Functional food promotes digestive functions and healthy growth of animals nurtured in confinement

^{1,2}Ajibola, A. and ¹Erlwanger, K. H.

¹School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, Johannesburg, South Africa.

²Department of Human Physiology, Faculty of Medicine, Kaduna State University, P.M.B 2339, Kaduna 800001, Kaduna State, Nigeria.

Corresponding Author: ajibola66@gmail.com

Phone Number: 0803 780 3988, 0805 521 8256

Abstract

In the study, the growth pattern, morphometric and morphological changes in the gastrointestinal tract of growing male and female Sprague-Dawley rats fed crude honey (CH)-supplemented diets as measures of nutrient utilization, digestive functions and healthy growth were investigated. Thirty-five suckling (7-day old male and female) rats were fed CH either as low (10mLkg⁻¹BW) or high (20mLkg⁻¹BW) dose daily via stomach tube for 14 days, while the control group was gavaged with distilled water. Rats were kept with their dams to nurse freely between gavages. On weaning, CH was mixed with commercial rat feed as low (20%) or high, 50% (volume/weight, v/w), while 20% (v/w) tap water was added to the control diet. The feed intake of honey-fed male rats and control (infant: 6 – 10g; adult: 31 – 38g) was significantly higher ($p < 0.05$) than their high dose-diet mates (infant: 5 – 8g; adult: 31 – 38g). All the rats were killed at 13 weeks old for gross and microscopic measurements of the abdominal viscera. Grossly, there were no significant differences ($p = 0.05$) in the relative lengths (%BW) and weight: length ratio (g cm⁻¹) of the small and large intestines in both sexes. Crude honey increased the absolute and relative weights of the caecum (1.73 ± 0.05 g; $0.38 \pm 0.02\%$ BW) and pancreas (2.52 ± 0.11 g; $0.55 \pm 0.03\%$ BW), with significant influence ($p < 0.05$) in the male rats. In addition, dietary inclusion of CH at low dose enhanced intestinal villi growth in height ($84.0 \pm 4.0\mu\text{m}$) and width ($25.2 \pm 1.5\mu\text{m}$). Dietary supplementation with crude honey also enhanced body weight gain of male rats (495.52 ± 8.98 g) and females (242.52 ± 6.87 g), improved abdominal organs' functional size: liver (10.92 ± 0.32 g; $2.72 \pm 0.13\%$ BW); spleen (1.25 ± 0.06 g; $0.27 \pm 0.02\%$ BW), devoid of pathological changes, as shown by liver histomorphology (1.2 ± 0.3) and normal hepatocellular architecture thereby conferring nutritional and health benefits on animals nurtured in confinement.

Keywords: Caecum, pancreas, morphometry, morphology, digestive functions, crude honey, rats.

Introduction

Laboratory and domestic animals are housed in different cages and used severally for production and research (Yamauchi and Isshiki 1991; Fasuyi *et al.*, 2005; Ajibola, 2006; Ajibola and Boomker, 2007; Adedeji *et al.*, 2008; Ajibola *et al.*, 2010; Zomrawi *et al.*, 2011; Qiao *et al.*, 2013; Azeez *et al.*, 2016). Apart from subjecting these animals to various forms of experimental

manipulation, their being restricted to the research environment imposes additional constraint on them. Nutritional studies entail dietary manipulation and oral administration of substances hitherto alien to the animals and far from their conventional diets (Ajibola *et al.*, 2007; Azeez *et al.*, 2016). This can alter gastrointestinal functions (Satchithanandam *et al.*, 1990), with consequent

histopathological changes in the visceral organs (Guth *et al.*, 1984; Rutledge and Adeli, 2007; Ajibola *et al.*, 2013). However, the use of functional food as dietary supplement in nutritional studies can make positive alterations, as it has immense health benefits (Ajibola, 2015; Ajibola *et al.*, 2016).

Crude honey (CH) is a liquid, sweet and flavourful natural product known to be a typical functional food with high nutritive value (Steinberg *et al.*, 1996; Ajibola, 2015). Hence, the present study was carried out to investigate the effects of oral administration of CH on growing Sprague-Dawley rats nurtured from infancy in confinement.

Materials and methods

The study was approved by the Animal Ethics Screening Committee (AESC approval number – 2010/29/2B) of the University of the Witwatersrand, South Africa, and performed according to the humane handling rules contained in the “Guidelines for the use and care of animals in Experimental, Education and other Scientific Procedures” of the University of the Witwatersrand, South Africa. The experimental procedures were done in the animal unit of Central Animal Services (CAS) and the School of Physiology at the University of the Witwatersrand, South Africa.

