P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.033

Evaluation of Sonia (BMR Mutant Sorghum and *Tithonia diversifolia*) Usage as Sustainable Alternative Feed to Reduce Concentrate Dependency in Ruminant Diet: *In Vitro* Study

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Article History: 25-011 Received: 15-Jan-25 Revised: 17-Mar-25 Accepted: 20-Mar-25 Online First: 29-Mar-25

ABSTRACT

This study aimed to evaluate the potential of Sonia, a novel feed blend comprising BMR mutant sorghum and *Tithonia diversifolia*, as a sustainable alternative to conventional concentrates in ruminant diets. A Randomized Block Design (RBD) was employed in this research, incorporating four different treatment levels: 60% Sonia+40% concentrate, 70% Sonia+30% concentrate, 80% Sonia+20% concentrate, and 90% Sonia+10% concentrate. The variable determined in the present study were, hence, acid detergent fibre (ADF), neutral detergent fibre (NDF), hemicellulose, crude fibre, cellulose, nitrogen-free extract (NFE), crude fat, and total gas production, microbial biomass. In the present study the findings revealed that percentage variation in the proportion of sonia did not reflect the following main nutritional parameters. From the results of the study, the fairly steady digestibility and fermentation values obtained in the various treatments suggest that Sonia can potentially be a cheap source of feed. Crude fiber, crude fat, IVNFED, IVCLD and IVHLD digestibility varies between 60.30-63.72% suggesting lower digestibility as the content increases, While IVNDFD and IVADFD levels of digestibility fall between 53.65-59.01%. Nevertheless, the total gas production and microbial biomass were also unaffected by treatments. This research can relate Sonia's ability to improve the sustainability and effectiveness of feed in ruminant production systems. The application of Sonia can help reduce demand for traditional animal concentrates without reducing the quality of animal feed.

Key words: Digestibility, Fermentation rumen, Microbial biomass, Sorghum, Tithonia, Total gas.

INTRODUCTION

Ruminant livestock consuming forages productivity is highly dependent on the quality forages, and in Indonesia this is affected by seasons. Forage production can therefore be a challenge in the development of sustainable livestock production by the farmers (Guyader et al. 2016). Due to the variation in weather, forages are available much during rainy season and very little during dry season hence there is need to look for other feedstuffs that are rich in nutrients, productive, and sustainable throughout the year (Guyader et al. 2016; Wahyono et al.

Cite This Article as: Pazla R, Agustin F, Ikhlas Z, Ardani LR, Sari A, Cahyana PT, Iswari K, Ardinal, Jumjunidang, Marlina L, Haloho JD, Susanti I, Syaputri M and Fitri SY, 2025. Evaluation of Sonia (BMR mutant sorghum and *Tithonia diversifolia*) usage as sustainable alternative feed to reduce concentrate dependency in ruminant diet: *In vitro* study. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2025.033

2023). Another potential afordable feed is “Sonia”, which comprises of BMR (Brown Midrib) mutant sorghum (*Sorghum bicolor*) and *Tithonia diversifolia*.

Sorghum is a multipurpose crop that is used for fodder, food and also for biofuel production (Laingen 2015; Cruickshank 2016; Prasad et al. 2016). This makes it appropriate wherever water is limited and it can grow in impoverished soils, making the crop suitable for cultivation in areas of impoverishment (Almeida et al. 2019). The BMR mutant variety of sorghum is called a ‘super’ sorghum for ruminant feed as it contains more nutritive value and lesser lignin content as compared to normal sorghum hence improves the feed digestibility (Oliver et al. 2005; Umakanth et al. 2014; Wahyono et al. 2023). Wahyono et al. (2023) evaluated different sorghum varieties for GH2 capacity and found that GH2.3 has higher *in vitro* digestibility and nutrient composition compared to other varieties. The results showed that the *in vitro* digestibility was also high while the lignin content was low when all 3 varieties were compared. As for BMR mutant sorghum, it is used as an energy source while *T. diversifolia* is rich in protein which is important in feeding ruminant livestock. *T. diversifolia* is highly rich in protein and can therefore be used as an important part of feed (Rivera et al. 2021; Pazla et al. 2022). Nonetheless, high phytic acid content leads to the fact that its taste is bitter which is why it has low palatability (Pazla et al. 2022; Pazla et al. 2024b). Hence the ration between sorghum and Tithonia must be determined in a way that would improve animal’s feed consumption. A previous study shows that the ratios of 60% BMR, 40% *T. diversifolia* are the most appropriate proportion of the two materials in terms of feeding value and palatability (Pazla et al. 2024b; Putri et al. 2024).

