Effects of Breed, Sex and Source Within Breed on the Blood Bilirubin, Cholesterol and Glucose Concentrations of Nigerian Goats

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\textbf{Abstract:} Effects of breed, sex and source within breed on the blood bilirubin, cholesterol and glucose concentrations of the Nigerian goats were studied using eighty-one yearly goats, comprising equal numbers of the Saheliont Goat (SG), Red Sokoto Goat (RSG) and West African Dwarf goat (WADG) breeds. The studies indicate that there were no significant breed differences in total serum bilirubin concentrations and the range of 0.63-0.85 mg/100 ml was observed. However, the conjugated (direct) bilirubin is generally lower in the male goats than in the female goats, while the unconjugated (indirect) bilirubin is higher in the male goats than in their female counterparts. Source within each breed exhibited no pronounced effects on the Sahelian goats, but had effects on the RSG and WADG breeds. The study further revealed that the Sahelian goat breed has the highest concentration of serum glucose and the lowest level of serum cholesterol, the reverse is the case for the West African Dwarf goats, while the Red Sokoto goats recorded moderate concentrations of both cholesterol and glucose in the serum. The serum cholesterol level in goat is inversely proportional to the glucose concentration.

\textbf{Key words:} Goat breeds, sex, source within breed, serum bilirubin, cholesterol and glucose

\section*{INTRODUCTION}
Bilirubin, a brownish yellow substance, is one of the end products of haemoglobin catabolism. After the protein and iron are broken away from haemoglobin, a green pigment, biliverdin, remains, which becomes reduced to bilirubin (Harper\textit{ et al.}, 1977; Frandsen, 1981; Singh, 2004). Bilirubin tests measure the amount of the bilirubin in the blood sample and it is considered the true test for the liver function, as it reflects the ability of the liver to take up, process and secrete bilirubin into the bile (Frandsen, 1981; Singh, 2004). Total bilirubin in the blood stream may be partitioned into two forms namely, direct (unconjugated/water soluble) and indirect (conjugated/non-water soluble). Usually, the conjugated form travels through the blood to the liver where it is converted to the soluble form (Harper\textit{ et al.}, 1977; Frandsen, 1981; Singh, 2004). The serum total bilirubin ranges of 0.00-0.9 mg/100 ml of serum have been reported for both man and livestock (Harper\textit{ et al.}, 1977; Frandsen, 1981; Singh, 2004). High bilirubin concentration in the body causes jaundice in man, a condition in which the skin and the white part of the eyes appear yellowish. Singh (2004) maintained that the high blood bilirubin is caused by liver disease, blood disorder and or blockage of the tube.

The blood sugar level in man and livestock is maintains at a constant range through the action of several hormones (Scott, 1999; Singh, 2004; Randox, 2006). Scott (1999), Zubicic (2001), Lazzaro (2001) and Tambuwal\textit{ et al.} (2002) have reported the range of 80-100 mg of glucose per 100 ml of blood for different breeds of goat. Similar values have been documented for man (Harper\textit{ et al.}, 1977; Singh, 2004; Randox, 2006), for cat (Nottidge\textit{ et al.}, 1999), for Sheep (Hunter, 1996; Kelly, 1974) and for poultry (Schalm\textit{ et al.}, 1975). Insulin and glycogen are the principal hormones involved in the regulation of blood sugar. While insulin is hypoglycemic, glycogen is hyperglycemic. Generally when the sugar in the blood reaches the range of 160-180 mg, it appears in the urine indicating abnormality caused by excess sugar in the blood, which could lead to health hazard (Harper\textit{ et al.}, 1977; Singh, 2004; Randox, 2006). Very low level of glucose in the blood is typified in livestock by gross reduction in weight gain, milk yield and alteration in the fatty acid composition of the milk (Scott, 1999; Zubicic, 2001; Lazzaro, 2001; Tambuwal\textit{ et al.}, 2002).

Cholesterol is a combination of sterol and lipids, which plays a key role in formation of cell membrane, production of hormones, cortisone and bile and metabolism of fat soluble vitamins, as well as acting as antioxidant (Macdonald and Low, 1995; Ritter, 1996). They noted that excess cholesterol in the serum blocks blood vessels thereby leading to angina and or heart attack in man and very low level is equally dangerous. The work therefore was designed to determine the levels of bilirubin, cholesterol and sugar in the blood of Nigerian goats and to study the effects of breed, sex and source within breed on these blood biochemical indices.

