy sources, may help to spare protein and fatty acids that would normally be used for gluconeogenesis so that embryo growth may be optimized (Foye *et al*., 2006). It had been suggested that an *in ovo* injection of a 1.0 ml volume of various combinations of carbohydrates at a concentration of 0.18 to 0.25 g/ml can improve the energy status of the livers and bodies of subsequent hatchlings (Smirnov *et al.,* 2006). Ingram *et al*. (1997) investigated the effect of *in ovo* injection of glucose and reported that glucose applied at levels lower than 25 mg increased egg hatchability in broiler chicken breeders.

2.3 Effect of *in ovo* glucose injection on chick embryo development

Towards the later stages of incubation the embryo begins to adjust its metabolism for utilization of exogenous carbohydrates and proteins derived from feed sources post hatch. This metabolic transition includes a progressive increase in the use of glucose carbon as an energy source and also gradual up-regulation of the lipogenic machinery in the liver. The degree to which the gastrointestinal tract (GIT) is developed, the content of the residual yolk at hatch and energy reserves (glycogen stores, subcutaneous fat depots) are vital in aiding the metabolic shift from *in ovo* to post-hatch development (Sunny, 2008). Glucose injected in the egg during incubation plays an important role in the initiation of embryonic development and further as an energy substrate via anaerobic catabolism (Moran, 2007). The glucose is then stored as glycogen which is a very important energy resource for maintaining normal metabolism and body growth (Christensen *et al.,* 2000). *In ovo* injection of glucose improves the

energy needed for hatching by elevating glycogen reserves, moderating the use of muscle proteins which, thus, contributes towards enhancing body weight of newly hatched-chicks (Sunny, 2008). It, also, reduces the use of liver glycogen reserves and the depletion of muscle protein (Ebrahimnezhad *et al.,* 2011).

Glucose synthesis, primarily, occurs in the liver which can produce glucose both by gluconeogenesis and glycogenolysis. The contribution of the kidney to gluconeogenesis ranges from 5-50% (Corssmit *et al*., 2001) which is significant during periods of nutrient deprivation or prolonged fasting. Glucose can be metabolized through glycolysis, pentose phosphate pathway, uridine diphosphate –glucose - glycogen synthetic pathway or uronic acid pathway. In general, glycolysis and the activities of glycolytic enzymes increase with embryonic development, thus, preparing the embryonic metabolism to utilize a high carbohydrate diet post-hatch (Pearce, 1977). Goodridge (1968) demonstrated that glucose oxidation in embryonic liver slices were low. However, the oxidation rates were found to be 20 times greater in liver slices from 4-week old chicks. Pentose phosphate pathway is not known to play any major role in degradation of glucose during embryonic development except during the initial stages of incubation (Pearce, 1977).

2.4 Effect of *in ovo* injection of nutrients on embryo development

Shafey *et al*. (2012) indicated that *in ovo* feeding of carbohydrates improved chick hatch-weight as a proportion of egg weight without any effect on hatchability traits. These results were similar to the findings of Tako *et al.* (2004), who found that *in ovo* feeding of 1 ml of carbohydrates (25 g of maltose/l, 25 g of sucrose/l, 200 g of dextrin/l, and 5 g of NaCl/l) or carbohydrates plus beta-hydroxy-beta-methylbutyrate (HMB) (1 g of HMB/l in 5 g of NaCl/l) per egg at Day 17 of incubation improved the weight and intestinal development of hatched chicks. Smirnov *et al.* (2006) concluded that the presence of carbohydrates in the intestinal lumen of the chick embryo improved intestinal morphology and consequently nutrient absorption. Zhai *et al.* (2011) reported that *in ovo* feeding of 0.1 to 1 ml of carbohydrates (0.25 g/ml of glucose, fructose, sucrose, maltose or dextrin) per egg at Day 19 of incubation had no influence on the hatching rate of chicken eggs but weight of hatched chicks was positively related to injection volume when expressed on an absolute value or as a proportion of egg weight. However, Uni *et al.* (2005) found that *in ovo* injection of carbohydrates plus

HMB (1 ml of a solution containing 25 g/l maltose, 25 g/l sucrose, 200 g/l dextrin, 1 g/l HMB and 5 g/l NaCl) increased hatchability, liver glycogen and pectoral muscle size among chickens up to 25 days post-hatch. Abiola (1999) observed a positive correlation between egg size and chick hatch-weight. The higher weight of hatched chicks from large eggs is due to the surplus supply of nutrients and the size of the residual yolk sac at hatch when compared with those hatched from small eggs (Lourens *et al*., 2006).

Nowaczewski *et al.* (2011) reported no significant differences with *in ovo* injection of vitamin C (ascorbic acid) on egg hatchability, although the best results were recorded following the injection of 6 mg of ascorbic acid on days 13 and 15 of incubation. Good results were observed by Zakaria and Al-Anezi (1996) on egg hatchability when 3 mg of vitamin C per egg were injected on Day 15 of incubation. A favourable influence on hatchability of injection of 3 mg of vitamin C per egg into broiler breeder eggs on Day 13 of incubation was also reported by Ipek *et al*. (2004). On the other hand, the results of trials performed by Zakaria and Al-Latif (1998) showed that premature and excessively high doses of ascorbic acid injected *in ovo* can lead to the deterioration of chick hatchability. However, Nowaczewski and Kontecka (2005) showed a decreasing trend in the number of hatched but crippled chicks in comparison to the control in pheasants fed a complete diet supplemented with vitamin C. Ghonim *et al*. (2009) carried out a trial on Muscovy ducks in which they analysed the impact of the introduction of 3 mg of ascorbic acid per egg on Day 14 of incubation, the results demonstrated a statistically significant improvement in hatchability following treatment with vitamin C in comparison with the control group. A similar significantly favourable impact of the introduction of the above-mentioned dose of ascorbic acid in eggs of Muscovy ducks was demonstrated by the lower proportion of dead embryos.

2.5 Effect of *in ovo* injection of glucose on hatchability, chick hatch-weight and productivity of chickens

Ebrahimnezhad *et al.* (2011) suggested that *in ovo* injection of glucose solution in the albumin can be an effective tool in increasing the chick hatch-weight without a negative effect on hatching rates of the chicks. In the same study it was shown that *in ovo* injection of glucose in the albumin improved weight of newly hatched chicks compared

with the control group. Leitao *et al*. (2008) investigated the effect of *in ovo* injection of glucose in varying levels on broiler chicken egg hatchability. They reported that the utilization of 0.6 ml of glucose per egg decreased the hatch rate. Adriana *et al.* (2006) also found that decreased hatchability was observed when embryos received *in ovo* injection of glucose at Day 16 of chicken egg incubation. Bhanja *et al.* (2008) concluded that glucose injected into eggs had higher chick hatch-weight than sham (*in ovo* injected with water) and un-injected control. Amitav *et al*. (2007) showed that chick hatch-weight was significantly higher when glucose was injected in the yolk sac or amniotic sac than un-injected control group. Ebrahimnezhad *et al.* (2011) observed that there was a positive relationship between broiler chick hatch-weight and blood glucose concentration. Christensen *et al.* (2000) also reported that hatching weights were significantly and positively correlated with blood glucose concentrations in newly hatched chicks.

Ipek *et al.* (2004) investigated the effect of *in ovo* injection of different levels of glucose in broiler chicken eggs on hatchability. They reported that eggs injected with 0.5 ml of deionized sterile water containing 5, 10 and 15 mg of glucose did not differ significantly. However, Salmanzadeh (2012) found that injection of glucose in the albumen reduced hatchability. Bhanja *et al.* (2008) also showed that the injection of glucose in the albumen reduced the hatchability of newly-hatched chicks compared with the control group. Pedroso *et al.* (2006) also observed that chick embryos from *in ovo* glucose injected eggs at 16 days of incubation had low hatchability. Accelerated embryo development and improved nutritional status afforded by *in ovo* feeding improved hatch-weight and growth rate of broiler chickens (Al-Murrani, 1982; Ohta *et al.*, 1999; Bhanja *et al.*, 2004). Chen *et al.* (2009) stated that *in ovo* injection of carbohydrates improved duck weight gain in the early days of post-hatch. Salmanzadeh (2012) reported that the weight of newly hatched chicks was significantly higher with *in ovo* injection of glucose compared with that of the control and sham groups. Amitav *et al.* (2007) demonstrated that *in ovo* injection of glucose in the eggs of small white turkeys had significantly higher body weight throughout the experimental period and, at 6 weeks of age there was a difference of 76-78 g in body weight between those *in ovo* injected with glucose and the control treatment. Leitao *et al.* (2008) concluded that the *in ovo* injection of glucose had no effect on the broiler

chicken performance. However, Salmanzadeh (2012) reported that broiler chicks hatched from eggs injected with glucose had better weight gain and feed conversion ratio compared with those hatched from eggs of the control and sham groups throughout the experimental rearing period. Bhanja *et al.* (2008) also showed that feed conversion ratio during early post-hatch period was better in the *in ovo* glucose injected treatments than the control treatment. However, Leitao *et al*. (2008) concluded that *in ovo* glucose injection had no effect on growth rate of broiler chickens.

Salmanzadeh (2012) reported that broiler chickens hatched from eggs injected with glucose had higher carcass percentage and breast weight than the control and sham treatments. However, *in ovo* glucose injection had no effect on the legs, wings and neck weights of broiler chickens. *In ovo* injection of glucose had no effect on liver, heart and gizzard weights of broiler chickens aged 42 days (Salmanzadeh *et al*., 2011). Pilarski *et al.* (2005) reported that *in ovo* injection of oligosaccharides at a dose of 1.763 mg/egg had no effect on carcass and breast weights of broiler chickens. *In ovo* injection of a mixture of carbohydrates dissolved in saline on Days 17 or 18 of incubation improved embryonic development and increased chick hatch-weight (Uni and Ferket, 2003; Uni *et al.,* 2005; Smirnov *et al.,* 2006). Uni and Ferket (2003) showed that *in ovo* carbohydrate injection improved chick hatch-weight by 5 to 6 %. These body weight differences continued until Day 42. Salmanzadeh *et al.* (2011) showed that *in ovo* injection of glucose at Day 7 of incubation improved early growth, carcass and breast weights of broiler chickens.

2.6 Conclusion

Embryo development requires a lot of nutrients. Deficiency of such nutrients results in low hatchability, chick hatch-weight and growth rate, and high mortality rates of broiler chickens. The effects of *in ovo* glucose injection on productivity of the chickens are not conclusive. No study on the effect of *in ovo* glucose injection on productivity of indigenous chickens was found. The objective of this study was, therefore, to determine *in ovo* glucose injection levels for optimal growth and carcass characteristics of Potchefstroom koekoek aged 1 to 91days.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

This study was conducted at the University of Limpopo, Turfloop campus, South Africa. The area has a longitude of 23.886°S and a latitude of 29.738°E. The ambient temperatures around the study area range between 20 and 36 °C during summer and between 5 and 28 °C during winter months (Shiringani, 2007).

3.2 Preparation of the house

The experimental house was thoroughly cleaned with water and disinfectant and fumigated with formalin (NTK Company, Polokwane). To break the life cycle of any disease causing organisms that were not killed by the disinfectant, the house was left empty for two weeks. The experimental house was divided into 20 floor pens of 4.0 m^2 per pen. Fresh saw dust and wood shavings were placed on the floor to a level of 7 cm.

3.3 Acquisition of materials and eggs

All the required materials (feed, chemicals, medicines and vaccines) for the experiment were purchased before the commencement of the experiment (NTK Polokwane). Potchefstroom koekoek eggs used in the study were collected from the University of Limpopo Experimental farm. The eggs were from Potchefstroom koekoek chickens aged 30 weeks. The hens were artificially fertilized by semen from one cock.

3.4 Experimental designs, dietary treatments and procedures

The first part of the study determined the effect of *in ovo* injection of glucose on egg hatchability and chick hatch-weight. Five hundred Potchefstroom koekoek eggs were collected, fumigated, weighed and placed in a commercial multi-stage incubator. The eggs were candled on Days 7, 14 and 18 of incubation. Unfertilized eggs and those containing dead embryos were removed. On Day 18 the developing eggs were randomly assigned to six dietary treatments, having three replicates. Each replicate had twenty eggs. Thus, 360 eggs were used. A complete randomized design was used. The treatments were as indicated in Table 3.1. Glucose was injected into the yolk sac of the embryos aged 18 days. The yolk sac was identified through egg candling. The injection was made through a pinhole which was made at the broad end of the egg, using a 25 mm needle (Selim *et al*., 2012). Prior to *in ovo* injection, the

injection site was disinfected with methylated spirit. Immediately after the injection, the site was sealed with paraffin wax and the eggs were returned to the incubator. On Day 19 all the eggs were placed in hatching trays according to their treatments and replicates. The hatched chicks were weighed and transferred to the floor pens, according to their replicates, for growth monitoring.

Table 3.1 Treatments for the first part of the study (egg incubation)

Diet code	Diet description
EG_{0-}	Potchefstroom koekoek eggs not injected with distilled water or glucose during incubation (negative control)
EG_{0+}	Potchefstroom koekoek eggs injected with 0.1 ml of distilled water per egg but not glucose during incubation (positive control)
EG_{5}	Potchefstroom koekoek eggs injected with 0.1 ml distilled of water plus 5 mg of glucose per egg during incubation
EG_{10}	Potchefstroom koekoek eggs injected with 0.1 ml distilled of water plus 10 mg of glucose per egg during incubation
EG_{15}	Potchefstroom koekoek eggs injected with 0.1 ml distilled of water plus 15 mg of glucose per egg during incubation
EG_{20}	Potchefstroom koekoek eggs injected with 0.1 ml distilled of water plus 20 mg of glucose per egg during incubation

The second part of the study determined the effect of *in ovo* injection of glucose on feed intake, digestibility, growth rate, feed conversion ratio, metabolisable energy, live weight and mortality of unsexed Potchefstroom koekoek chickens aged 1 to 49 days (Table 3.2). Unsexed day-old chicks from the first part of the experiment (according to their initial treatments and replicates) were used in a complete randomized design having six treatments, replicated three times with ten birds per replicate. Each pen had an area of 4.0 m^2. Feed and fresh water were provided *ad libitum* and light was provided 24 hours throughout the experimental period. All the chickens were offered the same grower feed. The grower feed contained 18 % CP and 12 MJ of ME per kg DM (Table 3.3).

Table 3.2 Treatments for the second part of the study (chickens aged 1 to 49 days)

Diet code	Diet description
UCG_{0-}	Unsexed Potchefstroom koekoek chickens hatched from eggs not injected with distilled water or glucose during incubation
UCG_{0+}	Unsexed Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water but not glucose during incubation
UCG_5	Unsexed Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water plus 5 mg of glucose per egg during incubation
UCG_{10}	Unsexed Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water plus 10 mg of glucose per egg during incubation
UCG_{15}	Unsexed Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water plus 15 mg of glucose per egg during incubation
UCG_{20}	Unsexed Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water plus 20 mg of glucose per egg during incubation

The third part of the study determined the effect of *in ovo* injection of glucose on feed intake, digestibility, growth rate, feed conversion ratio, metabolisable energy, live weight, mortality and carcass characteristics of female Potchefstroom koekoek chickens aged 50 to 91 days (Table 3.4). Only female chickens were used because there were not enough cocks. The chickens were used in a complete randomized complete design (SAS, 2008). There were six treatments, replicated three times with five chickens per replicate. The treatments and replicates were as assigned in the first part of the experiment. Each pen had an area of 4.0 m^2. Feed and fresh water were provided *ad libitum* and light was provided 24 hours throughout the experimental period. All the chickens were offered the same grower feed (Table 3.3).

