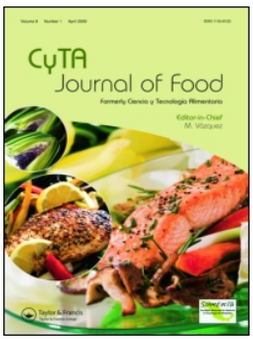

CyTA - Journal of Food

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tcyt20

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To cite this article: Koen Venema , Jessica Verhoeven , Ingrid S. Surono , Priyo Waspodo , Abraham Simatupang & Pratiwi D. Kusuma (2020) Differential glucose bioaccessibility from native and modified taro-starches in the absence or presence of beet juice, CyTA - Journal of Food, 18:1, 670-674, DOI: 10.1080/19476337.2020.1829073

To link to this article: https://doi.org/10.1080/19476337.2020.1829073

Published online: 20 Oct 2020.

CYTA - JOURNAL OF FOOD
2020, VOL. 18, NO. 1, 670–674
https://doi.org/10.1080/19476337.2020.1829073

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ARTICLE

Differential glucose bioaccessibility from native and modified taro-starches in the absence or presence of beet juice

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ABSTRACT

The speed of starch-digestion defines peak blood-glucose concentrations. Slow digestion is beneficial for diabetic individuals. To investigate the effects on blood-glucose it is important to be able to predict the amount of digestible starch. Therefore, the aim of this study was to compare the digestibility of different starches in a validated *in-vitro* model of the upper gastrointestinal (GI) tract. Digestion was performed in the TNO dynamic, computer-controlled *in-vitro* model of the upper GI-tract (TIM-1). Release of glucose was measured over time. Products tested were taro-flour, native-, and modified taro-starch. The latter two were also tested with beet-juice adsorbed. These were compared to wheat-flour and a glucose-solution. Modified taro-starch showed a similar glucose-bioaccessibility as wheat-flour (81–83%), while the other products tested had a lower bioaccessibility (60–75%). Adsorption of beet-juice affected digestibility of the modified taro-starch, but not the native-starch. Taro-based products can be used to lower plasma glucose concentrations in diabetic individuals.

ARTICLE HISTORY
Received 27 May 2020
Accepted 20 September 2020

KEYWORDS
Taro; starch; *in vitro* digestibility; *in vitro* GI tract model; plasma glucose concentrations; glycemic response; diabetes

Bioaccesibilidad de la glucosa diferencial obtenida del almidón de taro [malanga] nativo y modificado en ausencia o presencia de jugo de remolacha

RESUMEN

La velocidad de la digestión del almidón define las concentraciones máximas de glucosa en la sangre. La digestión lenta es beneficiosa para los individuos diabéticos. Para investigar los efectos del almidón digerible sobre la glucosa en sangre, es importante poder predecir la cantidad del mismo. Por esta razón, el objetivo de este estudio fue comparar la digestibilidad de diferentes almidones en un modelo *in vitro* validado del tracto gastrointestinal superior (GI). El proceso de digestión se realizó en un modelo *in vitro* dinámico y controlado por computadora del tracto gastrointestinal superior (TIM-1). En el estudio se midió la liberación de glucosa a lo largo del tiempo. Los productos analizados fueron harina de taro, almidón de taro nativo y modificado. Los dos últimos también se probaron con jugo de remolacha adsorbido. Estos fueron comparados con harina de trigo y una solución de glucosa. Se constató que el almidón de taro modificado tiene una bioaccesibilidad de glucosa similar a la de la harina de trigo (81-83%), mientras que los otros productos probados muestran menor bioaccesibilidad (60-75%). La adsorción del jugo de remolacha afectó la digestibilidad del almidón de taro modificado, más no la del almidón de taro nativo. Por lo tanto, se concluye que los productos elaborados a base de taro pueden utilizarse para reducir las concentraciones de glucosa en el plasma de las personas diabéticas.

