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Seasonal fluctuations of antioxidant enzymes and biochemical compositions of *Apis mellifera adansonii* L. from three ecological zones of Nigeria

Julius Akolawole Bamidele[1*], Adewumi Babatunde Idowu[1], Kehinde Olutoyin Ademolu[1], Adedoyin Adebola Osipitan[2] and Samson Adisa Rahman[3]

Abstract

Background The ability of honeybees to survive and sustain honey production depends on their ability to adapt to their environment. This study evaluated the seasonal fluctuations of antioxidant activities and biochemical compositions of honeybees, *Apis mellifera adansonii* L. from rainforest, guinea savannah and derived savannah zones of Nigeria during three seasonal periods (onset of rain, wet season and dry season) from January to December, 2017. Honeybees were collected from fully colonized colonies of two different apiaries from each of the ecological zones during the seasonal periods. Amino acid composition was determined using high-performance liquid chromatography. Antioxidant enzymes activity, body protein, glucose and lipid profile were evaluated using standard protocols.

Results Seasonal periods showed significant ($p < 0.05$) effect on the eighteen amino acids, superoxide dismutase (SOD), triglycerides and fat-free nitrogen recorded in the honeybees. Amino acids were higher in the honeybees from the rainforest zone, regardless of the seasonal period. SOD and body protein were higher during the wet season in the three ecological zones. However, seasonal periods showed no significant ($p > 0.05$) influence on the body glucose, protein pattern, total cholesterol, activities of malondialdehyde, reduced glutathione, glutathione peroxidase and catalase. Body glucose content in the honeybees from the rainforest and guinea savannah zones was highest during the dry season.

Conclusions Antioxidant activities and biochemical compositions of honeybees are differently affected by both ecological zones and seasonal fluctuations.

Keywords Honeybees, Environment, Oxidative stress, Adaptation, Physiology, Lipids

*Correspondence:
Julius Akolawole Bamidele
julius.bamidele@yahoo.com
Full list of author information is available at the end of the article

Background

The honeybees (Hymoneptera: Apidae) have been regarded as social insect and the most economically treasured insect due its role in pollination and honey production (Lawal & Banjo, 2010). Crop pollination activities of the honeybees were also described as the link between the honeybees and man which have been of both ecological and conservation implication (Greenleaf et al., 2007). Therefore, reports have identified the honeybees as a principal insect pollinator, pollinating wide variety of crops in orchards and other agricultural systems (Morse & Calderone, 2000; Sharma et al., 2004; Williams, 1994). However, Abou-Shaara et al. (2012) suggested that the foraging patterns and daily activities of the honeybees could be influenced by environmental weather conditions. For example, reports have shown that the activity level of honeybee is low during cold weather and survival is mainly by feeding on the honey stored in the colony (Sudarsan et al., 2012) or on supplemented food material (Fasasi et al., 2007).

Although the honeybee colony is active all year round, the level of activities of the colony varies with seasons (Kovac & Stabentheiner, 2011; Tirado et al., 2013). The variations in honeybee activities in the different seasons was associated with the abundance of forage plants and foraging activities. For instance, at the onset of the spring months, the blossoming of bee forage plants also begin (Bayir & Albayrak, 2016). Similarly, Harris (2016) reported that honeybee colony growth depends largely on the abundance and quality of pollen resources for the period of high blossoming in the spring and autumn. This increased foraging activities of the honeybees largely depends on the body tissues and biochemistry. The report of Schippers et al. (2006) showed that the flight muscle, being a locomotive tissue in the honeybees, is energetically expensive to control and maintain. Ji (1999) also submitted that the locomotive muscle tissues are affected by reactive oxygen species (ROS). Increased flight and mental alertness in the honeybee foragers result in oxidative stress and leads to the production of more ROS (Williams et al., 2008). However, the antioxidant enzymes play important roles in the prevention of oxidative stress by counteracting the ROS (Lijun et al., 2005; Seehuus et al., 2006; Williams et al., 2008) and protect the cells against the detrimental effects of ROS (Saint-Denis et al., 1998).

In their notes on pollinator's biodiversity research and policy across the African continent, the International Centre of Insect Physiology and Ecology (2014), affirmed that climate condition such as weather, forests and forage composition affect the rate at which honeybees get out to forage. However, their report called for research on the impact of climate on the health and physiology of the honeybees. Up till date, the underlining factor influencing the fluctuations in honeybee activities with respect to environmental conditions is not clear. This presents the need for the evaluation of the health of the Nigerian honeybee, *Apis mellifera adansonii* with respect to ecological zones and weather parameters using physiological parameters as indices. Therefore, this study evaluated the antioxidant enzymes activity, amino acids composition, body biochemical composition and lipid profile of the honeybee, *A. m. adansonii* from rainforest, guinea savannah and derived savannah zones of Nigeria during the onset of rain, wet season and dry season periods.

Methods

Study locations

The present study was conducted between January and December, 2017 in the rainforest zone (Ogun state), Guinea savannah zone (Oyo state) and the Derived savannah zone (Kwara state) of Nigeria. Ogun and Oyo states are located in the south-western region of the country while Kwara state is located in the middle-belt region of the country. Two apiaries each were selected from the ecological zones and used for this study. These apiaries were selected based on approved consent by the owners as well as the suitability of its location to the desired ecological zone. Map of the study locations was constructed using ArcGIS version 9.3 and is shown in Fig. 1. The coordinates of the different locations used in the ecological zones are also shown in Table 1.