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MATERIALS AND METHODS

The study was conducted at the Goat Research Unit of the Department of Animal Science, Faculty of Agriculture, Delta State University, Asaba Campus, Asaba, Delta State. Delta State falls within the humid tropic. Each breed was sourced from three different sources based on areas its predominance in the country. A total of nine goats (comprising three males and six females) were selected from each location.

Design of the experiment: The experiment was conducted under a 2 x 3 factorial in CRD to test the effects of sex, breed and their interactions on the blood bilirubin, cholesterol and glucose concentrations of the Nigerian indigenous goats. Sex was tested on two levels with unequal replicates of twenty-seven bucks and fifty-four does; while there were three breeds with twenty-seven goats for each breed. In addition, effect of source of goat within each breed on these parameters was tested using one-way classification.

The statistical model used:

\[ Y_{ij} = \mu + B_i + S_j + (BS)_{ij} + e_{ijk} \]

Where:
- \( Y_{ij} \) = is the observed blood bilirubin, cholesterol or glucose
- \( \mu \) = The population mean
- \( B_i \) = The effect of the breed, \( i = 1, ... , 3 \)
- \( S_j \) = The effect of the sex of the animal, \( j = 1, 2 \)
- \( (BS)_{ij} \) = is the interaction between breed and sex and
- \( e_{ijk} \) = is the error term associated with the observations

Assumptions; error term is independently, identically and normally distributed, with zero mean and constant variance, that is, and (0, \( \delta^2 \)).

Analytical procedure: 10 ml of blood was collected from each goat via the jugular vein; properly identified and centrifuged at 4000 revolutions per minute for 15 min. Then, the parameters were determined as follow.

Determination of total bilirubin: The number of test tubes to represent all the samples was set up on a test tube stand. All were properly labeled against each sample. One tube was labeled blank. To the blank, 0.2 ml of sulphanilic acid, 1 ml of caffeine and 0.2 ml of sample were added. In each sample tube, 0.2 ml sulphanilic acid, 1 ml caffeine, 1 drop sodium nitrate and 0.2 ml respective samples were added. They were thoroughly mixed and allowed to stand for 10 min at 25°C. Thereafter, 1 ml of titrate was added to both the sample blank and rest of the tubes. They were again mixed and allowed to stand for 15 min at 25°C. The absorbance values (A.V.) of the samples against the sample blank were read in a colorimeter at 540 mn.

Then, the total bilirubin value was derived from the equation:

Total bilirubin (mg/dl) = 10.8 x A.V. (540 mn)

Determination of direct bilirubin: The test tubes were set as in total bilirubin determined in 1.1 above. To the sample blank, 0.2 ml of sulphanilic acid, 2 ml of normal saline acid and 0.2 ml of the sample were added. Then, in each sample 0.2 ml of sulphanilic acid, 1 drop of sodium nitrate, 2 ml of normal saline and 0.2 ml of required sample were added. They were all mixed and allowed to stand for exactly 5 min at 25°C. At the end of this period the absorbance values (A.V.) of the samples were read against sample blank at 546 mn. Then,

Direct bilirubin = 14.4 x A.V. (546 mn)

Estimation of indirect bilirubin: Indirect bilirubin was calculated as a difference between the values of total and direct bilirubin. That is:

Indirect bilirubin = Total bilirubin - Direct bilirubin

Determination of cholesterol: Test tubes were prepared and labeled as blank, standard and sample specimen. 10 ml of distilled water and 1000 ml of working reagent (4-Aminoantipyrine 0.30 mmol/l, phenol 6 mmol/l, peroxidase = 0.5 µl/ml, cholesterol esterase = 0.15 µl/ml, cholesterol oxidase = 0.1 µl/ml and pipe buffer 80 mmol/l all mixed together) were pipetted into the test tube labeled blank. 10 ml of standard solution and 1000 ml of the working reagent were added to the test tube labeled standard. Again, 1000 ml of the working reagent and 10 ml of the required samples were pipetted into the appropriate test tubes. Each tube was thoroughly mixed and incubated for 5 min at 37°C. The absorbencies of the samples were measured against the blank. From this, the cholesterol value was determined from the equation:

Cholesterol conc. = \( \frac{\Delta \text{Sample} \times \text{conc. of standard}}{\Delta \text{Standard}} \)

