Research Article

Cardioprotective Effects of Insect *Apis melifera*-Based Complementary Foods Using an *In Vivo* Mouse Model

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Background. Cardiovascular disease is the cause of one-third of deaths worldwide because of increased risk factors, such as intake of cholesterol and saturated fat. Atherosclerosis begins in childhood; therefore, nutritional prevention should begin at an early stage. This study assessed the lipid profile, atherogenic, and castill’s risk index intake of *Apis melifera*-based complementary foods using an *in vivo* mouse model. Methods. The experiment was conducted for 28 days. A total of 75 male white albino mice were randomly assigned to five diets in triplicate. The diets were Diet 1 = casein diet; Diet 2 = (57% maize, 29% tef, 14% soybean); Diet 3 = (58% maize, 29% tef, 13% bee larvae); Diet 4 = commercial wean mix; Diet 5 = basal diet alone. Mouse blood samples were collected by cardiac puncture. The lipid profiles of TC, TG, HDL-C, and LDL-C were analyzed using an automated pentra C400 made in France. Results. Biochemical (mg/dl) parameters showed that mice fed Diet 3 had high (P≤0.001), TG (167.79), HDL-C (67.18), and low LDL-C (71.73) levels. The atherogenic indices CRI-I (1.84), CRI-II (1.07), and AC (0.84) were low in Diet 3. The atherogenicity indices showed a significant positive correlation (P≤0.001) with one another as follows: CRI-I vs. CRI-II (r=0.919), CRI-I vs. AC (r=1), and CRI-II vs. AC (r=0.919). Conclusion. The results of the present investigation confirm that intake of an *Apis melifera*-based diet could prevent children from atherosclerotic cardiovascular disease in a mouse model.

1. Introduction

Cardiovascular disease (CVD) is currently the main factor contributing to morbidity and mortality [1, 2], representing 30% of all deaths worldwide [3]. Coronary heart disease (CHD) is a common disease caused by increased intake of cholesterol and saturated fat, decreased intake of polyunsaturated fatty acids (PUFAs), and increased obesity [4]. Additionally, CHDs are more common in low-birth-weight infants [5]. Atherosclerosis is an inflammatory disease that contributes to the major incidence and mortality of CVD [3]. In the process of atherosclerosis, lipids accumulate on the walls of blood vessels and cause inflammatory reactions, thereby stimulating the progression of atherosclerosis [2]. However, the atherosclerotic process starts before birth, progresses through childhood in the so-called “first thousand days,” and eventually leads to CHD if unstopped. Therefore, it is important to start prevention in the earliest stages of life [6, 7].

The lipid profile (dyslipidemia) is a significant risk factor for CVD and early atherosclerosis [8, 9]. Dyslipidemia is a significant contributor to the risk of both coronary artery disease and stroke and is defined as an elevated plasma concentration of lipids (triglycerides and total cholesterol).
and their related blood-transporting lipoproteins: high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) [10–12]. However, the associations between the relative amount of cholesterol and total cholesterol in individual lipoprotein classes and CHD are complicated [13].

In the absence of an unfavorable lipid profile, the possible consequence of CVD cannot be ruled out [14]. Additionally, dyslipidemia is a target for the prevention and treatment of many CVDs. As a result, identifying persons at risk of CVD is necessary for early detection and prevention [15]. The internalization of lipids, especially LDL, in the intima contributes to the early stages of atherosclerosis, which leads to endothelial dysfunction [16]. An increase in LDL and triglycerides is the traditional indicator of dyslipidemia [17] particles and a decrease in HDL levels [18]. Lowering LDL-C levels is well established as an intervention for reducing CVD [18].