Animals and dietary treatments

Four nursing Sprague-Dawley (SD) rats with their 7-day old litters of 35 male and female pups (body weight 17.9 ± 0.28 g, [range 14 – 22]) g were used for the study. During the pre-weaning period, the pups were housed with their respective dams as a family in solid bottom plastic cages (425 x 270 x 140 mm) with beddings of wood shavings. The dams were given commercial rat feed (Epol, Johannesburg, South Africa)

and tap water *ad libitum*. After weaning, the dams were returned to stock and the weaned rats were housed in pairs in the plastic cages. The animals were placed on a 12 hour light-dark cycle (lights on 07:00–19.00 h) and environmental temperature of $22 \pm 2^\circ\text{C}$. The pups were gavaged with 50 % honey solution as low dose (10 mL/kg BW) or high dose (20 mL/kg BW); and distilled water for control rats, in addition to suckling for two weeks. The rats were weaned at 22 days of age and fed with 20% honey-enriched diet (volume/weight, v/w) as natural honey low (NHL); 50% honey-enriched diet (v/w) as natural honey high (NHH); and 20% tap water (v/w) was added to rat feed as control diet (CON). The diets were prepared daily, weighed, freshly served in clean bowls and fed to the experimental rats *ad libitum*. The chemical composition of the raw honey and the treatment diets as determined by an accredited Analytical laboratory according to AOAC (2011) is given in Table 1. The rats were supplied with tap water *ad libitum*, and were weighed twice weekly to monitor growth. The feed consumption was also measured daily and recorded (Table 2).

Visceral organs' measurements

At the age of 13 weeks, the rats were euthanized by intra-peritoneal injection of sodium pentobarbitone ($150\text{mgkg}^{-1}\text{BW}$) (Euthanaze®, Centaur Laboratories, Johannesburg, South Africa), and dissected for gross and microscopic measurements. The lengths of the small intestine (SI) and large intestine (LI) were recorded by dissecting the intestines and arranging each on a straight line with minimum stretching on a board according to the technique of Leopold (1953). The weights of the SI, LI, liver stomach, caecum, pancreas and spleen were obtained with Precisa 310M digital balance (Precisa®, Vadodara, Switzerland). Small intestinal sections (8µm thick) were stained with haematoxylin and eosin

Table 1: Chemical composition (g/100g DM) of crude honey and diets used during the 12-week study on rats

Proximate analyses	Crude honey	CON	CHL	CHH
Water (%)	15.92 ± 0.07	25.21 ± 0.09	16.51 ± 0.21	23.39 ± 0.02
Carbohydrate (g/100g)	88.97 ± 1.71	38.57 ± 1.33	42.84 ± 1.54	41.34 ± 0.79
Glucose (g/100g)	37.64 ± 0.01	2.12 ± 0.02	14.71 ± 0.02	12.72 ± 0.07
Fructose (g/100g)	43.35 ± 0.02	1.31 ± 0.02	16.53 ± 0.00	17.33 ± 0.02
Sucrose (g/100g)	2.03 ± 0.05	0.56 ± 0.02	2.65 ± 0.02	2.46 ± 0.02
Maltose (g/100g)	2.75 ± 0.02	0.65 ± 0.07	2.95 ± 0.03	2.92 ± 0.05
Glycaemic sugars (%)	76.71 ± 1.55	32.81 ± 0.61	44.44 ± 0.86	51.06 ± 1.50
Protein* (%)	0.42 ± 0.06	19.33 ± 1.49	17.50 ± 0.46	11.85 ± 0.30
Fat (%)	0.53 ± 0.01	3.71 ± 0.01	3.51 ± 0.02	2.45 ± 0.01
Energy (MJ/Kg)	15.56 ± 0.21	16.47 ± 0.04	16.56 ± 0.06	17.87 ± 0.01
Ash (%)	0.53 ± 0.00	7.74 ± 0.04	6.30 ± 0.07	4.42 ± 0.02

Note: Samples were analysed in duplicates; data are expressed as mean ± SD. *Obtained by multiplying nitrogen content by the factor of 6.25

(H&E); SI villi and crypts measured with micrometer mounted on a light microscope, LM (Reichert®, Austria). The jejunum was used for histology due to its absorptive importance. Morphometric measurements were done with an eye piece micrometer mounted on a light microscope, LM (Reichert®, Austria) at 100x magnification. Mean villus height per segment was estimated as the mean of the measurements (of 3 – 5 sections per rat) according to the procedures of Guth *et al* (1984). In an attempt to minimise under-estimation error due to angle and position of section planes, only the tallest profiles in each segment were measured (McAnuff *et al.*, 2003).

Portions of the caudate lobe of the liver were cut from each liver sample and was used for consistency of observation. These liver sections were also fixed in 10% formal-saline and later stained with H&E stain to prepare histological sections 5µm in thickness. The sections were examined under light microscope (magnification 100X; 400X) for cellular damage and fatty degeneration. A minimum of 10 fields for each liver slide were observed for severity of changes according to Korkmaz and Kolankaya (2008), and mean value of observations recorded. Severity of hepatic changes was scored based on a scale of 0 to

5, where 0 indicated absence of pathological changes and 5 that of chronic changes (Bacon *et al.*, 1984).

Liver histo-morphological changes were assessed by scoring the 5µm-thick H&E stained liver sections under LM on the scale 0-5, with 0 as no pathology, and 5 severe degenerative changes.

Data analyses

Data of the chemical composition of the raw honey and the experimental diets were expressed as mean. Student's t-test was used for the analysis of the data. All other data were expressed as mean ± standard error of the mean (Mean ± SEM). Graph Pad Prism version 5.02 (Graph Pad, San Diego, California, USA) was used as the statistical tool for the data analyses. The data from BW changes were analysed by repeated measures two-way analysis of variance (ANOVA) with Bonferonni's post hoc test. The means of the other parameters were analysed by one-way ANOVA with Neuman-Keul's post hoc used as a multiple comparison test. Level of significance was set at a p value of less than 0.05 (p < 0.05).

