Physiological response of WAD sheep fed different combinations of Guinea grass and ensiled Alternanthera brasiliana (L.) Kuntze based diets: Intake, haematology and serum biochemical indices

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ABSTRACT

Animal feed shortages characterize the long dry season in most Sub-Saharan African countries. Studies exploring alternative feedstuffs with similar or complementary nutritional efficacy to the conventional ones are seriously canvassed. Therefore, this study aimed to evaluate the effect of feeding West African Dwarf (WAD) sheep with different combinations of guinea grass and ensiled A. brasiliana based diets on their intake, haematology, and biochemical indices. A total of 30 growing female WAD sheep (BW =10.5 ± 0.92 kg; mean ± SD), 12–18 months of age were allocated to five dietary treatments containing different inclusion levels of ensiled A. brasiliana (0%, 30%, 45%, 60% and 90%) in a trial that lasted for 90 days. Intakes were estimated daily and body weight changes taken weekly. Blood concentrations of haemoglobin, packed cell volume, white blood cells, differential white blood counts (lymphocytes, monocytes, neutrophils, eosinophils), red blood cells, total protein, albumin, globulin, creatinine and cholesterol were also determined. The total dry matter intake (DMI) increased (P < 0.05) with increasing inclusion levels of A. brasiliana. While the feed conversion ratio decreased (P < 0.05), the final weight and average daily gain significantly increased (P < 0.05) with increasing levels ensiled A. brasiliana. The red blood cell, creatinine, globulin, cholesterol, white blood cell and its differentials were not affected (P > 0.05) by the inclusion levels of A. brasiliana. However, the total protein and albumin were influenced (P < 0.05) by inclusion levels of A. brasiliana. The inclusion levels of A. brasiliana up to 90% promote growth and were not harmful to the ewes.

1. Introduction

The role of livestock, especially small ruminants, to most developing nations’ socio-economic development and food security cannot be overemphasized. Apart from their preference over other livestock due to their ability to withstand harsh environmental conditions, sheep production forms an integral part of rural farming life, providing them with an animal protein source and income from sales (Akinmoladun, Muchenje, Fon, & Mpendulo, 2019). In Nigeria, the long dry season characterized by animal feed shortages (both in quantity and quality) and poor nutritional value of forages is a major factor limiting their sustainable development and productivity. Poor nutrition, occasioned by low voluntary intake and reduced digestibility, are the root cause of low reproductive performance, depression in growth rate, loss of body condition and decreased immunity in livestock. A possible solution is to identify and explore alternative and non-conventional feed resources of no feeding value to humans, locally available and with high nutritional potentials (Akinmoladun, Adejoro & Jimoh, 2018a). Such non-conventional feeds can be supplemented, either wholly or combined with natural pastures and/or crop residues to alleviate the nutritional problems that sheep production faces. According to Olivares-Perez, Aviles-Nova, Rojas-Hernández, Albarrán-Portillo and Castelán-Ortega (2011), browse fodders are not only abundant all year round but are capable of yielding high protein during the dry seasonal periods and thus improve the nutritional quality of crop residues when supplemented. The non-conventional feed resources adapted (not limited to) to this study area are Alternanthera brasiliana (A. brasiliana) and guinea grass.

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A. brasiliana (L.) O. Kuntze, an evergreen perennial plant, is an example of a browse species available in the tropics which can be used for feeding ruminants. Although it was originally found in the tropical and subtropical regions of Australia and South America (Duarte & Debur, 2004), it has become naturalized in tropical countries like Cuba, Nigeria, India, Kenya, Colombia and Vietnam (Sanchez-Del, 2012). Its alternative names, Brazilian joy weed, penicillin or Terracina, Joseph’s coat, calico plant, and indoor clover, were due to its relative abundance in Brazil (Duarte & Debur, 2004; Macedo et al., 2004). Despite the plant’s appreciable nutritional and chemical constituents for animal productivity, the biological effects of the leaves of this plant (e.g., wound healing, lymphocyte proliferation, anti-diabetic and anti-microbial), which are due to the inherently abundant phytochemicals are well documented (Arigbodo, 2020; Barua et al., 2012).