In tropical regions of the world, bee broods (larvae and pupae) were known to be accepted by numerous ethnic populations [19]. Bee brood serves as a food source for humans in many countries [20]. In Ethiopia, the use of edible insects in the production of CF has not been scientifically studied. Honeybees at all stages of development are an excellent food source due to their high protein content and optimal composition of saturated and MUFA [19, 20]. Palmitic, stearic, and oleic acids are the dominant fatty acids in all stages of bee development [19, 21]. A high level of SFAs is related to atherosclerosis and heart failure; however, some SFAs, such as stearic acid, which is found in bee larvae, help to lower LDL cholesterol levels [22]. As a result, blending bee larvae with other dietary ingredients can be used to develop nutritious and healthful complementary foods (CFs) [23] for infants and young children (IYC). The increased levels of TC, LDL-C, and TG, as well as HDL-C, have traditionally been associated with atherosclerosis. Currently, the atherogenic index of plasma (AIP) [24], Castelli’s risk index I (CRI-I), and II (CRI-II) and the atherogenic coefficient (AC) parameters are used for a better prognosis in cases of CVDs [13, 25]. The AIP was discovered to be one of the most effective markers for predicting the risk of CVD [11]. However, there is no research output on the association of insect bee larvae-based CFs with dyslipidemia in infants and young children using an in vivo mouse model of serum lipid profiles. Therefore, this study evaluated the lipid profile, atherogenic index, and Castill’s risk index of Apis mellifera-based complementary foods using in vivo white albino mice.

2. Materials and Methods

2.1. Diet Formulation and Preparation. Five isonitrogenous (10% protein) experimental diets were formulated. The commercial wean mix and casein diet were purchased from a supermarket in Ethiopia. Insect bee larvae (Apis mellifera) were collected from modern beehives, maize (Zea mays L.), red teff (Eragrostis tef (Zucc.)), and soybean seeds (Glycine max) were purchased and collected from the Ethiopian Agricultural Research Center and local market. The basal diet was used as a control [26]. In summary, the basal diet comprised 610 g/kg maize starch, 20 g/kg bone meal, 50 g/kg wheat bran, 100 g/kg vegetable oil, 50 g/kg mineral and vitamin premix, 60 g/kg glucose, 88 g/kg sucrose, 20 g/kg oyster shell, and 2 g/kg NaCl. The two developed complementary diets were developed using NutriSurvey software (version, 2007), i.e., diet 1 = maize (57%), teff (29%), and soybean (14%), whereas diet 2 = maize (58%), teff (29%), and bee larvae (13%), and then extrusion processes were performed [23]. The commercial wean mix was purchased from the local market in Ethiopia. Table 1 shows the nutritional composition of the developed and commercial complementary diets. The proximate analysis of experimental diets and commercial wean mix data (Table 1) that used to support the findings of this study were published previously [27].

The composition of the experimental diets with the basal diets was calculated using (1) [26].

\[
IS = \frac{X}{100} x Y = \frac{100}{100} x Z,
\]

where \(X\) = original protein content of the sample as analysed \(Y\) = weight of sample required for the new feed mixture \(Z\) = total weight of the mixture \(IS\) = Isonitrogenous.

Finally, each group of mice was randomly assigned to one of the experimental diets (Table 2).

2.2. Experimental Animals. All aspects of animal care and experimentation were performed according to the Guide for Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and followed the EEC directive of 1986 (86/609/EEC) and were approved by the ethical committee of the University of Gondar, Ethiopia. A total of seventy-five white albino male mice with an average age of 26–28 days and an average body weight of 31.57 ± 1.42 g were used. The mice were randomly assigned to one of five groups (\(n = 15\)) with five animals per cage. The experiment was conducted for twenty-eight days and seven days of acclimatization under the same environmental conditions (room temperature 26 ± 0.42°C, RH 55 ± 5%, and 12 h light-dark cycle). Clean tap water and diet were offered ad libitum throughout the experiment.

2.3. Blood Sample Collection and Analysis. Fasted blood serum was used for the determination of the biochemical lipid profiles of TC, TG, HDL-C, and LDL-C. The experimental animals were fasted for 12 hrs before blood sample collection. Before sacrifice, the mice were anaesthetized using ketamine-xylazine anaesthesia (mixed in the ratio 4:1) with 0.25 mL/100 g body weight using a 25-gauge needle and a syringe intraperitoneally and then terminated by cardiac puncture injection [28, 29]. The collected blood samples were immediately administered into heparin-coated tubes in serum containers and placed on wet ice for further lipid analyses [30]. Sera were isolated from whole blood and centrifuged at 3000 rpm for 10 min on a 4°C digital laboratory centrifuge (TD4C dc brushless motor centrifuge, Hunan, China).
Table 1: Proximate analysis of experimental diets and commercial wean mix (on a dry matter base).

