Glucose Tolerance and Lipid Absorption in Guinea Fowl (Numida meleagris) and Domestic Fowl (Gallus gallus var. domesticus)

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ABSTRACT
Research on metabolism in poultry is mainly conducted on domestic fowl and the same physiological response is assumed in Guinea fowl (Numida meleagris). Glucose tolerance and lipid absorption were compared between chickens (Gallus gallus var. domesticus) and Guinea fowl. Twelve Guinea fowl and Chickens were randomly divided into subgroups of 6 each and subjected to an intravenous and oral glucose tolerance test or fat loading tests with medium and long chain fatty acids triglycerides (coconut oil and olive oil, respectively). The mass, glycogen content and lipid content of the livers was also determined. The Guinea fowl had higher (p<0.001) basal glucose levels than chickens. In the Guinea fowl, plasma glucose levels peaked 15 min after the oral glucose load and returned to basal levels (13.9±1.2 mmol L⁻¹) by 30 min whereas in the chicken plasma glucose levels remained elevated and returned to basal levels (11.2±0.8 mmol L⁻¹) after 90 min. Plasma glucose levels peaked 10 min after the intravenous administration of glucose in both species and returned to basal levels at 30 min in the chickens and 60 min in the Guinea fowl. Triglyceride levels in the chicken remained below 0.8 mmol L⁻¹ even after the oral fat loading. In the Guinea fowl, plasma triglyceride levels increased after 30 min, peaking at 1.7±0.4 mmol L⁻¹ (basal level 0.9±0.1 mmol L⁻¹) at 300 min and although it dropped it did not return to basal levels by 420 min after the administration of the coconut oil. Administration of the olive oil caused non-significant increases (p>0.05) in the triglyceride levels of the Guinea fowl. Chicken livers had a greater mass (p = 0.0002, t-test) and glycogen content (p = 0.0003, t-test) than Guinea fowl livers. The differences in glucose tolerance and lipid absorption indicate that data from chicken cannot be directly extrapolated to Guinea fowl.

Key words: Guinea fowl, chicken, glucose tolerance, fat absorption, liver

INTRODUCTION
Poultry farming is an important activity in Africa and Asia. Most small-scale farmers cannot afford to keep large livestock, such as cattle, sheep and goats, as a result poultry has therefore become a major source of protein in human diets and contribute to their food security (Mbajorgo, 2011). Many smallholder farmers rear Guinea fowl (N. meleagris) as an alternative source of meat protein, eggs and income (Abubakar et al., 2008; Mwale et al., 2008;
Obike et al., 2011). Guinea fowl reportedly have many advantages over chickens (G. gallus var. domesticus), such as their increased disease resistance, an increased ability to scavenge for food as well as a high meat-to-bone ratio (Kozaczynski, 1998; Saina et al., 2005). In order to decrease production costs by maximizing feed utilization, farmers need to be in a position to tailor the diet of the Guinea fowl to meet its specific nutritional needs. There is a dearth of information on the protein and energy requirements of Guinea fowl (Oke et al., 2003). Research on the gastrointestinal tract (GIT) and handling of metabolic substrates is most often conducted on chickens and the same physiological response is assumed in Guinea fowl.

Starch is the main dietary carbohydrate consumed by poultry. Starch digestion begins in the oropharynx as a result of the salivary amylase (Berradi et al., 2007; Pollock, 2002) and is ultimately hydrolysed to glucose which is most often used for energy production (Braun and Sweazea, 2008). In the chicken, glucose is mainly absorbed from the duodenum and jejunum (Pollock, 2002). Glucose is then transported to the liver via secondary active transport, where it is then converted to glycogen.

Digestion and absorption of lipids occurs in the small intestine where bile salts and phospholipids play a major role in the emulsification of triglycerides, as well as any other fat-soluble nutrients. Emulsification of dietary fats takes place as soon as the chyme enters the small intestine and comes into contact with the bile and pancreatic secretions. Pancreatic lipase plays a role at the water-lipid interface by acting on the triglycerides (Bickerstaffe et al., 1970). Bile salt micelles are then formed in order to remove the products from the water-lipid interface (Krogdahl, 1985). Due to an absence of an intestinal lymphatic system in the chicken, absorbed fat is transported as a low-density lipoprotein directly to the liver via the portal blood (Bickerstaffe et al., 1970).