Specifically, this study aims to determine the feasibility of reducing the amount of concentrate fed to dairy cows while increasing the amount of Sonia to 90% *in vitro*. It is hoped that by reducing the amount of concentrates fed, feed costs can be controlled while still giving the herd the nutritional value it deserves. Admitted crude fiber, nitrogen-free extract (NFE), crude fat, acid detergent fiber (ADF), cellulose, neutral detergent fiber (NDF), hemicellulose and total gas production, microbial biomass will also be assessed in the view of *in vitro* digestibility study. This research study will help in the reduction of reliance on concentrates and the enhancement of feed conversion ratios in ruminant farming systems.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was not necessary for this experiment as it did not involve using live animals.

Research Materials

The primary materials utilized in this study included Sonia, a blend composed of BMR mutant sorghum and tithonia plants (*T. diversifolia*), and concentrates made from corn, rice bran, and palm kernel meal. The chemicals used for the research were McDougall's solution (aquadest, H_2SO_4 0.3 N, concentrated H_2S0_4, Selenium, and NaOH 1.5 N (Merck KGaA, 64271 Darmstadt, Germany), rumen fluid, and laboratory reagents such as sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH).

Study Area and Period

The experiment conducted at the Ruminant Nutrition Laboratory, Andalas University, Indonesia, over the period from September to December 2023.

Experimental Design

The design experimental adopted in the research was the Randomized Block Design, using four treatments, each replicated in 4 ruminant groups whereby the rumen fluids were different. The feed contains Sonia and concentrates in percentages according to treatment as follows: T1: 60% Sonia + 40% concentrate; T2: 70% Sonia + 30% concentrate; T3: 80% Sonia + 20% concentrate and T4: 90% Sonia + 10% concentrate. This study followed a modified *in vitro* rumen incubation method from Tilley and Terry (Tilley and Terry 1963).

Materials Preparation

Both BMR mutant sorghum and *T. diversifolia* were chopped, sun-dried, and ground into flour to prepare the feed samples. The chemical composition of the dietary ingredients and each treatment is shown in Tables 1-4. McDougall's solution was prepared the day before the experiment. Anaerobic conditions were maintained by storing the solution at 39°C in a shaking water bath while carbon dioxide (CO_2) was added. Meanwhile, rumen fluid was collected from beef cattle, filtered through cheesecloth and stored anaerobically in a thermos flask. The rumen fluid was maintained at a temperature of 39°C and the thermos flask infused with CO_2 before use.

Table 1 : Composition of ration treatments

Feed Ingredients	Treatments (%)			
	T1	T2	T3	T4
Sonia	60	70	80	90
Palm kernel meal	19	14	7	1
Bran	15	10	5	1
Corn	5	5	7	7
Salt	0.5	0.5	0.5	0.5
Minerals	0.5	0.5	0.5	0.5
Total	100	100	100	100

T1: 60% Sonia + 40% concentrate; T2: 70% Sonia + 30% concentrate; T3: 80% Sonia + 20% concentrate and T4: 90% Sonia + 10% concentrate.

Table 2: Chemical composition of feed ingredients

Chemical components (%)	Feed ingredients			
	Sonia	Corn	Rice Bran	PKM
DM	90.86	85.55	85.87	85.91
OM	86.63	95.87	88.99	96.13
Crude protein	13.79	13.87	8.94	22.29
Crude fat	2.86	3.09	8.76	10.99
Crude fiber	26.88	9.61	15.62	29.96
Ash	13.37	4.13	11.01	3.87
NFE	43.1	69.30	55.67	32.89
TDN	63.89	77.69	68.97	69.18
NDF	68.21	58.62	48.11	72.59
ADF	39.30	34.66	23.08	50.33
Cellulose	27.18	19.70	11.05	29.10
Hemicellulose	28.91	23.96	25.03	22.26
Lignin	8.70	7.5	3.78	9.25
Silica	3.42	0.70	2.28	14.28

DM: Dry Matter; OM: Organic Matter; NFE: Nitrogen-Free Extract; TDN: Total Digestible Nutrient; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; PKM: Palm Kernel Meal.