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Commercial wean-mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/100 g)</td>
<td>417.93 ± 3.23</td>
<td>427.18 ± 2.42</td>
<td>385.25 ± 1.77</td>
</tr>
<tr>
<td>Carbohydrate (g/100 g)</td>
<td>64.02 ± 0.41</td>
<td>62.87 ± 0.23</td>
<td>79.19 ± 0.55</td>
</tr>
<tr>
<td>Fat (g/100 g)</td>
<td>12.4 ± 0.1</td>
<td>14.3 ± 0.1</td>
<td>2.82 ± 0.36</td>
</tr>
<tr>
<td>Fiber (g/100 g)</td>
<td>4.52 ± 0.04</td>
<td>3.47 ± 0.08</td>
<td>2.75 ± 0.17</td>
</tr>
<tr>
<td>Protein (g/100 g)</td>
<td>12.56 ± 0.17</td>
<td>11.75 ± 0.15</td>
<td>10.78 ± 0.29</td>
</tr>
</tbody>
</table>

Diet 1: soybean (14%), teff (29%), and maize (57%); Diet 2: bee larvae (13%), teff (29%), and maize (58%).

Table 2: Experimental diet formulation and preparation.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Protein content (%)</th>
<th>Wt. of basal diet (g)</th>
<th>Wt. of food (g)</th>
<th>Total (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 1</td>
<td>99</td>
<td>898.99</td>
<td>101.01</td>
<td>1000</td>
</tr>
<tr>
<td>Diet 2</td>
<td>12.56</td>
<td>203.82</td>
<td>796.18</td>
<td>1000</td>
</tr>
<tr>
<td>Diet 3</td>
<td>11.75</td>
<td>148.94</td>
<td>851.06</td>
<td>1000</td>
</tr>
<tr>
<td>Diet 4</td>
<td>10.78</td>
<td>72.36</td>
<td>927.64</td>
<td>1000</td>
</tr>
<tr>
<td>Diet 5</td>
<td>10</td>
<td>1000</td>
<td>—</td>
<td>1000</td>
</tr>
</tbody>
</table>

Diet 1: casein diet + basal diet; Diet 2: soybean (14%), teff (29%), and maize (57%) + basal diet; Diet 3: bee larvae (13%), teff (29%), and maize (58%) + basal diet; Diet 4: commercial wean mix (enriched mama's choice) + basal diet; Diet 5: basal diet alone (610g/kg corn starch, 50g/kg corn starch, 50g/kg wheat bran, 100g/kg vegetable oil, 50g/kg mineral, and vitamin premix, 60g/100g glucose, 88g/kg sucrose, 20g/kg oyster shell, 2g/kg NaCl, and 20g/100kg bone meal).

2.3.1. Lipid Profile Analysis. The lipid profile test was conducted briefly, and 50-μl blood samples were aspirated and distributed to the various chambers for sample analysis [31]. The lipid profiles of TC, TG, HDL-C, and LDL-C were evaluated using an automated pentra C400 manufactured in France.

2.3.2. Atherogenic and Castelli’s Risk Indices. The atherogenic indices of plasma, Castelli’s risk indices I, and II, and the atherogenic coefficient were calculated using equations (2)–(5), respectively [15, 32, 33].

\[
AIP = \log \left( \frac{\text{TG}}{\text{HDL} - c} \right),
\]

\[
CRI-I = \frac{\text{TC}}{\text{HDL} - c},
\]

\[
CRI-II = \frac{\text{LDL} - c}{\text{HDL} - c},
\]

\[
AC = \frac{\text{TC} - \text{HDL} - c}{\text{HDL} - c}.
\]