Determination of glucose: Tubes were prepared and labeled as blank, standard and sample specimen. Then, 1.5 ml of working reagent (glucose oxidase 15 µl/ml, peroxidase 1.2 µl/ml, mutarotase 40 µl/ml, 4-aminoantipyrine 0.38 mM and pyridobenzensulfonate 10 mM) was added to all the tubes. They were incubated in water bath at 37°C for 5 min. Thereafter, 0.01 ml of the sample was pipetted to the respective tubes, thoroughly mixed and incubated at 37°C for exactly 10 min. Colorimeter was zeroed with the reagent blank and the absorbencies of the sample tubes were read and recorded at 540 mn. Glucose concentration was determined from the equation given below:
Glucose conc. = \frac{Abs \times conc. \ of \ standard}{Ab \ standard}

**Data analysis:** All the data collected were subjected to Analysis of Variance (ANOVA) appropriate for a 2 x 3 factorial in a CRD to test the effects of breed, sex, and their interactions on the measured parameter. However, percentage values were transformed into Arcsine angles prior to analysis. The differences between means were separated using Duncan's New Multiple Range Test (DNMRT). SPSS (2004) Statistical Package was used for data analysis.

**RESULTS**

The breed effects on the blood bilirubin, glucose and cholesterol levels of the Nigerian Goats studied are given in Table 1.

Total bilirubin were similar (p>0.05) in all the Nigerian goat breeds. However, the SG recorded high direct bilirubin value but a low indirect bilirubin level. The reverse is true for the RSG, that is, the RSG scored high indirect bilirubin and less (p<0.05) direct bilirubin. The WADG on the other hand contained almost equal levels of direct bilirubin and indirect bilirubin. The level of glucose was significantly higher (p<0.05) in the RSG and SG than in the WADG. Significantly low (p<0.05) level of cholesterol was recorded in the SG compared to the levels in other breeds.

Table 2 presents sex effect on the blood bilirubin fractions, glucose and cholesterol levels of the Nigerian goat breeds. The total bilirubin fraction did not differ (p>0.05) between the male and female goats. The buck recorded higher levels of indirect bilirubin than did the doe, while the reverse was the case for direct bilirubin (p<0.05). The glucose level is significantly higher (p<0.05) in the doe than in the buck while cholesterol is higher in the buck than in the doe.

The mean levels of total bilirubin, direct bilirubin, indirect bilirubin, glucose and cholesterol as affected by sex x breed interaction are in Table 3. Except for the RSG, the total bilirubin is significantly the same (p>0.05) in the males and females goats studied. However, males recorded consistently higher numerical values in indirect (conjugated) bilirubin than their females' counterparts in all the goat breeds, but were significant (p<0.05) only in the RSG breed. Similarly, the Does persistently outscored the Bucks' counterparts in direct (unconjugated) bilirubin, but were significant (p<0.05) in the RSG and WADG breeds. There was no significant sex x breed interaction effects on the glucose level of the goats no matter the breed. It is only in the RSG group that the Bucks gave higher values of cholesterol (p<0.05) than did the Does. No other sex x breed interaction effect was exhibited on both glucose and cholesterol fractions of the goats, breeds and gender not withstanding.

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<tr>
<th>Table 1: The mean levels of bilirubin, glucose and cholesterol in SG, RSG and WADG breeds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
</tr>
<tr>
<td>----------------</td>
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<tr>
<td>Total bilirubin (mg/dl)</td>
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<td>Direct bilirubin (mg/dl)</td>
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*Means bearing different superscripts in the same row are significantly different (p<0.05). Where: SG = Sahel Goat, RSG = Red Sokoto Goat and WADG = West African Dwarf Goat

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<th>Table 2: The effect of sex on the total bilirubin, direct and indirect bilirubin, glucose and cholesterol levels across breed and source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
</tr>
<tr>
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</table>

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The mean values of total bilirubin, direct and indirect bilirubin, glucose and cholesterol levels of the experimental breeds of goat obtained from different sources for each breed are presented in Table 4. The Sahel goats have statistically the same levels (p<0.05) of unconjugated bilirubin, glucose and cholesterol, irrespective of source of the breed. Except for the Sahelian goat ecotype from Gumel, the same is true for total and conjugated bilirubin.

The RSG derived from Sokoto were statistically low in total bilirubin, direct and indirect bilirubin, and cholesterol level compared to ecotypes from Katsina and Gusau. RSG from Gusau were remarkably high in cholesterol, indirect bilirubin and total bilirubin, but low (p<0.05) in glucose level.