Table 3.3 Feed (%) and nutrient composition of grower feed for Potchefstroom koekoek chickens (the units are in g/kg feed for dry matter, MJ/kg DM diet for

metabolisable energy and g/kg DM diet for crude protein, calcium, sodium, lysine, methionine and threonine)

	Treatment					
	UCG_{0-}	UCG_{0+}	UCG_5	UCG_{10}	UCG_{15}	UCG_{20}
Feed						
Maize	40.69	40.69	40.69	40.69	40.69	40.69
Wheat	15	15	15	15	15	15
Lucerne meal	6.8	6.8	6.8	6.8	6.8	6.8
Soya bean meal	18	18	18	18	18	18
Fish meal (2-8% fat)	5	5	5	5	5	5
Maize gluten meal	3.40	3.40	3.40	3.40	3.40	3.40
Full fat	2.13	2.13	2.13	2.13	2.13	2.13
Soya oil	3	5	5	5	5	5
DI sodium phosphate	0.11	0.11	0.11	0.11	0.11	0.11
Calcium carbonate	0.86	0.86	0.86	0.86	0.86	0.86
Salt	0.18	0.18	0.18	0.18	0.18	0.18
DI calcium phosphate	1.47	1.47	1.47	1.47	1.47	1.47
DL- Methionine	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.20	0.20	0.20	0.20	0.20	0.20
Threonine	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100
Nutrients						
Dry matter	923	923	923	923	923	923
Calcium	0.05	0.05	0.05	0.05	0.05	0.05
Sodium	0.20	0.29	0.20	0.20	0.20	0.20
Lysine	1	1	1	1	1	1
Methionine	0.84	0.84	0.84	0.84	0.84	0.84
Threonine	0.05	0.05	0.05	0.05	0.05	0.05
Crude protein	180	180	180	180	180	180
*ME	12	12	12	12	12	12

***** Laboratory deremined ME(NIRA)

Table 3.4 Treatments for the third part of the study (female chickens aged 50 to 91 days)

Diet code	Diet description
FCG_{0-}	Female Potchefstroom koekoek chickens hatched from eggs not injected with distilled water or glucose during incubation
FCG_{0+}	Female Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water but not glucose during incubation
FCG_{5}	Female Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water plus 5 mg of glucose per egg during incubation
FCG_{10}	Female Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water plus 10 mg of glucose per egg during incubation
FCG_{15}	Female Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water plus 15 mg of glucose per egg during incubation
FCG_{20}	Female Potchefstroom koekoek chickens hatched from eggs injected with 0.1 ml of distilled water plus 20 mg of glucose per egg during incubation

3.5 Data collection

Hatched chicks were counted. Percent hatchability was calculated as the number of hatched eggs per number of fertile eggs times a hundred. The initial live weights were taken when the chicks were a day old and thereafter average live weight per chicken was measured weekly by weighing the chickens in each pen, and the total live weight was divided by the number of chickens in the pen to determine the average live weight per chicken. Feed intake per chicken was measured by subtracting feed refusals from the feed offered per week, and the difference was divided by the total number of birds per pen. Feed conversion ratio was calculated by dividing the average feed intake by the average weight gain in each pen. This was calculated as the amount of feed consumed divided by the total weight of live chickens plus those of dead or culled chickens minus initial weight of all the chickens in the pen. Apparent digestibility was carried out when the chickens were between the ages of 42 and 49 days, and 84 and 91 days for the second and third parts of the study, respectively. It was conducted in specially designed metabolic cages having separated watering and feeding troughs.

Two chickens were randomly selected from each replicate and transferred to metabolic cages for the measurement of apparent digestibility (McDonald *et al.,* 2002). A three-day acclimatization period was allowed prior to a three-day collection period. Droppings voided by each chicken were collected on a daily basis at 09.00 hours. Care was taken to avoid contamination from feathers, scales, debris and feeds.

Mortalities were recorded as they occurred per pen. At 91 days of age all the remaining chickens were weighed and slaughtered. Thereafter, carcass weights of the chickens were measured. Dressing percentage was calculated by dividing carcass weight by live weight and then multiplied by one hundred. Carcass, breast meat, wing, thigh, drumstick, liver, heart and gizzard weights were determined. At the end of each slaughtering, meat samples from breast part of the slaughtered bird dried was in the oven for 24 hours at a temperature of 105 °C and later analysed for nitrogen content.

3.6 Sensory evaluation

Meat samples which had been frozen at 4 °C were thawed for 24 hours in a cooler room for sensory evaluation. The samples were broiled on an oven rack set at 160 °C and allowed to preheat for 20 minutes.

The meat samples were broiled for approximately 50 minutes and turned every 25 minutes. Tongs were used for turning to avoid piercing that could let the moisture escape. The samples were cut into 1.5 cm thick, according to their treatments and replicates. Lemon juice and water were used to rinse and cleanse the palate before tasting to the next samples. Each member of the trained panel had a chance to taste all the samples. The panel consisted of 20 trained people. The meat was evaluated for its tenderness, juiciness and flavour using a 5-point ranking scale (Table 3.5).

Table 3.5 Evaluation scores used by the sensory panel

	Sensory Attributes		
Score	Tenderness	Juiciness	Flavour

1	Too tough	Much too dry	Very bad flavour
2	Tough	Dry	Poor flavour
3	Neither tough nor tender	Neither dry nor juicy	Neither bad nor good flavour
4	Tender	Juicy	Good flavour
5	Too tender	Too juicy	Very good flavour

3.7 Chemical analysis

Dry matter of feeds, feed refusals, faeces and meat samples were determined by drying the samples in the oven for 24 hours at a temperature of 105 °C. Ash contents of the feeds, feed refusals and faeces were analysed by ashing a sample at 600 °C in a muffle furnace overnight (AOAC, 2010). Gross energy values for feeds and faeces were measured in a bomb calorimeter (AOAC, 2010) at the Animal Production Laboratory of the University of Limpopo. The apparent metabolisable energy and nitrogen retention contents of the diets for each experiment were calculated as described by AOAC (2010). Calcium and sodium in the feeds were determined with an inductively coupled plasma emission (ICP) Perkin-Elmer spectrometer (University of Limpopo Laboratory, South Africa). Lysine, methionine and threonine were determined by high performance liquid chromatography (HPLC).

3.8 Data analysis

Data on egg hatchability, chick hatch-weight, feed intake, digestibility, live weight, growth rate, feed conversion ratio, mortality rate, carcass characteristics and meat quality were analyzed using the General Linear Model procedure for statistical analysis of variance (SAS, 2008). Duncan test for multiple comparisons was used to test the significance of differences between treatment means ($P<0.05$) (SAS, 2008). The responses in feed intake, feed conversion ratio, growth rate, live weight, carcass characteristics and meat quality to *in ovo* injection of glucose was modelled using the following quadratic equation:

$$Y = a + b_1x + b_2x^2$$

Where Y = optimum feed intake, feed conversion ratio, growth rate, live weight, mortality rate and carcass characteristics; a = intercept; b_1 and b_2 = coefficients of quadratic equation; x = *in ovo* glucose injection level and $-b_1/2b_2$ = x value for optimal response. The quadratic model was used because it gave the best fit.

The relationship between dry matter intake and feed conversion ratio and *in ovo* injection of glucose level were modelled using a linear regression equation (SAS, 2008) of the form:

$$Y = a + bx$$

Where Y= Hatchability %, chick hatch-weight, chick to egg weight ratio, growth rate, feed conversion ratio, live weight, metabolisable energy, nitrogen retention or meat quality; a = intercept; b = coefficient of the linear eqaution and x = *in ovo* glucose injection level.

CHAPTER 4

RESULTS

4.1 Effect of *in ovo* injection of glucose on egg hatchability, chick hatch-weight and chick to egg weight ratio of indigenous Potchefstroom koekoek chickens

Results of the effect of *in ovo* injection of glucose on egg hatchability, chick hatch-weight and chick to egg weight ratio of indigenous Potchefstroom koekoek chickens are presented in Table 4.1. The eggs used in the treatments had similar (P>0.05) weights. Potchefstroom koekoek eggs injected with 10 mg of glucose per egg (EG_{10}) had higher (P<0.05) hatchability values than those not injected with anything (EG_0), those injected with 0.1 ml of water per egg (EG_{0+}), those injected with 5 mg of glucose per egg (EG_5), those injected with 15 mg of glucose per egg (EG_{15}) or those injected with 20 mg of glucose per egg (EG_{20}). Potchefstroom koekoek eggs injected with 5 mg of glucose per egg (EG_5) had higher (P<0.05) hatchability values than those on EG_{0-}, EG_{0+}, EG_{15} or EG_{20} treatments. Eggs not injected with anything (EG_{0-}), had higher (P<0.05) hatchability values than those on EG_{15} or EG_{20} treatments. However, eggs not injected with anything (EG_{0-}), or those injected with 0.1 ml of water per egg (EG_{0+}), had similar (P>0.05) hatchability values.

Potchefstroom koekoek eggs injected with 10 mg of glucose per egg (EG_{10}), produced chicks with higher (P<0.05) hatch-weights than the hatch-weights of chicks from eggs on EG_{0-}, EG_{0+}, EG_5, EG_{15} or EG_{20} treatments (Table 4.1). Eggs injected with 15 mg of glucose per egg (EG_{15}), produced heavier (P<0.05) chicks than those hatched from eggs on EG_{0-}, EG_{0+}, EG_5 or EG_{20} treatments. Eggs injected with 5 mg of glucose per egg (EG_5) produced chicks with higher (P<0.05) hatch-weights than those hatched from eggs on EG_{0-}, EG_{0+} or EG_{20} treatments. Eggs injected with 20 mg of glucose per egg (EG_{20}) and eggs not injected with anything (EG_{0-}) produced heavier (P<0.05) chicks than those hatched from eggs on EG_{0+} treatment. However, eggs not injected with anything (EG_{0-}) and those injected with 20 mg of glucose per egg (EG_{20}) produced chicks with similar (P>0.05) hatch weights.

Potchefstroom koekoek eggs injected with 10 mg of glucose per egg (EG_{10}) had higher (P<0.05) chick to egg weight ratio than those on EG_{0-}, EG_{0+}, EG_5, EG_{15} or EG_{20} treatments (Table 4.1). Eggs injected with 15 mg of glucose per egg (EG_{15}) had higher (P<0.05) chick to egg weight ratio than those on EG_{0-}, EG_{0+}, EG_5 or EG_{20} treatments. Eggs injected with 5 mg of glucose per egg (EG_5) had higher (P<0.05) chick to egg

weight ratio than those not injected with anything (EG_{0-}), from eggs injected with 0.1 ml of water per egg (EG_{0+}) or from eggs injected with 20 mg of glucose per egg (EG_{10}).

Hatchability, chick hatch-weight and chick to egg weight ratio of Potchefstroom koekoek chickens were optimized at *in ovo* glucose injection levels of 4.50 ($r^2 = 0.714$), 10.43 ($r^2 = 0.746$) and 12.00 ($r^2 = 0.710$) mg per egg, respectively (Figures 4.01, 4.02 and 4.03, respectively and Table 4.2).

Table 4.1 Effect of *in ovo* injection of glucose on egg weight (g/egg), hatchability (%), chick hatch-weight (g/chick) and chick to egg weight ratio of indigenous Potchefstroom koekoek chickens

	Treatment						
Variable	EG_{0-}	EG_{0+}	EG_5	EG_{10}	EG_{15}	EG_{20}	SEM
Egg weight	50.07	50.10	50.00	50.03	50.00	50.00	0.010
Hatchability	72.97^{c}	73.03^{c}	74.20^{b}	76.00^{a}	66.00^{d}	66.00^{d}	0.952
Chick hatch-wt	30.07^{d}	29.92^{e}	32.20^{c}	38.16^{a}	33.19^{b}	30.29^{d}	0.699
Chick to egg wt ratio	0.60^{d}	0.60^{d}	0.64^{c}	0.76^{a}	0.66^{b}	0.61^{d}	0.014

a, b, c, d, e : Means with different superscripts within a row are significantly different ($P<0.05$)

SEM : Standard error of the mean

$Y = 73.478 + 0.363\ x - 0.040x^2$

$r^2 = 0.714$

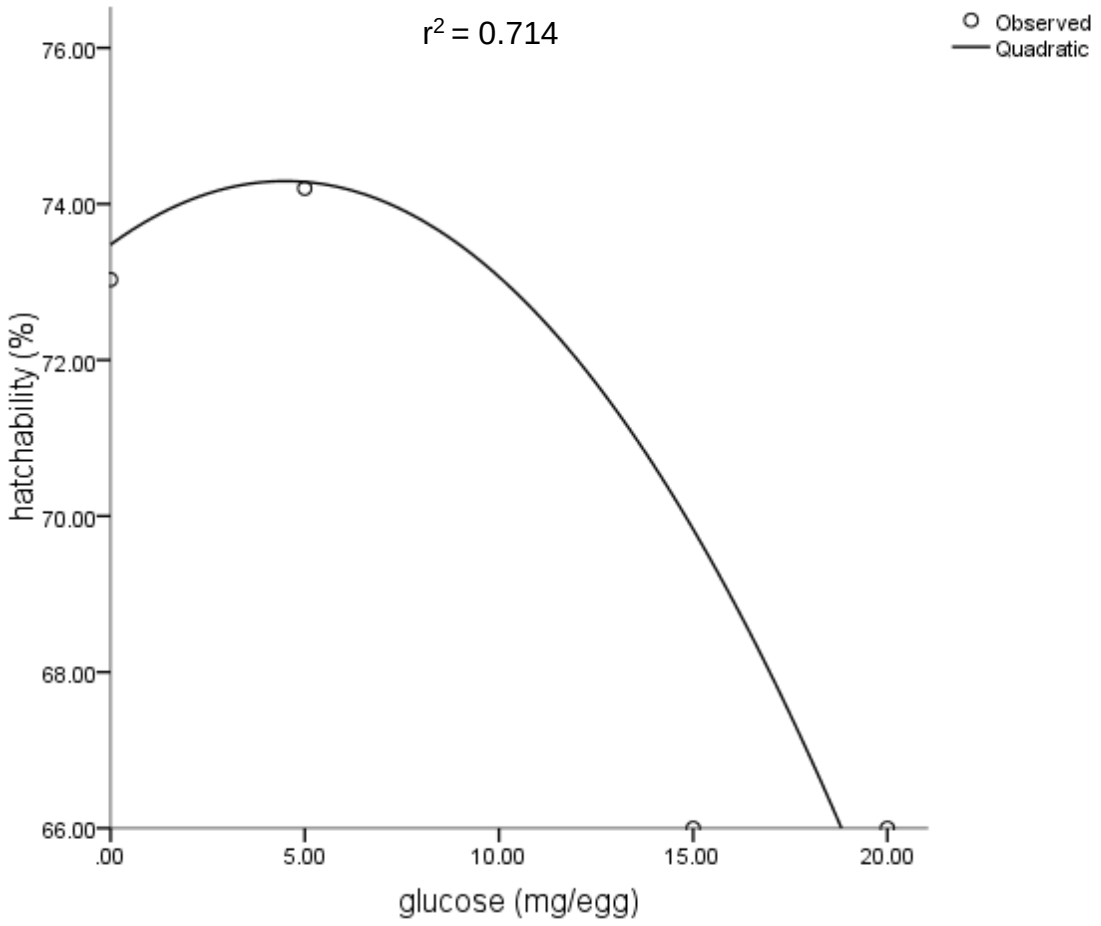

Figure 4.01 Effect of *in ovo* injection of glucose on hatchability of indigenous Potchefstroom koekoek eggs

