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Manuscript Evaluation Report- Referee 2

Manuscript ID: JJBS 126/R2/22 **Due date: Oct 29, 2022**

MS Title: **The Potential of Cashew Apple Waste as a Slimming Agent**

Type of Article: ☐ Review Article ☒ **Research Paper** ☐ Case Report

PART A:

On a scale of 1 – 5 (1 being lowest and 5 being highest), rate the manuscript based on the following criteria;

NO.	Criteria	Score
1	**Is the topic of the manuscript within the scope of the journal?**	5
2	**Does the title clearly and sufficiently reflect its content?**	5
3	**Are the keywords and abstracts sufficient and informative?**	5
4	**What is the scholarly quality of the manuscript?**	5
5	**Is this a new and/ or original contribution?**	4
6	**Is the research methodology utilized appropriate and properly administered?**	4
7	**Are the methods of data analysis acceptable?**	5
8	**Are the results and conclusions clear, adequately presented, and organized in relation to rest of manuscript?**	5
9	**Are the illustrations and tables necessary and in an acceptable format?**	5
10	**Are the interpretations/ conclusions sound and justified by the data?**	5
11	**Are the References in a proper format according to JJBS author Instructions?**	3
12	**Is the MS written in correct and satisfactory English?**	4

Please rate the priority for publication of this article (10 is the highest priority, 1 is the lowest priority)

9

PART B: Comments per Section of Manuscript:

Abstract	sufficient
Introduction	good
Methodology	good
Results	is in accordance with the methodology and what is being researched
Discussion and Conclusion	the discussion is quite good and the conclusion has concluded what was discussed
References	less thorough, there are things that need to be written in the references, but there are also things that are not in the script

PART C: Recommendation (Kindly Mark With An ✓)

Acceptable in its Present Form	
Acceptable with Minor Revision	√
Reconsidered after Major Revision	
Reject on Ground of (Please be Specific)	

PART D: Additional Comments:

Please add any other additional comments or specific suggestions on the enclosed comments sheet:

Researchers use cashew nut shells for feed where for this feed it is necessary to analyze the digestibility of protein and starch, as well as the calcium content in feces as well as body weight.

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Manuscript Evaluation Report- Referee 1

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MS Title: **The Potential of Cashew Apple Waste as a Slimming Agent**

Type of Article: ☐ Review Article ☒ **Research Paper** ☐ Case Report

PART A:

On a scale of 1 – 5 (1 being lowest and 5 being highest), rate the manuscript based on the following criteria;

NO.	Criteria	Score
1	**Is the topic of the manuscript within the scope of the journal?**	4
2	**Does the title clearly and sufficiently reflect its content?**	4
3	**Are the keywords and abstracts sufficient and informative?**	4
4	**What is the scholarly quality of the manuscript?**	3
5	**Is this a new and/ or original contribution?**	4
6	**Is the research methodology utilized appropriate and properly administered?**	3
7	**Are the methods of data analysis acceptable?**	2
8	**Are the results and conclusions clear, adequately presented, and organized in relation to rest of manuscript?**	2
9	**Are the illustrations and tables necessary and in an acceptable format?**	3
10	**Are the interpretations/ conclusions sound and justified by the data?**	3
11	**Are the References in a proper format according to JJBS author Instructions?**	4
12	**Is the MS written in correct and satisfactory English?**	4

Please rate the priority for publication of this article (10 is the highest priority, 1 is the lowest priority)

7

PART B: Comments per Section of Manuscript:

Abstract	-The research method used needs to be clearly described. This abstract only explains the variables observed in the study. Therefore, defining the method used for analyzing the observed data is advisable. -Correction the abbreviation of CAJC and CAJT on abstract and consistent with that abbreviation in text - How many percent of the tannins in cashew apple juice reduce the digestibility of protein and feed starch, increase the excretion of calcium in the feces, and suppress the rate of weight? That is the essential data to show in the abstract
Introduction	-The research problem has not been clearly defined -There is a statement that high tannin content in cashew fruit juice has the potential as a slimming agent. However, it is better to add the data of the research results on this matter because it is the basis for the treatment used in the study.
Methodology	- Why was the Cashew Apple Juice with a tannin content of 0.14 % mentioned as a control? - Add an explanation of the calculation of Average Intake Feed in methodology.
Results	-The study results for all observational variables are always compared with the placebo group treatment, and what is the function of the control treatment? If comparing the average treatment with a placebo, use Dunnett because LSD is used to compare the mean between treatments. The comparison test of the average treatment used is adjusted to the data analysis written in the methodology - The paragraph after Table 3 is the repetition of the statement after Table 2, and that statement did not become relevant to the data in Table 3 - Correction for calculating the percentage of weight gain in table 5. Percentage on CAJ. Control = 62.18 % instead of 38.27 %. So discussion after Table 5 is to be revised.
Discussion and Conclusion	Revise the discussion in Table 5, adjusted for the revised calculation results
References	

PART C: Recommendation (Kindly Mark With An ✓)

Acceptable in its Present Form	
Acceptable with Minor Revision	✓
Reconsidered after	

Major Revision	
Reject on Ground of (Please be Specific)	

PART D: Additional Comments:

Please add any other additional comments or specific suggestions on the enclosed comments sheet:

Suppose the results of data analysis in all tables are a comparison of the mean treatments using LSD. In that case, it is better if the table title does not use sentences compared to the placebo group.

Title: The Potential of Cashew Apple Waste as a Slimming Agent

Authors and Affiliations:

126/R2/22
Running Title:
The Consumption of Cashew Apple Juice and Its Nutritional Effects

Keywords:
Anacardium occidentale (L), Bioactive compound, Caju, Herbs, *In vivo* test, *Rattus norvegicus* (Berkenhout, 1769), Tannin, Waste to functional food

The Potential of Cashew Apple Waste as a Slimming Agent

ABSTRACT

The cashew apple (*Anacardium occidental* L.) is a tropical fruit that is a byproduct of the cashew nut processing industry. Rich in vitamins, polyphenols, sugars, minerals, amino acids, and dietary fiber, it contains bioactive compounds and several active components (ascorbic acid, anacardic acid, carotenoids, condensed tannins, quercetin, and other phenolic) that serve as antioxidants. This study aims to determine the effect of giving cashew apple juice and its nutritional impact on Wistar rats (*Rattus norvegicus* – Berkenhout 1769). The study adopted 21 male Wistar rats for 7 d fed with AIN 76 (American Institute of Nutrition 1976) diet, divided into three groups of seven rats. The first group was administered fresh cashew apple juice treatment 0.14 % (CAJT 0.14 %), the second group with cashew apple juice 0.12 % (CAJT 0.12 %), and the last group with aqua

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dest (Placebo) per day orally for 28 d. Employing an experimental method, variables of protein using Kjeldahl method, starch using the direct acid hydrolysis method, and calcium levels in the feces of experimental rats were observed. The data was obtained through analysis of variance, and differences among samples were tested using Least Significant Different (LSD). The results showed that the cashew apple juice treatment 0.14 % significantly affected protein digestibility and starch feed, calcium excretion in feces, and the total weight of experimental rats than cashew apple juice treatment 0.12 % and Placebo treatments. Tannins in cashew apple juice treatment 0.14 % (CAJT 0.14 %) have a solid presence to reduce the digestibility protein in the feces (11.49 %), feed starch in feces (0.69 %), and increase the excretion of calcium in the feces (0.44 %), and suppress the rate of weight 38.34 % (78.85 g), followed by cashew apple juice treatment 0.12 % (CAJT 0.12 %) with the digestibility protein in the feces (11.36 %), feed starch in feces (0.68%), and increase the excretion of calcium in the feces (0.44 %), and suppress the rate of weight 38.64 % (88.74 g) and the placebo treatment does not have affect digestibility of variable research. The effect of consuming cashew apple juice regarding nutrient digestibility shows its potential for a commercial process as a functional food and a slimming agent, which answers the environmental need for waste utilization.