Table 3: Chemical composition of treatments

Chemical components (%)	Treatments			
	T1	T2	T3	T4
DM	88.00	88.49	88.98	89.48
OM	88.38	87.79	87.19	86.53
Crude protein	14.54	14.36	14.01	13.69
Crude fiber	24.64	25.05	25.05	25.32
Crude fat	5.27	4.57	3.71	2.99
Ash	10.62	11.21	11.81	12.47
NFE	43.92	43.81	44.42	44.53
TDN	65.71	65.19	64.84	64.32
NDF	64.87	65.65	66.16	66.70
ADF	38.34	38.60	38.54	38.53
Cellulose	24.48	25.19	25.71	26.24
Hemicellulose	26.53	27.05	27.61	28.17
Lignin	7.92	8.14	8.32	8.49
Silica	5.14	4.66	3.90	3.29

T1: 60% Sonia+40% concentrate; T2: 70% Sonia+30% concentrate; T3: 80% Sonia+20% concentrate, T4: 90% Sonia+10% concentrate; DM: Dry Matter; OM: Organic Matter; NFE: Nitrogen-Free Extract; TDN: Total Digestible Nutrient; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber.

Table 4: Chemical composition of 100% concentrate

Chemical components (%)	Treatments 100%			
	T1	T2	T3	T4
DM	83.70	82.97	81.48	77.06
OM	91.02	90.50	89.45	85.62
Crude protein	15.67	15.69	14.89	12.83
Crude fat	8.89	8.56	7.12	4.14
Crude fiber	21.29	20.79	17.75	11.29
Ash	6.48	6.16	5.55	4.38
NFE	4516	45.46	49.68	57.37
TDN	68.44	68.23	68.65	68.20
NDF	59.85	59.68	57.95	53.10
ADF	36.89	36.96	35.52	31.60
Cellulose	20.43	20.55	19.84	17.81
Hemicellulose	22.95	22.72	22.43	21.50
Lignin	6.75	6.83	6.81	6.55
Silica	7.73	7.54	5.81	2.15

Calculated based on table 1 and table 2. T1: 60% Sonia + 40% concentrate; T2: 70% Sonia + 30% concentrate; T3: 80% Sonia + 20% concentrate, T4: 90% Sonia + 10% concentrate.

In Vitro Evaluation

For *in vitro* rumen fermentation, 2.5g of each feed sample was placed in Erlenmeyer flask (250mL). Each flask was then filled with McDougall's solution (200mL) and rumen fluid (50mL). CO_2 was used to achieve anaerobic conditions. The flash was placed on a water bath shaker for 48h at 39°C. When incubation was complete, the flask was placed in a block of ice to stop microbial activity, followed by centrifuge at 3000rpm for 5min. The liquid/supernatant was used to measure microbial biomass. However, the resulting residue was filtered, dried, and analyzed. Total gas production was measured during 48h of fermentation. The dried residue was then dried in an oven at 60°C for 12h. It continues to determine chemical content, such NFE, crude fiber, ADF, crude fat, NDF, cellulose and hemicellulose. *In vitro* digestibility was calculated using the following formulas:

Crude Fiber Digestibility =

$$\frac{Crude\ fiber\ samples-(Crude\ fiber\ residue-Crude\ fiber\ blanko)}{Crude\ fiber\ sample}\ x\ 100\%$$