Glucone is a major hepatic substrate and is the direct precursor for glycogen and fat; which are most commonly stored in the liver (Bennett et al., 2007). In the chicken, the liver is the main site of triglyceride synthesis and fatty acids are used as an energy substrate during fasting (Colin et al., 2009). After ingestion of a carbohydrate-rich meal, liver glycogen is replenished and hepatic lipogenesis is activated. In birds, leptin stimulates lipogenesis (Proszkowiec-Weglarz et al., 2009). Glycogen in the liver is most often derived from the glucose found in the hepatic portal vein (Stanley et al., 1984). The rate of glucose uptake by the liver is controlled by glucokinase which plays an important role in the conversion of glucose into glucose 6-phosphate (Stanley et al., 1984). A decrease in food intake, leads to decreases in blood glucose levels, resulting in a decrease in liver glycogen content (Braun and Sweazea, 2008). When a large proportion of glucose is consumed, it is stored in the form of glycogen until it is needed (Pollock, 2002).

Glucose absorption capacities and homeostasis are often determined using oral and/or intravenous glucose tolerance tests. The changes in blood glucose levels in oral glucose tolerance tests depend largely on the amount absorbed as well as the rate of intestinal absorption. An intravenous glucose tolerance test eliminates any changes caused by gastrointestinal factors as the rise in blood glucose levels is immediate.

An oral fat loading test is routinely used to determine digestive and absorptive capacities in animals (Simpson and Doxey, 1983). Fats used in this test are divided into two groups, according to the number of carbons they contain. Medium chain triglycerides, for example coconut oil and dairy fat (Marten et al., 2006), contain between eight and twelve carbon atoms. Long chain triglycerides, for example corn oil, olive oil and sunflower oil, contain fourteen or more carbon atoms (Simpson and Doxey, 1983). Medium chain triglycerides are hydrolysed by lipase in the small intestine or can be absorbed intact across the intestinal wall. Conversely, long chain triglycerides
cannot be transported intact and therefore need to be hydrolysed in order for absorption to take place (Simpson and Doxey, 1983).

There are physiological differences between chickens and Guinea fowl, in particular their response to water deprivation (Mafuvadze et al., 2008) and gastric acid secretion (Erlwanger et al., 2000) and hematology (Nalubamba et al., 2010). Even though there are reported differences between these two types of birds, most farmers treat Guinea fowl in the same way they do chickens, by feeding them the same food. Feed is a major cost in poultry production, therefore in an attempt to reduce costs and maximize profit more knowledge on GIT function and handling of metabolic substrates is required to employ the most efficient feed strategy for the type of poultry being farmed. The aim of this study was therefore to investigate glucose tolerance and lipid absorption in chickens and Guinea fowl.

**MATERIALS AND METHODS**

The study was approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, South Africa, AESC approval No. 2010/11/2A. The study was conducted at the Central Animal Services animal unit at the University of the Witwatersrand, in 2010.

**Animals:** Twelve male and female Guinea fowl (*N. meleagris*) as well as twelve male and female Domestic fowl (*G. gallus* var. *domesticus*) were obtained from a commercial farmer at the age of five weeks and one week, respectively. Rearing methods used during the study were similar that of the farm.