Results

Feed consumption

The daily feed intake of the experimental rats during the post weaning period was as

Functional food promotes healthy growth in animals

shown in the Table 2. The male rats fed with low dose-diet (CHL) and the control (CON) group consumed significantly higher ($p < 0.05$) amount of feed than their high dose-diet litter mates (CHH). This ranges from 6 – 10g for CON and CHL groups compared to 5 – 8g for CHH at infancy, and 31 – 38g for CON and CHL relative to 26 – 27g for

CHH at adulthood. In the females, the disparity in feed intake was observed starting from 6 weeks of age to termination (Table 2). The male groups were also observed to have consumed more feed than their corresponding female litter mates from age 6 weeks to end of the study at 13 weeks.

Table 2: Daily feed intake of male and female rats fed with crude honey-supplemented diets for 12 weeks (mean $\pm$ SEM)

Age (weeks)	Sex	CON	CHL	CHH
3 – 4	M	6.37 $\pm$ 0.98	6.85 $\pm$ 1.64	5.60 $\pm$ 0.65
	F	6.52 $\pm$ 1.08	6.93 $\pm$ 1.46	5.68 $\pm$ 0.89
4 – 5	M	9.39 $\pm$ 1.81	10.07 $\pm$ 2.01	8.13 $\pm$ 1.76
	F	9.48 $\pm$ 1.86	9.61 $\pm$ 0.99	8.52 $\pm$ 2.02
5 – 6	M	15.60 $\pm$ 2.05	17.33 $\pm$ 1.21	13.18 $\pm$ 1.98*
	F	12.83 $\pm$ 1.29 [§]	12.42 $\pm$ 1.87 [§]	11.92 $\pm$ 1.68 [§]
6 – 7	M	21.27 $\pm$ 0.68	22.25 $\pm$ 2.04	15.71 $\pm$ 1.33*
	F	16.67 $\pm$ 1.84 [§]	15.93 $\pm$ 1.77 [§]	13.24 $\pm$ 1.05*
7 – 8	M	26.01 $\pm$ 1.79	26.07 $\pm$ 1.67	18.01 $\pm$ 2.48*
	F	19.77 $\pm$ 1.41 [§]	19.46 $\pm$ 1.96 [§]	15.86 $\pm$ 1.06* [§]
8 – 9	M	29.16 $\pm$ 2.14	29.59 $\pm$ 1.57	20.43 $\pm$ 1.84*
	F	20.29 $\pm$ 1.58 [§]	21.39 $\pm$ 1.93 [§]	17.04 $\pm$ 1.22* [§]
9 – 10	M	29.56 $\pm$ 2.38	32.87 $\pm$ 2.12	22.91 $\pm$ 2.41*
	F	20.88 $\pm$ 2.15 [§]	24.21 $\pm$ 1.43 [§]	19.29 $\pm$ 1.86* [§]
10 – 11	M	30.36 $\pm$ 2.36	34.25 $\pm$ 1.93	23.97 $\pm$ 1.96*
	F	22.73 $\pm$ 1.27 [§]	26.02 $\pm$ 1.82 [§]	21.19 $\pm$ 1.86* [§]
11 – 12	M	31.53 $\pm$ 1.76	35.78 $\pm$ 1.34	26.09 $\pm$ 1.04*
	F	24.07 $\pm$ 1.49 [§]	27.50 $\pm$ 1.42 [§]	21.84 $\pm$ 1.47* [§]
12 – 13	M	33.57 $\pm$ 1.84	38.12 $\pm$ 1.87	27.14 $\pm$ 1.88*
	F	27.35 $\pm$ 2.09 [§]	29.06 $\pm$ 2.51 [§]	23.73 $\pm$ 1.75* [§]

*significantly different ($p < 0.05$) along the row; [§]significantly different ($p < 0.05$) from the corresponding value of male group

Body weight

The growth pattern records showed that the rats in all groups had significant body weight gain (BWG) from the commencement to the end of the 12-week study (Figures 1 and 2). However, the final body weight (BW) and BWG of the CHL

male group was significantly higher ($p < 0.01$) than that of the other male groups, and normal slightly higher (5.0%) relative to CHH rats (Figure 1). There was also significant difference ($p < 0.05$) in the final BW among the female groups (Figure 2).

Initial body weight (BW) at day 7; Weaning BW (day 21); and Final BW at 13 weeks old: *BW significantly higher ($p < 0.001$) than BW of previous phase. **Significantly higher ($p < 0.05$) than control.

Figure 1: Growth pattern of male experimental rats fed with crude honey-supplemented diets for 12 weeks

Initial body weight (BW) at day 7; Weaning BW (day 21); and Terminal BW at 13 weeks old: *BW significantly higher ($p < 0.001$) than BW of previous phase. **Significantly higher ($p < 0.05$) than control.