PALABRAS CLAVE
taro; almidón; digestibilidad *in vitro*; modelo de tracto gastrointestinal *in vitro*; concentraciones plasmáticas de glucosa; respuesta glucémica; diabetes

1. Introduction

The digestion of food and absorption of the resulting digested nutrients are spatiotemporal and dynamic processes, which involve complex enzymatic systems in addition to specific transport reactions. To simulate all these biochemical and physiological events in a model is challenging. Nevertheless, several *in vitro* digestion models, from basic batch systems to sophisticated dynamic models, have been developed in order to (mechanistically) study digestibility of food (Fassler et al., 2006; Lebet et al., 1998; Minekus et al., 1995). Advantages of using *in vitro* instead of *in vivo* models are amongst others: low costs, no ethical constraints, and the possibility to compare different substrates under standardized conditions. The TNO (Dutch Organization for Applied Scientific Research) *in vitro* model of the stomach and small intestine (TIM-1; (Minekus et al., 1995)) is a dynamic, computer-controlled model that simulates the successive dynamic conditions occurring in the upper gastrointestinal (GI) tract during transit of food. It has been used extensively to study food digestion, including foods rich in starch (Fassler et al., 2006; Nalin et al., 2015). Combined with *in silico* modelling, the system has been validated (Bellmann et al., 2018; Fassler et al., 2006), and can be used to predict plasma glucose concentrations. The *in vitro* predictions showed a correlation coefficient for glucose incremental area under the curve $(iAUC)_{0-120}$ and glucose C_{max} of 0.89 and 0.94, respectively, after testing 22 different foods.

Frequent high blood glucose concentrations are associated with increased risk of metabolic diseases (Alam

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et al., 2014). Knowledge about the glycemic response after food intake is essential in relation to human health, particularly diabetes. Information about the postprandial glycemic response can be used to assess whether the intake of a carbohydrate product results in a low or high, and in a slow or rapid increase in the blood glucose concentration. Development of a product that is (s)lowly digestible is important, particularly for type II diabetic (T2D) patients, since the global incidence of T2D increases rapidly and is predicted to reach 360 million cases by the year 2030 (Wild et al., 2004).

Indonesia is rich in biodiversity, including a variety of local tubers which are still not widely utilized. This includes Cocoyam or taro, which belongs to the monocotyledonous family Araceae (the aroids), and which is an important ethnic root crop throughout Asia. Taro is used as an alternative starch source to reduce dependence on rice. Processing taro into flour can also be an alternative substitute for wheat flour which has been widely used but depends on imports. Furthermore, taro use increases efforts to diversify food and support food security. In addition to potentially lowering blood glucose concentrations, taro may also contain resistant starch, which reaches the colon and can contribute to the modulation of the composition and/or activity of gut microbiota (Fassler et al., 2006; Kovatcheva-Datchary et al., 2009), and through this increases human health, e.g., by increasing the production of butyrate (Rose et al., 2010).

Polyphenols have been suggested to be able to modify postprandial hyperglycaemia and prevent reactive hyperinsulinaemia (Ishikawa et al., 2007; Rahimi et al., 2005). The mechanism may be through inhibition of carbohydrate digestion, reducing glucose absorption, stimulation of insulin release, modulation of hepatic glucose secretion, activation of insulin receptors, or modulation of glucose uptake in insulin-sensitive cells (Rahimi et al., 2005). Beetroot juice has received attention because it contains a number of compounds including phenolic acids, flavonoids and betalains (Kujala et al., 2002), and beetroot juice has a high total antioxidant capacity and total polyphenol content (Wootton-Beard & Ryan, 2011).

The aim of the current experiments was to study starch digestion, which was determined in TIM-1 through the bioaccessibility (the amount available for absorption) of glucose. Taro-starch was tested in multiple forms, namely as taro-flour, native taro-starch, modified taro-starch, native taro-starch with beet juice adsorbed, and modified taro-starch with beet juice adsorbed. Glucose bioaccessibility was compared to wheat flour and a glucose solution (maximum bioaccessibility).

2. Materials and methods

2.1. Starch substrates

"HASILBUMIKU" taro-starch was purchased from a local supplier in Bantul, Yogyakarta (Indonesia). For modification, the method of Zhao and Lin (Zhao & Lin, 2009) was used, with slight modifications. In brief, taro starch was blended with distilled water (1:3.5 w/w), and then gelatinized using pressure-heating at 121°C for 30 minutes. Subsequently, the slurry was cooled at 4°C. Afterwards, the obtained retrograded starch was dried in an oven (60°C for 16 hours) and then allowed to cool to room temperature for 24 hours. Then the starch was grinded, and sieved using a 60 mesh. Beet juice was adsorbed to both native and modified taro starch by adsorbing beetroot juice, at a ratio of 1:1, and then drying in an oven at 40°C for16 hours. The reference substrate wheat flour (Jumbo basic) was purchased from Jumbo supermarket in Roermond (the Netherlands), and glucose was purchased from Merck (Darmstadt, Germany).