**Housing and treatments:** The birds were separated according to species and housed in groups in wire cages with wood shavings as bedding. Environmental enrichment was provided in the form of logs as perches. All birds were given *ad-libitum* access to both water and standard poultry feed (Epol®, Sure-lay, South Africa). The feed was formulated for specific age groups, that is, chick mash (recommended for chickens from hatching to six weeks of age) and grower feed (recommended for chickens from six weeks to twenty weeks of age). The birds were provided with supplementary heating, in the form of infrared lighting, during the first four weeks of life. Lighting was restricted to 12 h in each 24 h period, lights on from 07:00. The ambient temperature was set at 22±1°C during the experimental period and the relative humidity ranged from 30 to 40%. The birds were handled for a couple of min, several times a day, so that they could get used to being handled in an attempt to prevent handling stress hyperglycaemia when the interventions took place (Simon and Rosselin, 1979). As this was an open, randomized study, the birds were randomly divided into four groups, consisting of six birds with 3 males in each group (Group I: six Guinea fowl, Group II: six domestic fowl, Group III: six Guinea fowl and Group IV: six domestic fowl). Groups I and II were subjected to glucose tolerance tests while groups III and IV underwent oral fat loading tests. For groups I and II the birds were allowed a seven day recovery period between the oral glucose tolerance test and the intravenous glucose tolerance test and similarly for group III and IV between the medium and long chain fat absorption tests. The birds were weighed on a weekly basis in order to monitor growth and to enable accurate dosing of the test substances. This was done by placing the birds into pre-weighed cages and then placing these cages onto a scale.

**Glucose tolerance and fat loading tests:** The oral glucose tolerance and medium chain fat loading tests commenced at eight weeks of age and after the seven day recovery period the intravenous glucose tolerance and long chain fat loading tests took place.
**Oral glucose tolerance test:** The oral glucose tolerance test was performed after a 16 h (overnight) fast (Gueritault et al., 1990). The birds in group I and II were given a 5 mL kg⁻¹ b.wt. dose of glucose (50% weight by volume glucose solution, saarchem D (+) glucose anhydrous, Merck Chemicals (Pty) Ltd., Gauteng, South Africa) orally via oesogastric intubation into the crop (Simon and Rosselin, 1979). Fasting levels of glucose in the blood were measured prior to the administration of the glucose load. Before the venipuncture was to take place, the area surrounding the wing vein was clipped of its feathers and sterilised with alcohol soaked gauze swabs. Blood was collected after a pin-prick and placed on to a glucometer (Ascensia Elite™ Blood glucose meter, Bayer Corporation, Mishawaka, USA) for the determination of blood glucose levels. Blood was collected at fixed time intervals of 0, 15, 30, 60, 90 and 120 min to determine blood glucose levels after the administration of the oral glucose load (Muellenbach et al., 2009; Loxham et al., 2007).

**Intravenous glucose tolerance test:** The intravenous glucose tolerance test was performed after a 16 h (overnight) fast. The birds in group I and II were given a 2 mL kg⁻¹ body weight bolus of glucose (intramed sterile 50% anhydrous dextrose, Intramed, Port Elizabeth, South Africa) intravenously via injection into the wing vein (Myers and Klasing, 1999). Fasting levels of glucose in the blood were measured prior to the administration of the glucose load, as per the method described above. Blood for glucose determination was collected from the opposite wing to infusion. Blood was collected at fixed time intervals of 0, 10, 30, 60 and 120 min for blood glucose determination after the administration of the intravenous glucose load (Myers and Klasing, 1999). Blood glucose concentrations were determined using a glucometer (Ascensia Elite™ Blood glucose meter, Bayer Corporation, Mishawaka, USA).

**Oral fat loading tests:** After a period of fasting (16 h), the birds in group III and IV received a dose (10 mL kg⁻¹ b.wt.) of a medium chain triglyceride, in the form of cold pressed extra virgin certified organic coconut oil (Absolute Organix, Johannesburg, South Africa) orally, via intubation of the crop. One week later, the birds were given a long chain triglyceride, in the form of extra virgin olive oil (R.M. S.p.A, Lucca, Italy). No response was seen when the birds were given a dose of 5 mL kg⁻¹ b.wt. (Simpson and Doxey, 1983), data not shown and the dose was therefore increased to 10 mL kg⁻¹ body weight. In order to determine triglyceride levels, 0.2 mL of blood was collected using sterile hypodermic needles (25 G) and syringes (1 mL). Fasting triglyceride levels were determined prior to the administration of the coconut oil. Blood samples were collected at 30 min and every h for eight h thereafter. The triglyceride concentrations were determined using a GCT meter (Accutrend® Plus GCTL Roche, Manheim, Germany).