Sample collection

Honeybee, *Apis mellifera adansonii* workers were collected by modifying the method of Ajao and Babatunde (2013). In this study, honeybee sample collection was made using a round shaped 1 l volume plastic container with the diameter of 14.5 cm and 7.5 cm height. Each of the honeybee hives was gently tapped from the side. As soon as the honeybees began to rush out of the hive, the plastic container was used to trap the honeybees from the entrance of the hive. Where the entrance was not obvious, the container was swirled to collect the bees. Half-filled volume of plastic container with honeybee samples were collected and carefully covered to prevent the trapped honeybees from escaping. One container each was collected per honeybee colony. A total of fifty-four (54) honeybee colonies were used in this study, comprising of six (6) colonies each from the rainforest, guinea savannah and derived savannah zones of Nigeria during the onset of rain, wet season and dry season periods. Collected honeybee samples were transported in ice box to the laboratory for antioxidant enzymes activities and biochemical composition analyses. Collected honeybee samples were carefully sorted in the laboratory to remove any

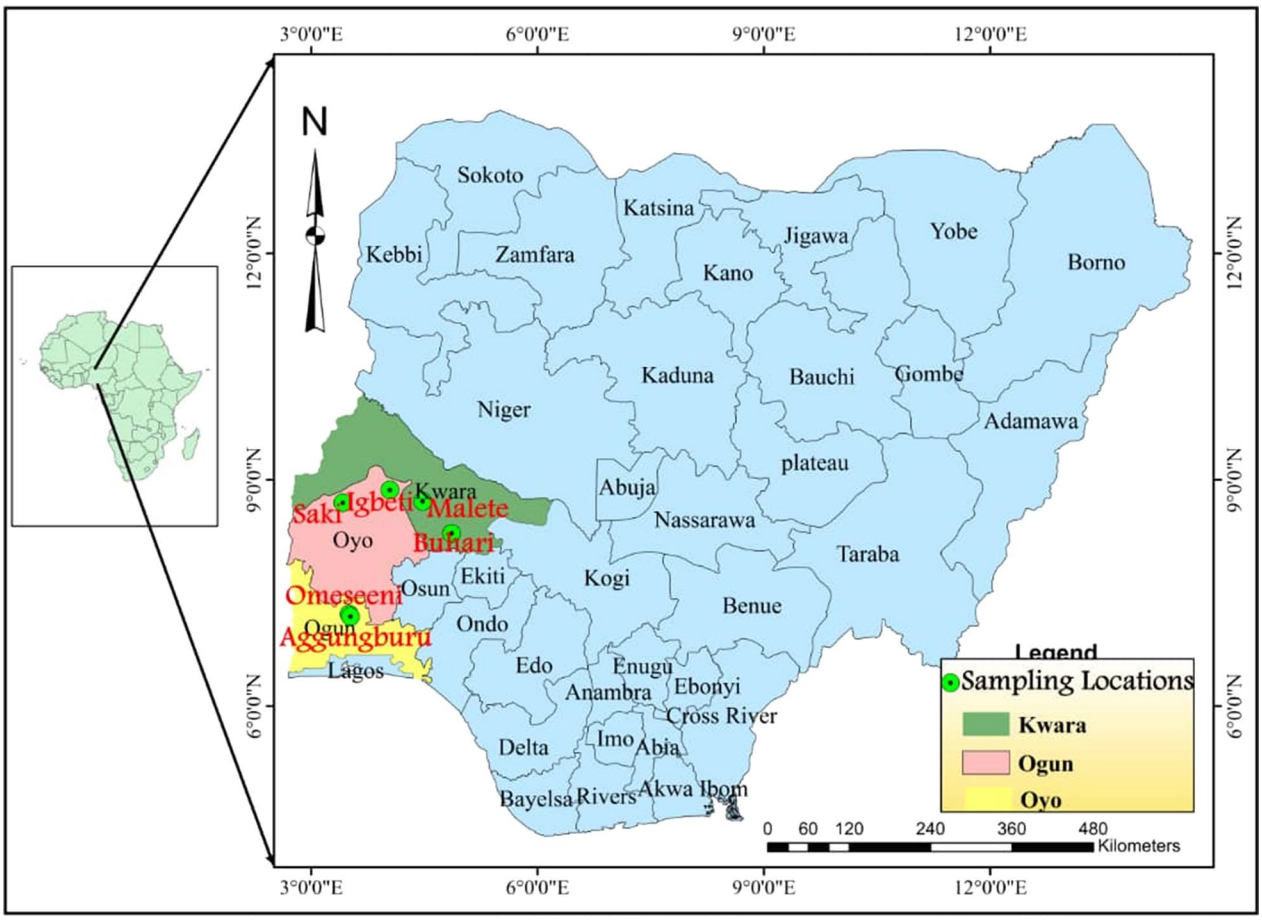

Fig. 1 Map showing the study locations

Table 1 Coordinates of the study locations

Zones	Apiary locations	Coordinates
Rainforest	1. Agbungburu village, Osiele	3° 31′ 13.6″ E 7° 11′ 11.6″ N
	2. Omiseeni village, Odeda	3° 30′ 05.5″ E 7° 13′ 07.6″ N
Guinea savannah	1. Tesi Garba village, Igbeti	4° 02′ 34.1 ″E 8° 51′ 56.1″ N
	2. Aba-Ogbomoso, Saki	3° 25′ 10.4″ E 8° 41′ 47.3″ N
Derived savannah	1. Malete-KWASU-CBTR	4° 28′ 34.4″ E 8° 42′ 57.5″ N
	2. Buari-BTRC	4° 56′ 13.8″ E 8° 28′ 39.8″ N

Buari-BTRC Beekeeping Training and Research Centre, Buari, Ifelodun Local Government, Kwara State; *KWASU-CBTR* Kwara State University Centre for Beekeeping Training and Research, Malete, *RF* Rainforest, *GS* Guinea savannah, *DS* Derived savannah

incidence of non-worker caste prior to chemical analyses. Honeybee samples were identified at the Entomology Unit of the Pure and Applied Zoology Department, Federal University of Agriculture, Abeokuta and confirmed at the Zoological Unit Laboratory of Kwara state University, Malete, Kwara state Nigeria.