$Y = 29.365 + 1.251x - 0.061x^2$

$r^2 = 0.746$

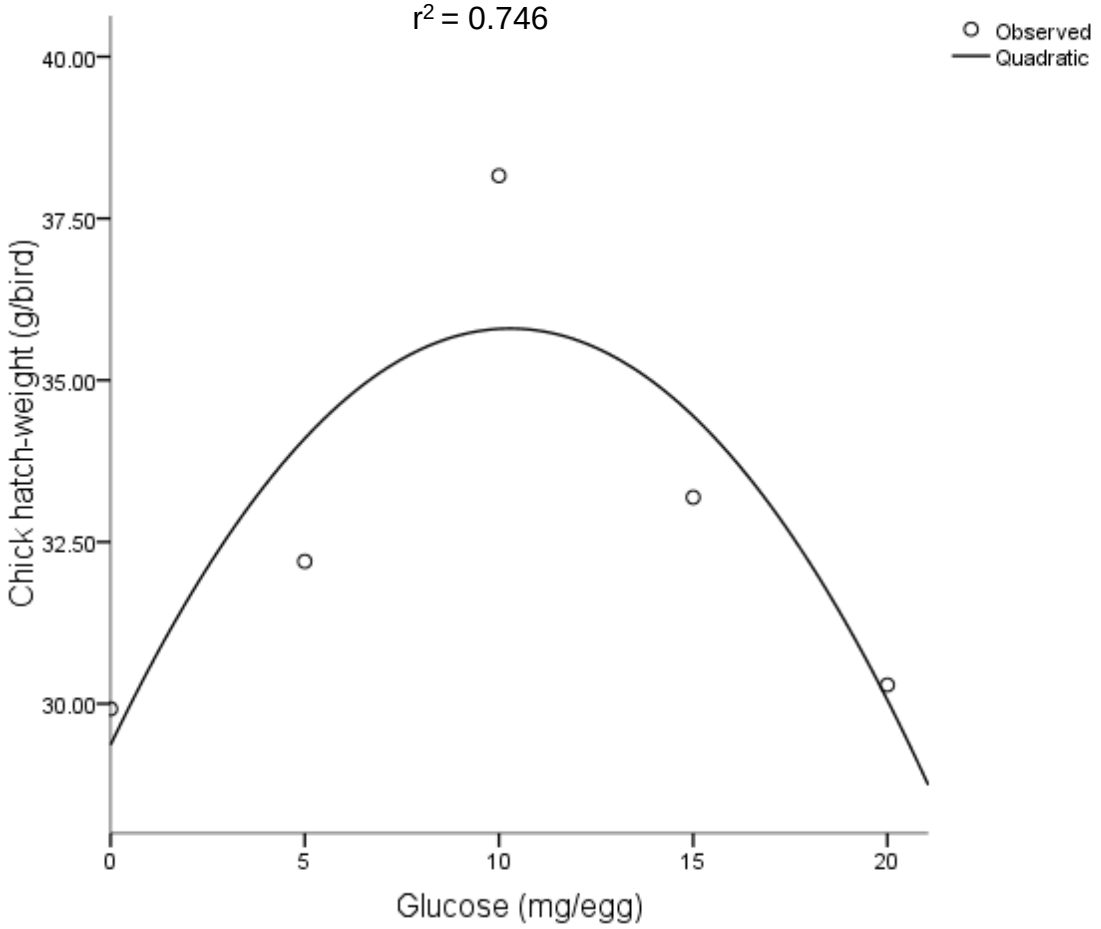

Figure 4.02 Effect of *in ovo* injection of glucose on chick hatch-weight of indigenous Potchefstroom koekoek chickens

$Y= 0.589 + 0.024x - 0.001x^2$

$r^2 = 0.710$

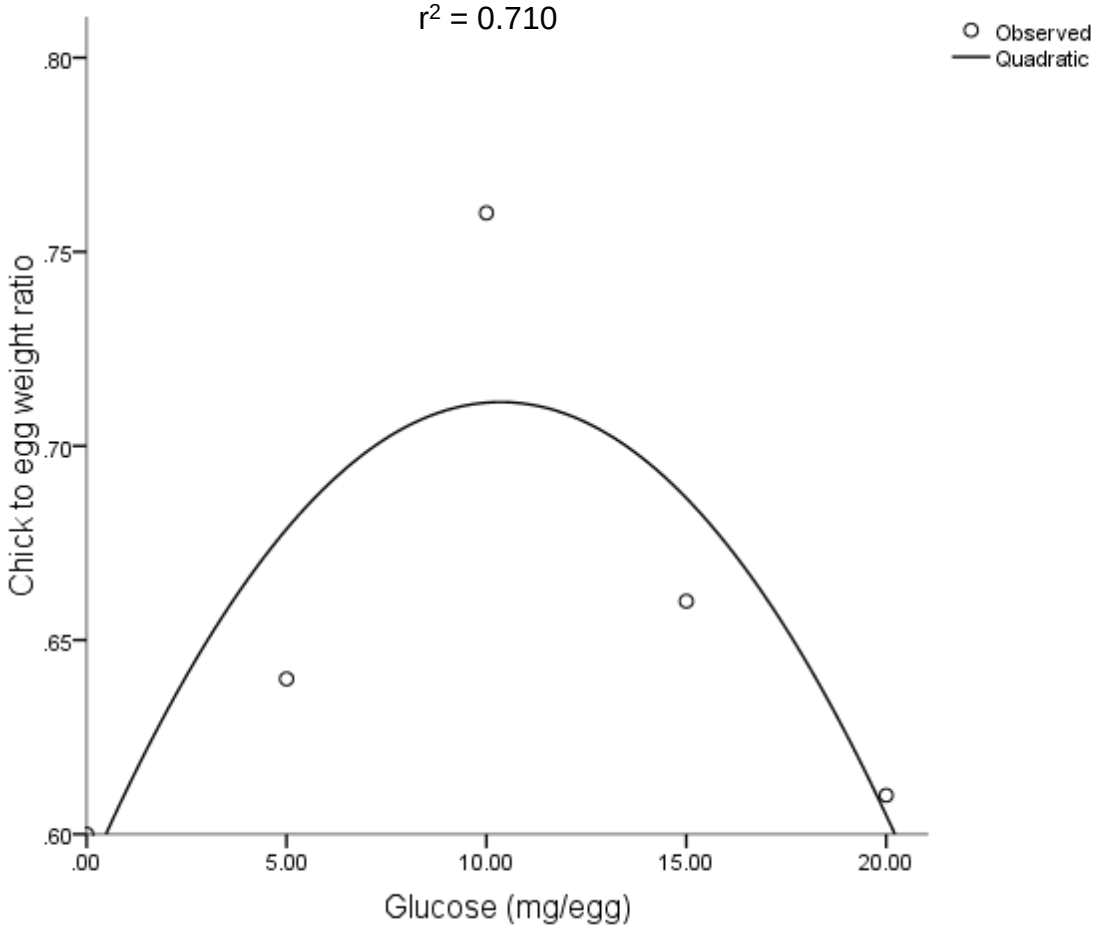

Figure 4.03 Effect of *in ovo* injection of glucose on chick to egg weight ratio of indigenous Potchefstroom koekoek chickens

Table 4.2 *In ovo* glucose injection levels for optimal egg hatchability (%), chick hatch-weight (g/chick) and chick to egg weight ratio of indigenous Potchefstroom koekoek chickens

Trait	Formula	Glucose	Y-Value	r^2	P
Hatchability (%)	$Y = 73.4 + 0.360x - 0.040x^2$	4.50	74.21	0.714	0.286
chick hatch-weight	$Y = 29.365 + 1.251x - 0.061x^2$	10.43	35.78	0.746	0.254
chick to egg weight ratio	$Y = 0.589 + 0.024x - 0.001x^2$	12.00	0.87	0.710	0.290

Glucose : *In ovo* glucose level for optimal variable
Y- Value : Optimal Y-Value
r^2 : Regression coefficient
P : Probability

4.2 Effect of *in ovo* injection of glucose on intake, growth, feed conversion ratio, apparent metabolisable energy intake, nitrogen retention and live weight of female Potchefstroom koekoek chickens aged one to 91 days

Results of the effect of *in ovo* injection of glucose on intake, growth, feed conversion ratio, apparent metabolisable energy intake, nitrogen retention and live weight of indigenous Potchefstroom koekoek chickens aged 1 to 49 days are presented in Table 4.3. Unsexed Potchefstroom koekoek chickens that hatched from eggs injected with 15 mg of glucose per egg (UCG_{15}) ate more ($P<0.05$) feed than those that hatched from eggs not injected with anything (UCG_{0-}), those from eggs injected with 0.1 ml of water per egg (UCG_{0+}), those from eggs injected with 5 mg of glucose per egg (UCG_5), those from eggs injected with 10 mg of glucose per egg (UCG_{10}) and those from eggs injected with 20 mg of glucose per egg (UCG_{20}). Potchefstroom koekoek chickens that hatched from eggs injected with 20 mg of glucose per egg (UCG_{20}) had higher ($P<0.05$) dry matter intake than those that hatched from eggs on UCG_{0-}, UCG_{0+}, UCG_5 or UCG_{10} treatments. Unsexed chickens that hatched from eggs injected with 0.1 ml of water per egg (UCG_{0+}) had higher ($P<0.05$) dry matter intakes than those that hatched from eggs on UCG_{0-}, UCG_5 or UCG_{10} treatments. The chickens that hatched

from eggs injected with 5 mg of glucose per egg (UCG_5) had higher ($P<0.05$) dry matter intakes than those that hatched from eggs on UCG_{0-} or UCG_{10} treatments. Similarly, unsexed chickens that hatched from eggs injected with 10 mg of glucose per egg (UCG_{10}) had higher ($P<0.05$) dry matter intakes than those that hatched from eggs not injected with anything (UCG_{0-}).

Unsexed Potchefstroom koekoek chickens that hatched from eggs injected with 10 mg of glucose per egg (UCG_{10}) had higher ($P<0.05$) growth rates than those that hatched from eggs on UCG_{0-}, UCG_{0+}, UCG_5, UCG_{15} or UCG_{20} treatments (Table 4.3). Potchefstroom koekoek chickens that hatched from eggs injected with 5 mg of glucose per egg (UCG_5) had better ($P<0.05$) growth rates than those that hatched from eggs on UCG_{0-}, UCG_{0+}, UCG_{15} or UCG_{20} treatments. The chickens that hatched from eggs injected with 0.1 ml of water per egg had higher ($P<0.05$) growth rates than those that hatched from eggs on UCG_{0-}, UCG_{15} or UCG_{20} treatments. Unsexed Potchefstroom koekoek chickens that hatched from eggs not injected with anything had higher ($P<0.05$) growth rates than those that hatched from eggs on UCG_{15} or UCG_{20} treatments. However, unsexed chickens that hatched from eggs injected with 20 mg of glucose per egg (UCG_{20}) and those that hatched from eggs injected with 15 mg of glucose per egg (UCG_{15}) had similar ($P>0.05$) growth rates.

Potchefstroom koekoek chickens that hatched from eggs injected with 10 mg of glucose per egg (UCG_{10}) had better ($P<0.05$) feed conversion ratio than those that hatched from eggs on UCG_{0-}, UCG_{0+}, UCG_5, UCG_{15} or UCG_{20} treatments (Table 4.3). Unsexed chickens that hatched from eggs injected with 5 mg of glucose per egg (UCG_5) had better ($P<0.05$) feed conversion ratio values than those that hatched from eggs on UCG_{0-}, UCG_{0+}, UCG_{15} or UCG_{20} treatments. Unsexed chickens that hatched from eggs injected with 0.1 ml of water per egg (UCG_{0+}) and those that hatched from eggs not injected with anything (UCG_{0-}) had better ($P<0.05$) feed conversion ratio values than those that hatched from eggs on UCG_{15} or UCG_{20} treatments. Unsexed chickens that hatched from eggs injected with 20 mg of glucose per egg (UCG_{20}) had better ($P<0.05$) feed conversion ratio than those that hatched from eggs injected with 15 mg of glucose per egg (UCG_{15}). However, chickens that hatched from eggs injected

with 0.1 ml of water per egg and those that hatched from eggs not injected with anything (UCG_{15}) had a similar ($P>0.05$) feed conversion ratio.

Unsexed Potchefstroom koekoek chickens that hatched from eggs injected with 10 mg of glucose per egg (UCG_{10}) and those that hatched from eggs injected with 5 mg of glucose per egg (UCG_5) had higher ($P<0.05$) live weights at 49 days of age than those that hatched from eggs on UCG_{0-}, UCG_{0+}, UCG_{15} or UCG_{20} treatments (Table 4.3). Potchefstroom koekoek chickens that hatched from eggs injected with 0.1 ml of water per egg (UCG_{0+}) had higher ($P<0.05$) live weights than those that hatched from eggs on UCG_{0-}, UCG_{15} or UCG_{20} treatments. Similarly, unsexed chickens that hatched from eggs not injected with anything (UCG_{0-}) had higher ($P<0.05$) live weights than those that hatched from eggs injected with 15 mg of glucose per egg (UCG_{15}) or those from eggs injected with 20 mg of glucose per egg (UCG_{20}). However, chickens that hatched from eggs injected with 15 mg of glucose per egg (UCG_{15}) and those that hatched from eggs injected with 20 mg of glucose per egg (UCG_{20}) had similar ($P>0.05$) live weights.

Potchefstroom koekoek chickens that hatched from eggs injected with 5 mg of glucose per egg (UCG_5) had higher ($P<0.05$) metabolisable energy (ME) intakes at 7 weeks of age than those that hatched from eggs on UCG_{0-}, UCG_{0+}, UCG_{10}, UCG_{15} or UCG_{20} treatments (Table 4.3). Unsexed chickens that hatched from eggs not injected with anything (UCG_{0-}) had higher ($P<0.05$) ME intakes than those that hatched from eggs on UCG_{0+}, UCG_{10}, UCG_{15} or UCG_{20} treatments. The chickens that hatched from eggs injected with 0.1 ml of water per egg (UCG_{0+}) and those that hatched from eggs injected with 15 mg of glucose per egg (UCG_{15}) had higher ($P<0.05$) ME intakes than those that hatched from eggs on UCG_{10} or UCG_{20} treatments. However, chickens that hatched from eggs injected with 0.1 ml of water per egg (UCG_{0+}) and those that hatched from eggs injected with 15 mg of glucose per egg (UCG_{15}) had similar ($P>0.05$) ME intakes. Unsexed chickens that hatched from eggs injected with 20 mg of glucose per egg (UCG_{20}) had higher ($P<0.05$) ME than those that hatched from eggs injected with 10 mg of glucose per egg (UCG_{10}).