**Hepatic glycogen and lipid contents:** One week after the last interventions and feeding with standard poultry feed, the birds were fasted for 16 h and euthanized by anaesthetic overdose of sodium pentobarbital (Eutha-naze, Centaur Labs, South Africa), 100 mg kg⁻¹ into the wing vein. The livers were carefully removed, weighed and frozen (-5°C) until analysed for glycogen and lipid content. Liver lipids were extracted by standard procedures (Bligh and Dyer, 1959) whilst the liver glycogen was determined indirectly by hydrolysis to glucose (Passonneau and Lauderdale, 1974).

**Data analysis:** All data are expressed as Mean±SD. A two-way analysis of variance (ANOVA) was used to compare blood glucose concentrations between different species at specific time points. A repeated measures ANOVA was used to assess blood glucose and blood triglyceride concentrations
within the same species to determine whether there were any differences at specific time points, followed by a Bonferroni post hoc test. An unpaired Student’s t-test was used for comparisons of liver masses, hepatic lipid and glycogen content between the two species of birds. In all statistical tests, p<0.05 was considered significant. All statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, USA).

RESULTS

**Oral glucose tolerance test**: Basal blood glucose concentrations were significantly (p<0.0001) lower in chickens (11.2±0.8 mmol L⁻¹) than in the Guinea fowl (13.9±1.2 mmol L⁻¹). The peak in blood glucose concentrations (Fig. 1) occurred at 15 min after gavage in the Guinea fowl versus 30 min in the chicken, representing a 27% increase in blood glucose concentrations from basal levels in the Guinea fowl versus a 54% increase for the chicken. The blood glucose returned to normal at 30 min in the Guinea fowl versus 90 min in the chicken but in the Guinea fowl it dropped significantly (p<0.05) below the basal levels at 120 min.

**Intravenous glucose tolerance test**: During the intravenous glucose tolerance test, the peak in plasma glucose levels occurred at 10 min after the injection in both the chicken and the Guinea fowl and this represented a 120.8% increase from basal levels in the chicken versus a 92.5% increase for the Guinea fowl (Fig. 2). Blood glucose levels returned to normal after 30 min in the chicken and 60 min in the Guinea fowl.

**Oral fat loading test**: The meter used to determine plasma triglyceride levels had a detection range of 0.8-6.86 mmol L⁻¹. The triglycerides in the chicken were below the detectable range even after the administration of both the long chain triglyceride (olive oil) and the medium chain

![Graph showing blood glucose levels over time](image)

Fig. 1: Comparison of blood glucose levels in Guinea fowl (*N. meleagris*) and chickens (*G. gallus* var. *domesticus*) after an oral glucose load. Birds were fasted for 16 h and then gavaged with 5 mL kg⁻¹ glucose (50% w/v) solution. Values are Mean±SD, n = 6 group⁻¹, *, **: Significant differences between Guinea fowl and chickens (p<0.0001 and p<0.05, respectively)
Fig. 2: Comparison of blood glucose levels in Guinea fowl (N. meleagris) and chickens (G. gallus var. domesticus) after an intravenous glucose load. Birds were fasted for 16 h and then injected with 2 mL kg⁻¹ glucose (50% dextrose) solution in the wing vein. Values are Mean±SD, n = 6 group⁻¹, *, **: Significant differences between Guinea fowl and chickens (p<0.0001 and p<0.001, respectively), †: Significant differences between 10 min and all other time points for both Guinea fowl and chickens (p<0.05)

Fig. 3: Plasma triglyceride levels in Guinea fowl (N. meleagris) after an oral fat loading test. Birds were fasted for 16 h and then gavaged with 10 mL kg⁻¹ olive oil (long chain fatty acid). Values are Mean±SD, n = 6 group⁻¹, Triglyceride levels in the chicken (G. gallus var. domesticus) were below the detection limit of the meter used.

triglyceride (coconut oil). After the Guinea fowl received the olive oil, small increments in the plasma TG levels were observed at 120, 240 and 360 min however the peaks were not statistically significantly higher (p>0.05) than the basal levels (Fig. 3). However following the administration of the coconut oil, an increase in plasma triglyceride levels was noted 30 min after gavage in the Guinea fowl (Fig. 4). Basal triglyceride levels were 0.9±0.1 mmol L⁻¹, with an 88.9% change, resulting in peak plasma triglyceride levels of 1.7±0.4 mmol L⁻¹. The plasma triglyceride levels remained elevated until 360 min and although they dropped thereafter, they did not return to basal levels by 480 min.