Crude Fat Digestibility =

$$\frac{Crude\ Fat\ samples-(Crude\ Fat\ residue-Crude\ Fat\ blanko)}{Crude\ Fat\ sample}\ x\ 100\%$$

$$\text{IVNFED} = \frac{NFE\ samples-(NFE\ residue-NFE\ blanko)}{NFE\ sample}\ x\ 100\%$$

$$\text{IVNDFD} = \frac{NDF\ samples-(NDF\ residue-NDF\ blanko)}{NDF\ sample}\ x\ 100\%$$

$$\text{IVADFD} = \frac{ADF\ samples-(ADF\ residue-ADF\ blanko)}{ADF\ sample}\ x\ 100\%$$

$$\text{IVCLD} = \frac{CLD\ samples-(CLD\ residue-CLD\ blanko)}{CLD\ sample}\ x\ 100\%$$

$$\text{IVHLD} = \frac{HLD\ samples-(HLD\ residue-HLD\ blanko)}{HLD\ sample}\ x\ 100\%$$

Where:

acid detergent fiber (ADF), neutral detergent fiber (NDF), nitrogen-free extract (NFE), cellulose (CLD), hemicellulose (HLD). IVNDFD: *in vitro* neutral detergent fiber digestibility; IVNFED: *in vitro* nitrogen-free extract digestibility; IVCLD: *in vitro* cellulose digestibility; IVHLD: *in vitro* hemicellulose digestibility; IVADFD: *in vitro* acid detergent fiber digestibility.

Statistical Analysis

Experimental data were analysed using analysis of variance (ANOVA). Data that showed a significant difference were further tested using the Duncan's test. The Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp., NY, USA) was used for the data analysis conducted in this study.

RESULTS AND DISCUSSION

The results of using Sonia and concentrates in different proportions are presented in terms of nutrient digestibility (Table 5), total gas production (Fig. 1), and microbial biomass (Fig. 2). The digestibility of crude fiber, NFE and crude fat ranges from 60.34 to 63.72%, while the digestibility of NDF and ADF ranges from 53.55 to 59.01%. Meanwhile, total gas production and microbial biomass did not show significant differences.

Nutrient Digestibility

The findings of this experiment showed that the Sonia doses used in the feed composition did not significantly influence (P>0.05) nutrient digestibility parameters, including crude fiber, crude fat, IVNFED, IVNDFD, IVADFD, IVCLD, and IVHLD, as shown in Table 5. These findings emphasize that the use of Sonia can replace concentrates in optimal doses. Crude fat, crude fiber, and NFE have digestibility rates ranging from 60.34 to 63.72%. In comparison, the IVNDFD and IVADFD are somewhat lower, at about 53.65 to 59.01%. Meanwhile, the digestibility of cellulose and hemicellulose, falls between 60.30 to 63.22%.

The findings of this study indicate that the *in vitro* digestibility of crude fiber, crude fat, and NFE fell within the ranges of 62.00 to 63.72%, 60.34 to 61.77%, and 61.55 to 63.35%, respectively. These values showed no significance (P>0.05) across the different treatments, as shown in Table 5. This lack of significance was caused by the similarity of lignin content across treatments, which ranged from 7.92 to 8.49%. Lignin is known to inhibit enzymatic accessibility to cellulose and hemicellulose, thereby limiting the digestibility of crude fiber (Shao and Zhao, 2016; Marlida et al. 2023). High lignin levels in feed reduce the ability of rumen microbes to digest crude fiber (Pazla et al. 2021; Lacayo et al. 2023). Apart from lignin content, the balance of energy and protein in the ration

Table 5: Nutrient digestibility of the treatments (%)

Treatments	Crude fiber Digestiblity	Crude fat Digestibility	IVNFED	IVNDFD	IVADFD	IVCLD	IVHLD
T1	62.47	61.20	63.00	58.07	55.13	61.65	62.46
T2	62.00	61.77	61.55	57.38	54.34	60.63	61.82
T3	63.72	60.34	63.35	56.92	53.65	60.30	61.59
T4	62.86	61.73	62.35	59.01	56.01	62.02	63.22
SE	0.80	0.84	0.80	0.74	0.83	0.70	0.71

T1: 60% Sonia+40% concentrate; T2: 70% Sonia+ 30% concentrate; T3: 80% Sonia+20% concentrate, T4: 90% Sonia + 10% concentrate; SE: Standard Error; IVNFED: In Vitro Nitrogen-Free Extract Digestibility; IVNDFD: In Vitro Neutral Detergent Fiber Digestibility; IVADFD: In Vitro Acid Detergent Fiber Digestibility; IVCLD: In Vitro Cellulose Digestibility; IVHLD: In Vitro Hemicellulose Digestibility. NS: Non Significant ($P>0.05$).