The WADG ecotype from Akure statistically gave lower (p<0.05) blood concentrations of total bilirubin, direct bilirubin and cholesterol, while they are comparable to the ecotype from Ughelli in glucose and indirect bilirubin levels. WADGS from Umuahia were exceptionally high (p<0.05) in cholesterol, total bilirubin and indirect bilirubin. However, they were statistically lower (p<0.05) in glucose level when compared to the ecotypes from Ughelli and Akure. The WADG goats from Ughelli were remarkable high (p<0.01) in conjugated bilirubin. It appears that the higher the glucose level, the lower the cholesterol concentration and vice versa.

**DISCUSSION**

The concentrations of cholesterol obtained for the SGs are numerically lower than the range of 70-116 mg/100 ml documented by researchers (Jovanovic et al., 1989; Macdonald and Low, 1995; Ritter, 1996; Scott, 1999; Zubic, 2001; Aikhuomobhogbe and Oheruatu, 2006), but those of the RSGs and WADGs fall within the range.
The low cholesterol concentration observed in the SGs indicates poor cell membrane and vitamin utilization, which might be responsible for the poor meat yield and quality often reported in the breed. In addition, the roughness and lightness of their skins could be attributed to the extreme low level of cholesterol in the breed. Higher levels of cholesterol recorded by bucks compared to the does could be physiological. The sahelian goats recorded similar values in cholesterol concentrations, irrespective of source. It could be that the sahelian goat breed is more homogenous compared to other breeds and extreme high levels of cholesterol recorded by the RSG goats from Gusau and WADG goats from Umuahia might be responsible for neonatal deaths often reported by farmers in those areas. However, there is lack of literature reports to buttress these facts.

The concentrations of glucose recorded for the Nigerian goats are within the literature values. Scott (1999) reported the range of 60-100 mg/100 ml; Jovanovic et al. (1989) gave the range of 68-82 mg/100 ml, while Grabkowski and Rutkowski (1989) documented the range of 60-78 mg/100 ml for different breeds of goat. Blood glucose level is of great value in determining the efficiency of sugar absorption in the body. The level of glucose in the WADG is lower than the referral ranges, and indicates high glucagon and low insulin concentrations in the blood. Schalm et al. (1975), Harper et al. (1977), Kaneko et al. (1997), Mohammed et al. (1991) and Zapata et al. (2003) reported that very low level of glucose in the blood will lead to reductions in weight gain and milk yield and to a change in the fatty acid composition of the milk. Consequently, the low weight gain and poor milk yield in the WADGs compared to other breeds might be as a result of low level of glucose in their blood. The males have lower glucose levels than the females, which could be due to catabolism as the bucks frequently engage in exercise. The exceptional low level of glucose recorded by the WADG goats from Umuahia (South-East) accounts for the miniature size of the goats from the area. The study further revealed that the higher the blood glucose, the lower the cholesterol concentration in the blood and vice-verse.

The total serum bilirubin is similar in the three breeds of goats studied and lies within the range of 0.00-0.90 mg/100 ml reported by notable researchers for goats and other classes of livestock (Harper et al., 1977; Frandsen, 1981; Singh, 2004). However, the SG breed has the highest serum concentration of unconjugated (direct) bilirubin, followed by the WADG and the least being the RSG. This implies that the Sahelian goats have the greatest inherent tendency to suffer from liver disease and or more of blocked blood vessels. Consequently, the SG breed kids are prone to neonatal jaundice. Significantly higher levels of unconjugated bilirubin recorded by does in this study could be as a result of the fact that bucks engage in more exercise than the does and as such, the conjugated bilirubins are broken down, though there is scarcity of information to buttress these findings.
Conclusion: The cholesterol level in Nigerian goats is inversely proportional to the glucose concentration. WADG should be the meat of choice for people suffering from diabetes or family with chronological records of diabetes, while the Sahelian chevron is preferable for individuals with high levels of cholesterol (cholesterol arum). Nigerian goat breeds have similar concentrations of total bilirubin, but sex plays key role in the fraction of the conjugated (direct) and unconjugated (indirect) bilirubin. The Sahelian goats are more homogeneous, irrespective of source. Again, poor weight gain and milk yield often reported in WADG are as a result of low level of glucose in their blood, and miniature size of the WADG ecotype from South-East is a function of their blood glucose.

REFERENCES