2.4. Statistical Analysis. Data on lipid profiles of TC, TG, HDL-C, LDL-C, and atherogenic and Castelli’s risk indices are presented as the means and standard deviations. Pearson correlations between lipid profiles and atherogenic and Castelli’s risk indices were calculated. Data were analysed using SPSS for Windows version 23.0. The data obtained were subjected to a one-way ANOVA, and Tukey’s HSD test was used to examine the similarities among all experimental groups. A \( p \)-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Lipid Profile. The results of the lipid profile (mg/dl) among experimental diets are indicated in Table 3. The lipid profiles of TC, TG, HDL-C, and LDL-C were significantly different (\( P \leq 0.001 \)) between experimental treatments. Mice fed diet 4 had low TC (112.52 mg/dl), TG (97.83 mg/dl), and LDL-C (47.87 mg/dl) levels. Additionally, mice assigned to diet 5 had high serum TG and LDL-C levels and low HDL-C levels, which were 153 mg/dl, 102 mg/dl, and 50.12 mg/dl, respectively. Mice receiving bee larvae containing CF (diet 3) had high \( (P \leq 0.001) \) levels of TG (167.79 mg/dl) and HDL-C (67.18 mg/dl) and low LDL-C (71.73 mg/dl) compared to the other treatments. The increase in plasma HDL-C is considered to reduce the risk of coronary heart disease.

Among the experimental group’s mice, intake, Diet 2, Diet 3, and Diet 5 had high TC (mg/dl) in the blood, which were 131.23, 123.34, and 121.06, respectively. However, based on the present findings, it is difficult to be very certain that diet caused CVDs. Therefore, further atherogenic indices should be calculated to identify a predictive indicator of coronary artery disease.

3.1.1. Atherogenic and Castelli’s Risk Indices. The atherogenic index of plasma (AIP) is a critical index that can be used as a stand-alone index for cardiac risk estimation [34]. The results of AIP, CRI-I, CRI-II, and AC were found to be significantly different (\( P \leq 0.001 \)) between the experimental diets (Table 4). High AIP, CRI-I, CRI-II, and AC ratios were recorded on Diet 5, which were 0.49, 2.50, 2.04, and 1.50, respectively. Additionally, Diet 4 was recorded with high CRI-I (2.35), CRI-II (1.98), and AC (1.35). However, in Diet 3, they had low CRI-I (1.84), CRI-II (1.07), and AC (0.84) but a high AIP (0.40) compared with the mice that consumed CVDs. Therefore, further atherogenic indices should be calculated to identify a predictive indicator of coronary artery disease.

3.1.2. Pearson Correlation between Atherogenic Indices and Risk Factors. The results of the correlation between the atherogenic indices of AIP, CRI-I, CRI-II, and AC and the risk factors for TC, TG, HDL-C, and LDL-C are illustrated in Table 5. The results showed that there was a positive correlation of AIP with TC (\( r = 0.68 \); \( r = 0.115 \)) and LDL-C (\( r = 0.59 \); \( r = 0.151 \)) and a significant positive correlation with TG (\( P \leq 0.001; r = 0.737 \)). However, AIP was inversely...
correlated \((P = 0.73; r = -0.096)\) with HDL-C. Therefore, AIP was positively correlated with TC, TG, and LDL-C and had a negative correlation with HDL-C. The TC had no significant difference \((P > 0.05)\) but a positive correlation between AIP \((r = 0.115)\), CRI-I, and AC \((r = 0.161)\) and a negative correlation with CRI-II \((r = -0.190)\). However, plasma TG had a negative association \((P > 0.05; r = -0.429)\) between the CRI-I and AC. Similarly, HDL-C levels showed a significant difference \((P \leq 0.001)\) and were negatively correlated with CRI-I \((r = -0.872)\) and CRI-II \((r = -0.971)\), and AC \((r = -0.872)\). However, LDL-C levels showed a significant \((P \leq 0.001)\) positive correlation between CRI-I \((r = 0.875)\), CRI-II \((r = 0.931)\), and AC \((r = 0.875)\). The correlation of individual atherogenicity indices revealed significant positive associations \((P \leq 0.001)\) with one another as follows: CRI-I vs. CRI-II \((r = 0.919)\), CRI-I vs. AC \((r = 1)\), and CRI-II vs. AC \((r = 0.919)\). Although the AIP indices were not significantly correlated, they were positively correlated with CRI-I \((P = 0.47; r = 0.204)\), CRI-II \((P = 0.57; r = 0.16)\), and AC \((P = 0.47; r = 0.204)\).