Figure 2: Growth pattern of female experimental rats fed with honey-supplemented diets for 12 weeks

Functional food promotes healthy growth in animals

Measurements of the intestines

The absolute weights (g) and lengths (cm) of the small intestine (SI) and large intestine (LI) showed significant differences ($p < 0.05$) amongst the male as well as the female groups (Table 3). However, there were no significant differences ($p > 0.05$) in

the relative lengths (%BW) and weight: length ratio (g cm^{-1}) of these organs (SI and LI) in both sexes as shown in Table 3. There were sex differences shown in these parameters with the females having lower values relative to corresponding male values.

Table 3: Gross measurements of the small intestine (SI) and large intestine (LI) in male and female rats fed with crude honey-supplemented diets for 12 weeks

Organ	Unit	Sex	CON	CHL	CHH
SI	G	M	$8.09 \pm 0.30^{\text{a,b}}$	$9.05 \pm 0.20^{\text{a}}$	$7.84 \pm 0.30^{\text{b}}$
		F	$6.42 \pm 0.16^*$	$6.70 \pm 0.29^*$	$6.19 \pm 0.14^*$
	Cm	M	$131.75 \pm 0.98^{\text{b}}$	$138.33 \pm 0.67^{\text{a}}$	$130.50 \pm 2.43^{\text{b}}$
		F	$123.00 \pm 2.22^*$	$124.00 \pm 2.06^*$	$113.40 \pm 4.86^*$
	g cm^{-1}	M	0.06 ± 0.00	0.07 ± 0.00	0.06 ± 0.00
		F	0.05 ± 0.00	$0.05 \pm 0.00^*$	0.06 ± 0.00
LI	%BW	M	1.86 ± 0.04	1.96 ± 0.09	1.92 ± 0.05
		F	$2.45 \pm 0.09^*$	$2.41 \pm 0.07^*$	2.27 ± 0.08
	G	M	$2.29 \pm 0.11^{\text{a}}$	$2.18 \pm 0.11^{\text{a}}$	$2.03 \pm 0.06^{\text{b}}$
		F	$1.60 \pm 0.10^*$	$1.80 \pm 0.09^*$	$1.67 \pm 0.05^*$
	cm	M	24.00 ± 0.33	23.33 ± 0.69	24.33 ± 0.42
		F	$20.70 \pm 0.73^*$	$20.20 \pm 0.53^*$	$21.60 \pm 0.86^*$
	g cm^{-1}	M	0.10 ± 0.01	0.09 ± 0.00	0.08 ± 0.00
		F	0.08 ± 0.00	0.09 ± 0.01	0.08 ± 0.00
	%BW	M	0.53 ± 0.02	0.47 ± 0.03	0.50 ± 0.01
		F	0.61 ± 0.04	0.65 ± 0.02	0.61 ± 0.03

Length (cm), absolute (g) and relative (%BW) weights, and weight : length ratio calculated as g cm^{-1} of SI and LI in male and female experimental rats. Data are expressed as mean $\pm$ SEM. Data in the same row with different superscripts are significantly different ($p < 0.05$). *Values different at $p < 0.05$ from the corresponding values of male group

Histology of SI and liver

Microscopic examinations of the small intestinal villi and crypts showed increased ($p < 0.05$) height and width of the villi, and crypt depth, as well as the SI villus height: crypt depth ratio in the CHL male rats relative to the CHH and control groups (Table 4). However, only the SI villus height and crypt depth among the histological parameters of the low dose crude honey-supplemented (CHL) female rats were higher ($p < 0.05$) than values obtained from the female CHH and control animals (Table 4). The high dose crude honey fed (CHH)

female rats did not differ in SI villus width relative to female control (CON) rats; whilst the SI villus height: crypt depth ratio did not differ ($p > 0.05$) amongst the female rats.

The hepatic changes observed under the LM were also recorded in Table 4. Rats fed with commercial rat feed (control) and the two doses of crude honey-supplemented diets (CHL & CHH) had liver sections showing normal cytology. The normal hepatic cytology of the experimental animals is presented graphically in Figures 3 and 4.

Table 4: Histological characteristics of the small intestine (SI) and the liver in male and female rats fed with crude honey-supplemented diets for 12 weeks

Measurements	Sex	CON	CHL	CHH
SI villus height (μm)	M	63.5 ± 3.6^b	84.0 ± 4.0^a	64.8 ± 1.1^b
	F	$45.5 \pm 1.5^{b*}$	$56.8 \pm 0.8^{a*}$	$47.2 \pm 2.0^{b*}$
SI villus width (μm)	M	16.8 ± 0.3^b	25.2 ± 1.5^a	16.8 ± 0.9^b
	F	16.0 ± 0.9^b	$18.8 \pm 0.6^{a*}$	$17.2 \pm 0.8^{a,b}$
SI crypt depth (μm)	M	20.2 ± 1.0^b	24.0 ± 1.0^a	20.8 ± 0.5^b
	F	21.8 ± 1.4^b	27.5 ± 1.1^a	22.2 ± 0.6^b
SI villus height/crypt depth	M	3.2 ± 0.1^b	3.5 ± 0.1^a	3.1 ± 0.1^b
	F	$2.1 \pm 0.1^*$	$2.1 \pm 0.1^*$	$2.1 \pm 0.1^*$
Liver histo-morphology	M	0.7 ± 0.2	1.2 ± 0.3	1.3 ± 0.4
	F	0.7 ± 0.2	1.2 ± 0.3	1.2 ± 0.3

Data are expressed as means $\pm$ SEM. Data in the same row with different superscripts are significantly different ($p < 0.05$). *significantly different at $p < 0.05$ from the corresponding value of male group

Scale bar is 100 μm ; magnification (mag) of control (CON) liver is 100 x; treatment (CHL & CHH) groups' livers mag is 400 x.