Unsexed Potchefstroom koekoek chickens that hatched from eggs injected with 15 mg of glucose per egg (UCG_{15}) had higher ($P<0.05$) nitrogen retention values than those

that hatched from eggs on UCG_{0-}, UCG_{0+}, UCG_5, UCG_{10} or UCG_{20} treatments (Table 4.3). Potchefstroom koekoek chickens that hatched from eggs not injected with anything (UCG_{0-}) and those that hatched from eggs injected with 5 mg of glucose per egg (UCG_5) had higher ($P<0.05$) nitrogen retention values than those that hatched from eggs on UCG_{0+}, UCG_{10} or UCG_{20} treatments. Unsexed chickens that hatched from eggs injected with 10 mg of glucose per egg (UCG_{10}) and those that hatched from eggs injected with 20 mg of glucose per egg (UCG_{20}) had higher ($P<0.05$) nitrogen retention values than those that hatched from eggs not injected with anything. However, chickens that hatched from eggs injected with 10 or 20 mg of glucose per egg (UCG_{20}) had similar ($P>0.05$) nitrogen retention values (Table 4.3).

A positive relationship was observed between *in ovo* glucose injection level and feed intake of unsexed Potchefstroom koekoek chickens aged 1 to 49 days (Figure 4.04). There was, also, a positive relationship between *in ovo* glucose injection level and feed conversion ratio of unsexed Potchefstroom koekoek chickens aged 1 to 49 days (Figure 4.05). Growth rate, live weight, ME intake and nitrogen retention of unsexed Potchefstroom koekoek chickens were optimized at *in ovo* glucose injection levels of 3.92 ($r^2 = 0.713$), 4.36 ($r^2 = 0.729$), 10.67 ($r^2 = 0.627$) and 13.50 ($r^2 = 0.526$) mg per egg, respectively (Figures 4.06, 4.07, 4.08 and 4.09, respectively and Table 4.4). There were no deaths observed during this part of the study.

Table 4.3 Effect of *in ovo* injection of glucose on feed intake (g DM//bird/day), growth rate (g/bird/day), feed conversion ratio (FCR) (g DM feed/g weight gain/bird), live weight (g/bird aged 49 days), apparent metabolisable energy intake (ME) (MJ ME/kg

DM) and nitrogen retention (g/bird/day) of indigenous Potchefstroom koekoek chickens aged 1 to 49 days

	Treatment						
Variable	UCG_{0-}	UCG_{0+}	UCG_5	UCG_{10}	UCG_{15}	UCG_{20}	SEM
Feed intake	45^{f}	57^{c}	54^{d}	51^{e}	72^{a}	67^{b}	2.262
Growth rate	10.2^{d}	12.7^{c}	13.7^{b}	14.0^{a}	8.9^{e}	9.2^{e}	0.502
FCR	4.4^{c}	4.6^{c}	4.0^{d}	3.6^{e}	8.1^{a}	7.3^{b}	0.416
Live weight	565^{c}	651^{b}	701^{a}	720^{a}	470^{d}	473^{d}	25.0
ME	9.7^{b}	9.6^{c}	9.9^{a}	9.4^{e}	9.6^{c}	9.5^{d}	0.037
N-retention	1.5^{b}	1.3^{d}	1.5^{b}	1.4^{c}	1.6^{a}	1.4^{c}	0.021

a, b, c, d, e, f : Means with different superscripts within a row are significantly different ($P<0.05$)

SEM : Standard error of the mean

$Y = 0.765x + 52.592$

$r^2 = 0.443$

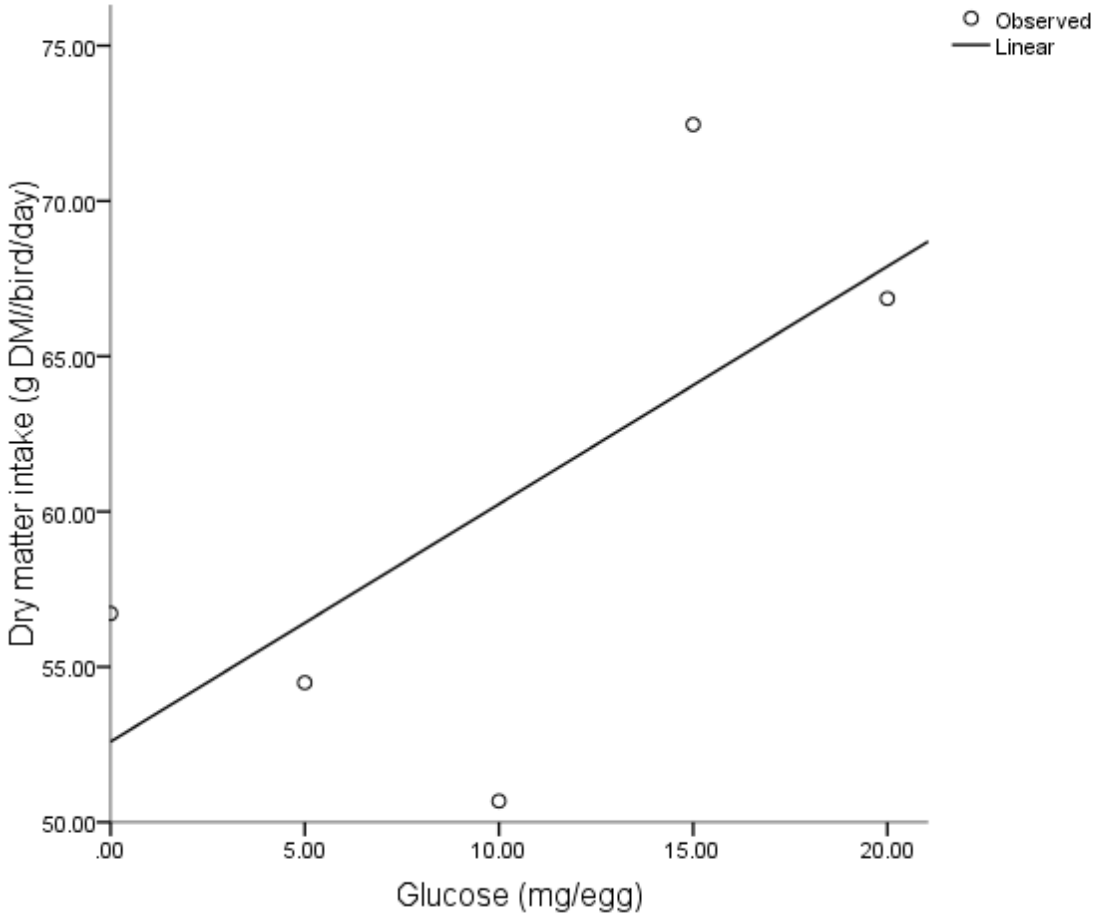

Figure 4.04 Relationship between *in ovo* injection of glucose level and dry matter intake of indigenous Potchefstroom koekoek chickens aged 1 to 49 days

Y = 0.189x + 3.644

r^2 = 0.539

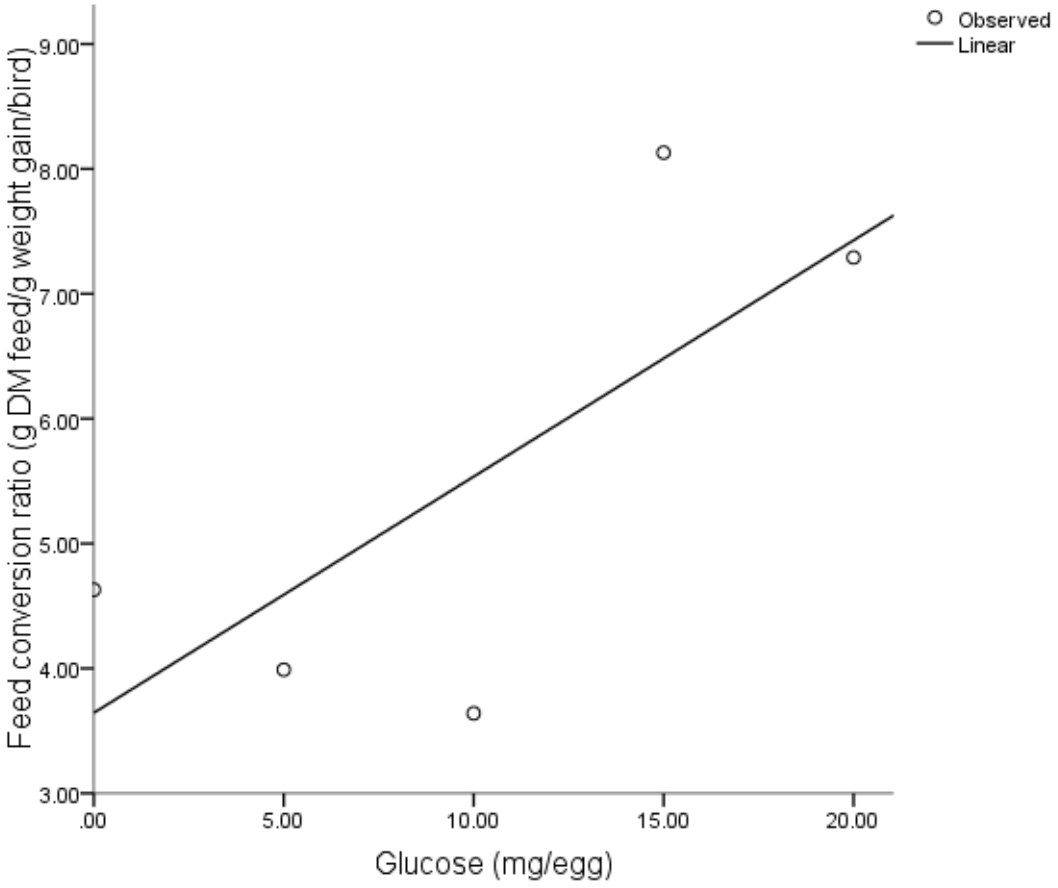

Figure 4.05 Relationship between *in ovo* injection of glucose level and feed conversion ratio of indigenous Potchefstroom koekoek chickens aged 1 to 49 days

$Y = 13.052 + 0.149x - 0.019x^2$

$r^2 = 0.713$

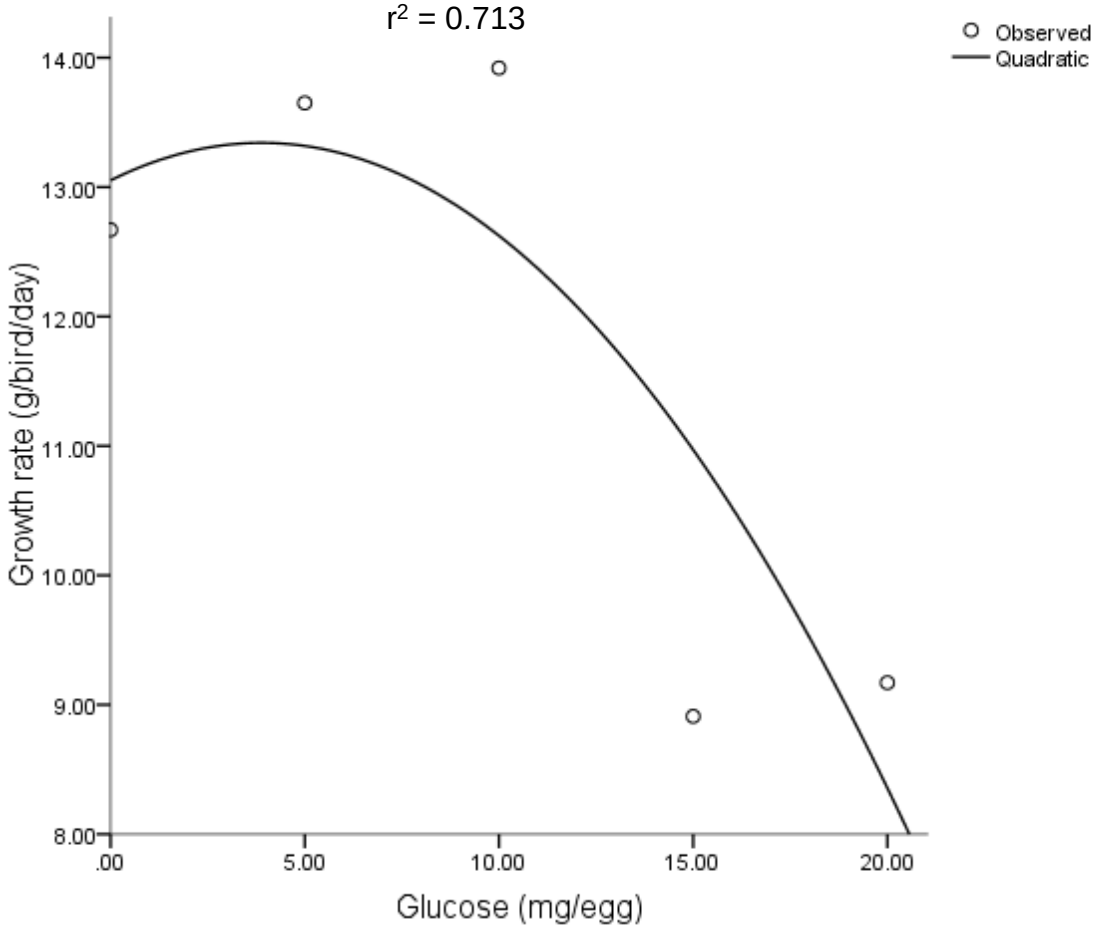

Figure 4.06 Effect of *in ovo* injection of glucose on growth rate of indigenous Potchefstroom koekoek chickens aged 1 to 49 days

