

# Influence of Dietary Avocado on Gut Health in Rats

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**Abstract** This study investigated the impact of diets containing various levels of avocado (5, 10 and 15%) on gut health in rats fed for six weeks. Avocado-fed rats had significantly higher food intakes while their body weights remained similar to the control diet-fed rats. No significant changes in intestinal bacterial populations (ileum, cecum and colon) were found in rats fed avocado diets compared to the control diet. Ileum and colon tissues of rats fed avocado diets showed significantly higher expression of genes ( $\beta$ -defensin 1, mucin 3 or mucin 4) and a greater number of mucin-producing goblet cells in the colon. The percentage of avocado in the diet had varying effects in altering the biomarkers, whereby diet containing 15% avocado was the more effective diet. This study delivers new knowledge on the role of avocado on gut health in rats.

**Keywords**  $\beta$ -defensins · Gene expression · Goblet cells · Microbiota · Mucin · Plant-based foods

## Introduction

Avocado (*Persea americana*) is a native fruit of Central and South America and was first cultivated in Mexico in 500 BC.

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Avocado has several health benefits including cardiovascular health, anti-inflammatory effects and blood glucose regulation. Avocados are a medium energy dense fruit rich in vitamins, minerals and dietary fiber. Half an avocado (68 g) provides approximately 5 g of dietary fiber consisting of 70% insoluble and 30% soluble fiber [1]. Dietary fiber exerts several health benefits, one of them is enteric bacterial fermentation of fiber and the production of short-chain fatty acids (SCFAs) [2]. Avocados also have a diverse fatty acid profile with high percentages of monounsaturated fatty acids (MUFAs), saturated and polyunsaturated fatty acids. These dietary fats and fibers in avocados can influence the gut ecosystem as it is well documented that macronutrients alter gut microbiota composition and metabolic activity [3]. Currently, no reported studies have examined the influence of avocado on gut health, particularly in relation to the gut microbiota and gut health biomarkers. Gut health research is rapidly evolving with food alternatives being sought to alleviate disease symptoms and promote health. In the present study, we aim to investigate the influence of diets supplemented with avocado on intestinal bacterial populations and fermentation end-products, gene expression and colon morphology in healthy rats.

## Materials and Methods

Avocados (*P. americana* var. Hass) at edible ripeness were purchased from a commercial supplier in Palmerston North, New Zealand, and the flesh was removed, mashed and kept frozen until added to the diet. The nutrient profile of Avocado is available in The Concise New Zealand Food Composition Tables [4]. Animal procedures were approved by AgResearch Grasslands Animal Ethics Committee (Palmerston North) according to the Animal Welfare Act 1999, New Zealand. Male Sprague-Dawley rats (4 weeks old) were housed in individual

cages and fed the experimental diets: control diet and diet containing avocado at 5, 10 or 15% for 6 weeks ( $n = 10/\text{diet}$ ; Table 1). Rats were given *ad libitum* access to food and water. After 6 weeks, rats were euthanized by  $\text{CO}_2$  asphyxiation and digesta from the ileum, cecum and colon were removed for microbiota quantification and SCFAs analysis. Ileum and colon tissues were excised for gene expression analysis and histological examination. See supplementary text for additional information on the methodology. All the data were analysed using one-way analysis of variance followed by *post-hoc* analysis by Duncan's multiple range test (GenStat 14th edition, VSN International, UK). A value of  $P < 0.05$  was considered statistically significant.

## Results and Discussion

Avocado fed rats consumed significantly more food, but their body weights remained similar to those rats fed the control diet (Table 2). There were no significant differences in ileal,

**Table 1** Ingredient compositions of experimental diets (g/kg)

	Control	Avocado %		
		5	10	15
Lactic casein <sup>a</sup>	120	120	120	120
Vitamin mixture <sup>b</sup>	50	50	50	50
Mineral mixture <sup>c</sup>	50	50	50	50
Corn oil <sup>d</sup>	100	86.7	73.4	60.1
Starch <sup>e</sup>	580	545.7	511.4	477.1
Sucrose <sup>f</sup>	50	50	50	50
Cellulose <sup>g</sup>	50	47.6	45.2	42.8
Avocado <sup>h</sup>		50	100	150
Energy (kcal/kg)	4063.69	3914.14	3764.58	3615.03

<sup>a</sup> Acid casein, New Zealand Milk Products Ltd., Wellington, New Zealand

<sup>b</sup> Mixture contains the following components: (mg/kg diet) – retinol acetate 5, DL- $\alpha$ -tocopheryl acetate 100, menadione 3, thiamin hydrochloride 5, riboflavin 7, pyridoxine hydrochloride 8, D-pantothenic acid 20, folic acid 2, nicotinic acid 20, D-biotin 1, myo-inositol 200, choline chloride 1500; ( $\mu\text{g/kg}$  diet) – ergocalciferol 25, cyanocobalamin 50