Figure 3: Photomicrographs of the livers (Haematoxylin & Eosin stain) from experimental male rats fed with crude honey -supplemented diets for 12 weeks showing the normal liver architecture

Scale bar is 100 μm ; magnification (mag) of control (CON) liver is 100 x; treatment (CHL & CHH) groups' livers mag is 400 x.

Figure 4: Photomicrographs of the livers (Haematoxylin & Eosin stain) from female rats fed crude honey-supplemented diets for 12 weeks showing the normal liver architecture

Functional food promotes healthy growth in animals

Visceral organs measurements

The weights of the other abdominal visceral organs in the rats are shown in Table 5. There were significant differences ($p < 0.05$) in the absolute (g) and relative

(%BW) weights of the male abdominal visceral organs namely caecum and pancreas, while there were not significant differences in gross measurements of same organs amongst the female groups.

Table 5: Absolute (g) and relative (%BW) weights of the abdominal visceral organs in male and female rats fed with crude honey-supplemented diets for 12 weeks

Organ	Unit	Sex	CON	CHL	CHH
Caecum	G	M	1.62 ± 0.06	1.73 ± 0.05*	1.49 ± 0.09
		F	1.23 ± 0.08	1.19 ± 0.04 ^β	1.05 ± 0.04
	%BW	M	0.37 ± 0.01	0.38 ± 0.02*	0.37 ± 0.02
		F	0.46 ± 0.02	0.43 ± 0.02	0.39 ± 0.02
Stomach	G	M	2.31 ± 0.10	2.46 ± 0.13	2.33 ± 0.06
		F	1.85 ± 0.07	1.77 ± 0.05	1.61 ± 0.12
	%BW	M	0.54 ± 0.03	0.55 ± 0.02	0.55 ± 0.02
		F	0.71 ± 0.03	0.64 ± 0.02	0.60 ± 0.06
Liver	G	M	11.16 ± 0.34	10.92 ± 0.32	10.24 ± 0.40
		F	7.02 ± 0.33 ^β	7.49 ± 0.30 ^β	7.20 ± 0.28 ^β
	%BW	M	2.57 ± 0.04	2.72 ± 0.13	2.51 ± 0.06
		F	2.67 ± 0.10	2.70 ± 0.06	2.63 ± 0.04
Pancreas	G	M	1.80 ± 0.10	2.52 ± 0.11*	2.25 ± 0.09*
		F	1.41 ± 0.08	1.68 ± 0.13*	1.41 ± 0.10 ^β
	%BW	M	0.42 ± 0.03	0.55 ± 0.03*	0.56 ± 0.04*
		F	0.54 ± 0.03	0.61 ± 0.04	0.52 ± 0.04 ^β
Spleen	G	M	1.16 ± 0.04	1.25 ± 0.06	0.96 ± 0.04*
		F	0.93 ± 0.03	0.91 ± 0.02	0.78 ± 0.02
	%BW	M	0.27 ± 0.01	0.27 ± 0.02	0.23 ± 0.01
		F	0.36 ± 0.02	0.33 ± 0.01	0.29 ± 0.01

Data are expressed as means ± SEM. *significantly different ($p < 0.05$) along the row.^βValue significantly different at $p < 0.05$ from the corresponding value of male group

Discussion

Feed intake and utilization

In this study, low dose-diet group ate more feed than their high dose-diet counterparts (Table 2). The discretionary intake of diets containing low amount of solutions demonstrated rodent's preference for dry feed to pasty or liquid material. The preferential dietary intake by rats had been previously reported (Davis *et al.*, 1976). The nature and fluidity of the high dose diets might have also influenced the pattern of feed intake, as one author (Davidson, 2005) opines that feed viscosity influences calorific intake. Another probable explanation is that these animals regulate

their calorific intake based on fast-acting specific gustatory signals (Prabhakar and Rao, 1985). In a study, it is established that early life events influence life-long patterns of behavioural and physiological responsiveness (McEwen, 2007). In the light of this, the preferential consumption of low dose diet by the experimental rats could have resulted from the introduction of the dietary treatments to the rats in their neonatal period. Since both groups of rats were exposed simultaneously to their different dietary treatments from infancy, another mechanism will adduce for higher feed intake by CHL rats. It can be inferred that sweet receptors evolved in ancestral

environments poor in sugars in most mammals including rats, and are thus not adapted to high concentrations of sweet taste stimulants (Lenoir *et al.*, 2007). Hence, these rats might have evoked some self-control mechanisms due to supra-normal stimulation of some receptors in order to deal with dose dependency and avoid over consumption of the high dose (CHH) diet. This assumption is supported by the hypotheses of some workers in the field of neuroscience that early life experience has influence on physiological and behavioural patterns (Berridge, 1996; Drewnowski, 1997; Sclafani, 2004). This can be adduced for the higher feed intake by low dose honey-fed rats.