Abdel fattah, Carlos and Shibu (2020) reviewed that most studies reported improved animal performance when fed plant-based diets as an alternative feed resource. However, the efficient utilization of most browse plants is hampered due to the presence of phytochemicals such as saponins, condensed tannins and phytate. The high abundance of such phytochemicals may cause bitterness, reduce nutrient availability, palatability, and induce toxicity in animals when consumed beyond threshold levels (Akinmoludun, Sabi & Adedayo, 2018b; Ladipo & Akinfenmi, 2014). When animals are fed non-conventional feeds containing high levels of nutrional compounds, and for an extended period, their growth rate, health status and disease resistance are negatively affected. Although the animal’s death may result after a while, the long processes leading to death are a collapse of normal physiology and homeostasis. Hence, the need to check the physiological and health indicators of feed consumption from non-conventional sources. The present study was designed to determine the intake, haematology and biochemical indices of WAD ewes fed ensiled A. brasiliana (L.) O. Kuntze and Guinea grass-based diets

2. Materials and methods

2.1. Study site and animal protocol

The experiment was carried out at the goat unit of the Teaching and Research Farm, Tai Solarin University of Education, Ijagun, Ijebu-Ode (6.80’N, 3.92’E). The climate is tropical and with significant rainfall (average 2020 mm) in most months of the year. The short dry season (about three months) has little effect on the overall climate and is marked with a temperature average of 26.0°C. The study was conducted in conformity with the routine care of animal management, approved by the Research and Ethics Committee on Animal Use, Animal Science Unit, Department of Agricultural Science, Tai Solarin University of Education, Ijebu-Ode, Nigeria.

2.2. Experimental diet

A concentrate diet was formulated to meet the demand of a growing sheep (National Research Council NRC, 1981) (Table 1). The diet treatments consisted of various combinations of ensiled A. brasiliana (AB), Guinea grass (GG) and concentrate (CC) on a dry matter basis (Table 2). Briefly, A. brasiliana was harvested, chopped and ensiled with wheat offal at a ratio of 80:20% w/w respectively for 42 days (Akinwande, Mako & Babayemi, 2011). Polythene bags with a capacity ≥ 30 kg of AB were used as silos. A 25 kg sandbag was used to seal the mouth of the polythene bag by gently placing it on each bag after proper tying. Five different combinations of experimental diets were thereafter formulated using the ensiled A. brasiliana.

2.3. Animal feeding and experimental design

Thirty (30) growing female WAD sheep, 12–18 months of age and weighing 9–12 kg (mean 10.5 ± 0.62 kg SE), were allocated to five groups of comparable body weight. The feeding experiment was conducted for 121 days, with the first 21 days for acclimation. A labelled tag was placed on each animal for easy identification and separation corresponding to the pen number. The sheep were vaccinated against PPR (Peste des petit ruminant) and treated with ivermectin (against endo and ectoparasite) before the commencement of the study. The sheep were housed individually and provided daily feed (5% of BW: 1% concentrate; 4% of A. brasiliana and Guinea grass) and water. Body weight changes were taken weekly and in the morning (08:00 h) before they had access to feed and water while the daily feed intake was taken in the morning after subtracting the refusals from the amount offered. Daily feed refusals were collected and mixed for the entire duration of the study. The dry matter, crude protein, fat, ash, neutral detergent fibre and acid detergent fibre of the concentrate diet, guinea grass, and ensiled A. brasiliana were determined following standard methods of the (AOAC, 2000).

2.4. Blood collection and analysis

At the end of the feeding trial, two blood samples were collected from the jugular vein of each animal into plain (serum) and ethylenediaminetetraacetic acid (EDTA) tubes. The samples were transported in an icebox to the laboratory for the determination of red blood cell count, packed cell volume, haemoglobin, mean corpuscular haemoglobin concentration (MCHC), white blood cell count and differential white blood cell (neutrophils, lymphocytes, eosinophils, and monocytes) count using the procedure described by Lamb (1981). The blood serum tubes were centrifuged (10 min, 3500 rpm) to allow for separation. The separated serum samples were transferred into 5 ml vials and frozen (−20°C) until analysis. The extracted sera were evaluated for total protein, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SPGT) and creatinine using Diagnostic Limited kits, India.