**Liver mass, lipid and glycogen content:** Liver masses, expressed as both an absolute and relative to body mass, were significantly higher in chickens (p = 0.0002) compared to Guinea fowl.
Fig. 4: Plasma triglyceride levels in Guinea fowl (\textit{N. meleagris}) after an oral fat loading test. Birds were fasted for 16 h and then gavaged with 10 mL kg$^{-1}$ coconut oil (medium chain fatty acid). Values are Mean±SD, \( n = 6 \) group$^{-1}$, *: Significant differences when compared to 0 min after gavage (\( p < 0.05 \)). Triglyceride levels in the chicken (\textit{G. gallus var. domesticus}) were below the detection limit of the meter used.

![Graph showing triglyceride levels over time after gavage](image)

Table 1: Liver mass, hepatic lipid and glycogen concentrations in chickens (\textit{G. gallus var. domesticus}) and Guinea fowl (\textit{N. meleagris})

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Chicken</th>
<th>Guinea fowl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute liver mass (g)</td>
<td>19.83±2.420</td>
<td>13.08±1.61*</td>
</tr>
<tr>
<td>Relative liver mass (% body mass)</td>
<td>2.22±0.230</td>
<td>1.72±0.14F</td>
</tr>
<tr>
<td>Liver lipid (mg g$^{-1}$ liver)</td>
<td>47.50±11.22</td>
<td>42.12±2.60</td>
</tr>
<tr>
<td>Liver glycogen (mol L$^{-1}$ homogenate)</td>
<td>1.15±0.130</td>
<td>0.78±0.10F</td>
</tr>
</tbody>
</table>

Data are represented as Mean±SD. *\( p = 0.0002 \), F\( p = 0.001 \), F\( p = 0.0003 \) when compared to chicken, F: Expressed as glucose equivalent, \( n = 6 \)

(![](image)

(Table 1). Liver glycogen, expressed as a glucose equivalent, was greater (\( p = 0.0003 \)) in the chickens than the Guinea fowl. However, no significant differences were noted in the liver lipid concentrations in the chickens and Guinea fowl.

**DISCUSSION**

Chickens are often used as a reference species during studies comparing avian nutrition and metabolism, due to their homogenous genetic line (Myers and Klasing, 1999). In the current study, layer type chickens were used as they do not put on as much fat as do broilers (Griffin \textit{et al.}, 1991). Guinea fowl are naturally lean birds and therefore a more accurate comparison could be made when layers were used. This study showed that differences exist in the glucose tolerance and plasma triglyceride levels between chickens and Guinea fowl.

With regards to the difference in basal blood glucose levels between the chickens and Guinea fowl, the Guinea fowl appeared to be more anxious during handling which could have led to the increases in basal blood glucose levels observed in these birds. Stress such as that experienced during handling, causes increases in corticosteroids and catecholamines which in turn results in hepatic glycogenolysis and, an increase in lipolysis and gluconeogenesis (Elram, 2000).
Measurements of stress hormones should therefore be considered in future studies. The intravenous glucose tolerance test showed no differences in the time taken for the blood glucose levels to reach peak levels but the Guinea fowl took longer to return to basal levels, suggesting differences in the clearance of glucose from the blood in the two species of birds.