Growth pattern

The growth patterns of male and female rats from the age of 7 days old to termination at 13 weeks in the present study showed the treatment diets induced significant BWG in both sexes. However, there was a notable differential response to dietary treatments and sex influence at termination (13 weeks) as shown in Figures 1 and 2. As it is normal for Sprague-Dawley rats, a highly significant gender differences in growth response with the males having 43% final BW gain than females was noticed, despite their similar weight at birth, as well as being subjected to the same dietary treatments and experimental conditions. Sprague-Dawley male rats are noted for faster growth and higher body weight than females (Klinger *et al.*, 1996). The eating of honey caused increased terminal body weight gain than control and induced healthy growth of rodents. This observation was in conformity with the previous findings that honey improves growth of rats (Ajibola *et al.*, 2007; 2016). In a previous comparative nutritional study, Cheplius and Starkey (2008) had a different opinion on growth response by honey-fed rodents. These

authors reported low body weight (BW) gain of honey-fed and those fed sugar-free (control) diet adult rats relative to sugar-fed animals. The conflicting observations might be due to the difference in growth phases of the rats used for the studies. In the studies reporting the growth influence of honey, young rats in their active growing phase were used, while Cheplius and Starkey used two months old rats for their study, and also administered the honey at a probably sub-pharmacological and lower (10%) dose than the amount used in the present study (20% and 50%). In addition, it was not known whether the high BW of the sucrose-fed rats by these authors was due to muscle growth or high fat weight (Cheplius and Starkey, 2008). It is noteworthy that, the other aspect of the findings from the same study suggests the latter, as these authors report a significantly higher level of body fat in the sugar-fed rats than the honey eaters (Cheplius and Starkey, 2008). The observations from other studies in our nutrition laboratory also confirmed that excess consumption of refined sugars such as sucrose and fructose is culpable in increased visceral fat, fatty liver and obesity (Ajibola *et al.*, 2013; 2016). These pathological conditions were not seen in the honey-fed rats in the present study, as the treated rats presented hepatic tissue with normal cytology and distinct architectural layout similar to control rats in both sexes (Figures 3 and 4). This strengthened healthy growth of honey-fed rats.

Morphometric and morphological changes

The determination of organ weights is a long established practice to ascertain their functional integrity and establish health status of experimental animals (Baltrop and Brueton, 1990). The study showed that there were differences in the absolute weights and lengths of the small and large intestines

Functional food promotes healthy growth in animals

amongst all the groups of experimental animals (Table 3). The use of absolute organ weight may be misleading as it does not account for the differences in body weight of the animals (Bailey *et al.*, 2004). The differences in body weight often occur between and within groups of experimental animals due to the changes in some parameters. These include alterations in growth caused by modification of hormone secretion; hormonal status being modified by maturational patterns; feed consumption due to changes in neurotransmitters or reduced palatability of diets; and other non-specific systemic factors (Bailey *et al.*, 2004). Hence, the ratio of the organ weight to body weight (expressed as % BW), and referred to as relative organ weight is commonly used for the analysis of organ weight. When these organs' weights were evaluated relative to the rats' body weights, the differences were found to be insignificant in both sexes (Table 3). Further evaluation of the weight: length ratio (g cm^{-1}) of the intestines also showed no significant difference amongst the male rats as well as the female groups. This plausibly suggested that the variations observed in the absolute weights and lengths were of no biological significance. Thus, no experimental diet had any undue advantage over the other diets to influence the gross anatomical configuration and functional disposition of the GIT.

The differential impact of the dietary treatments on the gastrointestinal tract was observed at the microscopic level (Table 4). As discussed elsewhere, the intake of honey supplemented diets produced trophic effects on the intestinal morphology of male rats (Ajibola *et al.*, 2013). Satchithanandam *et al.* (1990) reported an influence of high dietary fibre in their experimental rats where they found an increase in the number of intestinal cells. Honey has been shown to

contain the biologically active soluble fibre, propolis (Steinberg *et al.*, 1996; Ajibola, 2015), which could have produced the trophic effects in the intestinal villi (Table 4). The influence of high dietary fibre on intestinal villi growth has also been documented in different domestic and laboratory animals (Yamauchi and Isshiki, 1991; Yu and Chiou, 1996; Ajibola and Boomker, 2007). The CH-enhanced villi and crypts dimensions could have provided more surface area for digestive functions and enzymes activities in the GIT. In the words of other workers, the broader villi provide a greater surface area and, therefore, more brush border for nutrients absorption (Yamauchi and Isshiki, 1991). This could be attributable for improved growth of the honey-fed rodents. These findings were emphasized by Yamauchi and Isshiki (1991), when they fed their broiler chickens with high fibre diet, and made similar observations of larger intestinal villi resulting in faster growth than the laying birds used in their study. This agreed with the opinion of McAnuff *et al.* (2003) in one dietary supplementation study. These workers demonstrate that a decrease in the villus height to crypt depth ratio suggests reduced overall capacity for digestion and absorption of nutrients, and vice versa. In addition, crude honey contains prebiotics including oligosaccharides which competitively activate beneficial intestinal bacteria flora and inhibit infectious agents in consumers (Kunz and Rudloff, 1993; Busserolles *et al.*, 2002) thereby enhancing digestive functions (Ajibola *et al.*, 2013). This improves metabolic activities and brings about healthy growth in honey eaters.