Keywords: *Anacardium occidentale* (L.), Bioactive compound, Caju, Functional food, *In vivo* test, *Rattus norvegicus* (Berkenhout, 1769), Tannin, Utilization waste.

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1. Introduction

A tropical plant commonly found at an altitude of about 1 000 m above sea level, cashew tree or *caju* (*Anacardium occidental* L.) can grow well on various types of soil, even the dry ones with poor nutrients (Runjala and Kella, 2017). The fruit produced by this plant consists of two edible parts: cashew nut and cashew apple – while the first is its actual fruit, the latter is pseudo fruit formed from an enlarged fruit stalk (Balandrán-Quintana *et al.*, 2019). Oliveira *et al.* (2020) have highlighted that cashew nut, the main commodity of the plant, represents only 10 % of the total fruit weight. So far, cashew apple has only been used as animal feed if not disposed of as waste (Aidoo *et al*., 2022). Several researchers (Gadikar *et al*., 2021; Prabhudessai *et al*., 2013; Setyobudi

et al., 2021a) suggest using this waste as feedstock for biogas. However, considering the low pH, a two-stage digester technology is recommended (Abdullah *et al*., 2020; Hendroko *et al*., 2013).

Cashew apple contains water (83.6 g 100 g^{-1}) and nutritional substances such as vitamin C (126 mg 100 g^{-1} to 372 mg 100 g^{-1}), which is 6 to 7 times higher than in citrus fruits, dietary fiber (312 mg), carbohydrates (11.1 g), and calcium (0.9 mg 100 g^{-1} to 21.4 mg 100 g^{-1}) (Damasceno *et al.*, 2008; Bhakyaraj and Singaravad, 2012), cashew apple should be able to serve as a good source of energy (Cristina *et al*., 2012; Honorato *et al*., 2007). Rich in bioactive compounds of polyphenols (gallic acid, protocatechuic acid, cryptoxanthin, zeinoxanthin, and lutein 214.8 mg 100 mL^{-1} to 215.1 mg 100 mL^{-1}) and organic acids (malic, citric, and lactic acids 0.1 g 100 g^{-1} to 0.36 g 100 g^{-1} (Sucupira *et al*., 2020), tannins 0.22 g 100 g^{-1} to 0.58 g 100 g^{-1} (Sobhana and Mathew, 2015), carotene 0.03 mg 100 g^{-1} to 0.74 mg 100 g^{-1} (Lopes *et al*., 2012), anacardic acid 1.1 g (Nambelaa *et al.*, 2022), the fruit is therefore packed with antioxidants (Andayanie *et al*., 2019; Laddha *et al*.,2020). Specifically, chemically active components of ascorbic acid, anacardic acid, carotenoids, condensed tannins, quercetin, and other phenolic compounds are essential in anti-mutagenic mechanism (Onuh *et al.*, 2017; Setyobudi *et al*. 2019) with ability to stimulate DNA repair or reverse DNA damage. The details above should prove that cashew apple is nutritious and healthy to consume (Tai *et al*., 2020).

The other positive impact of consuming cashew apple juice is that it can reduce the concentration of total cholesterol, LDL triglycerides T6, and increase the concentration of HDL (cholesterol in the blood) (Asmawati *et al.*, 2021; Carvalho *et al*., 2018). Several previous researches also reported the presence of tannin, which is known for its capacity to form insoluble complexes with macromolecules (proteins, fats, and carbohydrates) as well as micro-components (vitamins and minerals) to decrease availability and bioavailability (Emmanuelle *et al*., 2016; Setyobudi *et al*., 2021b and 2022; Soltan *et al.*, 2013), Total tannin (hydrolysable) in cashew is about 0.64 mg 100 g^{-1} while condensed tannin is about 0.18 mg 100 g^{-1}. Tannins content are common known for protein binding and leather-forming activities. Apart from the ability to precipitate protein, tannin equally decreases digestibility and palatability (Aliyu and Hammed, 2008; Dabonne *et al*., 2015). High concentrations of these compounds were discovered in experimental animals' feces, consequently suppressing their growth and weight gain rates, which