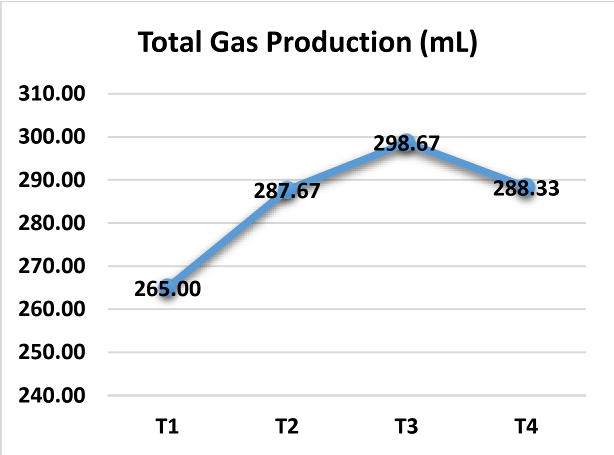

Fig. 1: Total gas production of the treatments.

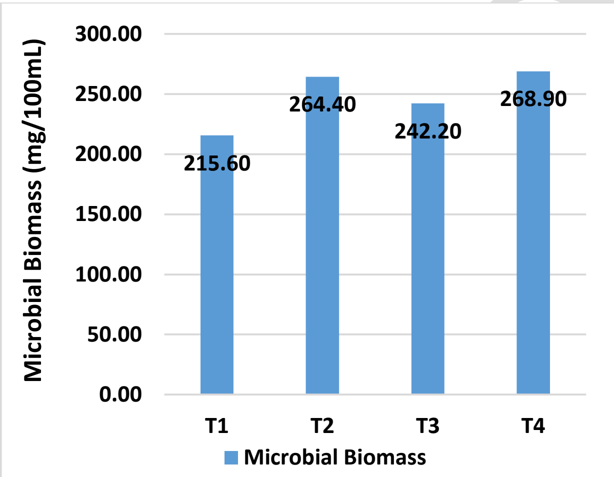

Fig. 2: Microbial biomass of the treatments.

plays a vital role in how well crude fiber is digested. Energy derived from fermentable carbohydrates supports the growth and fermentation processes of rumen microbes, while protein provides essential nitrogen for microbial protein synthesis (Elihasridas et al. 2024; Laura et al. 2024).

Therefore, a proper balance of energy and protein in the diet is essential for the growth of rumen microbes that are effective in digesting crude fiber. Meanwhile, factors that can affect crude fat digestibility include the type of fat in the feed and its interaction with crude fiber. Although crude fat serves as an important energy source, high fat levels can inhibit the growth of rumen microbes that ferment fiber (Montesqrit et al. 2024). In this study, the similar fat content in treatments did not result in any significant differences in crude fat digestibility. Easily digestible carbohydrates, such as starch and sugars, are part of NFE and supply rapidly available energy for livestock. The combination of Sonia and concentrate in various proportion ratios provided sufficient substrates for rumen microbes, thereby supporting optimal NFE digestibility.

Meanwhile, the fiber fraction also did not show significant digestibility results due to treatment (Table 5). The digestibility of IVADFD and IVNDFD was not significantly different between the treatments ($P<0.05$), with averages between 53.65-56.01% and between 56.92 to 59.01%, respectively. The similar fiber composition in each treatment likely explains this lack of significance. Pazla et al. (2021) report that NDF consists of lignin, cellulose and hemicellulose, which are the major structure components of plant cell walls. High lignin content can hinder NDF digestibility by obstructing enzymatic access to other fibers (Marlida et al. 2023). Furthermore, ADF contain cellulose, lignin, and silica, where cellulose can be digested by rumen microbes, but lignin and silica are more resistant (Van Soest et al. 2020). Despite the variation in the use of Sonia in the diet components, the IVADFD and IVNDFD remained stable. This indicates that the combination of sorghum and the Tithonia plant in Sonia effectively maintains the digestibility of the fibre components that are difficult to digest.