### 4. Discussion

Nutrient consumption in infancy influences the lipid profile [35]. A diet of high lipids increases TC, TG, and LDL-C and decreases HDL-C, which markedly causes hyperlipidemia [36] and is the key factor in CVD progression [37]. Additionally, according to the reports of Chandrashekar et al. [38], LDL-C is proatherogenic, and oxidation of LDL-C within the arterial wall would be an important early step in atherogenesis. Prolonged and sustained arterial exposure to elevated LDL-C increases cholesterol deposition and enhances the atherosclerotic process, eventually leading to CHD [6]. Serum cholesterol (and hence LDL-C) has consistently been shown to be a significant risk factor for CHD and other major CVDs as well.

According to the findings of Haber et al. [21], honey bees had an increased amount of palmitic and oleic acids, which are the dominant fatty acids determined in the larvae. Therefore, diets enriched in oleic and palmitic acid, such as Diet 3, increase HDL-C levels and decrease LDL-C or lower

| Table 3: Comparison of serum lipid profile (mg/dl) of experimental diets. |
|-------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Lipid profile          | Diet 1          | Diet 2          | Diet 3          | Diet 4          | Diet 5          | 
| TC                     | 113.07 ± 4.13b  | 131.23 ± 1.19a  | 123.34 ± 2.23b  | 112.52 ± 5.25b  | 121.06 ± 1.77a  | ≤0.001          |
| TG                     | 141.25 ± 0.79a  | 123.04 ± 2.23d  | 167.79 ± 3.85a  | 97.83 ± 1.06b   | 153.96 ± 1.52b  | ≤0.001          |
| HDL-C                  | 59.53 ± 2.63b   | 59.28 ± 0.85b   | 67.18 ± 0.67a   | 47.87 ± 2.15a   | 50.12 ± 1.15a   | ≤0.001          |
| LDL-C                  | 86.66 ± 2.11i   | 92.12 ± 1.96b   | 71.73 ± 0.60d   | 94.74 ± 1.40b   | 102.30 ± 2.12a  | ≤0.001          |

Results are presented as mean ± SD. means with different superscripts (alphabets) in the same row are significantly different \((P < 0.05)\); Diet 1 = casein diet + basal diet; Diet 2 = maize, teff with soybean + basal diet; Diet 3 = maize, teff with bee larvae + basal diet; Diet 4 = commercial wean mix (enriched mama’s choice) + basal diet; Diet 5 = basal diet alone; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

| Table 4: The distribution of atherogenic indices of plasma, castelli’s risk indices (I and II), and atherogenic coefficient among experimental treatments. |
|-------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Indices                | Diet 1          | Diet 2          | Diet 3          | Diet 4          | Diet 5          | 
| AIP                    | 0.38 ± 0.03b    | 0.32 ± 0.01c    | 0.40 ± 0.01b    | 0.31 ± 0.02c    | 0.49 ± 0.01a    | ≤0.001          |
| CRI-I                  | 1.90 ± 0.08c    | 2.21 ± 0.04b    | 1.84 ± 0.04c    | 2.35 ± 0.04b    | 2.50 ± 0.08a    | ≤0.001          |
| CRI-II                 | 1.46 ± 0.08b    | 1.55 ± 0.02b    | 1.07 ± 0.00c    | 1.98 ± 0.12a    | 2.04 ± 0.04a    | ≤0.001          |
| AC                     | 0.90 ± 0.08c    | 1.21 ± 0.04b    | 0.84 ± 0.04c    | 1.35 ± 0.04b    | 1.50 ± 0.08a    | ≤0.001          |