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showed potential as slimming agents (Menci *et al.*, 2021). The above findings have become the bases of research on cashew apple juice's nutritional digestibility, aimed to see if it's possible to be a source of functional food serving as a slimming agent.

2. Materials and Methods

2.1 Materials

2.1.1 Cashew apple juice

The raw material of yellow-orange cashew apples was obtained from North Lombok, Indonesia. After sorting and washing, the fruit was blanched for 1 min to soften the texture and then extracted for its juice to serve as first treatment of cashew apple juice contains of tannin content was 0.14 % (CAJT 0.14 %). As for the other treatment, the fruit was soaked in a calcium hydroxide solution [Ca (OH_2) 3 %] for 15 min after being washed and blanched, then extracted for its juice with a tannin content of 0.12 % (CAJT 0.12 %).

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2.1.2 Experimental animal

The animals involved in the experiment were Wistar male rats (*Rattus* norvegicus – Berkenhout 1769), 6 wk old with an average body weight of 104 g ± 8 g, obtained from the Experimental Animal Development Unit (UPHP) of Universitas Gadjah Mada, Yogyakarta, Indonesia. The rats were fed under the standard feed set by the American Institute of Nutrition 1976 (AIN 76) made in the nutritional laboratory of the Faculty of Agricultural Technology of Universitas Gadjah Mada, Yogyakarta, Indonesia.

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2.2 Research Procedure

The research procedure has been approved by the Ethical Commission of the Faculty of Medicine of Al-Azhar Islamic University, West Nusa Tenggara, Indonesia (Number 29/EC/FK-06/UNIZAR/VIII/2020) and carried out consistent with the steps in Figure 1. First, the AIN 76 standard feed was allotted to determine the levels of protein, carbohydrates, calcium, and initial body weight of experimental rats before treatment administering. Then, 21 male Wistar rats were led to 7 d of adaptation, fed AIN 76, and distilled drinking water. Once the period was over, the rats were divided into three equal groups:

- **Group 1** was given Cashew Apple Juice Treatment 0.14 % (CAJT 0.14 %) with tannin content.
- **Group 2** was assigned to drinking Cashew Apple Juice Treatment 0.12 % (CAJT 0.12 %) with reduced tannin content.
- **Group 3** was given aqua dest (placebo group).

The aforementioned rats received cashew apple juice of control and treatment and aqua dest of 2 mL d^{-1} two times a day (morning and evening) by force-feeding while still fed with AIN 76 standard meal for 28 d (4 wk). In addition, all rat feces collected during the study (28 d) was analyzed for their protein, carbohydrate, and calcium levels to determine digestibility.