The oral glucose tolerance test also revealed differences between the two species whereby in the Guinea fowl, blood glucose levels peaked and returned to basal levels earlier than the chicken indicating that absorption of glucose in the small intestine took place relatively quickly, as did clearance from the blood. Plasma glucose levels did, however, drop below basal levels after a period of time, indicating hypoglycaemia as the glucose in the blood was cleared. The chickens had an increase in glucose later than Guinea fowl and the levels remained elevated for a longer period, indicative of a slower clearance from the blood and/or absorption rate. Myers and Klasing (1999) found that the ability of barn owls (Tyto alba) to handle a glucose load differed significantly from that of the chicken, thus indicating differences in glucose tolerance of avian species. Compared to Guinea fowl, chickens have been shown to have a higher expression of Insulin like growth factor-II (IGF-II) which is associated with growth (Goyal et al., 2010) and its role in metabolism of the two birds needs to be compared. Future studies are necessary to investigate the action of hormones regulating glucose metabolism in Guinea fowl and the differences in glucose absorption and clearance could perhaps then be explained.

The plasma triglyceride levels in the chickens were below the range detected by the meter used when the birds were given both the medium chain triglycerides and the long chain triglycerides. In the chicken, it is reported that the physiological requirements for fat digestion are not well developed (Meng et al., 2004) and this may be why the chickens had much lower plasma triglyceride levels. Future studies are recommended to determine lipase levels in these birds. A more sensitive triglyceride meter that is able to detect very low levels of triglycerides in the blood could also be used.

Administration of the long chain triglycerides, in the form of olive oil, did result in increases in plasma triglycerides in the Guinea fowl. Olive oil consists of oleic acid, linoleic acid, palmitic acid, stearic acid and linolenic acid (Tripoli et al., 2005); all of which are long chain fatty acids. Long chain fatty acids need to be digested before they can be absorbed (Simpson and Doxey, 1983); therefore we expected that the triglycerides to only be present in the blood after an extended period of time. In other species, such as dogs, a significant increase in triglycerides is seen 180 min after the administration of a long chain triglyceride (Simpson and Doxey, 1983). We had to stop the blood sampling after eight h post administration of the oils because the birds had been without food for an extended period of time. Fatty acids have to be synthesized into triglycerides before they can be detected, it would thus have been better to determine the concentrations of free fatty acids in the birds' blood.

Coconut oil, a medium chain triglyceride (Marten et al., 2006), consists of 85% medium chain fatty acids, namely capric acid, caprylic acid and capric acid. As this is a medium chain triglyceride, it can immediately be absorbed across the intestinal wall (Simpson and Doxey, 1983) and therefore we expected to see increased levels of triglycerides in the blood after a shorter period of time than that expected for the olive oil which was the case. In the Guinea fowl the triglycerides levels remained significantly elevated up to 6 h and by seven h, they had not returned to basal levels. Guinea fowl are naturally omnivorous, consuming both insects, such as ticks, bees and grasshoppers; as well as weeds, grasses and seeds (Ayeni, 1983). They therefore could be innately more capable of handling a triglyceride load than are chickens, as they normally consume more fats in their natural diet.
This study showed that chickens had heavier livers than the Guinea fowl. This could be attributed to the increased glycogen stores that were present in the livers of the chickens. The lower glycogen levels in the Guinea fowl could be due to increased glycogenolysis as a result of increases in stress hormones, further evidence of this is exhibited in the higher fasting glucose levels noted in the Guinea fowl. Even though this study has shown marked differences in the glucose tolerance and lipid absorptive capacities in chickens and Guinea fowl, further studies are necessary to elucidate the mechanisms behind these differences. Watford (as cited in Simon et al., 2000) stated that gluconeogenesis takes place in both the liver and kidneys of chickens but only that taking place in the kidney is regulated by insulin. Future studies could perhaps look at the gluconeogenic capacities in both the liver and the kidney in chickens and Guinea fowl. Investigations into the glucose tolerance and lipid absorptive and digestive capacities in broilers could also be of interest.

CONCLUSION

The current study has shown that there are in fact physiological differences in the ability of chickens and Guinea fowl to tolerate an oral and intravenous glucose load and an oral fat load. These differences should therefore be taken into consideration during the feeding of these birds. The dietary inclusion of fats and simple sugars, such as glucose, should be optimized to ensure maximal feed conversion efficiency and improved growth performance.

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