Trophic effects on visceral organs

The oral administration of crude honey increased the absolute and relative weights of some visceral organs which include

caecum, stomach, pancreas and spleen in males, but the increase did not attain any statistical significance to influence BWG in the females. However, the organs' growth of honey-fed male rats was shown to have contributed positively to the animals' BWG. The predominant influence of crude honey on organs' macroscopic growth was shown by the caecum and pancreas in the male rats (Table 5). The underlying mechanistic factors attributable for these organs' growth and probable effects had been extensively discussed elsewhere (Ajibola *et al*, 2013; Ajibola, 2015).

In addition to greater intestinal villi of rodents fed high fibre diets, increasing the fibre content of laboratory animals reduces the susceptibility to gastric infections, and also found to ameliorate gastroenteritis. In one study, it was found that the gastric emptying of saccharides after ingestion of honey was slower than that after ingestion of a mixture of glucose and fructose (Pokornand Vukmirovic, 1978), and thus, mitigating diarrhoea. This observation was confirmed by Cheeke (1982) who found a decrease in diarrhoea in rabbits fed with high dietary fibre. This suggests a potential nutritional and health benefits of supplementing animal feed with honey.

Conclusion

Dietary supplementation with crude honey induced body weight gain and healthy weight gain of the pancreas and caecum. Crude honey at low dose-diet caused enhanced intestinal villi growth of the experimental rats. These could improve their digestive functions and confer nutritional benefits on the animals, with consequent improved growth. Nonetheless, there is need to probe further to unravel the mechanisms behind the trophic effects and health benefits of honey. One could suggest animal experimentation involving feeding of crude honey for a more prolonged period

of time in future studies.

References

- Adedeji, O. S., Farimi, G. O., Ameen, S. A. and Olayemi, J. B. 2008.** Effects of bitter cola (*Garcinia kola*) as growth promoter in Broiler Chicks from day old to four weeks old. *Journal of Animal and Veterinary Advances*, 5 (3), 191-193.
- Ajibola, A. 2006.** Nitrogen retention and water balance in animals fed a medium protein diet amidst limited water supply. *Afr. J. Biomedical Res.* 9, 225–228.
- Ajibola, A. and Boomker, E. A. 2007.** Effect of water deprivation on feed intake, nutrient digestibility and nitrogen balance in domestic animals. *Nig J. Nutr. Sci.* 28 (1), 36–41.
- Ajibola, A., Idowu, G. O., Amballi, A. A., Oyefuga, O. H. and Iquot, I. S. 2007.** Improvement of some haematological parameters in albino rats with pure natural honey. *J. Biol. Sci. Res.* 2, 67–69.
- Ajibola, A., Chamunorwa, J. P. and Erlwanger, K. H. 2013.** Comparative effect of cane syrup and natural honey on abdominal viscera of growing male and female rats. *Indian J. Exp. Biol.* 51, 303 – 312.
- Ajibola, A. 2015.** Physico-chemical and physiological values of honey and its importance as a functional food. *Int. J. Food Nutr. Sci.* 2 (6), 1–9.
- Ajibola, A. Olusakin, J. and Oyewale, A. A. 2016.** Growth and metabolic response of suckling rats fed with natural honey supplements. *Int. J. Food Nutr. Sci.* 3(1), 1-5.
- AOAC. 2011.** Official methods of analysis (18th Edition). *Association of*