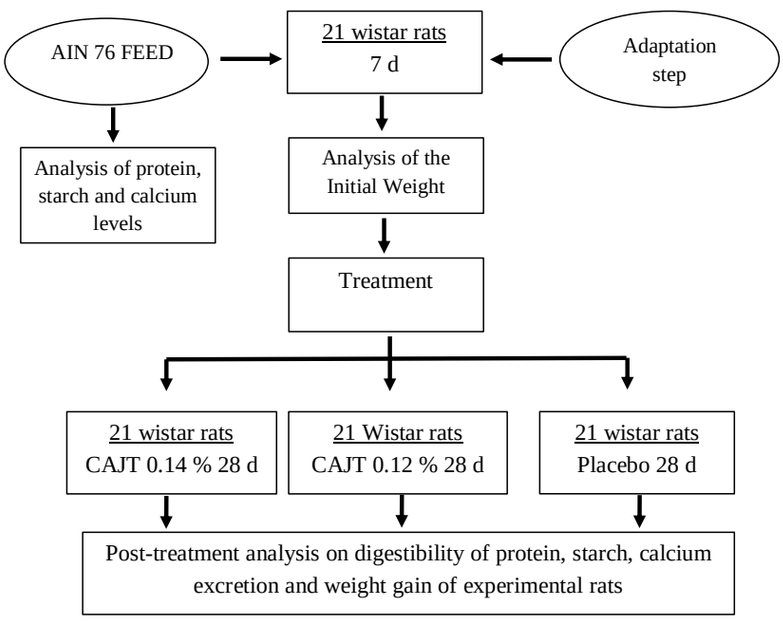

Figure 1. Scheme of the research

2.3 Measurement of research variables

2.3.1 Feed protein digestibility

Feed intake was weighed, and feces samples from the last 3 d (26 d to 28 d) were collected. To determine the effect of tannins on protein digestibility, 1 g of diet and feces samples were analyzed for nitrogen content using the micro Kjeldahl method (% N × 6.25). The 1 g of diet or feces sample

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was mixed with a digestion flask with 1.9 g ± 0.1 g potassium sulfate, 80 mg ± 10 mg mercuric oxide, and 2 mL H_2SO_4. Boiling chips were added to digest the sample and turn the solution colorless. Once cooled, the digest was diluted with distilled ammonia-free water before being transferred to the distillation apparatus. The tip of a 10 mL conical flask containing 5 mL boric acid solution and drops of an indicator variant was dipped into the solution, and 10 mL of sodium hydroxide-sodium thiosulphate solution was inserted. The ammonia produced by the boric acid was distilled and collected. The emulsion was then titrated until a violet color appeared at the tip of the condenser and rinsed before the titration process. Next, the reagent blank was run with an equal volume of aqua dest to one of the titration results. Finally, the titration result volume was subtracted from the sample's original volume. The results were calculated per Equation (1) (Kara *et al*., 2018; Mæhre *et al*., 2018).

$$\text{Protein digestibility (\%)} = \frac{\text{N intake} - \text{N feces} \times 100}{\text{N intake}} \quad (1)$$

2.3.2 Feed starch digestibility

Feed intake was weighed, and feces samples from the last 3 d (26 d to 28 d) were collected. To determine the effect of tannins on starch digestibility, diet and feces samples were analyzed for starch content using the direct acid hydrolysis method (Kim *et al*., 2012; Kumar *et al*., 2022). Amount of 15 g dry basis of starch was added in a mixture of sulphuric acid and water (100 mL, 3.16 M), stirred, and left at a temperature of 35 °C for different lengths of time (0 d to 15 d). The solution was then cooled to 5 °C to recover non-hydrolyzed materials and centrifuged (6 000 *x g*) for 15 min. The precipitates formed during the process were rinsed with aqua dest to reach pH 7 (neutral), and the solid products were air-dried at 35 °C for 24 h before being stored in a sealed glass container at 4 °C. The hydrolysis recorded in the form of percentages were of suspended solids and dissolved non-hydrolyzed starch relative to the original starch solids, of which results were calculated as per Equation (2):

$$\text{Digestibility of starch (\%)} = \frac{\text{Starch intake} - \text{starch stool} \times 100}{\text{Starch intake}} \quad (2)$$

2.3.3 Calcium content

Calcium contained in diet and feces was determined quantitatively. 0.5 g of sample and 25 mL of 6 M HCl were mixed in a 250 mL beaker and boiled for approximately 30 men to make a 5 mL reduction. An amount of 5 mL of hot deionized water was added and boiled further, then filtered in a 50 mL volumetric flask. Deionized water was then mixed in to reach a 50 mL end solution. Ca content was analyzed using Buck Scientific 210VGP – Atomic Absorption Spectrophotometric (USA) at a wavelength of 422.2 nm (Nehad *et al.*, 2018). This study's chemicals and reagents are classified as an analytical grade, purchased from Sigma Aldrich Chemical Co. (St Louis, Mo, USA).