Determination of amino acid profile

Amino acid profile in the honeybee samples was evaluated using High-performance liquid chromatography (HPLC) using to the methods of Benitez (1989). The honeybee samples (5 g each) were dried to constant weight, defatted, hydrolyzed, evaporated and loaded into the Applied Biosystems PTH Amino Acid Analyzer (Applied Biosystems Inc. USA) 120A model.

De-fatting Dried samples were defatted using chloroform/methanol mixture of ratio 2:1. 500 mg of the sample was put in extraction thimble and extracted for 15 h in soxhlet extraction apparatus (A.O.A.C., 2006).

Nitrogen determination 115 mg of honeybee were weighed, wrapped in Whatman filter paper (No.1) and

put in the Keidjhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added. The flask was then put in Kjeldhal digestion apparatus for 3 h until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 m1) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected. The distillate was then titrated with standardized 0.01 N hydrochloric acid to grey coloured end point.

Hydrolysis A known weight (100 mg to 300 mg) of the defatted sample was weighed into glass ampoule. 7 ml of 6 N HCL was added (10 ml of 4.2 M NaOH was used on samples prepared for tryptophan analysis) and oxygen was expelled by passing nitrogen into the ampoule in order to avoid possible oxidation of some amino acids such as methionine and cystine during hydrolysis. The glass ampoule was then sealed with Bunsen burner flame and put in an oven pre-set at 105 ± 5 °C for 22 h. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. This filtrate was evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into analyzer Sixty (60) microlitre was dispensed into the cartridge of the analyzer. The analyzer was designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

Evaluation of antioxidant enzymes

Fresh honeybee samples (2 g) were homogenized with homogenizing buffer using a ceramic mortar and pestle in ice. The homogenized samples were centrifuged. The supernatant was frozen for the antioxidant enzymes analyses. The supernatants were analysed for Catalase (CAT), Glutathione peroxidase (GPx), Superoxide dismutase (SOD), reduced glutathione (GSH) and Lipid peroxidation (Malondialdehyde—MDA). Catalase activity was carried out following the method of Sinha (1972), Glutathione peroxidise activity followed the methods of Rotruck et al. (1973), SOD activity according to the method of Zou et al. (1986) and reduced glutathione estimation according to Beutler et al. (1963). A product of lipid peroxidation (malondialdehyde—MDA) was measured using the method described by Buege and Aust (1978).

Estimation of glucose and total protein

Total protein content of honeybee samples was determined using Cypress Diagnostics Kit, Langdorp Belgium. Total protein level was estimated using Randox Diagnostic kit.

Protein analysis (gel electrophoresis)

Protein banding pattern of the honeybee samples was determined using the ProteoLadder 150 kit of the Norgen Biotek Corp, Canada as described in Bamidele et al (2015).

Protein extraction Fresh honeybee samples were homogenized for protein extraction. 0.3 g of honeybee sample was weighed into an Eppendorf tube and 500 µL of 0.1 M Tris-HCl pH 7.6 was added. This was homogenized and spun at 10,000 rpm for 10 min. The supernatant was collected into a fresh tube and refrigerated for SDS-PAGE gel electrophoresis.

SDS-Page gel electrophoresis Polyacrylamide gel of 12% was prepared as the separation gel. Stacking gel of 3% was also prepared. Sample extract (5 µL) and loading buffer (5 µL) were mixed, boiled at 95 °C for 5 min and loaded on the gel. This was run for 60 min at 150 V.

SDS-Page gel staining The plate was dismantled and the gel removed. The gel was stained in 0.1 M Coomassie blue solution for 1 h. The gel was removed and distained in several rinses of Ethanol/Acetic acid solution until it became clear for viewing. Picture of the gel was taken for documentation.

Estimation of lipid profile

The estimation of triacylglycerol, total cholesterol, HDL concentration and LDL concentration were carried out according to the method of Tietz et al. (1990) using Randox Diagnostic kit.

Statistical analyses

Data obtained were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corporation, 2011). Mean values were compared using the Multivariate analysis of the General Linear Model. The ecological zones and the seasonal periods were used as grouping factors. Results were presented as mean ± standard deviation. Post hoc test was done using the Student–Newman–Keuls (SNK). *P*-value less than 0.05 was considered to be statistically significant.

Results

Amino acid profile of the honeybees

A total of eighteen (18) amino acids were detected in the honeybees (Table 2). These include Leucine, Lysine, Isoleucine, Phenylalanine, Trytophan, Valine, Methionine, Proline, Arginine, Tyrosine, Histidine, Cystine, Alanine, Glutamic acid, Glycine, Threonine, Serine and Aspartic acid. Out of all the eighteen amino acids detected, proline was the highest in the body of the bees, regardless of the ecological zones and the seasonal periods. This was followed by gluthamic acid and leucine, respectively.

During the onset of rainfall period, no significant difference ($p > 0.05$) was recorded in the levels of Leucine, Phenylalanine, Proline, Tyrosine, Histidine and Cystine in the honeybees from the three ecological zones. These were however higher in the honeybees from the rainforest zone. Also, all the other amino acids tested were significantly higher ($p < 0.05$) in the honeybees from the rainforest zone. During the wet season period, there was no significant difference recorded in the levels of amino acids between the three ecological zones. On the other hand, levels of amino acids were significantly higher in the honeybees from the rainforest zone than those from the guinea savannah and derived savannah zones during the dry season period. Seasonal periods also showed significant interaction on each of the amino acids recorded in the honeybees.