2.2. Dynamic in vitro model of the stomach and small intestine (TIM-1)

The dynamic model used in this study has been described in detail before for carbohydrate digestion experiments (Bussolo de Souza et al., 2018; Fassler et al., 2006; Nalin et al., 2015). In brief, the model is comprised of four serial compartments simulating the stomach, and the three parts of the small intestine: duodenum, jejunum, and ileum (Figure 1). The *in vitro* digestion was performed for 6 h at 37°C. A mixture of 20 g of test-product (glucose or one of the starches), electrolyte solution, water, pepsin (Merck, P7012), lipase (*Rhizopus* lipase, Amano Pharmaceutical Co. F-AP 15, Ltd. Japan), and gastric start-residue was introduced into the gastric compartment (for details see Bussolo de Souza et al., 2018; Fassler et al., 2006; Nalin et al., 2015). Mixing through peristalsis was accomplished by computer-controlled peristaltic valve pumps, which also controlled meal transit through the individual compartments. The pH values in the compartments were computer monitored by adding HCl (1 M; stomach) or $NaHCO_3$ (1 M; small intestinal compartments), respectively. In the stomach the values were preset to pH 5.5, 5.0, 4.2, 2.8, 2.1, 1.8 and 1.7 at 0, 10, 20, 40, 60, 90, and 120 min, respectively. The pH was maintained at 6.2, 6.5, and 7.4 in the duodenum, jejunum, and ileum compartments, respectively. Porcine bile (Merck B8631; 4 g/100 g in water) and pancreatic solution (Pancrex-V powder, Paines & Byrne, Greenford, UK; 7 g/100 g in water) were secreted into the duodenal compartment at 0.5 and 0.25 ml/min, respectively (Bussolo de Souza et al., 2018; Fassler et al., 2006; Nalin et al., 2015). Using hollow-fiber membranes with a cut-off 5–10 kDa, absorption of digestion products and water from the jejunal and ileal compartments was simulated. Samples were collected from the dialysates every hour for 6 hours. These samples mimic the bioavailable fractions that are normally absorbed in the body, and which can be compared with blood glucose (Bellmann et al., 2018). Ileal efflux (indigestible fraction) were also collected every hour. At the end of the experiments, the residue in the system was collected to be able to create a mass-balance. Because the model is computer-controlled and therefore highly reproducible, experiments were performed in duplicate.

2.3. Glucose analyses

Samples from the dialysates were quantitatively hydrolyzed to glucose with amyloglucosidase (AMG). The free glucose was subsequently determined enzymatically using the Hexokinase/Glucose-6-phosphatedehydrogenase assay as described before (Fassler, Arrigoni, Venema, Brouns et al., 2006). Starch and ileal efflux samples were also hydrolyzed, but using sulfuric acid as described before (Bussolo de Souza et al., 2018), after which the liberated glucose was measured as described above. These analyses were performed on a Cobas-Mira plus autoanalyser (Roche, Almere, The Netherlands) by Bio-aNAlytiX (Zoetermeer, The Netherlands).