2.4 Body Weight and Food Intake

All rats were feed one times a day (morning), Each rat was provided with approximately 15 g fresh fed with AIN 76 standard meal for 28 d (4 wk) using feeder jar to preventing it from being title or dislodged. Approximately 1 g to 2 g food remained when the feeder jar was removed and another jar provided at the same time on the subsequent day. Providing the same amount for each day. During this period body weight was measured every other day (Wolden *et al*., 2000; Serrano *et al*., 2017).

2.5 Data analysis

This research was conducted in proportion to the experimental method, and the plan was of completely randomized design. The data was obtained through analysis of variance, and differences among samples were tested using Least Significant Different (LSD) with the significance level set at $P < 0.05$ (Adinurani, 2016).

3. Results and Discussion

The overall results came out positive, meaning that cashew apple juice significantly affected the digestibility of protein and carbohydrates, absorption of calcium, and weight gain of experimental rats.

3.1 Protein digestibility

Table 1 shows how protein digestibility in experimental rats has decreased significantly after drinking cashew apple juice ~~administratio~~n for 28 d.

Table 1. Protein digestibility of experimental rat with cashew apple juice

Treatment	Protein Digestibility Feed (%)	Protein Content Feces (%)
CAJT 0.14 %	33.05 b	11.49 a
CAJT 0.12 %	33.80 b	11.36 a
Placebo	52.39 a	8.17 b

Note: Numbers followed by the same letter in the same column are not significantly different at 5 %

CAJT 0.14 %: Pure Cashew Apple Juice

CAJT 0.12 %: Cashew Apple Juice with tannin reduction

Placebo: aqua dest

Feed's low protein digestibility rates were associated with high fecal protein contents. Cashew apple juice consumption has significantly increased the protein (nitrogen) contents in rat feces per trial due to the presence of tannins (CAJT 0.14 % and CAJT 0.12 %). An insoluble complex formed between protein and tannin inhibits protein digestibility (Osman and Gassem, 2013) and prevent the enzyme from breaking down, resulting in increased nitrogen removal through feces. This statement is compatible with the result of research by Kara *et al.* (2018).

3.2 Starch digestibility

Table 2 demonstrates how carbohydrate digestibility in experimental rats has decreased significantly after drinking cashew apple juice for 28 d. Yet, it is not as high as protein digestibility.

Table 2. Carbohydrate (starch) digestibility of experimental rat with cashew apple juice

Treatment	Carbohydrate digestibility Feed (%)	Carbohydrate content (starch) Feces (%)
CAJT 0.14 %	97.02 b	0.69 a
CAJT 0.12 %	97.05 b	0.68 a

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Placebo	97.31 a	0.62 b

Note: Numbers followed by the same letter in the same column are not significantly different at 5 %

CAJT 0.14 %: Pure Cashew Apple Juice

CAJT 0.12 %: Cashew Apple Juice with tannin reduction

Placebo: aqua dest

The significant increases in stool starch in Group 1 and Group 2 are evident in low carbohydrate digestibility rates. That tannic acid essentially inhibits glucose absorption in experimental rats' intestines, thus increasing their fecal starch levels, was compatible with the results of research conducted by Amoako and Awika (2016) and reported by Saha *et al.* (2018) that tannic acid and catechins could be associated with starch, resulting in decreased digestibility *in vitro.*

3.3. Calcium excretion

Table 3 records the significant increase of fecal calcium levels in experimental rats after drinking cashew apple juice for 28 d.