Results are presented as mean ± SD. means with different superscripts (alphabets) in the same row are significantly different \((P < 0.05)\); Diet 1 = casein diet + basal diet; Diet 2 = maize, teff with soybean + basal diet; Diet 3 = maize, teff with bee larvae + basal diet; Diet 4 = commercial wean mix (enriched mama’s choice) + basal diet; Diet 5 = basal diet alone; AIP = atherogenic indices of plasma; CRI = castelli’s risk indices; AC = atherogenic coefficient.

| Table 5: Pearson correlation between atherogenic indices and lipid profiles of experimental treatments. |
|-------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Parameters              | AIP             | CRI-I           | CRI-II          | AC              | 
| TC                      | 0.115           | 0.681           | −0.190          | 0.50            | 0.161           | 0.57            |
| TG                      | 0.737           | 0.0020**        | −0.429          | 0.11            | −0.535          | 0.04*           | −0.429          | 0.08            |
| HDL-C                   | −0.096          | 0.73            | −0.872          | ≤0.001**        | −0.971          | ≤0.001**        | −0.872          | ≤0.001**        |
| LDL-C                   | 0.151           | 0.59            | 0.875           | ≤0.001**        | 0.931           | ≤0.001**        | 0.875           | ≤0.001**        |
| AIP                     | 1               | 0.204           | 0.47            | 0.160           | 0.57            | 0.204           | 0.47            |
| CRI-I                   | 1               | 0.919           | ≤0.001**        | 0.875           | ≤0.001**        | 0.875           | ≤0.001**        |
| CRI-II                  | 1               | 0.919           | ≤0.001**        | 0.875           | ≤0.001**        | 0.875           | ≤0.001**        |
| AC                      | 1               | 0.919           | ≤0.001**        | 0.875           | ≤0.001**        | 0.875           | ≤0.001**        |

TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; AIP: atherogenic index of plasma; CRI: castelli risk indices; AC: atherogenic coefficient; *= correlation is significant at the 0.05 level; **= correlation is significant at the 0.01 level.
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Additionally, this monounsaturated fatty acid (MUFA) omega-9 fatty acid is important in the human diet; it has been proven effective in reducing LDL and TC levels [41]. High HDL-C protects the body by boosting reverse cholesterol transport by scavenging excessive cholesterol from peripheral tissues, which is then esterified with the aid of lecithin: a cholesterol acyltransferase and transported to the liver and steroidogenic organs for the synthesis of bile acids and lipoproteins and ultimate removal from the body [33, 42].

LDL-C is the primary cause of the link between elevated cholesterol and an increased risk of heart disease. HDL-C, on the other hand, has been inversely related to the risk of death from coronary heart disease [43, 44]. Therefore, the “cholesterol hypothesis” was born because of these studies, which hypothesized that LDL-C is responsible for the progression of atherosclerosis and that reducing LDL-C would lower the risk of myocardial infarction and other cardiovascular events [45]. Therefore, the present study showed that Diet 3 had low LDL-C and high HDL-C and low CRI-I, CRI-II, and AC. The correlation of HDL-C with other indices agreed with the report of [46] a strong inverse relationship between HDL-C levels and the risk of CVD.

The elevation of serum TC, TG, and LDL-C has been implicated as a principal risk factor for atherosclerotic CVD [47]. In this study, Diet 5 had a high record of LDL-C, and therefore, the probability of causing CVD would be higher than in the other diets. Hence, to minimize the risk of CVD, current recommendations rely on lowering LDL-C by consuming less saturated fat [48]. However, lower plasma HDL-C has indicated a risk of CVD [33, 49]. From the resulting perspective, the lower concentration of LDL-C in Diet 3 would be associated with the inclusion of bee larvae in the developed foods, which had high MUFA-s, and therefore, enrichment of the diet with MUFA-s lowers LDL-C [19, 50]. From these findings, isolated elevation in TG (Diet 3) increases CVD risk; however, according to the report of [51], these effects can be balanced by the cardioprotective lipoprotein HDL-C. Therefore, this finding was supported by a study [52] that reported that high levels of HDL-C were associated with reduced cardiovascular risk.