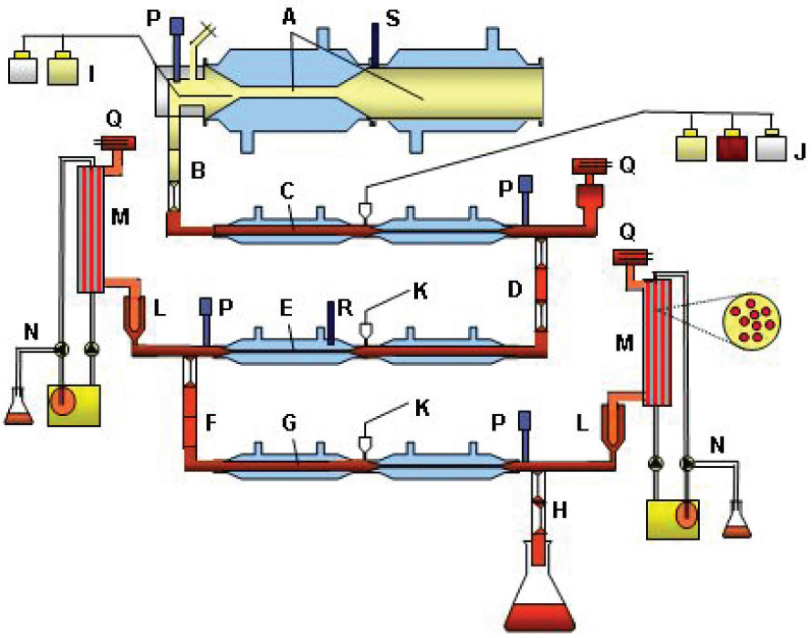

Figure 1. Schematic diagram of the dynamic, multi-compartmental TNO *in vitro* model of the stomach and small intestine (TIM-1). (a) stomach compartment; (b) pyloric sphincter; (c) duodenum compartment; (d) peristaltic valve; (e) jejunum compartment; (f) peristaltic valve; (g) ileum compartment; (h) ileo-caecal sphincter; (i) stomach secretion; (j) duodenum secretion; (k) jejunum/ileum secretion; (l) pre-filter; (m) semi-permeable membrane; (n) water absorption; (p) pH electrodes; (q) level sensors; (r) temperature sensor; (s) pressure sensor. Reprinted from Keller et al. (2017) with permission. (Keller et al., 2017).
Figura 1. Diagrama esquemático del modelo *in vitro* dinámico y multicompartimental de TNO del estómago y el intestino delgado (TIM-1). A. compartimento estomacal; B. esfínter pilórico; C. compartimento duodenal; D. válvula peristáltica; E. compartimento del yeyuno; F. válvula peristáltica; G. compartimento del íleon; H. esfínter ileocecal; I. secreción estomacal; J. secreción duodenal; K. secreción yeyuno/íleon; L. prefiltro; M. membrana semipermeable; N. absorción de agua; P. electrodos de pH; Q. sensores de nivel; R. sensor de temperatura; S. sensor de presión. Reimpreso de Keller et al. (2017) con permiso. (Keller et al., 2017).

3. Results

3.1. TIM-1 digestion experiments and glucose bioaccessibility

3.1.1. Glucose, wheat flour, and taro flour

The maximal glucose bioaccessibility, defined as the amount of glucose potentially available for uptake, was determined by using a glucose solution. When expressed as percentage of the amount of glucose ingested, cumulative glucose bioaccessibility in the model, which was calculated as the sum of the amount of glucose recovered in the jejunal and ileal dialysates in the 6 hours the experiment lasted, was 90% (Figure 2(a)), with 71% absorbed in the jejunum and 19% in the ileum (Figure 2(b)). Of the remaining 10%, 9% ended up in the ileal efflux, while 1% was found back in the residue after termination of the experiment. This is a typical absorption of glucose in the system (Bellmann et al., 2018). When wheat-flour (to which the taro-starches were benchmarked) was tested, total cumulative glucose bioaccessibility was 81% (Figure 2(a)), with 58% absorbed in the jejunum and 23% in the ileum (Figure 2(b)). Compared to free glucose, the absorption was "delayed": relatively more (28% (23%/81%)) of the absorbed glucose was absorbed in the ileum, compared to 21% (19%/90%) for free glucose (Table 1). Next, taro flour was examined. Glucose bioaccessibility reached 72% (Figure 2), of which 52% was absorbed in the jejunum and 19% in the ileum (Figure 2(b)). Similar to wheat flour, glucose absorption was delayed compared to glucose, with 27% absorbed in the ileum (Table 1). Since the total bioaccessibility (in percentage of intake) of taro flour was lower than for wheat flour, also the absolute amount of glucose absorption was lower (as the substrates were dosed at equivalent glucose amounts).