Table 1

| Ingredient | Composition (g/100 g DM) |
|------------|-------------------------|
| Maize      | 20.00                   |
| Wheat offal| 25.00                   |
| Corn offal | 18.00                   |
| Soybean meal| 8.00                  |
| Palm kernel cake| 10.00           |
| Brewer’s dry grain| 16.00     |
| Oyster shell| 1.75                   |
| Salt       | 1.00                    |
| Premix     | 0.25                    |
| Calculated CP| 18.00                  |
| Calculated ME (Kcal/kg)| 2400               |

Table 2

| Treatment       | Concentrate | A. brasiliana | Guinea grass |
|-----------------|-------------|---------------|--------------|
| 0%              | 10          | 0             | 90           |
| 30%             | 10          | 30            | 60           |
| 45%             | 10          | 45            | 45           |
| 60%             | 10          | 60            | 30           |
| 90%             | 10          | 90            | 0            |
| Total           | 100         | 100           | 100          |

*A Ca = 220 g/kg; P = 55 g/kg; Mg = 35 g/kg; S = 22 g/kg; Cl = 105 g/kg; Na = 70 g/kg; Mn = 1500 mg/kg; Fe = 500 mg/kg; Zn = 1550 mg/kg; Cu = 440 mg/kg; Co = 50 mg/kg; I = 40 mg/kg; Se = 20 mg/kg.

Statistical analysis

Data obtained on intakes and blood metabolites were analysed using the general linear model of the SAS procedure (SAS, 2007) version 9.1.3 of 2007. For all traits, the model included the fixed effect of treatments and the random effect of animals. The model used is
\[ Y_{ij} = \mu + T_i + V_k + e_{ijk} \]

Where \( Y_{ij} \) is the value of the dependant variable determined from each animal, \( \mu \) is the overall mean, \( T_i \) is the fixed effect of ith treatment (\( i = 1 \) to 5), \( V_k \) is the random effect of animals, and \( E_{ijk} \) is the random error. The least square means were generated and compared for significance using the same statistical package. Analysed data were considered significant at \( P < 0.05 \).

### 3. Results and discussion

The proximate analysis of guinea grass and ensiled \( A. \) brasiliiana revealed the following percentages respectively: dry matter (90.35, 90.81%); crude protein (8.34, 18.38%); EE (10.93, 3.54%); ash (13.32, 9.84%); neutral detergent fibre [NDF] (71.21, 61.51%) and acid detergent fibre [ADF] (48.70, 46.89). The intakes and body weight changes of WAD ewes fed ensiled \( A. \) brasiliiana based diets is shown in Table 3. While guinea grass intake decreased (\( P < 0.05 \)), the amount of \( A. \) brasiliiana voluntarily ingested by the sheep increased (\( P < 0.05 \)) with increasing inclusion levels. Similarly, the dry matter intake (DMI), final weight (FW), body weight gain, and average daily gain increased with increasing levels of \( A. \) brasiliiana. The relative and progressive increase in DMI (consistent with the FW) following the increasing inclusion levels of \( A. \) brasiliiana could be associated with its high crude protein contents and possibly high digestibility. A similar response of improved feed intake and final weight was observed when the Small East African goats were fed Leucaena leucocephala leaf meal, a notable plant with high crude protein content, compared to other browse (Acacia nilotica and Acacia polyacantha) (Rubanza, Shem, Bakengesa, Ichinohe (Rubanza, 2007)). Feeding ruminants with browse containing crude protein below the normal CP required for optimal rumen microbial function can reduce voluntary feed intake (Annisin & Bryden, 1998). Such reduced microbial activity implies a low nutrient supply to the animals, which reduces growth rate and body weight. The observed weight decrease with an increased inclusion level of guinea grass could be explained by the low CP and high fibre contents (NDF and ADF) due to their influence on feed digestibility. Feed intake, digestibility, and extension body weight are depressed. The continuous increase in weight gains of sheep with increasing levels of ensiled \( A. \) brasiliiana could be explained by the high protein content of the ensiled forage and high utilization efficiency. This is reflected in the feed conversion ratio, decreasing with an increasing inclusion level of \( A. \) brasiliiana.