$Y = 668.091 + 9.087x - 1.041x^2$

$r^2 = 0.729$

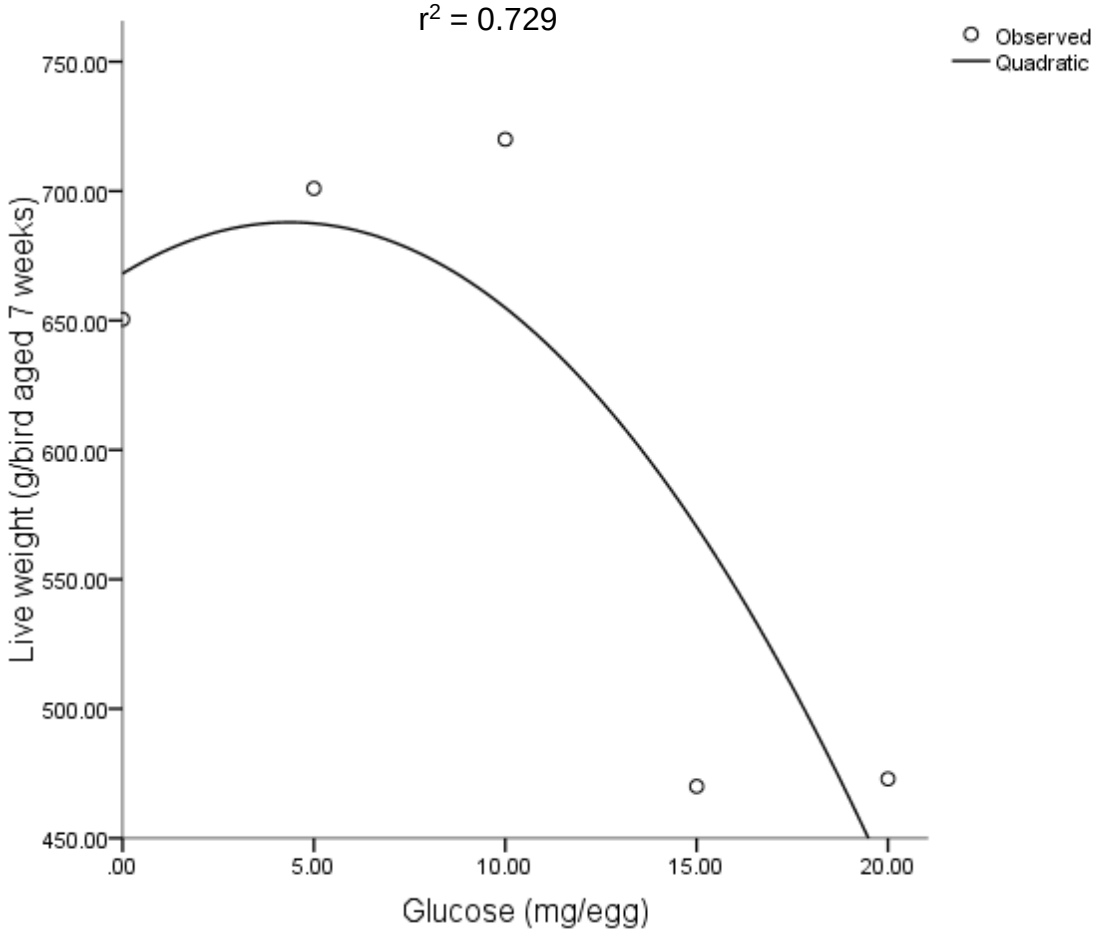

Figure 4.07 Effect of *in ovo* injection of glucose on live weight of indigenous Potchefstroom koekoek chickens aged 49 days

$Y = 9.477 + 0.064x - 0.003x_2$

$r^2 = 0.627$

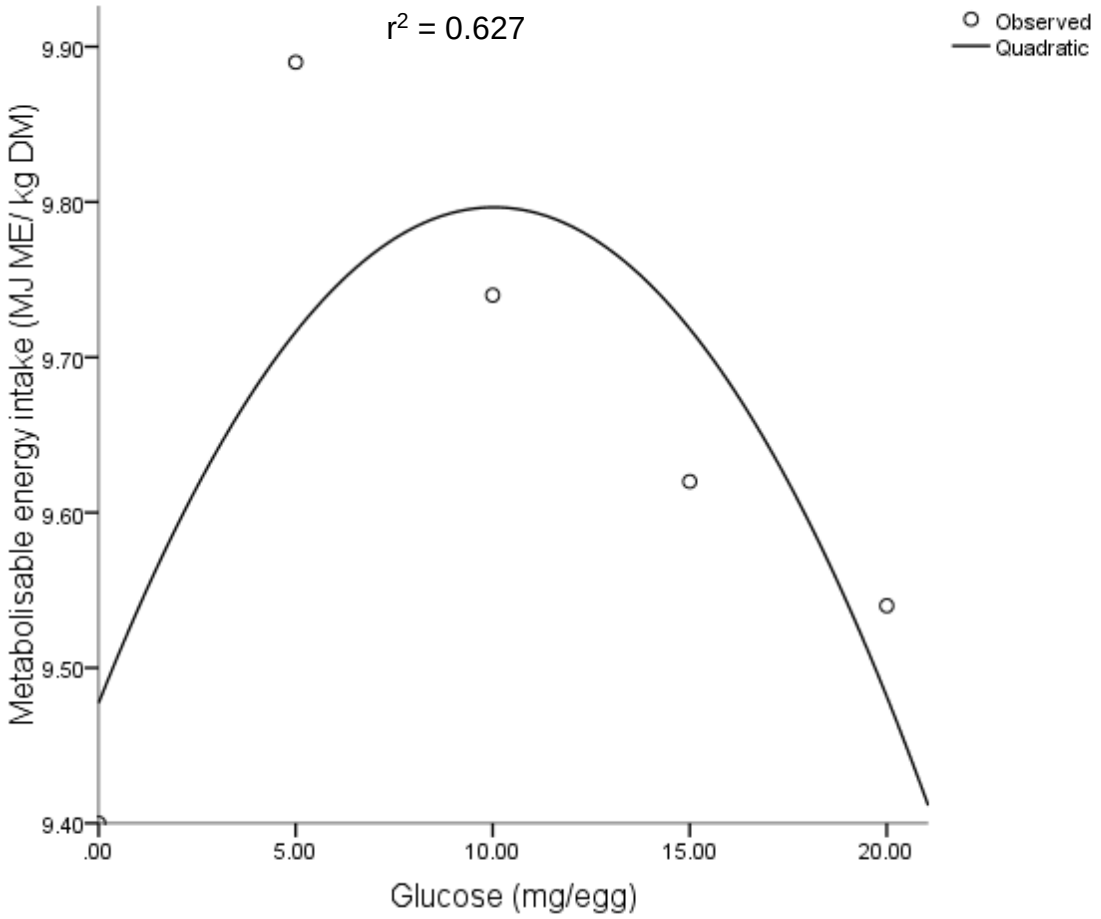

Figure 4.08 Effect of *in ovo* injection of glucose on metabolisable energy intake of indigenous Potchefstroom koekoek chickens aged 7 weeks

$Y = 1.313 + 0.027x - 0.001x^2$

$r^2 = 0.526$

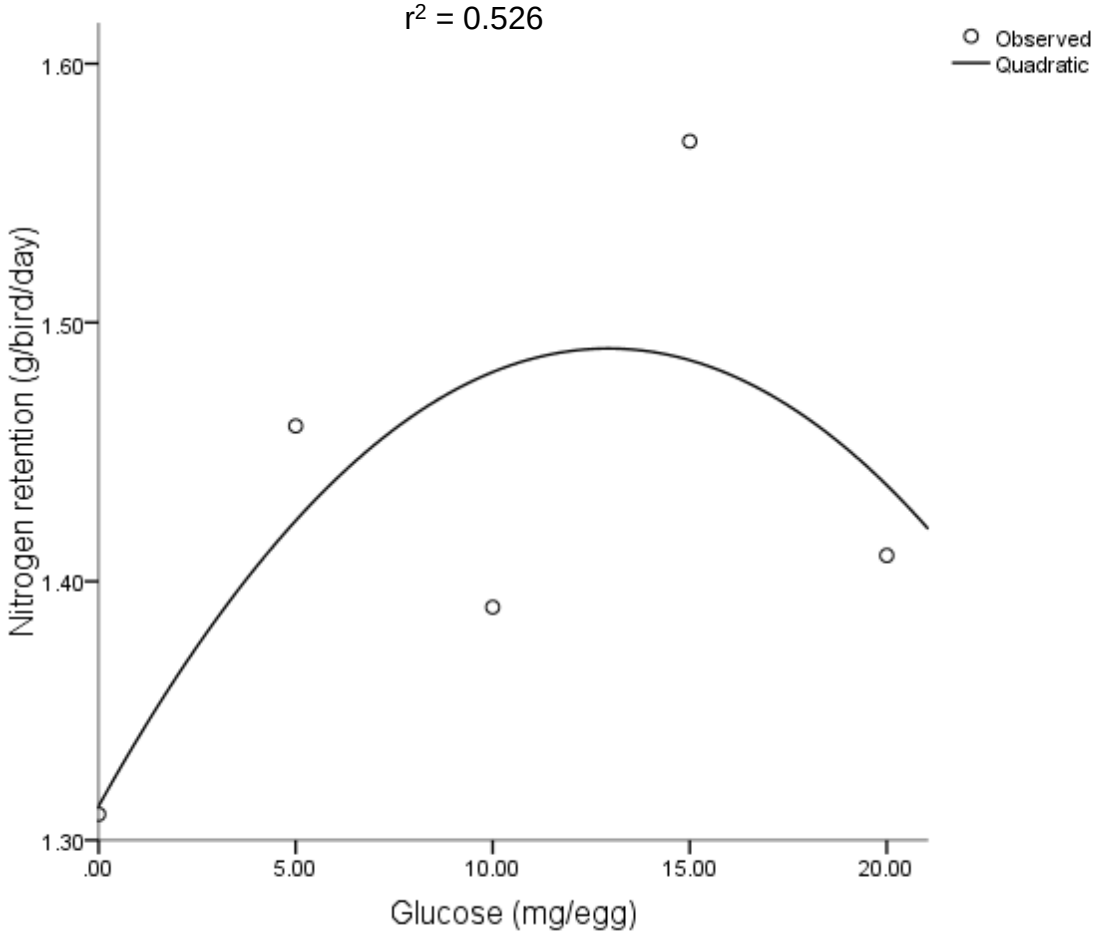

Figure 4.09 Effect of *in ovo* injection of glucose on nitrogen retention in indigenous Potchefstroom koekoek chickens aged 7 weeks

Table 4.4 *In ovo* glucose injection levels for optimal growth rate (g/bird/day), live weight (g/bird aged 49 days) and nitrogen retention (g/bird/day) of indigenous Potchefstroom koekoek chickens aged 1 to 49 days

Trait	Formula	Glucose	Y-Value	r^2	P
Growth rate	$Y = 13.052 + 0.149x - 0.019x^2$	3.92	13.34	0.713	0.287
Live weight	$Y = 668.091 + 9.087x - 1.041x^2$	4.36	688	0.729	0.271
ME	$Y = 9.477 + 0.064x - 0.003x^2$	10.67	9.82	0.627	0.373
N-ret	$Y = 1.313 + 0.027x - 0.001x^2$	13.50	1.49	0.526	0.474

Glucose : *In* ovo glucose level for optimal variable

Y- Value : Optimal Y-Value

r^2 : Regression coefficient

P : Probability

Results of the effect of *in ovo* injection of glucose on intake, growth, feed conversion ratio, apparent metabolisable energy intake, nitrogen retention and live weight of female Potchefstroom koekoek chickens aged 50 to 91 days are presented in Table 4.5. Female Potchefstroom koekoek chickens that hatched from eggs injected with 20 mg of glucose per egg ate (FCG_{20}) more ($P<0.05$) feed than those that hatched from eggs not injected with anything (FCG_{0-}), those from eggs injected with 0.1 ml of water per egg (FCG_{0+}), those from eggs injected with 5 mg of glucose per egg (FCG_5), those from eggs injected with 10 mg of glucose per egg (FCG_{10}) or those from eggs injected with 15 mg of glucose per egg (FCG_{15}). Female chickens that hatched from eggs injected with 5 mg of glucose per egg (FCG_5) had higher ($P<0.05$) dry matter intake than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_{10} or FCG_{15} treatments. Female Potchefstroom chickens hatched from eggs injected with 10 mg of glucose per egg (FCG_{10}) had higher ($P<0.05$) dry matter intakes than those that hatched from eggs on FCG_{0-}, FCG_{0+} or FCG_{15} treatments. Female chickens that hatched from eggs injected with 0.1 ml of water per egg (FCG_{0+}) had higher ($P<0.05$) dry matter intakes than those that hatched from eggs on FCG_{0-} or FCG_{15} treatments. Similarly, female chickens hatched from eggs injected with 15 mg of glucose per egg (FCG_{15}) had higher ($P<0.05$) dry matter intakes than those that hatched from eggs not injected with anything (FCG_{0-}).

Female Potchefstroom koekoek chickens that hatched from eggs injected with 20 mg of glucose per egg (FCG_{20}) had higher ($P<0.05$) growth rates than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_5, FCG_{10} or FCG_{15} treatments (Table 4.5). Female chickens that hatched from eggs not injected with anything (FCG_{0-}) or those that hatched from eggs injected with 5 mg of glucose per egg (FCG_5) had higher ($P<0.05$) growth rates than those that hatched from eggs on FCG_{0+}, FCG_{10} or FCG_{15} treatments. However, growth rates of female chickens that hatched from eggs not injected with anything (FCG_{0-}) or those that hatched from eggs injected with 5 mg of glucose per egg (FCG_5) were similar ($P>0.05$). Female chickens hatched from eggs injected with 0.1 ml of water per egg (FCG_{0+}) had higher ($P<0.05$) growth rates than those that hatched from eggs on FCG_{10} or FCG_{15} treatments. Similarly, female chickens that hatched from eggs injected with 10 mg of glucose per egg (FCG_{10}) had higher ($P<0.05$) growth rates than those that hatched from eggs injected with 15 mg of glucose per egg (FCG_{15}).

Female Potchefstroom koekoek chickens that hatched from eggs not injected with anything had better ($P<0.05$) feed conversion ratio than those that hatched from eggs on FCG_{0+}, FCG_5, FCG_{10}, FCG_{15} or FCG_{20} treatments (Table 4.5). Female chickens that hatched from eggs injected with 0.1 mg of water per egg (FCG_{0+}) had a better ($P<0.05$) feed conversion ratio than those that hatched from eggs on FCG_5, FCG_{10}, FCG_{15} or FCG_{20} treatments. Female chickens that hatched from eggs injected with 20 mg of glucose per egg (FCG_{20}) had better ($P<0.05$) feed conversion ratio values than those that hatched from eggs on FCG_5, FCG_{10} or FCG_{15} treatments. Female chickens that hatched from eggs injected with 5 mg of glucose per egg (FCG_{20}) had better ($P<0.05$) feed conversion ratio than those that hatched from eggs on FCG_{10} or FCG_{15} treatments. Similarly, female chickens that hatched from eggs injected with 10 mg of glucose per egg had a better ($P<0.05$) feed conversion ratio than those that hatched from eggs injected with 15 mg of glucose per egg (FCG_{15}).

Potchefstroom koekoek chickens that hatched from eggs injected with 5 mg of glucose per egg (FCG_5) had higher ($P<0.05$) live weights at 91 days of age than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_{10}, FCG_{15} or FCG_{20} treatments (Table 4.5). Female chickens that hatched from eggs injected with 20 mg of glucose per egg

(FCG_{20}) had higher ($P<0.05$) live weights than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_{10} or FCG_{15} treatments. Similarly, female Potchefstroom chickens that hatched from eggs not injected with anything (FCG_{0-}) had higher ($P<0.05$) live weights than those that hatched from eggs on FCG_{0+}, FCG_{10} or FCG_{15} treatments. Female chickens that hatched from eggs injected with 15 mg of glucose per egg (FCG_{15}) had higher ($P<0.05$) live weights than those that hatched from eggs on FCG_{0+} or FCG_{10} treatments. Similarly, female chickens that hatched from eggs injected with 10 mg of glucose per egg (FCG_{10}) had higher ($P<0.05$) live weights than those that hatched from eggs injected with 0.1 ml of water per egg (FCG_{0+}).