A high-fat diet has a significant influence on serum lipids [53] and has been related to metabolism and CVD [54]. Additionally, serum lipid levels, especially serum cholesterol, are a major risk factor for atherosclerosis and CVD that significantly contribute to mortality [55]. Mott and colleagues [56] reported elevated atherosclerotic lesions that were associated with elevated plasma TC levels, and these were related to increased cholesterol levels in children’s diets. Therefore, a decrease in serum lipids leads to good functioning of the heart muscle [57]. According to a report [58], a lower intake of PUFAs and a higher intake of dietary cholesterol and saturated fats raise blood total cholesterol levels.

In this experiment, based on the blood lipid profiles, it was difficult to conclude which experimental diet had cardioprotective potential and prevented infants or children from developing CVD. In addition to routine lipid investigations, the inclusion of atherogenic indices is a better index for screening for early detection of atherogenic CVD [59]. Calculating atherogenic indices would also be a good diagnostic tool if other atherogenic risk factors were normal [60]. Therefore, the lipid profile test has found a useful application in the assessment of malnutrition [61, 62]. The AIP [59, 63, 64] and AC [65] are also useful diagnostic tools to predict the risk of atherosclerosis and coronary heart disease; the higher the value, the higher the risk of developing CVD and vice versa.

During atherogenesis, lipids accumulate in the vascular wall and trigger inflammatory reactions that stimulate atherosclerosis progression [2]. However, the results of the mice assigned to both diets 4 and 5 predicted cardiovascular events [66]. In addition, CRI-II showed more predictive value than standard LDL- or HDL-C fractions [59]. Thus, this result is in line with the findings of [11, 64]. The results of TC and any indices of atherogenicity agreed with the report of [67], and a statistically significant association was observed.

There was a significant correlation between AIP and CVD risk factors (BMI, visceral fat, body fat, total cholesterol, LDL cholesterol, triglycerides, glucose, and HDL cholesterol) among the samples being studied. Based on these findings, for the prevention of CVD risk, early intervention programs such as dietary control and monitoring of AIP should be implemented regularly [11].

5. Conclusion

The findings of this investigation showed that the use of atherogenic indices is an indicator to confirm whether the diet is potentially caused or associated with CVD compared to the traditional lipid profile. The results of atherogenic indices, TC/HDL-C, LDL-C/HDL-C, and (TC–HDL-C)/HDL-C, showed that the intake of insect bee larvae-based complementary foods, i.e., Diet 3, could be used to prevent infants and young children from the risk of atherosclerotic CVD in a mouse model. However, further studies on the effect of developed complementary foods (Diet 3) on clinical and histopathological changes in the mouse model, and physiological and biological effects on infants and young children should be conducted.

Abbreviations

AC: Atherogenic coefficient
AIP: Atherogenic indices of plasma
CHD: Coronary heart disease
CRI-I: Castelli’s risk indices-I
CRI-II: Castelli’s risk indices-II
CVD: Cardiovascular disease
HDL-C: High-density lipoproteins cholesterol
LDL-C: Low-density lipoproteins cholesterol
MUFA-s: Monounsaturated fatty acids
PUFA-s: Polyunsaturated fatty acids
SFAs: Saturated fatty acids
SPSS: Statistical package for social science
TC: Total cholesterol
TG: Triglyceride.
Data Availability
All data generated or analysed during this study are included within the article.

Ethical Approval
All experimental procedures were approved by the Research Ethics Committee of the College of Veterinary Medicine and Animal Sciences, University of Gondar, Ethiopia (Ref. No. CVMAS/13/8064/2020).

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Conceptualization was given by S.A.M.; methodology was given by S.A.M., J.K., B.K., and M. W; software was designed by S.A.M.; validation was done by S.A.M., J.K., B.K., and M. W; formal analysis was done by S.A.M.; investigation was done by S.A.M.; resources were found by S.A.M.; data curation was done by S.A.M.; S.A.M. wrote the original draft preparation; S.A.M., J.K., B.K., and M. W. wrote the review and edited; S.A.M. visualized the study; J.K., B.K., and M. W supervised the study; S.A.M. administered the project; funding acquisition was done by S.A.M. All authors have read and agreed to the published version of the manuscript.

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