The haematological profile of WAD ewes fed different combinations of guinea grass and ensiled \( A. \) brasiliiana based diets is presented in Table 4. The result revealed significant (\( P < 0.05 \)) variations amongst treatment means for Haemoglobin (Hb) and Packed cell volume (PVC). It was observed that Hb and PVC values increased with increasing levels of ensiled \( A. \) brasiliiana. The Hb values ranged between 7.35 and 9.79 g/dl in ewes fed ensiled \( A. \) brasiliiana inclusion levels (0–90%). This result is comparable to the value range reported for healthy sheep (Fajemisin, Fadiyimu & Mokan, 2010) and also in agreement with the value range of 6.11–9.87 g/dl reported for WAD ram fed ensiled Mucuna pruriens foliage and whole maize stover (Alabi & Ososanya, 2017). The adequate haemoglobin concentrations for all the sheep fed various inclusion levels of \( A. \) brasiliiana is an indication that haemoglobin synthesis was not affected. The PCV values obtained in this study followed the same pattern with Hb and ranged between 29.5 and 33.12%. This value range is similar to the range reported for WAD sheep (29.9 – 33.6%) fed combinations of preserved elephant grass, layers droppings, and cassava peel diets (Sowande et al., 2008) as well as WAD rams (27.21 – 30.02%) fed silage combinations of maize forage and velvet beans (Alabi & Ososanya, 2017). The PCV provides a measure of toxicity in the blood, and a low value is often implicated in anaemia. This anaemic condition is characterized by reduced oxygen-carrying capacity of the blood, increased pulse rate, and consequently heart failure (Jiwuba, Ahamefule, Okechukwu & Ikunwze, 2016). The progressive increase in Hb and PVC with an increasing level of ensiled \( A. \) brasiliiana indicates a low presence of antinutritional factors or toxic elements, particularly phenols and condensed tannins, thus enhancing blood formation and supply (Olafadehan, 2011).

The effect of dietary treatments on white blood cells (WBC), red blood cells (RBC), and MCHC were not significant (\( P > 0.05 \)) and did not follow a particular pattern. The values for WBC in this study ranged between 6.63 and 8.25 × 10^3/μl. This result is similar to the value range of 7.43–8.85 × 10^3/μl reported for rams fed ensiled Mucuna pruriens and whole maize stover for healthy sheep (Alabi & Ososanya, 2017). The white blood cell differential counts (neutrophils, lymphocytes, monocytes, and eosinophils) were also not significantly different (\( P > 0.05 \)) amongst the treatment means. The values obtained here ranged between 36.24 and 40.82; 56.04 and 57.03; 2.00 and 4.00; 2.72 and 3.04 for neutrophils, lymphocytes, monocytes and eosinophils, respectively.

### Table 3

Intakes and body weight changes of WAD sheep fed ensiled \( A. \) brasiliiana based diets.

| Parameters                  | Inclusion levels of ensiled \( A. \) brasiliiana |
|-----------------------------|-----------------------------------------------|
|                             | 0%    | 30%   | 45%   | 60%   | 90%   | SEM   |
| Intake (g/d)                |       |       |       |       |       |       |
| DM (g/d)                    |        |       |       |       |       |       |
| Concentrate                 | 95.20± | 90.27b | 89.24d | 88.99d | 88.92d | 0.51  |
| \( A. \) brasiliiana         | 448.60d | 541.50c | 706.20d | 901.30c | 10.20  |
| Guinea grass                | 698.40e | 259.70b | 210.90d | 166.8d  | 12.70  |
| Total DMI (g/kg)            | 793.24c | 838.10b | 842.29c | 961.20c | 1020.50b | 22.10 |
| IW (g/kg)                   | 12.90c | 11.00c | 10.50c | 10.50c | 10.50c | 0.70  |
| FW (g/kg)                   | 15.00d | 15.00c | 14.50d | 15.50d | 16.00c | 0.33  |
| Weight gain (µg/day)        | 3.00d  | 4.00d  | 4.50d  | 5.00d  | 5.50d  | 0.25  |
| Daily gain (g/day)          | 53.57d | 71.43c | 71.43c | 89.39c  | 98.21c  | 7.89  |
| FCR                         | 14.81a | 11.73b | 11.79b | 10.76c  | 10.39d  | 0.91  |

Note: *a*, *b*, *c*, *d* means on the same row with different superscript differed significantly (\( P < 0.05 \)).

FCR = feed conversion ratio; SEM = standard error of mean; IW = initial weight; FW = final weight.