Female Potchefstroom koekoek chickens that hatched from eggs injected with 20 mg of glucose per egg (FCG_{20}) had higher ($P<0.05$) metabolisable energy (ME) intakes at 13 weeks of age than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_{5}, FCG_{10} or FCG_{15} treatments (Table 4.5). Female chickens that hatched from eggs injected with 10 mg of glucose per egg (FCG_{10}) had higher ($P<0.05$) ME intakes than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_{5} or FCG_{15} treatments. Chickens that hatched from eggs injected with 0.1 ml of water per egg (FCG_{0+}) had higher ($P<0.05$) ME intakes than those that hatched from eggs on FCG_{0-}, FCG_{5} or FCG_{15} treatments. Female chickens that hatched from eggs injected with 5 mg of glucose per egg (FCG_{5}) had higher ($P<0.05$) ME intakes than those that hatched from eggs on FCG_{0-} or FCG_{15} treatments. Similarly, female chickens that hatched from eggs not injected with anything (FCG_{0-}) had higher ($P<0.05$) ME intakes than those that hatched from eggs injected with 15 mg of glucose per egg (FCG_{15}).

Potchefstroom koekoek chickens that hatched from eggs injected with 5 mg of glucose per egg (FCG_{5}) had higher ($P<0.05$) nitrogen retention values at 13 weeks of age than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_{10}, FCG_{15} or FCG_{20} treatments (Table 4.5). Female chickens that hatched from eggs injected with 0.1 ml of water per egg (FCG_{0+}) or those injected with 10 mg of glucose per egg had higher ($P<0.05$) nitrogen retention values than those that hatched from eggs on FCG_{0-}, FCG_{15} or FCG_{20} treatments. However, female chickens that hatched from eggs injected with 0.1 ml of water per egg (FCG_{0+}) or 10 mg of glucose per egg (FCG_{10}) had similar ($P>0.05$) nitrogen retention values. Female chickens that hatched from eggs not injected with

anything had higher (P<0.05) nitrogen retention values than those that hatched from eggs on FCG_{15} or FCG_{20} treatments. Similarly, female chickens that hatched from eggs injected with 20 mg of glucose per egg (FCG_{20}) had higher (P<0.05) nitrogen retention values than those that hatched from eggs injected with 15 mg of glucose per egg (FCG_{15}) (Table 4.5).

Feed conversion ratio and ME intakes of female Potchefstroom koekoek chickens were optimized at *in ovo* glucose injection levels of 12.15 (r^2 = 0.847) and 5.57 (r^2 = 0.996) mg per egg, respectively (Figures 4.10 and 4.11, respectively and Table 4.6). There were no deaths during this part of the study.

Table 4.5 Effect of *in ovo* injection of glucose on feed intake (g DM//bird/day), growth rate (g/bird/day), feed conversion ratio (FCR) (g DM feed/g weight gain/bird), live weight (g/bird aged 91 days), apparent metabolisable energy intake (ME) (MJ ME/kg DM) and nitrogen retention (g/bird/day) of indigenous Potchefstroom koekoek chickens aged 50 to 91 days

	Treatment						
Variable	FCG_{0-}	FCG_{0+}	FCG_{5}	FCG_{10}	FCG_{15}	FCG_{20}	SEM
Intake	93^{f}	104^{d}	116^{b}	112^{c}	102^{e}	118^{a}	2.064
Growth	12.6^{b}	12.0^{c}	12.5^{b}	11.3^{d}	10.0^{e}	12.9^{a}	0.237
FCR	7.4^{f}	8.7^{e}	9.3^{c}	9.9^{b}	10.2^{a}	9.1^{d}	0.216
Lwt	1125^{c}	1045^{f}	1244^{a}	1068^{e}	1094^{d}	1214^{b}	18.0
ME	8.8^{e}	9.2^{c}	9.1^{d}	9.4^{b}	8.0^{f}	10.0^{a}	0.144
N-ret.	1.2^{c}	1.3^{b}	1.4^{a}	1.3^{b}	0.8^{e}	1.1^{d}	0.047

a, b, c, d, e, f : Means with different superscripts within a row are significantly different (P<0.05)

SEM : Standard error of the mean

$Y = 8.576 + 0.243x - 0.010x^2$

$r^2 = 0.847$

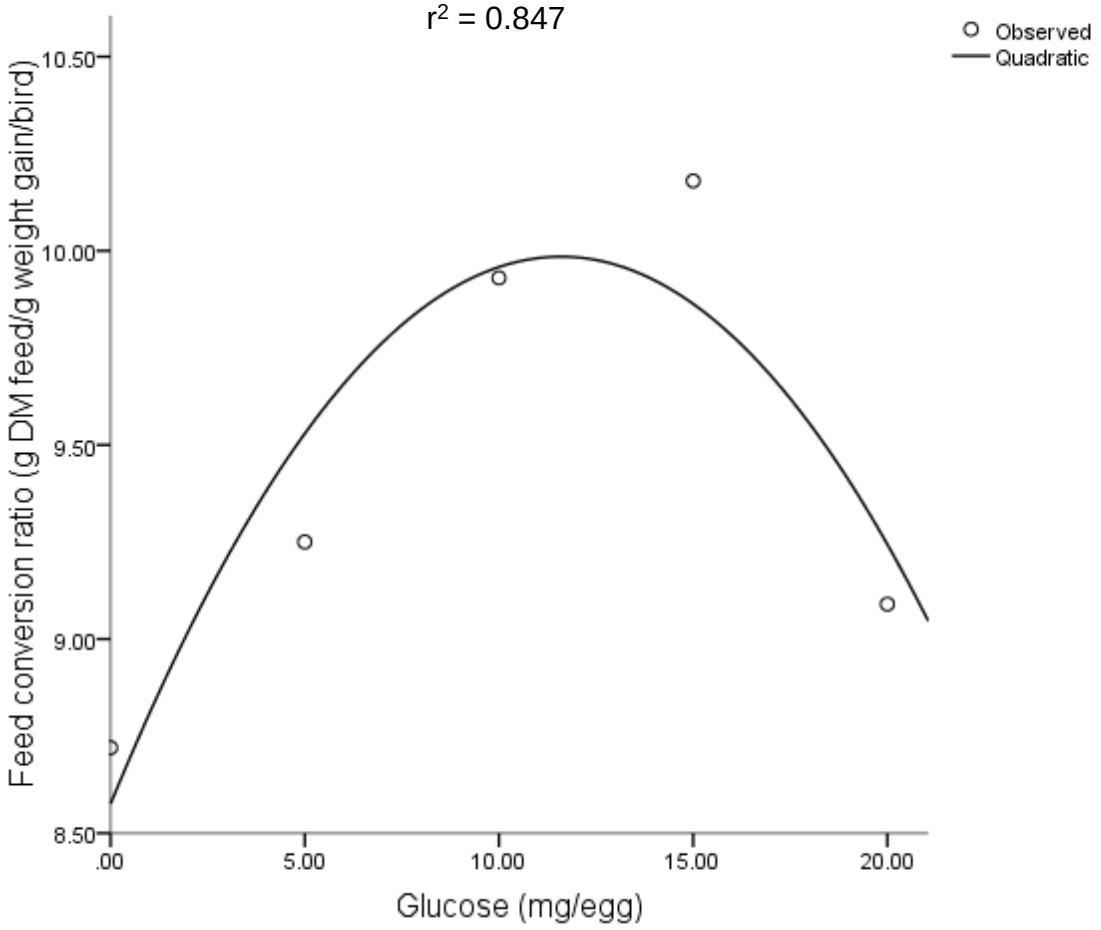

Figure 4.10 Effect of *in ovo* injection of glucose on feed conversion ratio of indigenous Potchefstroom koekoek chickens aged 8 to 13 weeks

$Y = 9.114 + 0.078x - 0.007x^2$

$r^2 = 0.996$

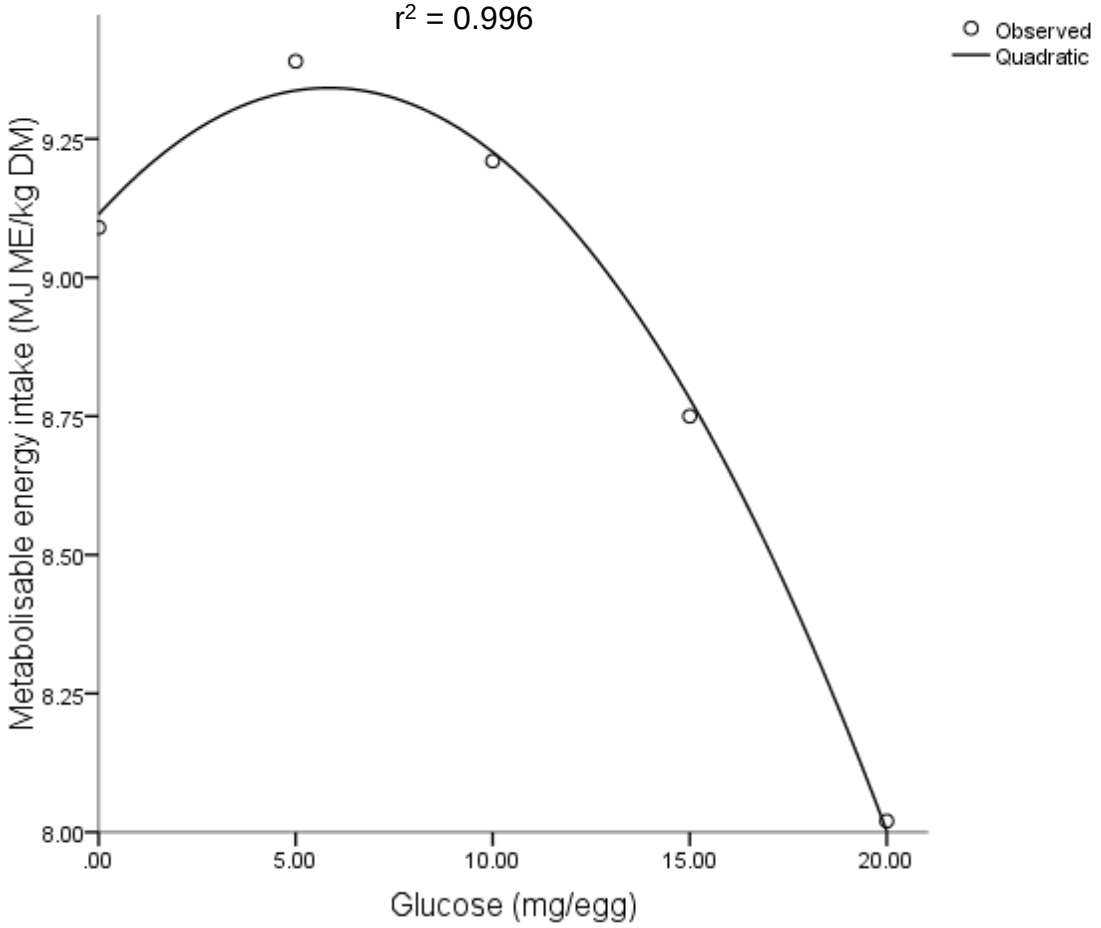

Figure 4.11 Effect of *in ovo* injection of glucose on metabolisable energy intake of indigenous Potchefstroom koekoek chickens aged 13 weeks

Table 4.6 *In ovo* glucose injection levels for optimal feed conversion ratio (FCR) and metabolisable energy (ME) intake of indigenous Potchefstroom koekoek chickens aged 50 to 91 days

Trait	Formula	Glucose	Y-Value	r^2	P
FCR	$Y = 8.576 + 0.243x - 0.010x^2$	12.15	10.05	0.847	0.153
ME	$Y = 9.114 + 0.078x - 0.007x^2$	5.57	9.33	0.996	0.004

Glucose : *In* ovo glucose level for optimal variable

Y- Value : Optimal Y-Value

r^2 : Regression coefficient

P : Probability

4.3 Effect of *in ovo* injection of glucose on carcass weight and carcass parts of Potchefstroom koekoek chickens aged 91 days

The effects of *in ovo* glucose injection on carcass, breast, drum, thigh, wing, gizzard, liver and heart weights of female Potchefstroom koekoek chickens aged 91 days are presented in Table 4.7. Female Potchefstroom koekoek chickens that hatched from eggs injected with 5 mg of glucose per egg had higher ($P<0.05$) carcass weights than those that hatched from eggs not injected with anything (FCG_{0-}), those from eggs injected with 0.1 ml of water per egg (FCG_{0+}), those from eggs injected with 10 mg of glucose per egg (FCG_{10}), those from eggs injected with 15 mg of glucose per egg (FCG_{15}) or those from eggs injected with 20 mg of glucose per egg (FCG_{20}). Female chickens that hatched from eggs injected with 10 mg of glucose per egg or eggs injected with 20 mg of glucose per egg had higher ($P<0.05$) carcass weights than those that hatched from eggs on FCG_{0-}, FCG_{0+} or FCG_{15} treatments. However, female chickens that hatched from eggs injected with 10 mg of glucose per egg or those that hatched from eggs injected with 20 mg of glucose per egg had similar ($P>0.05$) carcass weights. Female chickens hatched from eggs not injected with anything or those that hatched from eggs injected with 0.1 ml of water per egg had higher ($P<0.05$) carcass weights than those that hatched from eggs on an FCG_{15} treatment. Female chickens that hatched from eggs not injected with anything or those that hatched from eggs injected with 0.1 ml of water per egg had similar ($P>0.05$) carcass weights.

Potchefstroom koekoek chickens that hatched from eggs injected with 20 mg of glucose per egg had higher (P<0.05) breast meat weights than those that hatched from eggs on FCG_{0-}, $FCG_{0+,}$ FCG_5, FCG_{10} or FCG_{15} treatments (Table 4.7). Female chickens that hatched from eggs injected with 5 mg of glucose per egg had higher (P<0.05) breast meat weights than those that hatched from eggs on FCG_{0-}, $FCG_{0+,}$ FCG_{10} or FCG_{15} treatments. Female chickens that hatched from eggs not injected with anything and those from eggs injected with 10 or 15 mg of glucose per egg had higher (P<0.05) breast meat weights than those that hatched from eggs on an FCG_{0+} treatment. However, female chickens that hatched from eggs not injected with anything and those that hatched from eggs injected with 10 or 15 mg of glucose per egg had similar (P>0.05) breast meat weights.

The chickens that hatched from eggs injected with 20 mg of glucose per egg had higher (P<0.05) drum stick weights than those that hatched from eggs on FCG_{0-}, $FCG_{0+,}$ FCG_5, FCG_{10} or FCG_{15} treatments (Table 4.7). Female chickens that hatched from eggs injected with 5 mg of glucose per egg had higher (P<0.05) drum stick weights than those that hatched from eggs on FCG_{0-}, $FCG_{0+,}$ FCG_{10} or FCG_{15} treatments. The chickens that hatched from eggs not injected with anything and those injected with 10 or 15 mg of glucose per egg had higher (P<0.05) drum stick weights than those that hatched from eggs on an FCG_{0+} treatment. However, female chickens that hatched from eggs not injected with anything and those that hatched from eggs injected with 10 or 15 mg of glucose per egg had similar (P>0.05) drum stick weights.