Activities of antioxidant enzymes of the honeybees

Activities of Superoxide dismutase (SOD) and catalase in the honeybees from the three ecological zones were not significantly different ($p > 0.05$) at the onset of rain and dry season period (Table 3). However, honeybees from the guinea savannah zone had significantly lower activity of SOD and significantly higher activity of catalase during the wet season period. During the onset of rainfall period, activities of reduced glutathione (GSH) and Glutathione peroxidase (GPx) were significantly higher in the honeybees from the rainforest zone. However, honeybees from this zone (guinea savannah zone) had significantly lowest activities of GSH and GPx during the wet season period. On the other hand, during the dry season, activities of GSH and GPx were significantly lowest in the honeybees from the rainforest zone. Result of statistical interaction of the seasonal period on the antioxidant enzyme activities however showed significant interaction only on the activity of SOD in the honeybees.

Table 2 Amino acid composition (g/100 g protein) of the honeybees from the three ecological zones of Nigeria during the dry season, onset of rain and wet seasons

	Onset of rain			Wet season			Dry season		
	RF	GS	DS	RF	GS	DS	RF	GS	DS
Leucine	6.95 ± 0.04^{a}	6.77 ± 0.20^{ab}	6.55 ± 0.12^{a}	7.01 ± 1.00^{a}	6.77 ± 0.20^{a}	6.85 ± 0.03^{a}	6.77 ± 0.20^{a}	5.90 ± 0.20^{b}	5.60 ± 0.30^{b}
Lysine	4.93 ± 0.03^{a}	4.27 ± 0.02^{b}	4.06 ± 0.23^{b}	4.72 ± 0.02^{a}	5.04 ± 0.04^{a}	4.63 ± 0.31^{a}	4.03 ± 0.03^{a}	3.87 ± 0.02^{b}	3.63 ± 0.03^{b}
Isoleucine	5.11 ± 0.01^{a}	4.75 ± 0.02^{b}	4.36 ± 0.30^{b}	4.91 ± 0.01^{a}	4.94 ± 0.04^{a}	4.84 ± 0.00^{a}	4.52 ± 0.02^{a}	3.60 ± 0.30^{b}	3.30 ± 0.30^{b}
Phenylalanine	6.56 ± 0.02^{a}	6.39 ± 0.04^{a}	6.03 ± 0.31^{a}	6.65 ± 0.05^{a}	6.56 ± 0.20^{a}	6.50 ± 0.05^{a}	6.16 ± 0.01^{a}	5.68 ± 0.01^{b}	5.50 ± 0.30^{b}
Trytophan	0.53 ± 0.03^{a}	0.47 ± 0.02^{b}	0.42 ± 0.05^{b}	0.53 ± 0.03^{a}	0.50 ± 0.10^{a}	0.48 ± 0.02^{a}	0.50 ± 0.05^{a}	0.37 ± 0.02^{b}	0.39 ± 0.04^{b}
Valine	5.56 ± 0.02^{a}	5.00 ± 0.50^{b}	3.41 ± 1.50^{c}	5.44 ± 0.04^{a}	5.32 ± 0.02^{a}	5.26 ± 0.10^{a}	4.91 ± 0.01^{a}	3.86 ± 0.03^{b}	3.68 ± 0.01^{b}
Methionine	3.26 ± 0.01^{a}	2.99 ± 0.05^{b}	2.79 ± 0.16^{b}	3.31 ± 0.01^{a}	3.31 ± 0.01^{a}	3.24 ± 0.08^{a}	2.83 ± 0.03^{a}	2.78 ± 0.01^{a}	2.19 ± 0.02^{b}
Proline	12.90 ± 0.50^{a}	12.79 ± 0.10^{a}	12.05 ± 0.47^{a}	13.00 ± 0.10^{a}	12.79 ± 0.10^{a}	12.82 ± 0.12^{a}	11.98 ± 0.01^{a}	11.47 ± 0.10^{a}	10.56 ± 0.02^{b}
Arginine	6.88 ± 0.01^{a}	5.68 ± 0.01^{b}	5.65 ± 0.05^{b}	6.37 ± 0.10^{a}	6.71 ± 0.01^{a}	6.16 ± 0.54^{a}	6.02 ± 0.02^{a}	5.33 ± 0.03^{b}	4.99 ± 0.01^{c}
Tyrosine	3.79 ± 0.20^{a}	3.61 ± 0.01^{a}	3.27 ± 0.29^{a}	3.79 ± 0.20^{a}	3.78 ± 0.20^{a}	3.72 ± 0.10^{a}	3.27 ± 0.02^{a}	2.93 ± 0.03^{b}	2.41 ± 0.01^{b}
Histidine	2.46 ± 0.40^{a}	2.40 ± 0.40^{a}	2.18 ± 0.02^{a}	2.49 ± 0.20^{a}	2.30 ± 0.30^{a}	2.27 ± 0.12^{a}	2.17 ± 0.02^{a}	2.01 ± 0.01^{a}	1.88 ± 0.01^{b}
Cystine	2.54 ± 0.30^{a}	2.24 ± 0.04^{a}	2.14 ± 0.14^{a}	2.42 ± 0.02^{a}	2.48 ± 0.40^{a}	2.34 ± 0.03^{a}	2.18 ± 0.01^{a}	1.82 ± 0.02^{b}	1.99 ± 0.01^{b}
Alanine	5.61 ± 0.01^{a}	5.39 ± 0.30^{a}	4.93 ± 0.13^{b}	5.58 ± 0.20^{a}	5.46 ± 0.40^{a}	5.43 ± 0.09^{a}	5.01 ± 0.01^{a}	4.70 ± 0.20^{b}	4.02 ± 0.02^{c}
Glutamic acid	8.93 ± 0.03^{a}	8.63 ± 0.03^{a}	8.02 ± 0.27^{b}	8.93 ± 1.00^{a}	8.93 ± 0.03^{a}	8.78 ± 0.00^{a}	8.03 ± 0.03^{a}	7.42 ± 0.40^{b}	7.27 ± 0.02^{b}
Glycine	6.89 ± 0.10^{a}	5.94 ± 0.04^{b}	5.31 ± 0.44^{b}	6.03 ± 0.03^{a}	6.46 ± 0.03^{a}	6.02 ± 0.11^{a}	5.42 ± 0.02^{a}	4.89 ± 0.10^{b}	4.32 ± 0.02^{b}
Threonine	4.55 ± 0.03^{a}	3.61 ± 0.01^{b}	3.29 ± 0.37^{b}	4.11 ± 0.01^{a}	4.08 ± 0.01^{a}	3.92 ± 0.07^{a}	3.28 ± 0.01^{a}	2.83 ± 0.03^{b}	2.66 ± 0.20^{b}
Serine	5.19 ± 0.10^{a}	4.70 ± 0.20^{b}	4.17 ± 0.43^{b}	4.92 ± 0.02^{a}	4.99 ± 1.00^{a}	4.81 ± 0.00^{a}	4.21 ± 0.01^{a}	3.78 ± 0.01^{b}	3.51 ± 0.01^{b}
Aspartic acid	6.20 ± 0.20^{a}	5.89 ± 0.10^{b}	5.42 ± 0.36^{b}	6.08 ± 0.01^{a}	6.11 ± 0.01^{a}	6.01 ± 0.06^{a}	5.49 ± 0.10^{a}	4.99 ± 0.01^{c}	5.21 ± 0.01^{b}

