



(RESEARCH ARTICLE)



## Consumption of green tea infusion causes loss of nutrients through faeces in Wistar rats

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### Abstract

Relationship between consumption of green tea infusion and faecal nutrient content was studied. Twelve male Wistar rats (aged 10 to 12 weeks and weighed 87 to 120 g) were divided into two groups of six rats each. The control group was placed on tap water, while the test group was placed on green tea infusion (0.20 g/50 ml) daily as the only source of fluid; for 28 days. Both groups consumed rat feed *ad libitum*. Phytochemicals detected in the infusion were tannins, saponins, alkaloids, flavonoids and phytosterols. The infusion increased test animals' appetite for food (% coefficient of variation, % CV = 5.44) but-decreased their appetite for the infusion (% CV = 72.41). Animals placed on the infusion (test group) did not gain more weight (% CV = 3.86) nor had better feed conversion ratio (% CV = 1.41). Faecal ash and fibre contents were not affected by tea consumption (% CV = 3.38 and 2.03, respectively). Control rats lost more carbohydrate and moisture (% CV = 27.58 and 9.58, respectively) but test rats lost more protein and lipids. Energy lost by test rats through faecal matter was not appreciably higher (101.31 vs 96.24 kcal/ 100 g sample; % CV = 2.56). Loss of certain nutrients in the test rats may have been triggered by the inhibitory action of polyphenols such as tannins present in the infusion on their digestive enzymes. In conclusion, the tea infusion elicited increased faecal excretion of proteins and lipids in treated animals.

**Keywords:** Faeces; Green tea; Infusion; Nutrient loss; Wistar rats

### 1. Introduction

Weight loss, in the context of medicine, health, or physical fitness, refers to a reduction of the total body mass. Weight loss in individuals who are overweight or obese can reduce health risks, increase fitness, and may delay the onset of diabetes (LeBlanc *et al.*, 2011). Poor nutrient utilization can lead to weight loss, and can be caused by fistulae in the gastrointestinal tract, diarrhea, rug-nutrient interaction, enzyme depletion and muscle atrophy (Alibhai *et al.*, 2005).

*Camellia sinensis* (tea) is the most frequently consumed beverage worldwide besides water (Hayat *et al.*, 2015). It holds second position in consumption among beverages. Of the tea produced worldwide, 78% is black tea (fully fermented), which is usually consumed in western countries; 20% is green tea (unfermented), which is commonly consumed in Asian countries, and 2% is oolong tea which is produced by partial fermentation and is mainly consumed in southern China. Generally, tea possesses significant antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic and thermogenic properties (Butt and Sultan, 2009).

Digestion turns consumed food materials into products that can be assimilated. It is aided by digestive enzymes in the mouth, stomach and intestines (Goodman, 2010). However, controversial reports have surfaced regarding the beneficial effects and risks associated with excessive consumption of teas (Hayat *et al.*, 2013; Rosa-Sibakov *et al.*, 2018). The present study reports the loss of nutrients through faecal means in Wistar rats due to the consumption of green tea infusion.

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## 2. Material and methods

### 2.1. Materials

#### 2.1.1. Poultry feed, tea and rats

Palletized poultry feed (product of UAC Nigeria Plc., Jos Nigeria) and green tea were used in the study. Twelve male albino rats (*Rattus norvegicus*) of the Wistar strain (aged 10 to 12 weeks and weighed 87 to 120 g) were purchased from the Animal Colony of the Department of Veterinary Medicine, University of Nigeria Nsukka, Nigeria. The feed and tea used in the study were coded, and their ingredients listed according to the manufacturers' descriptions (Table 1).

**Table 1** Contents of Feed and Tea\*

Code/sample description	Contents
A (poultry feed)	Cereals/grains, vegetable, protein, premix (vitamin/minerals), probiotic and enzymes, essential amino acids, antioxidant, antitoxins.
B (green tea)	Polyphenols, amino acids, carbohydrate, protein, fluoride, aluminum, mineral and trace elements, vitamin, vitamin B, vitamin E and alkaloids.

\*Manufacturers' description

### 2.2. Methods

#### 2.2.1. Preparation of tea infusion

One hundred and fifty milliliters (150 ml) of boiled water was measured into a 500 ml beaker then 1.5 g of green tea sample was added and allowed to stand for 30 min. The infusion was filtered off the tea leaves and its concentration determined by evaporating 50 ml to dryness in a pre-weighed 50 ml beaker. The concentration of the infusion was 0.2 g/50 ml.