Cellulose and hemicellulose digestibility was not significant ($P<0.05$) (Table 5). IVCLD and IVHLD ranged from 60.30-62.02% and 61.59-63.22%, respectively. Previous studies report that cellulose is the major component of plant cell walls and efficiently digested by rumen microbes through enzymatic activity, producing VFA as the necessary energy source for ruminants (Wang et al. 2020; Weimer, 2022; Marlida et al. 2023). However, hemicellulose is less complex, so it is easier to digest than cellulose (Van Soest et al. 2020; Weimer 2022). Rumen microbes need the availability of balanced nutrients to optimize the rumen fermentation process which has an impact on livestock productivity (Elihasridas et al. 2023; Ardani et al. 2024). The results show that Sonia provides a sufficient substrate for the rumen microbiome to efficiently digest hemicellulose and cellulose, thereby reducing concentrate requirements without compromising nutritional quality.

Total Gas Production

This study found that the treatment had no significance ($P>0.05$) on total gas production (Fig. 1). The recorded total gas production was as follows: T1 (265mL), T2 (287.67mL), T3 (298.67mL), and T4 (288.33mL). The lack of significant differences suggests that all rations supported microbial fermentation activity in the rumen with similar efficiency. The nominal variation in total gas production is likely due to minor differences in nutritional composition and fermentability of each ration (Antonius et al. 2024). These findings imply that using BMR mutant sorghum in combination with Tithonia optimizes the nutritional balance necessary for effective rumen fermentation. High gas production typically indicates high fermentation activity and organic matter degradation by microbes (Ardani et al. 2023; Antonius et al. 2024), primarily resulting in carbon dioxide (CO_2) and methane (CH_4) (Antonius et al. 2024). The gas produced during rumen fermentation is considered an indicator of rumen digestibility (Pazla et al. 2021). Compared to earlier studies involving different feed combinations, this study recorded higher total gas production (Pazla et al. 2021, Ardani et al. 2023).

Microbial Biomass

Our findings in this study showed that microbial biomass, a crucial indicator of microbial activity and feed fermentation efficiency in the rumen, was not significantly affected ($P>0.05$) by the sonia-to-concentrate ratio (Fig. 2). The average microbial biomass for treatments was recorded as follows: T1 at 215.60mg/100mL, T2 at 264.40mg/100mL, T3 at 242.20mg/100mL, and T4 at 268.90mg/100mL. The similar nutritional composition across treatments probable provided adequate substrates for microbial growth, supporting microbial protein synthesis from the protein available in *T. diversifolia* and energy from BMR mutant sorghum. This combination effectively supported microbial growth without significant differences in biomass among treatments. Rumen microbes play an important role in degradation to produce secondary metabolites which will later be absorbed in the ruminant body such as VFA and NH_3 and converted into other forms (Antonius et al. 2023; Pazla et al. 2023). About 80% of ruminant energy comes from microbial biomass. Meanwhile, it contributes around 70 to 100% of ruminant protein (Pazla et al. 2023). The microbial biomass produced was higher than in previous studies with different fiber sources Agustin et al. (2024), indicating that Sonia and concentrate effectively support rumen microbial growth within the normal range for ruminant feed.

Conclusion

This *in vitro* study shows that Sonia, a blend of BMR mutant sorghum and *T. diversifolia*, can replace up to 90% of concentrate without affecting the digestibility of nutrients and ruminal fermentation. Its stable fermentation and digestibility values suggest that Sonia is a sustainable, cost-effective alternative feed that reduces reliance on traditional concentrates in ruminant production.

Funding

This research was conducted without external funding or financial support from any organization or institution.

Acknowledgement

The author sincerely appreciates the valuable contributions of the team members and expresses deep gratitude to Payakumbuh State Polytechnic, West Sumatra, Indonesia, for their encouragement and resources.

Conflict of interest

The authors declare that there are no conflicts of interest related to this study.

Authors contribution

RP, FA and ZI designed and conducted the laboratory experiments. MS and SYP collected the data in the laboratory. LRA, RP and AS analyzed data, drafted the original manuscript, and finalized it. PTC, KI and AA analyzed and interpreted data. JJ, LM, JDH, IS and provide manuscript revision. The final version of the manuscript has been approved by all authors.

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