RF rainforest; *GS* Guinea savannah; *DS* derived savannah

[abc] Means (± standard deviation) of amino acid components in the same row for each of the seasonal periods having similar superscripts are not significantly different at $p < 0.05$

Table 3 Levels of antioxidant enzymes in the honeybees from the three ecological zones of Nigeria during the dry season, onset of rain and wet seasons

Bee period	Zones	SOD (U/g)	GSH (µg/g)	GPx (µgmol/g)	CAT (U/g)
Onset of rain	RF	0.25 ± 0.05^{a}	6.83 ± 1.04^{b}	93.02 ± 9.88^{b}	10.75 ± 1.45^{a}
	GS	0.50 ± 0.00^{a}	10.02 ± 2.01^{a}	168.01 ± 3.39^{a}	9.50 ± 0.20^{a}
	DS	0.20 ± 0.10^{a}	5.79 ± 1.22^{b}	94.66 ± 38.09^{b}	8.50 ± 0.10^{a}
Wet season	RF	6.75 ± 0.75^{a}	4.41 ± 0.76^{b}	83.80 ± 12.20^{b}	8.75 ± 1.25^{b}
	GS	3.70 ± 2.10^{b}	2.93 ± 1.39^{c}	58.40 ± 27.50^{c}	11.20 ± 0.60^{a}
	DS	6.30 ± 2.90^{a}	6.25 ± 4.16^{a}	131.55 ± 92.85^{a}	8.20 ± 0.40^{b}
Dry season	RF	0.40 ± 0.10^{a}	6.93 ± 1.44^{c}	149.26 ± 15.01^{c}	8.40 ± 0.60^{a}
	GS	0.45 ± 0.05^{a}	8.60 ± 1.05^{b}	169.83 ± 22.92^{a}	8.75 ± 0.25^{a}
	DS	0.50 ± 0.00^{a}	10.20 ± 0.47^{a}	158.05 ± 10.08^{b}	8.30 ± 0.40^{a}
Seasonal interactions	*F* value	18.16	4.017	2.632	1.924
	P value	0.01*	0.06	0.13	0.20

SOD superoxide dismutase; *GSH* reduced glutathione; *CAT* catalase activity; *GPx* Glutathione peroxidase; *RF* rainforest; *GS* Guinea savannah; *DS* derived savannah

[abc] Means (± standard deviation) in the same column for each of the seasonal periods having similar superscripts are not significantly different at $p < 0.05$

*Interaction significant at $p < 0.05$

Table 4 Level of lipid peroxidation (Malondialdehyde [MDA] ($U/g \times 10^{-5}$)) of the honeybees from three ecological zones of Nigeria

	Seasonal interactions		
	Onset of rain	Wet season	Dry season
RF	6.30 ± 0.29^{a}	4.24 ± 0.41^{a}	0.13 ± 0.01^{b}
GS	3.57 ± 3.46^{c}	4.11 ± 0.43^{a}	7.72 ± 1.61^{a}
DS	5.11 ± 0.68^{b}	1.63 ± 0.30^{b}	6.71 ± 1.29^{a}
	$F = 1.342, p = 0.31$		

RF rainforest; *GS* Guinea savannah; *DS* derived savannah

[abc] Means (± standard deviation) of mean level of MDA in the same column for each of the seasonal periods having similar superscripts are not significantly different at $p < 0.05$

*Interaction significant at $p < 0.05$

Lipid peroxidation (malondialdehyde) of the honeybees

Lipid peroxidation (Malondialdehyde [MDA]) was significantly lower in the honeybees from the guinea savannah zone during the onset of rain period (Table 4). This was highest in the honeybees from the rainforest zone. During the wet season period, MDA activity was significantly lowest in the honeybees from the derived savannah zone. During this period, MDA activity was not significantly different in the honeybees from the rainforest and the guinea savannah zones. During the dry season period, MDA activity was not significantly different in the honeybees from the guinea savannah and derived savannah zones but significantly lowest in those from the rainforest zone.

Body glucose, fat free nitrogen and protein contents of the honeybees

Honeybees from the derived savannah zone recorded significantly higher ($p < 0.05$) body glucose level than those from the rainforest and guinea savannah zones during the onset of rain and the dry season period (Table 5). On the other hand, honeybees from the derived savannah zone had significantly highest body glucose level during the wet season. During this period (wet season), no significant difference ($p > 0.05$) was recorded in the body glucose level of honeybees from the rainforest and guinea savannah zones.