3.1.2. Native taro-starch (with and without beet juice)

Native, isolated taro-starch was tested as well. Compared to the flour, the isolated native starch showed a much slower and lower digestion. This was irrespective of the presence of adsorbed beet juice. In both cases, cumulative digestion was 60% (Figure 2(a)), with 22% and 20% absorbed in the ileum for native starch with and without beet juice, respectively (Figure 2(b)). The latter represents 38% and 33% of the total absorbed glucose (Table 1), indicating that digestion of the isolated starch is much delayed.

3.1.3. Modified taro-starch (with and without beet juice)

Native starch was also modified to change its characteristics and with the aim to slow digestion, according to Zhao and Lin (2009). However, while following the protocol of Zhao and Lin, our modified starch was digested much quicker and to a greater extent than the native starch (Figure 2(a)). Total cumulative glucose bioaccessibility was 83%. Adsorption of beet juice reduced this slightly to 76% (Figure 2(a)). Of this, 18% and 15% were absorbed in the ileum (Figure 2(b)), corresponding to 21.5% and 20% of the absorbed starch, respectively (Table 1), indicating that digestion was even quicker than for wheat flour, and absorption in the jejunum was also higher (Figure 2(a)).

4. Discussion and conclusion

Taro is an ethnic staple crop in Asia, although it is slowly being replaced by imported wheat. Here, we show that digestion of starch from taro, either as flour or as isolated starch, leads to a slower digestion and an overall lower digestion, which is beneficial for diabetic individuals and those with prediabetes, which also increases dramatically

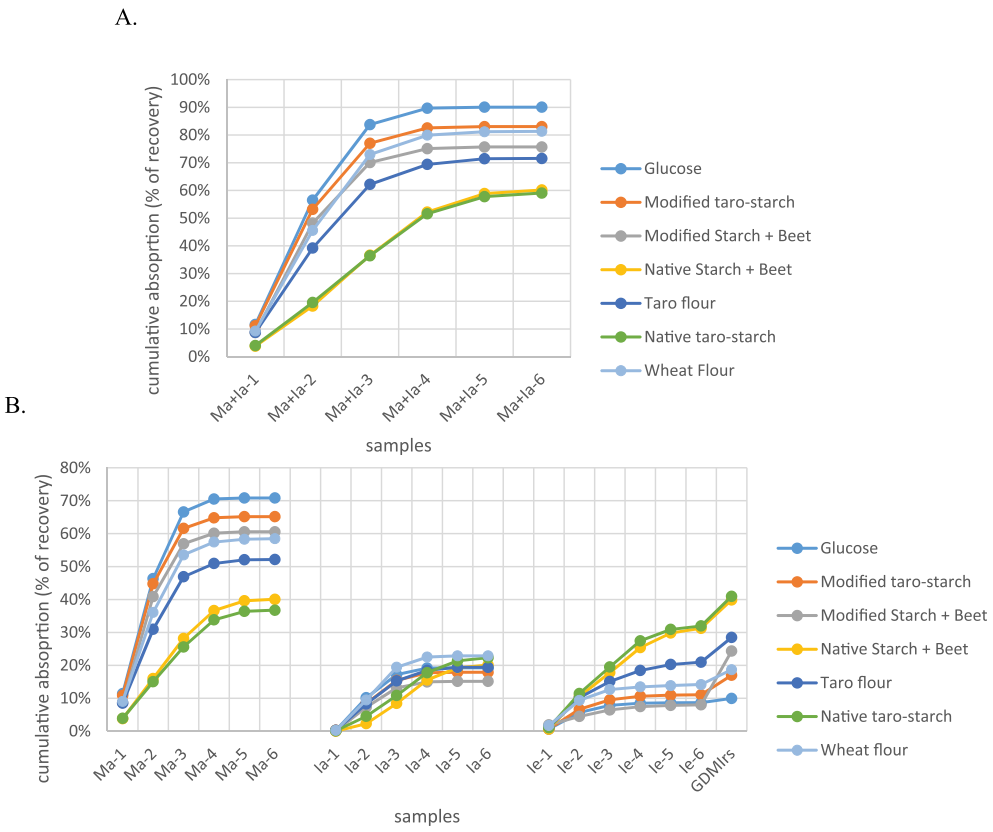

Figure 2. (a) Cumulative bioaccessibility of glucose in the summed jejunal (Ma) and ileal (Ia) dialysate fractions. (b) Cumulative glucose in the individual jejunal and ileal dialysates, and ileal efflux (Ie) and residue (GDMIrs) fractions.
Figura 2. A) Bioaccesibilidad acumulativa de la glucosa en las fracciones dializadas sumadas del yeyuno (Ma) e ileal (Ia). B) Glucosa acumulada en las fracciones dializadas individuales del yeyuno e ileal y de efluvio ileal (Ie) y residuo (GDMIrs).