### Table 4

Haematological indices of WAD sheep fed ensiled \( A. \) brasiliiana based diets.

| Parameters                  | Inclusion levels of ensiled \( A. \) brasiliiana |
|-----------------------------|-----------------------------------------------|
|                             | 0%    | 30%   | 45%   | 60%   | 90%   | SEM   |
| Haemoglobin (g/dl)          | 7.35d  | 8.18d  | 8.84d  | 9.23b  | 9.97b  | 0.31  |
| Packed cell volume (%)      | 29.52a | 29.98a | 31.60b | 32.45b | 33.12a | 0.25  |
| White blood cell (x 10^7/μl)| 7.84  | 7.09  | 6.63  | 7.91  | 8.25   | 0.53  |
| Neutrophils                 | 40.82 | 38.17 | 36.24 | 40.01 | 38.29 | 3.25  |
| Lymphocytes                 | 57.01 | 56.20 | 56.04 | 57.02 | 57.12 | 3.52  |
| Monocytes                   | 2.00  | 3.00  | 4.00  | 4.00  | 4.00   | 2.12  |
| Eosinophils                 | 3.00  | 2.78  | 2.72  | 2.81  | 3.04   | 0.18  |
| Red blood cell (x 10^6/μl)  | 12.86 | 11.60 | 11.67 | 13.01 | 13.23  | 2.19  |
| MCHC (g/dl)                 | 33.12 | 33.00 | 33.20 | 33.11 | 34.25  | 0.35  |

Note: *a*, *b* means on the same row with different superscript differed significantly (\( P < 0.05 \)).

MCHC = mean corpuscular haemoglobin concentration.
These values are similar to the range of values (35.38 and 41.23; 56.02 and 57.03; 2.00; 2.27 and 3.02 respectively) reported for neutrophils, lymphocyte, monocyte and eosinophil, respectively in WAD ram fed silage combination of maize forage and Mucuna pruriens (Alabi & Ososanya, 2017). The higher values of WBC reported, especially in the 90% inclusion of A. brasiliana based group, indicates a strong ability to fight disease (Nicholson, 2016). The implication of this is that the diet did not predispose the animals to any stress and disease. However, all the values obtained here are within the normal range of 5–8 × 10⁷/µl reported for normal healthy sheep (Mitruka & Rawnsley, 1997).

The MCHC values ranged between 33.00 and 34.25 g/dl from the 30% to 90% ensiled A. brasiliana inclusion levels. These values are within the range of 32.0–33.23 g/dl reported for WAD goats (Mako, Akinwande & Awobajo, 2015). However, all the RBC and MCHC values obtained in this study are within the normal range for normal sheep (Radostits, Blood & Gay, 1997). The result implies that the diets supported the sheep’s good health status and indicated the absence of anaemia amongst the experimental treatment groups.

The serum chemistry data of the study are presented in Table 5. The total protein (TP) and albumin differed significantly (p < 0.05) amongst the treatment means and did not follow a particular pattern. The TP for experimental sheep was between 5.71 and 6.22 g/l. These values were within the range reported by Carlos et al. (2015) in sheep with a body condition of 2.5. A similar range of TP values was also reported in goats fed Lablab purpureus and Vigna unguiculata as supplements (Washaya, Mupangwa, Muchenje & Mpendulo, 2019). Under extensive management, the serum TP values could be as high as 7.5 g/dl (Mohammed, Razaque, Omar, Albert & Al-Gallaf, 2016). Such elevated serum TP values could indicate dehydration, high temperature (Akinmoladun, Fon, Mpendulo & Okoh, 2020), kidney or liver disease, or increased intake of grains (Sandabe & Chaudhary, 2000). However, Akinrinmade and Akinrjide (2012) affirmed that TP values below 4.2 g/dl in small ruminants could induce rumen compaction. This was not the case in this study. The TP range in this study agrees with the normal range value (5.9 – 7.8 g/dl) reported for goats (Oloche, Oluremi & Ayuba, 2014), thus implying that the diet did not predispose the animals to disease.

The serum albumin values were significantly influenced (P < 0.05) by the dietary treatment methods without any influence pattern. However, according to Oyeyemi, Ekanade and Ogunsemoyin (2014), the values obtained were within the recommended range. As the most abundant type of protein, low serum albumin concentration is a sign of poor health (liver dysfunction). Nonetheless, other indicators of liver function (SGOT and SGPT) were not different amongst treatments and thus indicated no negative effect of diets on the liver.

The globulin content did not vary significantly (P > 0.05) amongst treatment means, with values ranging between 2.56 and 3.01 for ewes fed 90%, and 0% ensiled A. brasiliana, respectively. These values agree with the value range of 2.44–3.40 reported for goats (Mako et al., 2015). These values also fall within the normal range reported for healthy sheep (Mitruka & Rawnsley, 1997).