Results also showed significantly higher level of fat free nitrogen in the honeybees from the rainforest zone during each of the seasonal periods (onset of rain, wet season and dry season). During the onset of rain period, body protein level was not significantly different in the honeybees from the rainforest and the derived savannah zones. However, body protein level was significantly lowest ($p < 0.05$) in the honeybees from the guinea savannah zone during this period (onset of rain). On the other hand, honeybees from the guinea savannah zone had significantly higher body protein level during the wet season. During the dry season, no significant difference was recorded in the body protein level of the honeybees from the three studied ecological zones. Similarly, there was no significant difference in the mean number of protein bands recorded in the honeybees from the three ecological zones during each of the bee periods. The protein bands of the honeybee body (molecular weight ranged from 14.7 to 150.0 kDa) are shown in Fig. 2.

Result also revealed that protein bands of 14.7 kDa, 25.0 kDa and 100 kDa were only present in the honeybees

Table 5 Total body glucose, fat free nitrogen and protein composition of the honeybees from three ecological zones of Nigeria

		Glucose (mg/dl)	Fat free nitrogen (%)	Protein (g/dl)	Mean protein bands (*N*)
Onset of rain	RF	87.50 ± 5.50^{b}	16.50 ± 0.20^{a}	2.58 ± 0.28^{a}	10.0 ± 0.0^{a}
	GS	88.00 ± 6.00^{b}	11.91 ± 0.01^{b}	1.43 ± 0.04^{b}	8.5 ± 2.1^{a}
	DS	98.50 ± 1.50^{a}	11.00 ± 0.77^{b}	2.99 ± 1.17^{a}	9.0 ± 0.0^{a}
Wet season	RF	93.00 ± 6.00^{a}	15.43 ± 0.02^{a}	2.95 ± 0.45^{c}	8.5 ± 0.7^{a}
	GS	97.00 ± 3.00^{a}	12.66 ± 0.03^{b}	5.50 ± 2.30^{a}	7.5 ± 0.7^{a}
	DS	76.00 ± 4.00^{b}	12.45 ± 0.39^{b}	3.65 ± 2.55^{b}	8.0 ± 0.0^{a}
Dry season	RF	96.50 ± 10.50^{a}	11.29 ± 0.10^{a}	1.65 ± 0.18^{a}	9.0 ± 0.0^{a}
	GS	80.50 ± 7.50^{b}	9.88 ± 0.01^{b}	1.46 ± 0.20^{a}	9.5 ± 0.7^{a}
	DS	102.50 ± 9.50^{a}	7.81 ± 0.01^{c}	1.52 ± 0.09^{a}	10.0 ± 0.0^{a}
Seasonal interactions	*F* value	0.358	457.02	3.244	1.083
	P value	0.71	0.01*	0.09	0.38

RF rainforest; *GS* Guinea savannah; *DS* derived savannah

^{abc} Means ($\pm$ standard deviation) in the same column for each of the seasonal periods having similar superscripts are not significantly different at $p < 0.05$

*Interaction significant at $p < 0.05$

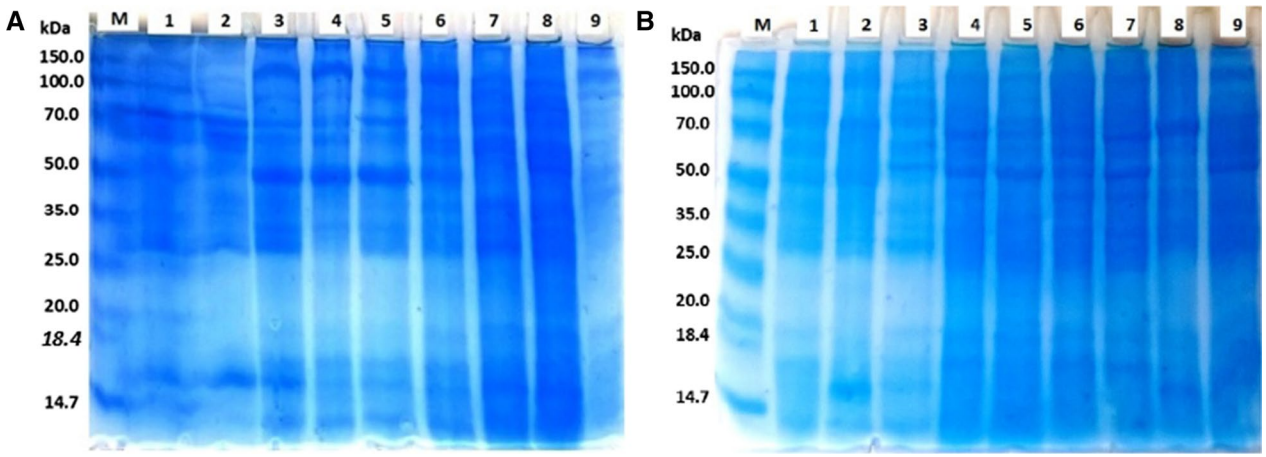

Fig. 2 SDS Gel page for honeybee samples from the three ecological zones during the onset of rain, wet season and dry seasons; M = Maker; Dry season (A1 and A2 = RF; A3 and A4 = DS; A5 and A6 = GS), Onset of rain (A7 and A8 = RF; A9 and B1 = DS; B2 and B3 = GS) and Wet season (B4 and B5 = RF; B 6 and B7 = DS; B8 and B9 = GS) periods; RF = rainforest; GS = guinea savannah; DS = derived savannah

during the onset of rain and dry season period, regardless of the ecological zone (Fig. 3). On the other hand, protein bands of 18.4 kDa, 20.0 kDa and 35.0 kDa were present in the honeybees only during the wet season period regardless of the ecological zone. However, protein bands of 50.0 kDa, 70.0 kDa and 150 kDa were present in the honeybees during the onset of rain, wet season and dry season periods.