Table 1. Total cumulative bioaccessibility (%) after the 6 hour experiment, and fraction of the total absorbed glucose that is absorbed in the ileum (% of total bioaccessibility).
Tabla 1. Bioaccesibilidad total acumulada (%) después del experimento de 6 horas y fracción de la glucosa total absorbida en el íleon (% de la bioaccesibilidad total).

Substrate	Total bio-accessibility (%)	Fraction of total bioaccessibility absorbed in the ileum
Glucose	90	21
Wheat flour	81	28
Taro flour	72	27
Native taro-starch	59	38
Native taro-starch with beet juice	60	33
Modified taro-starch	83	21.5
Modified taro-starch with beet juice	76	20

in prevalence in Asia. Since the TIM-system can be used to predict blood glucose concentrations with high accuracy (Bellmann et al., 2018), these results can be translated to the *in vivo* situation, and hence we predict that taro-derived starch will lead to a lower glycemic response than wheat flour-derived starch. Modification of the starch according to an earlier published protocol did not lead to an even slower digestion; in fact, digestion was increased. It is unclear why the protocol does not function in our hands, but our results are reproducible (data not shown).

Adsorption of beet juice to the taro-starch had no effect on native starch digestion, and only affected modified starch digestion to a limited degree. Therefore, combining native taro-starch with the polyphenols present in beet juice may provide additional benefit for diabetic individuals, as the polyphenols have been shown to have health benefits of their own (Ishikawa et al., 2007).

Reduction of glucose peaks as would be accomplished by taken isolated native starch would lead to a better insulin response and lower glycated HbA_1c. Individuals with T2D would greatly benefit from this. Apart from reducing the blood glucose concentrations, a lower overall digestibility also means that more resistant starch reaches the large intestine, where it can be fermented by the gut microbiota, which leads to increased production of the beneficial short-chain fatty acids (SCFA) (Hamer et al., 2008). In a preclinical rodent model, we have shown that feeding taro-starch leads to the modulation of the gut microbiota (Surono et al., manuscript under review), which may be a second, independent mechanism that may affect diabetes, as the microbiota has been shown to play a role in metabolic syndrome (Karlsson et al., 2013; Lynch et al., 2016; Qin et al., 2012; Shapiro et al., 2017).

Therefore, replacing rice or wheat-based starch-containing products with taro-based starch-containing products may be very beneficial for diabetic and prediabetic individuals. We are currently undertaking a cross-over clinical trial in 15 (pre) diabetic individuals to translate these interesting *in vitro* results to clinical practice.

Disclosure statement

The authors declare that they have no conflict of interests.

Funding

The study was funded through a grant from Insentif Riset Sistem Inovasi Nasional (INSINAS), Indonesian Ministry of Research, Technology and Higher Education, 2019; [grant number 20/INS-1/PPK/E4/2019]. This research has been made possible with the support of the Dutch Province of Limburg with a grant to [Centre for Healthy Eating & Food Innovation (HEFI)] of Maastricht University – campus Venlo.

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Dalam rangka melaksanakan Tridharma Perguruan Tinggi dan meningkatkan kualitas staf pengajar, maka dengan ini Dekan Fakultas Kedokteran Universitas Kristen Indonesia menugaskan staf pengajar tersebut di bawah ini :

Dr. Pratiwi Dyah Kusumo, S.Si., M.Biomed

Untuk melakukan penelitian dengan judul "*Differential Glucose Bioaccessibility From Native And Modified Taro-Starches In The Absence Or Presence Of Beet Juice*"

Demikian surat tugas ini diberikan kepada yang bersangkutan agar kiranya dapat dilaksanakan dengan sebaik-baiknya.

Jakarta, 8 September 2020
Dekan,

Dr. dr. Robert Hotman Sirait, Sp.An
NIP. UKI 031 545

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