#### 2.2.2. Detection of phytochemical contents of tea infusions

The qualitative determination of phytochemical content of tea infusions was carried out using the methods of Amadi *et al.* (2004).

- **Saponins:** An aliquot of the infusion was pipetted into a test tube, shaken vigorously for 1 minute and the solution observed for stable froth.
- **Flavonoids:** One milliliter of infusion was added to 1.0 ml of 10.0% ferric chloride. The solution observed for formation of black precipitate.
- **Tannins:** Three drops of lead acetate solution were added to 2.0 ml of infusion and observed for black precipitate formation.
- **Alkaloids:** One milliliter of the infusion was treated with Meyer's Reagent (potassium, mercuric-iodide solution) and observed for cream coloured precipitate.
- **Phytosterol:** One milliliter of infusion was added to 2.0 ml of chloroform, and then 2.0 ml of sulphuric acid was added to the solution and observed for coloured ring formation between the infusion and chloroform layer.

#### 2.2.3. Feeding study

- The rat feeding study was conducted in accordance with the protocols approved by the Experimental Animal Ethics Committee of the Department of Biochemistry, Federal University of Technology, Owerri, and in accordance with international standard for laboratory animal use and care as captured in NIH (1985). They were randomly allotted according to their weights into 2 groups of 6 rats each. The rats were housed in groups in metabolic cages with provision for food and fluid troughs and maintained under the same conditions of dark and lights cycles (circadian rhythm) and an ambient room temperature of  $25.0^{\circ} \pm 2.0^{\circ} \text{C}$  They were acclimatized for 14 days with supply of pelletized poultry feed and water *ad libitum*.

After the period of acclimatization, Group A animals which served as the normal control were fed pelletized poultry feed and tap water as the only source of fluid. Rats in group B served as the test group, and were fed pelletized poultry feed, and 0.2 g/50 ml of green tea infusion as the only source of fluid. Faecal droppings for each group were collected on a

daily basis, fallen rat hairs were removed, the droppings were dried in a hot-air oven at 60° C, ground into powder and stored in air-tight containers for analyses. All treatments lasted for a total of 28 days. The remnant feed and water for each group were collected, measured and recorded each day and total daily amount of feed and fluid consumed per group were calculated by subtracting the leftovers from what were initially served.

Body weight gained was calculated as the difference between the final body weight of each rat and the initial body weight (g). Growth rate was determined as the ratio of the weight gained to number of days of the study. Feed conversion ratio (FCR) was calculated as the ratio of the feed consumed to body weight gained (Yi *et al.*, 2018).

#### 2.2.4. Analysis of faecal matter

The feed and pooled dried daily faecal matter were analysed for their proximate compositions (ash, water, available carbohydrate, protein, lipid and fibre) using the methods of AOAC (2000). Energy content (kcal/ 100 g sample) was determined as: available carbohydrate content  $\times$  4) + (lipid content  $\times$  9) + (protein content  $\times$  4).

#### 2.2.5. Quantity and percentage of nutrient digested or retained

The quantity of nutrient (ash, water, available carbohydrate, protein, lipid and fibre) digested or retained was calculated as the difference got when the nutrient's value in faeces was subtracted from the nutrient's value in feed. Whereas lower value of a nutrient in faeces compared with its value in feed meant digestion; its increase in faeces meant retention. The percentage of nutrient digested or retained was computed as the ratio of the quantity of nutrient digested or retained to the value in the feed, multiplied by 100; positive value = digestion, while negative value = retention/ not digested.

#### 2.2.6. Statistical Analysis

Datum per parameter was analyzed relative to the control using percentage coefficient of variation (% CV); got by multiplying the ratio of the standard deviation to the mean by 100. Values  $\geq$  5 % were considered statistically significantly different.

## 3. Results

### 3.1. Phytochemicals present in tea

Table 2 shows the phytochemicals that were present in the tea infusions. The table shows the presence of flavonoids, saponins, tannins, alkaloids and phytosterols in the tea infusions.