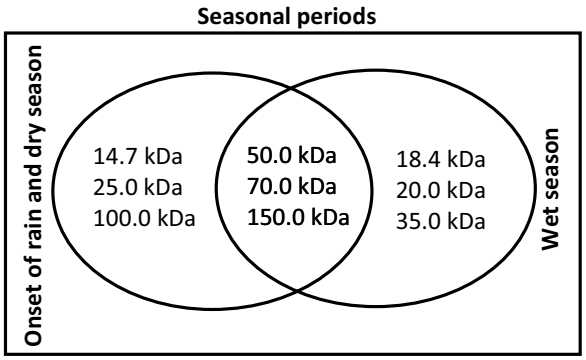

Fig. 3 Common protein bands of the study honeybees during the period of onset of rain, wet season and dry seasons

Lipid profile of the honeybees

Result of the lipid profile of the body of the honeybees revealed higher level of triglycerides than the other lipid components (Table 6). During the onset of rain, total

Table 6 Lipid profile (mg/dl) of the honeybees from the three ecological zones of Nigeria during the dry season, onset of rain and wet seasons

		Cholesterol (mg/dl)	TRIGS (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Onset of rain	RF	41.15 ± 0.55^{a}	28.60 ± 3.60^{b}	27.25 ± 1.35^{a}	8.20 ± 1.50^{a}	5.70 ± 0.70^{b}
	GS	27.50 ± 0.30^{b}	57.10 ± 3.20^{a}	11.35 ± 1.25^{b}	4.70 ± 0.30^{a}	11.45 ± 0.65^{a}
	DS	27.25 ± 4.45^{b}	35.80 ± 2.60^{b}	13.50 ± 3.90^{b}	6.58 ± 1.08^{a}	7.17 ± 0.53^{b}
Wet season	RF	33.60 ± 6.40^{b}	60.55 ± 2.65^{a}	12.70 ± 3.20^{b}	8.80 ± 2.70^{a}	12.10 ± 0.50^{a}
	GS	38.90 ± 11.70^{a}	57.80 ± 2.10^{a}	18.25 ± 9.35^{a}	9.10 ± 2.80^{a}	11.57 ± 0.43^{a}
	DS	34.20 ± 2.50^{b}	36.85 ± 2.65^{b}	20.30 ± 0.90^{a}	6.55 ± 1.05^{a}	7.35 ± 0.55^{b}
Dry season	RF	34.45 ± 6.15^{b}	57.50 ± 9.50^{c}	15.85 ± 3.05^{a}	7.10 ± 1.20^{a}	11.50 ± 1.90^{b}
	GS	37.50 ± 8.10^{a}	83.55 ± 1.35^{a}	14.55 ± 5.95^{a}	6.25 ± 1.85^{a}	16.70 ± 0.30^{a}
	DS	28.85 ± 0.55^{c}	76.95 ± 11.85^{b}	9.75 ± 0.45^{b}	3.72 ± 1.38^{b}	15.41 ± 2.39^{a}
Seasonal interactions	*F* value	0.285	25.616	0.823	1.603	25.368
	P value	0.76	0.01*	0.47	0.25	0.01*

Cholesterol total cholesterol; *TRIGS* triglycerides; *HDL* high density lipoprotein; *LDL* low density lipoprotein; *VLDL* very low density lipoprotein; *RF* rainforest; *GS* Guinea savannah; *DS* derived savannah

[abc] Means (± standard deviation) in the same column for each of the honeybee colony periods having similar superscripts are not significantly different at $p < 0.05$

*Interaction significant at $p < 0.05$

cholesterol was significantly higher in the honeybees from the rainforest zone than those from the guinea savannah and the derived savannah zones. On the other hand, honeybees from the guinea savannah zone had the highest ($p < 0.05$) total cholesterol during the wet season and the dry seasons. During the onset of rain and dry season, level of triglycerides and VLDL were significantly higher in the honeybees from the guinea savannah zone than those from the other studied ecological zones. During the wet season however, the level of triglycerides and VLDL were lowest in the honeybees from the derived savannah zone. Level of LDL recorded in the honeybees from the three studied ecological zones was not significantly different during the onset of rain, wet season and dry season. Result also showed significant interaction between the seasonal periods and levels of triglycerides and VLDL.

Discussion

This study evaluated the level of amino acids in the tissues of honeybees from three ecological zones of Nigeria during the onset of rain, wet and dry season periods. Out of the eighteen (18) amino acids recorded in the honeybees, proline, glutamic acid and leucine respectively had the highest composition. Mccaughey et al. (1980) earlier evaluated the amino acid composition of pollens from bee flora and reported that proline and glutamic acid were the most predominant. Also, Crailsheim and Leonhard (1997) identified proline as the predominant haemolymph amino acid of newly emerged honeybee workers. Although Crailsheim and Leonhard (1997) alleged that the role of high level of proline in the honeybee worker is not clear, other reports have supported the fact that in *A. mellifera*, proline may not be a significant metabolic fuel (Berger et al., 1997; Micheu et al., 2000; Rothe & Nachtigall, 1989). On the other hand, Campbell et al. (2016) suggested an increase in mitochondrial respiration of honeybees by proline. Studies on other insects have also shown that proline is the predominant amino acid in the body of many insect species (Consoli & Vinson, 2002; Lefevere et al., 1989; Sowa & Keeley, 1996). Proline is responsible for protein synthesis and metabolism, anti-oxidative reactions, immune response and wound healing (Wu et al., 2011). Also, glutamic acid (*L*-glutamic acid) with some other amino acids such as *L*-cysteine and glycine are responsible for the synthesis of glutathione which fights cellular oxidative stress in the body (Lu, 2013; Pastore et al., 2003). Hence, high levels of these amino acids in the honeybee workers from this study could be attributed to their roles in metabolism and cellular antioxidant defence system.