Table 3. Calcium excretion of experimental rat with cashew apple juice

Treatment	Ca cashew apple juice (%)	Ca content feces (%)
CAJT 0.14 %	0.23	0.44 a
CAJT 0.12 %	0.21	0.43 a
Placebo	-	0.39 b

Note: Numbers followed by the same letter in the same column are not significantly different at 5 %

CAJT 0.14 %: Pure Cashew Apple Juice

CAJT 0.12 %: Cashew Apple Juice with tannin reduction

Placebo: aqua dest

The high levels of calcium in the feces of Group 1 and Group 2 are evident that tannins can bind to calcium to form insoluble calcium-tanate; since the intestinal absorption of experimental

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rats was unable to absorb it, it was then excreted along with feces. This statement complies with Addisu (2016) that tannic acid could reduce the speed across the intestine due to the anti-nutritional inhibition of tannins. Further, the high loss of calcium through feces was due to decreased calcium absorption in the intestine triggered by the formation of complexes with tannins. This goes along with the assertion of Amalraj and Pius (2015) that calcium is very sensitive to even a tiny amount of tannins in feed or drink.

3.4 Rat weight gain

Table 4 logs the changes and percentages of experimental rats' weight gain after drinking cashew apple juice for 28 d.

Table 4. Weight gain of experimental rats with cashew apple juice

Treatment	Weekly weight gain (g)				
	0	I	II	III	IV
CAJT 0.14 %	126.81	142.37	166.47	186.10	205.66
CAJT 0.12 %	141.03	162.01	189.24	210.09	229.77
Placebo	112.14	133.17	164.69	187.74	213.63

CAJT 0.14 %: Pure Cashew Apple Juice

CAJT 0.12 %: Cashew Apple Juice with tannin reduction

Placebo: aqua dest

The result explains that the rats with cashew apple juice – both control and treatment – have lower weight gain rates than the placebo group. Focusing on the groups administered with cashew apple juice of different tannin contents, it is perceptible that the control group receiving more tannin has gained less weight than the treatment group. The ability of tannins to form insoluble complexes with protein (Table 1) and carbohydrates (Table 2) resulted in lower weight gain in rats. Details on weight gain percentage are revealed further in Table 5 below

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Table 5. Weight gain percentage of experimental rats with cashew apple juice

Treatment	Weight Gain		Average Intake
	%	g d^{-1}	Feed (g d^{-1})
CAJT 0.14 %	62.18 b	2.82 b	11.00 b
CAJT 0.12 %	62.92 b	3.17 ab	11.41 ab
Placebo	90.50 a	3.62 a	11.74 a

Note: Numbers followed by the same letter in the same column are not significantly different at 5 %

CAJT 0.14 %: Pure Cashew Apple Juice

CAJT 0.12 %: Cashew Apple Juice with tannin reduction

Placebo: aqua dest

The lowest weight gain occurred in the group treated with CAJT 0.14 % cashew apple juice at 62.18 % (78.85 g), followed by the group with CAJT 0.12 % at 62.92 % (88.74 g). In comparison, the placebo rat group was 90.50 % (101.49 g) and significantly different ($P < 0.05$). This corresponds to the low digestibility of protein (Table 2) and starch (Table 2) as a result of the occurrence of complexes with anti-nutritional compounds that can bind protein and carbohydrates and reduce the activity of digestive enzymes, causing the rat's body weight gain to be below optimal during the trial. This phenomenon is in line with the study of Rivera-Méndez *et al.* (2017), stating that tannins could affect experimental rats' growth and weight gain (Nwaneri *et al.*, 2016).

The rats' feed intake was affected by the rats' weight gain, which was lower on average at 11.00 g (CAJT 0.14 %) and 11.41 g (CAJT 0.12 %), while the placebo rat group was higher at 11.74 g. The low feed intake of the experimental rats is thought to be due to the astringent taste in the cashew juice that affects the experimental rats' appetite.

4. Conclusion

The tannin content in cashew apple juice can cut off protein and starch digestibility, increase calcium excretion, and suppress the rate of weight gain. Therefore, the results confirm cashew

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apple's potential to be a slimming agent. How cashew apple juice diminishes appetite and how to develop cashew apples from waste to functional food can be subjects for further research.

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