**Table 2** Phytochemicals Detected in Green Tea Infusion

Parameter	Green tea
Flavonoids	+
Saponins	+
Tannins	+
Alkaloids	+
Phytosterols	+

Key: + = Presence of phytochemicals

### 3.2. Performance characteristics

The effect of tea infusion on performance characteristics like body weight gain, growth rate, feed and fluid consumption and feed conversion ratio of the rats are shown in table 3. The table shows that animals fed the green tea infusion did not gain significantly more body weight or have higher growth rate when compared with the control rats but they consumed more feed. There was no difference between the feed conversion ratio (FCR) of the control rats and the test rats.

**Table 3** Effect of Green Tea Infusion on Performance Characteristics of the Rats

Group		Performance characteristics				
		Body weight gained (g)	Growth rate (g/day)	Feed consumed (g)	Fluid consumed (ml)	Feed conversion ratio
Water (control)		169.50	6.05	118.25	133.03	2.80
Green tea		183.10	6.53	131.86	21.28	2.90
	Mean	176.30	6.29	125.06	77.16	2.85
	SD	6.80	0.24	6.81	55.88	0.05
	% CV	3.79	3.81	5.44	72.41	1.75

SD = standard deviation; CV = coefficient of variation.

### 3.3. Faecal samples

The effects of the tea infusion on faecal proximate compositions are presented in Table 4.

Whereas the faeces of the control rats contained more water and available carbohydrate, those of the test rats contained more protein and lipid. There were no variations in their ash, fibre and energy contents.

**Table 4** Effect of Green Tea Infusion on Faecal Content of the Wistar Rats

Group		Parameter						
		Ash (%)	Moisture (%)	CHO (%)	Protein (%)	Lipid (%)	Fibre (%)	Energy content (kcal/100g sample)
Water (control)		5.00	12.70	8.16	10.50	2.40	61.24	96.24
Green tea		5.35	10.48	4.63	11.81	3.95	63.78	101.31
	Mean	5.18	11.59	6.40	11.16	3.18	62.51	98.78
	SD	0.18	1.11	1.77	0.66	0.78	1.27	2.54
	% CV	3.38	9.58	27.58	5.87	24.37	2.03	2.57

CHO = carbohydrate; SD = standard deviation; CV = coefficient of variation.

### 3.4. Effect of green tea on efficiency of digestion/accumulation of some nutrients

Tables 5(a-c) present the effect of the green tea infusion and control diet on the efficiency of digestion/retention of carbohydrates, proteins, lipids and fibre. Results indicate that the most digested nutrient was carbohydrate. Percentage digestion was in the order carbohydrates > lipids > protein. The most accumulated (undigested) nutrient was fibre.

**Table 5a** Efficiency of Digestion/Retention of Some Nutrients in the Control Rats

Nutrient	Feed (%)	Faeces (%)*	Amount digested/ retained (%)	Digestion efficiency/ retained (%)
CHO	43.62	8.16	35.46	81.29
Protein	12.69	10.50	2.19	17.26
Lipid	7.30	2.40	4.90	67.12
Fibre	19.99	61.24	-41.25	-206.35

\*Derived from Table 4 above; CHO = carbohydrate. Positive value = digestion. Negative value = retention/ not digested.

**Table 5b** Effect of Green Tea Infusion on Efficiency of Digestion/Retention of Some Nutrients

Nutrient	Feed (%)	Faeces (%)*	Amount digested/ retained (%)	Digestion efficiency/ retained (%)
CHO	43.62	4.63	38.99	89.39
Proteins	12.69	11.81	0.88	6.93
Lipids	7.30	3.95	3.35	45.80
Fibre	19.99	63.78	-43.79	-219.06

\*Values derived from Table 5a; CHO = carbohydrate. Positive value = digestion. Negative value = retention/ not digested.

**Table 5c** Digestion/Retention Efficiencies of the Control and Treated Rats\*

Nutrient	Control rats (%)	Treated rats (%)	Mean (%)	SD (%)	%CV
CHO	81.29	89.39	85.34	4.05	4.75
Protein	17.26	6.93	12.10	5.17	42.69
Lipids	67.12	45.80	56.46	10.66	18.88
Fibre	-206.35	-219.06	-212.71	-6.36	2.99

\*Values derived from Tables 5a and 5b; CHO = carbohydrate; SD = standard deviation; CV = coefficient of variation. Positive value = digestion. Negative value = retention/ not digested.