<sup>c</sup> Mixture contains the following components: (g/kg diet) – Ca 6.29, Cl 7.79, Mg 1.06, P 4.86, K 5.24, Na 1.97; (mg/kg diet) – Cr 1.97, Cu 10.7, Fe 424, Mn 78, Zn 48.2; ( $\mu\text{g/kg}$  diet) – Co 29, I 151, Mo 152, Se 151

<sup>d</sup> Tradewinds, Davis Trading, Palmerston North, New Zealand

<sup>e</sup> Wheaten cornflour, Golden Harvest, Primary Foods Ltd., Auckland, New Zealand

<sup>f</sup> Caster sugar, Chelsea, New Zealand Sugar Company Ltd., Auckland, New Zealand

<sup>g</sup> Ceolus PH-102, Asahi Kasei Chemicals Corporation, Tokyo, Japan

<sup>h</sup> *Persea americana* var. Hass

**Table 2** Food intake, body weight and gut health parameters of rats fed experimental diets

	Control	Avocado %			SEM	P values
		5	10	15		
Food intake (g)	111.1 <sup>a</sup>	123.0 <sup>b</sup>	123.8 <sup>b</sup>	127.2 <sup>b</sup>	3.28	0.008
Body weight (g)	316.4	348.7	337.0	336.6	8.80	0.091
Cecal short-chain fatty acids ( $\mu\text{mol/g}$ of digesta)						
Acetic acid	24.91 <sup>a</sup>	40.30 <sup>b</sup>	32.33 <sup>ab</sup>	39.06 <sup>b</sup>	3.21	0.006
Butyric acid	6.21	8.46	6.40	7.05	0.66	0.088
Propionic acid	6.38	9.05	7.64	7.58	0.67	0.062
Ileal gene expression (relative to reference genes)						
$\beta$ -defensin 1	1.16 <sup>a</sup>	2.56 <sup>b</sup>	2.49 <sup>b</sup>	0.98 <sup>a</sup>	0.46	0.029
Mucin 4	1.04 <sup>ab</sup>	1.51 <sup>b</sup>	1.70 <sup>b</sup>	0.64 <sup>a</sup>	0.24	0.013
Colonic gene expression (relative to reference genes)						
Mucin 3	1.42 <sup>ab</sup>	0.95 <sup>a</sup>	0.67 <sup>a</sup>	2.61 <sup>b</sup>	0.46	0.028
Mucin 4	1.20 <sup>ab</sup>	0.91 <sup>a</sup>	0.96 <sup>a</sup>	1.46 <sup>b</sup>	0.14	0.026
Colon morphology						
Goblet cells/crypt	19.16 <sup>a</sup>	19.88 <sup>a</sup>	22.05 <sup>ab</sup>	23.48 <sup>b</sup>	1.09	0.032
Crypt depth ( $\mu\text{m}$ )	183.7	185.0	192.9	198.9	11.46	0.765

Mean values with a different letter differ significantly. SEM – standard error of the mean. Expression of target genes was normalized to reference genes, glyceraldehyde 3-phosphate dehydrogenase and ribosomal protein L32

cecal and colonic bacterial populations between rats fed control and avocado diets (Table S1). The dietary fiber and large proportion of MUFAs in avocado had no effect on gut bacteria. A similar finding of MUFAs on gut bacterial populations was reported earlier [5]. Cecal acetic acid concentrations were significantly higher in rats fed 5 and 15% avocado (Table 2). Acetic acid is the most abundant SCFA produced by gut bacteria as a fermentation product or via the Wood-Ljungdahl pathway, and certain bacteria utilize acetic acid to produce butyric acid [6]. In general, rats fed avocado diets had higher concentrations of acetic, butyric or propionic acids in the gut (Tables 2 and S2). These SCFAs can facilitate intestinal epithelial cell expression of antimicrobial peptides called defensins, which have vital roles in host innate immune system [7, 8]. Rats fed 5 and 10% avocado diets had significantly higher expressions of  $\beta$ -defensin 1 gene in the ileum (Table 2). Among the avocado diets, colonic mucin 3 and 4 gene expression and the number of goblet cells (mucin-producing cells) were significantly higher in rats fed 15% avocado diet. Dietary fiber components increase the viscosity of the luminal contents as well as fecal bulk, which can contribute to mucus secretion [9]. This implies that avocados influence gut mucus secretion, and corroborates an earlier study in rats fed kiwifruit [10]. Ileal and colonic gene expression were similar between the diets as shown in Table S3.

## Conclusion

Avocado did not affect intestinal bacterial population numbers, but increased the acetic acid concentrations and expression of genes involved in gut barrier functions. The percentage of avocado in the diet influenced gut health measures with 15% dietary avocado being the most effective. This study provides new information on the effects of avocado on gut health biomarkers in using the rat as a model of the mammalian digestive tract.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare no conflict of interest.

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