Serum creatinine concentrations were not influenced (P > 0.05) by treatment means. This is contrary to the findings of Mako et al. (2015), who reported significant variations in the creatinine values of goats fed sun-cured water hyacinth. The range of creatinine values from this study (0.82 – 0.95 mg/dL) is similar to values reported by Oyeyemi et al. (2014) elsewhere. Serum thyroxine levels normally decrease following elevated creatinine levels (Yokus, Cakir, Kanay & Uysal, 2006). The non-significant effect of dietary treatments on creatinine suggests that the thyroxine levels were not affected, hence a better energy utilization.

Although there were no significant variations in the serum cholesterol and its fractions (high density lipoprotein [HDL] and low density lipoprotein [LDL]) across the dietary treatments, values obtained are within the recommended range (Taiwo & Ogunsanmi, 2003). The cholesterol range obtained in this study (55.94 – 57.23 mg/dl) is similar to the value range (58.61 – 59.81) reported for WAD ram fed silage combinations of maize forage and Mucuna pruriens foliage (Alabi & Ososanya, 2017). Serum cholesterol is a group of fats vital to the cell membrane and bile salts necessary for sex hormones (Alabi & Ososanya, 2017). A decrease in cholesterol concentration for an extended period could be attributed to the presence of phytochemicals in forages, such that the synthesis and absorption of cholesterol are reduced (Saxena, Saxena, Nema, Singh & Gupta, 2013). However, higher levels could indicate dehydration (Mpendulo, Akinmoladun, Ikusua & Chimonyo, 2020) or feeding ruminants with carbohydrates-rich diets (Alabi & Ososanya, 2017).

HDL and LDL values ranged from 41.94 – 43.82 mg/dL and 10.00 – 10.36 mg/dL in WAD ewes fed 90–90% inclusion of ensiled A. brasiliana respectively. LDL, the main carrier of cholesterol in the blood, delivers cholesterol from the liver to the peripheral tissues and are returned to the liver in HDL by reverse cholesterol transport (Kessler, Gross, Bruckmaier, & Albrecht, 2014) The normal range of HDL and LDL (McDonald, Edward, Greenhalgh & Morgan, 1995) values in this study suggest cholesterol transport and distribution were not impaired.

4. Conclusion

It can be concluded that feeding WAD ewes with ensiled Alternanthera brasiliana based diets up to 90% inclusion levels promote body mass growth with no harmful effect on blood parameters. This is indicative of good health status and optimal performance. WAD ewes can therefore survive on ensiled A. brasiliana based diet alone, especially during the dry season in the tropics.

Ethical statement

All authors stated the ethics procedure guidelines followed in the Methods section of their research and to detail any ethical review permissions that have been received. The ethics committee at Tai Solarin University approved the experimental procedure.

Declaration of competing interest

The authors declare no competing interest with any organization or financial group in the subject matter discussed in the manuscript.

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Table 5

| Parameters                         | Inclusion levels of ensiled A. brasiliana | 0% | 30% | 45% | 60% | 90% | SEM |
|-----------------------------------|------------------------------------------|----|-----|-----|-----|-----|-----|
| Total protein (g/dl)              | 6.01b                                    | 5.89b | 5.71b | 5.98b | 6.22b | 0.15 |
| Albumin                           | 3.54b                                    | 3.00b | 3.15b | 3.28b | 3.62b | 0.10 |
| Creatinine (mg/dl)                | 0.90                                      | 0.82 | 0.88 | 0.86 | 0.95 | 0.20 |
| Globulin (g/dl)                   | 2.56                                      | 3.01 | 2.59 | 2.64 | 3.52 | 0.29 |
| SGOT (µl/L)                       | 53.21                                    | 43.50 | 42.85 | 50.21 | 52.80 | 2.02 |
| SGPT (mg/dl)                      | 11.50                                     | 11.21 | 9.14 | 8.63 | 11.84 | 1.45 |
| Cholesterol (mg/dl)               | 57.23                                     | 56.91 | 56.72 | 56.53 | 55.94 | 1.20 |
| HDL (mg/dL)                       | 41.94                                     | 42.51 | 42.73 | 43.50 | 43.82 | 1.00 |
| LDL (mg/dL)                       | 10.00                                     | 10.13 | 10.24 | 10.33 | 10.36 | 0.15 |

b, c, d = means on the same row with different superscript differed significantly (p < 0.05); SGOT = Serum glutamic oxaloacetic transaminase; SGPT = Serum glutamic pyruvic transaminase, SEM = standard error of mean.
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