- Analytical Chemists*, Arlington, Virginia.
- Azeez, O. M., Adah, S. A., Adenkola, A. Y. and Ameen, S. A. 2016.** Changes in erythrocyte membrane properties following exposure to premium motor spirit (petrol vapour) and modulatory effects of *moringaoleifera* and vitamin c in wistar rats. *J. Afr. Ass. Physiol. Sci.* 4 (2), 102-108.
- Bacon, B. R., Park, C. H., Fowell, E. M. and McLaren, C. E. 1984.** Hepatic steatosis in rats fed diets with varying concentrations of sucrose. *Fundamentals Appl. Toxicol.* 4, 819 – 826.
- Bailey, S. A., Zidell, R. H. and Perry, R. W. 2004.** Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint? *Toxicologic Pathol.* 32, 448 – 466.
- Baltrop, D. and Brueton, M. J. 1990.** The gastrointestinal tract and short term toxicity tests. In: Bordeau, P.J. (Ed.), *Short-term Toxicity Tests for Non-genotoxic Effects*. John Wiley and Sons Ltd, pp. 99 – 110.
- Berridge, K. C. 1996.** Food reward: brain substrates of wanting and liking. *Neuroscience Biobehavioural Review* 20, 1 – 25.
- Busserolles, J., Gueux, E., Rock, E., Mazur, A. and Rayssiquier, Y. 2002.** Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidative effects of fructose. *J. Nutr.* 132(11), 3379 – 3382.
- Cheeke, P. R. 1982.** High roughage diets utilisation by rabbits. *Feed International* 3, 46 – 51.
- Chepulis, L. and Starkey, N. 2008.** The long-term effects of feeding honey compared to sucrose and a sugar-free diet on weight gain, lipid profiles, and DEXA measurements in rats. *J. Food Sci.* 73 (1), S1 – 7.
- Davis, J. D., Collins, B. J. and Levine, M. W. 1976.** Peripheral control of meal size: Interactions of gustatory stimulation and postingestional feedback. In: *Hunger: Basic Mechanisms and Clinical Implications*. Edited by D. Novin, W. Wgrwicka and G. Bray. New York: Raven Press. pp 395 – 408.
- Davidson, T. L. 2005.** Food viscosity influences caloric intake compensation and body weight in rats. *Obesity Res.* 13, 537 – 544.
- Drewnowski, A. 1997.** Taste preferences and food intake. *Annu. Rev. Nutr.* 17: 237-253.
- Fasuyi, A. O., Fajemilehin, S. O. K. and Omojola, A. B. 2005.** The egg quality characteristics of layers fed varying dietary inclusions of Siam weed (*Chromolaena odorata*) leaf meal (SWLM). *Int. J. Poultry Sci.* 4(10), 752-757.
- Guth, P. H., Paulsen, G. and Nagata, H. 1984.** Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat. Effect of prostaglandin cytoprotection. *Gastroenterol.* 87, 1083 – 1090.
- Klinger, M. M., MacCarter, G. D. and Boozer, C. N. 1996.** Body weight and composition in the Sprague Dawley rat: Comparison of three outbred stocks. *Lab. Anim. Sci.* 46, 7 – 70.
- Korkmaz, A. and Kolankaya, D. 2009.** Anzer honey prevents N-ethyl maleimide-induced liver damage in rats. *Exptal. Toxicol. Pathol.* 61, 333 – 337.
- Kunz C. and Rudloff S. 1993.** Biological functions of oligosaccharides in

- human milk. *Acta Paediatrica* 82, 903–912.
- Lenoir, M., Serre, F., Cantin, L. and Ahmed, S. H. 2007.** Intense sweetness surpasses cocaine reward. *PLoS ONE* 2(8): e698. doi:10.1371/journal.pone.000069
- Leopold, A. S. 1953.** Intestinal morphology of gallinaceous birds in relation to food habits. *J. Wildl. Manage.* 17, 197–203.
- McAnuff, M. A., Omoruyi, F. O., Gardner, M. T., Morrison, E. Y. and Asemota, H. N. 2003.** Alterations in intestinal morphology of streptozotocin induced diabetic rats fed Jamaican bitter yam (*Dioscorea polygonoides*) steroidal sapogenin extract. *Nutr. Res.* 23, 1569–1577.
- McEwen, B. S. 2007.** Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. *Physiological Reviews* 87, 873–904.
- Prabhakar, E. and Rao, B. S. 1985.** Rapid calorie metering in *ad lib* rats. *J. Bioscience* 9(1 & 2), 41–45.
- Pokorn, D. and Vukmirovic, V. 1978.** Velocity of gastric emptying of saccharides after administering honey and pure invert sugar. III International Apitherapy, Symposium 11–15 September 1978, Portoroz, Yugoslavia. Bukarest: Apimondia, pp 277 – 279.
- Qiao, J., Li, H. H., Zheng, Ch. J., Feng, Z. Y. and Wang, W. 2013.** Dietary supplementation with aloe vera polysaccharide enhances the growth performance and immune function of weaned piglets. *Journal of Animal Feed Science*, 22, 329–334.
- Rutledge, A. C. and Adeli, K. 2007.** Fructose and the Metabolic Syndrome: Pathophysiology and Molecular Mechanisms. *Nutr. Rev.* 65(6), S13–23.
- Satchithanandam, S., Apler, M. V., Calvert, R. J., Leeds, A. R. and Cassidy, M. M. 1990.** Alteration of gastrointestinal mucin by fibre feeding in rats. *J. Nutr.* 120, 1179 – 1184.
- Sclafani, A. 2004.** Oral and postoral determinants of food reward. *Physiol. Behav.* 81, 773–779.
- Sieck, G. C., Nance, D. M. and Gorski, R. A. 1978.** Regulation of feeding behaviour in the prepubertal female rat. *Physiol. Behav.* 21 (5), 727–733.
- Steinberg, D., Kaine, G. And Gedalia, I. 1996.** Antibacterial effect of propolis and honey on oral bacteria. *Am. J. Dentistry* 9, 236 – 239.
- Yamauchi, K. E. and Isshiki, Y. 1991.** Scanning electron microscopic observations on the intestinal villi in growing white leghorn and broiler chickens from 1 to 30 days of age. *Brit. Poultry Sci.* 32, 67 – 78.
- Yu, B. and Chiou, P. W. S. 1996.** Effects of crude fibre level in the diet on the intestinal morphology of growing rabbits. *Lab. Animals* 30, 143–148.
- Zomrawi, W. B., Abdel Atti, K. H. A. and Mahalam, A. G. 2011.** Effect of ginger root powder supplementation on broiler chick performance, blood and serum constituents, *Online J. Anim. Feed Res.* 1(6), 457–460.

Received: 24th November, 2016

Accepted: 4th March, 2017