#### 4. Discussion

Phytochemicals are bioactive plant chemicals found in fruits, vegetables, grains and other plant food and herbal preparations. They may provide desirable health benefits and could reduce the risk of major chronic diseases when ingested (Prakash, 2020). Table 2 showed that the tea infusion contained phyto-compounds such as tannins, saponins, alkaloids, flavonoids and phytosterols. These phytochemical substances are usually present in plant foods and herbal medicines. Both flavonoids and phenolic components have been reported to be effective antioxidants, antibacterial, anti-cancer, cardio-protective and anti-inflammatory agents and could protect the skin from UV radiation (Giada, 2013). Phytosterols present in vegetables, nuts, fruits and seeds, suppress diseases and tumors in cell lines via initiation of apoptosis and concomitant arrest of cell growth in the GI phase of the cell cycle (Prakash, 2020).

Tea infusions can alter appetite (Carter and Drewnowski, 2012). There was an increase in appetite for food in the animals fed the green tea and a decreased appetite for fluid as shown in table 3. Animals in the test group did not gain more weight and did not have a higher feed conversion ratio. These animals consumed far less quantity of fluid (green tea infusion) than the control rats. However, they consumed more feed. The enhanced feed intake may have been due to sudden withdrawal from the fluid (green tea infusion) and a biochemical need to compensate for body water need by producing metabolic water. Metabolic water is naturally produced in the body as a byproduct of metabolism; when oxygen serves as the final recipient of protons in oxidative phosphorylation. The withdrawal from green tea infusion intake may have been due to the astringent tastes of teas occasioned by the presence of polyphenols. Polyphenols are also known to hinder the activity of digestive enzymes thereby helping to reduce the digestion of nutrients in food, thus lowering energy uptake and consequently loss of body weight (Boccellino and D'Angelo, 2020). Plant-based phenolic compounds suppress appetite either by slowing down secretion of appetite-stimulating hormones, modulating melanin-concentrating hormone receptors, and inactivation of appetite sensors (Geoffrey *et al.*, 2017).

The dietary fibre in faecal samples of treated animals did not vary with those in the faecal samples of the control rats (Table 4). The energy contents from protein (47.24 kcal/ 100 g sample) and lipid (35.55 kcal/ 100 g sample) of the feed for the rats treated with green tea were wasted via excretion. Pan *et al.* (2016) in their study reported that the polyphenols present in tea suppress the digestion and absorption of lipid and complex sugars in diets. There was no increased excretion of minerals (ash content) through faeces upon treatment with the green tea.

There is growing interest on whether tea infusion can contribute to weight loss and the effects of these infusions. From the results of the various parameters studied, the green tea infusion was found to contain phytochemicals. Ingestion of the green tea infusion did not affect digestion and assimilation; for instance, conversion of feed mass to body mass. It

did not affect the body weight gain of the rats. From the results, green tea cannot be said to be a strong weight loss agent. The non-appreciable gain in body weight in rats fed green tea infusion may be linked directly to increased loss of protein and lipid, and their energy content through the faeces; ostensibly triggered by the action of polyphenols present in the tea. These polyphenols may have inhibited the activities of proteases and lipases and inexplicably did not affect the activities of amylases which are responsible for the digestion of available carbohydrate.

Digestion of nutrients is not a hundred percent perfect process where all nutrients are completely digested and absorbed from the bowel as noticed with control rats. That was why undigested nutrients still remained in the faecal matter of both treated and untreated rats. The study showed that carbohydrates were the most digested, followed by lipids and proteins. The fibre content of the feed was rather undigested while the other nutrients were digested. This seemed to suggest that the fibre content was spared or could not be digested, while the carbohydrates, proteins and lipids were digested at different rates. The polyphenols in the tea may have inhibited the activities of proteases and lipases, and inexplicably did not affect the activities of digestive amylases and cellulase.

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## 5. Conclusion

The green tea infusion promoted the excretion of protein and lipid in treated animals.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors have no conflict of interest to disclose.

### *Statement of ethical approval*

The rat feeding study was conducted in accordance with the protocols approved by the Experimental Animal Ethics Committee of the Department of Biochemistry, Federal University of Technology, Owerri, and in accordance with international standard for laboratory animal use and care as captured in NIH (1985).

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