This study also showed significant effect of the seasonal periods on the activities of superoxide dismutase (SOD) in the honeybees. This was highest during the wet season period. Elevated activity of SOD could be a good marker of oxidative stress. In the antioxidant system, SOD plays the leading role in the defence and protection against free radical-induced damage and keep lipid peroxide levels low (Handa, 1997; Sandhir & Gill, 1999). SOD acts first on superoxide radicals of the reactive oxygen species, converting it to hydrogen peroxide (H_2O_2) (Lijun et al., 2005). During the wet season period of this study, there was abundance of flowering plants in both the wild and cultivated fields for the honeybees to forage on. Reports

have also shown that the spring months is usually characterized by the blossoming of plants with the potential to produce nectar and pollen (Bayir & Albayrak, 2016) and that the abundance of pollen resources during this period depends largely on the honeybee colony (Harris, 2016). Thus, foraging activities of the honeybees would increase significantly during the blossoming period (Harris, 2016). However, foraging activities in the honeybees is energetic, relying more on the flight muscles for locomotion (Schippers et al., 2006). Korayem et al. (2012) also noted that flight activity of honeybees raise during the active season because of the increase in tissue demand for more oxygen and therefore subjecting the honeybees to increased loads of oxidative stress through ROS. Hence, higher activity of SOD recorded in the honeybees during the wet season period of this study could be a coping strategy against oxidative stress associated with foraging activity during this period. Korayem et al. (2012) also reported an elevated activity of SOD in the honeybees during the active season.

The honeybees (except those from guinea savannah zone) had the highest body glucose levels during the dry season period. The usable form of energy in the insect body is sugar. Reports have shown that levels of sugar (glucose) in the honeybee could provide some details on their physiological state of carbohydrate metabolism (Amdam et al., 2006; Wang et al., 2012). Likewise, honeybees use almost exclusively sugars as substrates for flight (Rothe & Nachtigall, 1989; Teulier et al., 2016) and their brain is highly specialised for carbohydrate oxidation (Tsacopoulos, 1995). Significantly higher body glucose level recorded in the honeybees during the dry season period as compared to the onset of rain and wet season periods of this study could be associated with the level of work done especially through foraging exercise. From the onset of rain to the wet season period, there is abundance of blossoming plants for the honeybees to forage and much of the honeybee energy (glucose) would be expended in flight, foraging from plants to plants. This foraging activities of the honeybees was described by the Ministry of Agriculture (2015) to require physical adaptations of the sense organs including vision, olfactory sense, taste and sense of time. According to Conley and Lindstedt (1998), these modifications, especially with regard to flight activities consumes energy. However, during the dry season period, foraging and flight activity is reduced and there is abundant supply of honey in the hive. Reports have also shown that this period (dry season period) which falls immediately after the spring (wet season period) is characterized by reduced foraging activity due to winter coldness and abundance of honey storage in the hive, making the bees to feed on stored honey (Cornell University, 2017; Manchester and District Beekeepers' Association, 2019). It is therefore possible that the higher body glucose content recorded in the honeybees during the dry season period was as a result of excessive accumulation of unused energy since there was reduced foraging activities of the honeybees during this period.

Similarly, body protein level of the honeybees was higher during the wet season, regardless of the ecological zone. According to Neil (2004), protein is very essential in the activities of the neuron. Also, protein synthesis takes place where it is needed, especially in the dendrite (Neil, 2004). Studies have equally shown that the dendrites contain all they need to synthesize proteins: ribosomes, messenger RNAs, transfer RNAs and various enzymes that participate in the process (Steward & Schuman, 2001; Tiedge & Brosius, 1996). Proteins are equally responsible for body building. As reported by Jahan-Mihan et al. (2011), proteins are involved in the regulation of food intake, glucose and lipid metabolism and immune function. Higher total body protein content recorded in the honeybees of this study during the wet season period could therefore be associated with its synthesis from higher muscular activities and higher energy required to survive the stress of foraging activities and flight during this period.

On the other hand, protein banding pattern (gel electrophoresis) of the tissues of the honeybees from the three ecological zones was not significantly different, regardless of the seasonal periods. Previous report has shown that genetic mutation begins at the protein level, through alteration in protein chains (Clancy, 2008). Hamdan and Magdy (2010) also associated changes in the protein banding pattern of animals of the same species to gene mutation. Similarly, Kordarfshari et al. (2010) described the appearance of new protein bands in animals of the same species to be based on a mutational event at the regulatory system of unexpressed gene(s) that activate them. The report of Bamidele et al. (2015) equally showed that the environments of organisms significantly affect their protein banding pattern, with the expression of more protein bands as a result of physiological adaptation. Thus, similar protein banding pattern recorded in the tissues of the honeybees of this study is an indication that the honeybees are of the same species and have not undergone any significant genetic mutation due to the environment they live in.

Conclusions

From the results of this study, both the ecological zones of the honeybee colony and seasonal variations differently affect the physiological state of the honeybee workers. However, seasonal variations tend to present more

effect, especially on the antioxidant defence system and body metabolism of the honeybees.

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Author contributions
JAB developed the study design, oversee laboratory analyses, analysed results data and was a major contributor in writing the manuscript. ABI conceived the research idea and provided technical support during field collection and laboratory work. KOA designed sample collection methods and materials, provided laboratory materials and assisted in laboratory analyses. AAO liaised with apiary owners, provided logistics for transportation and for collection of honeybee samples and lead the team for sample collection. SAR provided the laboratory, equipment and kits and conducted laboratory analyses. All authors read and approved the final manuscript.

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Availability of data and materials
The data set analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This research was done in compliance with the ethical standard set by the College of Biosciences of the Federal University of Agriculture, Abeokuta on the use of "Economic Insects" for research study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no potential conflict of interest in relation to the study in this paper.

Author details
[1]Department of Pure and Applied Zoology, College of Biosciences, P.M.B. 2240, Abeokuta, Nigeria. [2]Department of Crop Protection, College of Plant Science, P.M.B. 2240, Abeokuta, Nigeria. [3]Physiology Department, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, P.M.B. 2240, Abeokuta, Nigeria.

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