Potchefstroom koekoek female chickens that hatched from eggs injected with 20 mg of glucose per egg had higher (P<0.05) thigh weights than those that hatched from eggs on FCG_{0-}, $FCG_{0+,}$ FCG_5, FCG_{10} or FCG_{15} treatments (Table 4.7). Female chickens that hatched from eggs injected with 5 or 15 mg of glucose per egg had higher (P<0.05) thigh weights than the chickens from eggs on FCG_{0+} or FCG_{10} treatments. However, female chickens that hatched from eggs not injected with anything and those that hatched from eggs injected with 5 or 15 mg of glucose per egg had similar (P>0.05) thigh weights. Similarly, female chickens that hatched from eggs not injected with anything, those from eggs injected with 0.1 ml of water per egg

and those from eggs injected with 10 mg of glucose per egg had similar (P>0.05) thigh weights.

The chickens that hatched from eggs injected with 20 mg of glucose per egg had higher (P<0.05) wing weights than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_5, FCG_{10} or FCG_{15} treatments (Table 4.7). Similarly, chickens that hatched from eggs injected with 5 mg of glucose per egg or those that hatched from eggs injected with 20 mg of glucose per egg had similar (P>0.05) wing weights. Female chickens that hatched from eggs injected with 5 mg of glucose per egg had higher (P<0.05) wing weights than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_{10} or FCG_{15} treatments. Similarly, chickens that hatched from eggs not injected with anything had higher (P<0.05) wing weights than those that hatched from eggs injected with 0.1 ml of water per egg and those that hatched from eggs injected with 10 mg of glucose per egg.

Potchefstroom koekoek female chickens that hatched from eggs injected with 5 mg of glucose per egg had higher (P<0.05) gizzard weights than those that hatched from eggs on FCG_{0+}, FCG_{10}, FCG_{15} or FCG_{20} treatments (Table 4.7). However, chickens that hatched from eggs injected with 5 or 20 mg of glucose per egg had similar (P>0.05) gizzard weights. Female chickens that hatched from eggs injected with 20 mg of glucose per egg had higher (P<0.05) gizzard weights than those that hatched from eggs on FCG_{0+}, FCG_{10} or FCG_{15} treatments. Chickens that hatched from eggs not injected with anything and those that hatched from eggs injected with 20 mg of glucose per egg had similar (P>0.05) gizzard weights. Potchefstroom koekoek chickens that hatched from eggs injected with 10 or 15 mg of glucose per egg had higher (P<0.05) gizzard weights than those that hatched from eggs on an FCG_{0+} treatment. However, chickens that hatched from eggs injected with 10 or 15 mg of glucose per egg had similar (P>0.05) gizzard weights.

Female Potchefstroom koekoek chickens that hatched from eggs injected with 20 mg of glucose per egg had higher (P<0.05) liver weights than those that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_5, FCG_{10} or FCG_{15} treatments (Table 4.7). Female chickens that hatched from eggs injected with 0.1 ml of water per egg or 5 mg of

glucose per egg had higher (P<0.05) liver weights than those that hatched from eggs on FCG_{0-}, FCG_{10} or FCG_{15} treatments. Chickens that hatched from eggs injected with 0.1 ml of water or those that hatched from eggs injected with 5 mg of glucose per egg had similar (P>0.05) liver weights. Female Potchefstroom koekoek chickens that hatched from eggs not injected with anything had higher (P<0.05) liver weights than those that hatched from eggs on FCG_{10} or FCG_{15} treatments. Similarly, chickens that hatched from eggs injected with 15 mg of glucose had higher (P<0.05) liver weights than those that hatched from eggs injected with 10 mg of glucose per egg (Table 4.7).

Data obtained for carcass weight, breast, drum, thigh, wing, gizzard and liver weights could not fit into the quadratic model for obtaining optimal responses.

Table 4.7 Effect of *in ovo* injection of glucose on carcass weight (g) and carcass parts (g) of Potchefstroom koekoek chickens aged 91 days

	Treatment						
Variable	FCG_{0-}	FCG_{0+}	FCG_5	FCG_{10}	FCG_{15}	FCG_{20}	SEM
Carcass wt	762^{c}	747^{c}	841^{a}	797^{b}	726^{d}	807^{b}	19.582
Breast	172^{c}	161^{d}	192^{b}	177^{c}	173^{c}	203^{a}	5.871
Drum	60^{c}	54^{d}	64^{b}	58^{c}	58^{c}	69^{a}	1.922
Thigh	64^{bc}	59^{c}	66^{b}	60^{c}	69^{b}	101^{a}	4.184
Wing	56^{d}	53^{e}	60^{b}	53^{e}	58^{c}	62^{a}	1.343
Gizzard	53^{ab}	39^{d}	54^{a}	43^{c}	41^{c}	51^{b}	2.169
Liver	26^{c}	28^{b}	28^{b}	23^{e}	24^{d}	30^{a}	0.713

a, b, c : Means with different superscripts within a row are significantly different (P<0.05)

SEM : Standard error of the mean

Results of the effect of *in ovo* glucose injection on meat tenderness, juiciness and flavour of female Potchefstroom koekoek chickens aged 91 days are presented in Table 4.8. Meat of female chickens that hatched from eggs injected with 15 or 20 mg of glucose per egg was more (P<0.05) tender than the meat of chickens that hatched from eggs on FCG_{0-}, FCG_{0+} or FCG_5 or FCG_{10} treatments. However, meat of female chickens that hatched from eggs injected with 5 or 20 mg of glucose per egg had

similar (P>0.05) tenderness values. Meat of female chickens that hatched from eggs injected with 10 mg of glucose per egg was more (P<0.05) tender than the meat of chickens on FCG_{0-}, FCG_{0+} or FCG_5 treatments. Similarly, meat of female chickens that hatched from eggs injected with 5 mg of glucose per egg had higher (P<0.05) tenderness values than the meat of chickens on FCG_{0-} or FCG_{0+} treatments. However, meat of chickens that hatched from eggs not injected with anything or from eggs injected with 0.1 ml of water per egg had similar (P>0.05) tenderness values.

The chickens that hatched from eggs injected with 15 mg of glucose per egg produced meat which was more (P<0.05) juicy than the meat of chickens on FCG_{0-}, FCG_{0+}, FCG_5, FCG_{10} or FCG_{20} treatments (Table 4.8). Meat of female chickens that hatched from eggs injected with 5 or 20 mg of glucose per egg was more (P<0.05) juicy than the meat of chickens on FCG_{0-}, FCG_{0+} or FCG_{10} treatments. However, meat of female chickens that hatched from eggs injected with 5 or 20 mg of glucose per egg had similar (P>0.05) juiciness values. Meat of female chickens that hatched from eggs injected with 10 mg of glucose per egg was more (P<0.05) juicy than the meat of chickens that hatched from eggs not injected with anything or meat of chickens that hatched from eggs injected with 0.1 ml of water per egg. However, meat of chickens that hatched from eggs not injected with anything and meat of chickens that hatched from eggs injected with 0.1 ml of water per egg had similar (P>0.05) juiciness values.

Female Potchefstroom koekoek chickens that hatched from eggs injected with 10 mg of glucose per egg had better (P<0.05) flavour than the meat of chickens that hatched from eggs on FCG_{0-}, FCG_{0+}, FCG_5, FCG_{15} or FCG_{20} treatments (Table 4.8). Meat of female chickens that hatched from eggs injected with 5 or 15 mg of glucose per egg had better (P<0.05) flavour than the meat of chickens that hatched from eggs on FCG_{0-}, FCG_{0+} and FCG_{10} treatments. However, meat from female chickens that hatched from eggs injected with 5 or 15 mg of glucose per egg had similar (P>0.05) flavour. Meat of female chickens that hatched from eggs not injected with anything or meat of chickens that hatched from eggs injected with 0.1 ml of water per egg had better (P<0.05) flavour than the meat of chickens that hatched from eggs on an FCG_{0+} treatment. However, meat of female chickens that hatched from eggs not injected with

anything or those that hatched from eggs injected with 20 mg of glucose per egg had similar (P>0.05) flavour (Table 4.8).

Female Potchefstroom koekoek chicken meat tenderness, juiciness and flavour were optimized at *in ovo* injection levels of 13.50 (r^2 = 0.990), 19.25 (r^2 = 0.726) and 10.83 (r^2 = 0.950) mg of glucose per egg, respectively (Figures 4.12, 4.13 and 4.14, respectively and Table 4.9).

Table 4.8 Effect of *in ovo* injection of glucose on tenderness, juiciness and flavour of meat of female indigenous Potchefstroom koekoek chickens aged 91 days

	Treatment						
Variable	FCG_{0-}	FCG_{0+}	FCG_5	FCG_{10}	FCG_{15}	FCG_{20}	SEM
Tenderness	2.93^d	2.93^d	3.20^c	3.30^b	3.40^a	3.40^a	0.048
Juiciness	2.53^d	2.60^d	3.06^b	2.93^c	3.40^a	3.13^b	0.074
Flavour	3.13^c	3.20^d	3.50^b	3.60^a	3.50^b	3.40^c	0.041

a, b, c, d : Means with different superscripts within a row are significantly different (P<0.05)

SEM : Standard error of the mean

$$Y = 2.941 + 0.054x - 0.001x^2$$

$$r^2 = 0.990$$

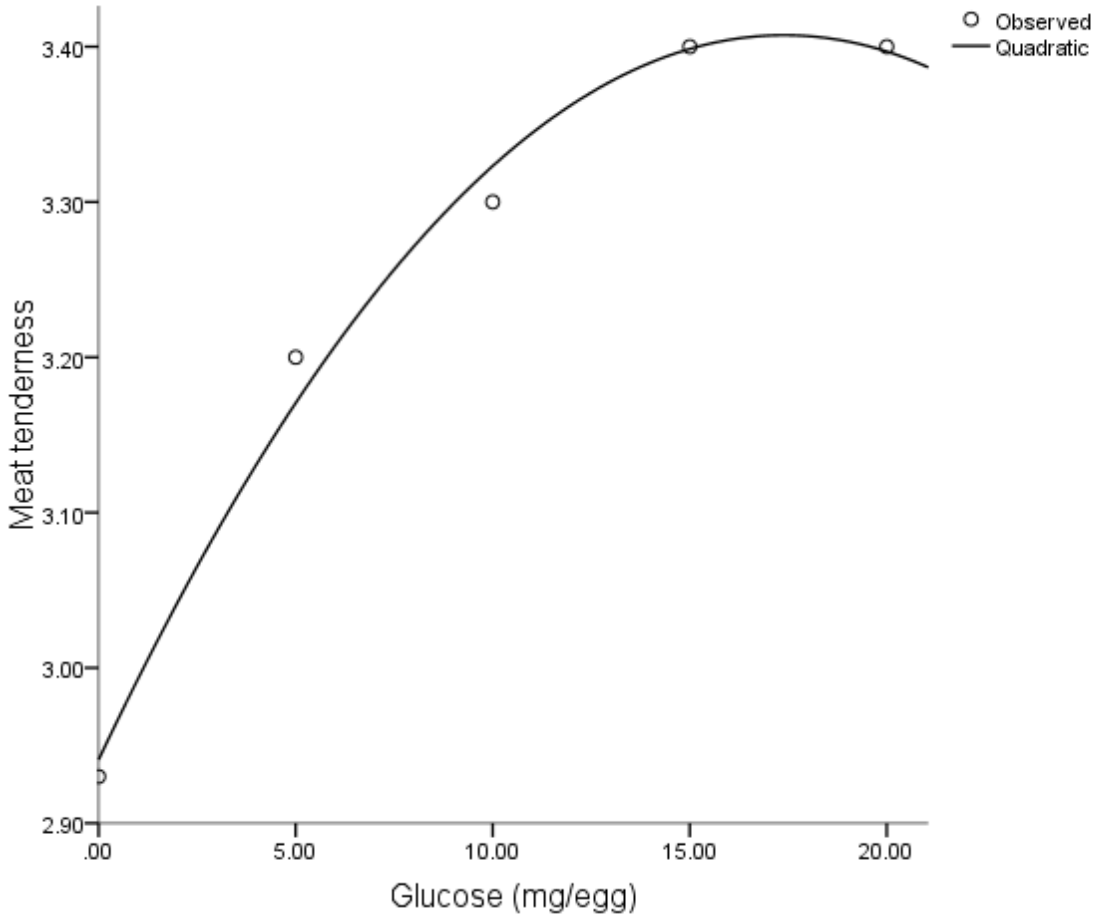

Figure 4.12 Effect of *in ovo* injection of glucose on tenderness of meat of indigenous female Potchefstroom koekoek chickens aged 13 weeks

$$Y = 2.621 + 0.077x - 0.002x^2$$

$$r^2 = 0.726$$

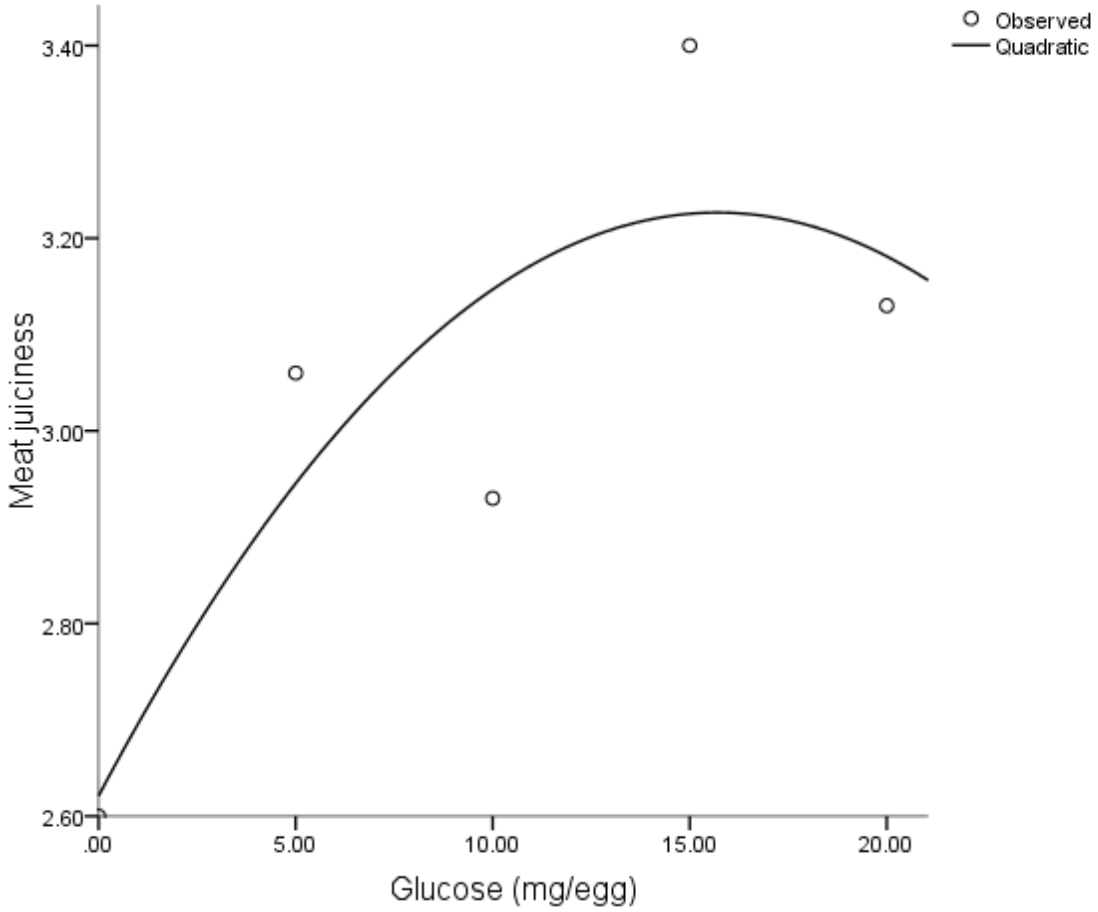

Figure 4.13 Effect of *in ovo* injection of glucose on juiciness of meat of indigenous female Potchefstroom koekoek chickens aged 13 weeks

$Y = 3.217 + 0.065x - 0.003x^2$

$r^2 = 0.950$

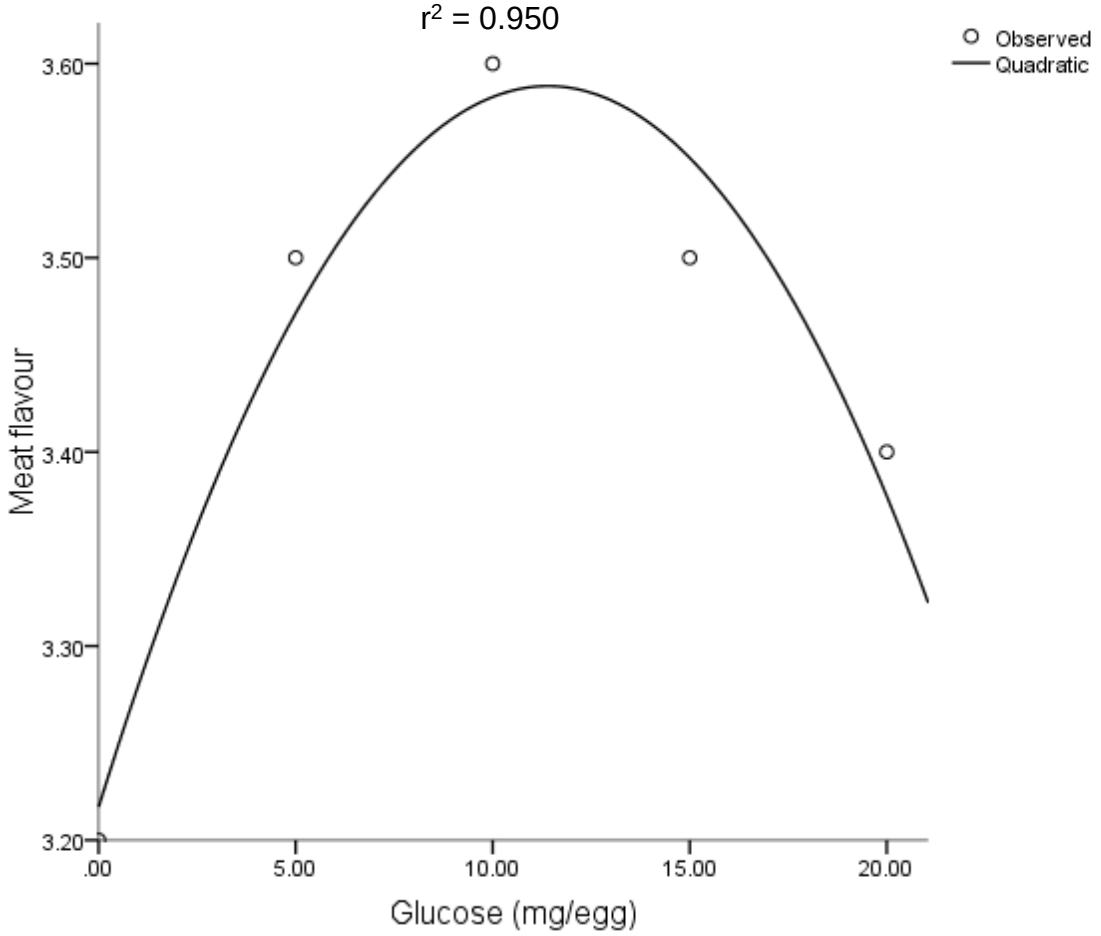

Figure 4.14 Effect of *in ovo* injection of glucose on flavour of meat of indigenous female Potchefstroom koekoek chickens aged 13 weeks

Table 4.9 *In ovo* glucose injection levels for optimal tenderness, juiciness and flavour of meat of female indigenous Potchefstroom koekoek chickens aged 91 days

Trait	Formula	Glucose	Y-Value	r^2	P
Tenderness	$Y = 2.941 + 0.054x - 0.002x^2$	13.50	3.31	0.990	0.010
Juiciness	$Y = 2.621 + 0.077x - 0.002x^2$	19.25	3.36	0.726	0.274
Flavour	$Y = 3.217 + 0.065x - 0.003x^2$	10.83	3.57	0.950	0.050

Glucose : *In* ovo glucose level for optimal variable

Y- Value : Optimal Y-Value

r^2 : Regression coefficient

P : Probability

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

In ovo injection of 10 mg of glucose per egg improved hatchability of indigenous Potchefstroom koekoek chicken eggs from 73 to 76 %; however *in ovo* injection of 15 and 20 mg of glucose per egg decreased hatchability. Hatchability was optimized at an *in ovo* glucose injection level of 4.50 mg per egg. Shafey *et al*. (2012) and Uni *et al.* (2005) also observed that *in ovo* carbohydrate injection improved hatchability of broiler chicken eggs. Uni *et al*. (2005) reported that *in ovo* injection of carbohydrates increased the level of available liver glycogen which enhanced hatchability. Towards the end of the incubation period embryos utilise their energy reserves to meet the high demand of glucose for hatching activities (Christensen *et al.,* 2001). Thus, *in ovo* injection of glucose increases the liver glycogen in the chick embryo which accelerates the hatching process by providing energy required during hatching. Contrary to the results obtained in the present study, Salmanzadeh (2012) and Ipek *et al.* (2004) observed that *in ovo* glucose injection did not affect hatchability of broiler chicken eggs. Ebrahimnezhad *et al.* (2011), on the other hand, observed that *in ovo* glucose injection reduced hatchability of broiler chicken eggs. Similarly, Leitao *et al*. (2008), Adriana *et al*. (2006) and Pedroso *et al*. (2006) reported that *in ovo* glucose injection reduced hatchability of broiler chicken eggs. The authors suggested that *in ovo* glucose injection may have caused allergic reaction under the sac and stopped respiration of the developing embryo which ultimately led to death of the embryos. It is also possible that in that case *in ovo* glucose injection may not have been done properly.

Chick hatch-weight of indigenous Potchefstroom koekoek chicks was improved by *in ovo* injection of 5, 10 and 15 mg of glucose per egg but injection with 20 mg of glucose per egg did not improve hatch-weight of the chicks. Similar results were observed by Salmanzadeh *et al.* (2012), Ebrahimnezhad *et al.* (2011), Salmanzadeh *et al*. (2011) and Bhanja *et al*. (2008) in broiler chickens. Shafey *et al.* (2012), also, observed that *in ovo* injection of carbohydrates (maltose, sucrose and dextrin) improved broiler chick hatch-weight. *In ovo* injection of carbohydrates in chicken eggs during incubation improves nutritional status and embryonic growth, resulting in increased chick hatch-weights (Smirnov *et al.,* 2006; Uni *et al.,* 2005; Uni and Ferket, 2004). Thus, increased

chick hatch-weight in the present study may have been due to surplus supply of glucose as reported by Lourens *et al.* (2006).

High chick to egg weight ratio indicates better utilization of the egg contents for the development of the chick (Enting *et al*., 2007). *In ovo* glucose injection improved Potchefstroom koekoek chick to egg weight ratio except at in ovo injection of 20 mg of glucose per egg. However, *in ovo* glucose injection level of 12 mg per egg optimized chick to egg weight ratio of Potchefstroom koekoek chickens. Improvements in broiler chick to egg weight ratio due to *in ovo* glucose injection were calculated from the data of Salmanzadeh (2012), Shafey (2012), Ebrahimnezhad (2011), Salmanzadeh *et al*. (2011) and Amitav *et al*. (2007). *In ovo* glucose injection levels for optimal chick to egg weight ratio observed in the present study are lower than those calculated from the studies of Shafey *et al.* (2012) and Uni *et al*. (2005) in broiler chickens. The possible reason for the difference may be that broiler chicken embryos are larger than Potchefstroom koekoek embryos and hence they require more nutrients for growth optimization. But higher glucose levels in the present study showed a negative effect.

It is concluded that *in ovo* glucose injection improved Potchefstroom koekoek egg hatchability, chick hatch-weight and chick to egg weight ratio. However, egg hatchability, chick hatch-weight and chick to egg weight ratio were optimized at different *in ovo* glucose injection levels of 4.50, 10.43 and 12.00 mg per egg, respectively. Thus, there was no single glucose injection level that optimized all the three production variables. It is, also, noted that chick to egg weight ratio was optimized at a higher *in ovo* glucose injection level than egg hatchability and Potchefstroom koekoek chick hatch-weight.

In ovo injection levels of 5 and 10 mg of glucose per egg increased growth rates of unsexed Potchefstroom koekoek chickens aged 1 to 49 days. However, injection levels of 15 and 20 mg of glucose per egg resulted in chicks having lower growth rates. Improved growth rates resulted in increased live weights of the chickens at 49 days of age. The increase in growth rate was due to improvements in diet intake, feed conversion ratio, metabolisable energy intake and nitrogen retention in the chickens that hatched from eggs injected with glucose. *In ovo* glucose injection, possibly,

accelerated maturation of the gastrointestinal tract which increased feed intake, digestibility and nutrient absorption (Sunny, 2008; Murakami *et al.,* 2007), resulting in increased growth rates and hence live weights of the chickens. Similar improvements were observed in feed intake, growth rate and live weight of broiler chickens that hatched from eggs injected with glucose (Salmanzadeh, 2012; Salmanzadeh *et al.*, 2011; Sunny, 2008; Bhanja, 2008; Amitav *et al*., 2007; Murakami *et al.,* 2007; Wilson, 1991). The authors attributed the improvements in feed intake, growth and live weight of broiler chickens to increased embryo nutritional status after *in ovo* glucose injection. However, other studies (Leitao *et al.*, 2008; Ingram *et al*., 1997) observed no improvements in feed intake, growth and live weights of broiler chickens hatched from eggs injected with glucose.

Growth rate, live weight, metabolisable energy intake and nitrogen retention of Potchefstroom koekoek chickens aged 1 to 49 days were optimized at *in ovo* glucose injection levels of 3.92, 4.36, 10.67 and 13.30 mg per egg, respectively. Thus, there was no single level that optimized all the production variables. *In ovo* glucose injection level for growth rate of Potchefstroom koekoek chickens was lower than those observed in broiler chickens by Salmanzadeh (2012). The reason for the difference may be due to breed differences. Broiler chickens require more nutrients for growth (Sklan and Noy, 2000). Thus, broiler chicken embryos require a higher *in ovo* glucose injection level for optimal growth.

In ovo glucose injection increased growth rate of female Potchefstroom koekoek chickens aged 50 to 91 days, resulting in increased live weight of the chickens at 91 days of age. Improvements in live weights were due to improvements in diet intake, feed conversion ratio, metabolisable energy intake and nitrogen retention in chickens hatched from eggs injected with glucose. Thus, *in ovo* glucose injection improved embryo development and subsequent performance of the chickens. However, FCR and ME intakes were optimized at different *in ovo* glucose injection levels of 12.15 and 5.57 mg per egg, respectively. Similar improvements were observed in feed intake, growth rate and live weight of broiler chickens hatched from eggs injected with glucose (Salmanzadeh, 2012; Salmanzadeh *et al.*, 2011; Chen *et al*., 2009; Sunny, 2008; Bhanja, 2008; Amitav *et al*., 2007; Murakami *et al.,* 2007; Ingram *et al*., 1997; Wilson,

1991). However, Leitao *et al.* (2008) observed no improvements in feed intake, growth and live weights of broiler chickens hatched from eggs injected with glucose.

Female Potchefstroom koekoek chickens that hatched from eggs injected with glucose had higher carcass, breast, drumstick, thigh, wing, gizzard and liver weights. Salmanzadeh *et al*. (2011) also reported that *in ovo* injection of glucose improved carcass and breast meat weights in broiler chickens. However, the authors found that *in ovo* glucose injection had no significant effect on other carcass parts like leg, wing, gizzard and liver weights. Similarly, Pilarski *et al*. (2005) observed that *in ovo* injection of oligosaccharides did not improve broiler carcass and breast meat weights.

In ovo glucose injection improved breast meat tenderness, juiciness and flavour of Potchefstroom koekoek chickens aged 91 days. However, breast meat tenderness, juiciness and flavour were optimized at different *in ovo* glucose injection levels of 13.50, 19.25 and 10.83 mg per egg. This means that the level of *in ovo* glucose injection for optimal productivity will depend on the variable of interest. Dransfield and Sosnicki (1999) reported that *in ovo* glucose injection increased broiler chicken breast meat tenderness by reducing the rate of pH decline and the toughening of the meat. Warris (2000) observed that *in ovo* glucose injection increased water holding capacity which improved juiciness of cooked broiler chicken meat.

5.2 Conclusion

Different *in ovo* glucose injection levels optimized egg hatchability, chick hatch-weight and chick to egg weight ratio of Potchefstroom koekoek chickens. Thus, there was no single glucose injection level that optimized all the three production variables. However, chick to egg weight ratio was optimized at a higher *in ovo* glucose injection level than egg hatchability and Potchefstroom koekoek chick hatch-weight. Thus, *in ovo* glucose injection level for optimal productivity will depend on the parameter of interest.

In ovo glucose injection increased feed intake, growth rate, feed conversion ratio, live weight, metabolisable energy intake and nitrogen retention of Potchefstroom koekoek chickens aged 1 to 91 days. These improvements were due to increased embryo

nutritional status after *in ovo* glucose injection which improved subsequent performance of the chickens.

In ovo glucose injection improved carcass characteristics and breast meat flavour, tenderness and juiciness of female Potchefstroom koekoek chickens aged 13 weeks. However, breast meat flavour, tenderness and juiciness were optimized at different injection levels.

6.2 Recommendation

Different production parameters of Potchefstroom koekoek chickens were optimized at different injection levels. Therefore, it is recommended that *in ovo* glucose injection levels for Potchefstroom koekoek chickens should depend on the parameters of interest. It is, also, recommended that more research is done to fully understand the effect of *in ovo* glucose injection on optimal productivity of Potchefstroom koekoek chickens.

CHAPTER 6

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