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Effects of Chestnut Tannin and in Combination with MGM-P Tannin on Early-Weaned Piglets

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Abstract

The present study evaluated the potential of Ch tannin and chestnut-quebracho tannin as alternatives to antibiotics; it investigated the effects of tannins on the growth performance, diarrhea, fecal moisture content, hematology, and common diarrhea-associated bacteria in weaned piglets. Thirty-six piglets weaned at 21 days of age were randomly assigned to three groups: C (basal diet), 0.75% N (basal diet + 0.75% chestnut tannin), and MN (basal diet + 0.75% chestnut tannin + 0.5% MGM-P). The experiment lasted for four weeks post-weaning. The results that both of tannin addition groups showed inhibiting trend for the growth performance. The average daily gain (ADG) in 0.75N group was significantly lower than in C group. The incidence of diarrhea was 41.7% in C group, 25% in MN group and 0% in 0.75N group. Besides, tannin treatments tended to decrease the fecal moisture content and did not exhibit the antimicrobial effect in the gut. Furthermore, tannin trended to decrease the level of red blood cell, hemoglobin and white blood cell. Together, the results indicated that although 0.75% Ch tannin effectively reduced PWD, this concentration could have a negative impact on the growth performance of piglets and carries a risk of anemia.

Keywords: chestnut tannin; post-weaning diarrhea; weaned piglets; growth performance; hematology

1. Introduction

Early weaning of piglets, typically at 3 to 4 weeks of age, not only enhance the reproductive performance of sows, reduces the risk of disease transmission from sows to piglets, and allows sows to return to estrus and conceive earlier, thereby boosting the overall herd productivity [1], but also improves the feed conversion and growth rates of piglets due to the better nutritional support from high-quality weaning diets [2,3]. While since the intestines and immune systems of piglets are not fully developed as they are weaned at a younger age, the stress of early weaning can exacerbate post-weaning diarrhea (PWD), bacterial infections, and even mortality [4,5]. The use of antibiotics is an effective measure to prevent bacterial diseases caused by stress and underdeveloped immune systems during early weaning [6]. However, such antibiotic use has led to the emergence of drug-resistant bacteria, making traditional antibiotics ineffective and posing health risks [7]. Residual antibiotics in livestock products can also increase human resistance and trigger allergic reactions [8]. Therefore, it is necessary to find alternatives to antibiotics. Plant-derived tannins, Anti-parasitic property, exhibit various pharmacological activities, including inhibiting bacterial growth and viral activity, reducing inflammation, and scavenging free radicals [9–12]. Our laboratory has found that a condensed tannin, MGM-P, derived from quebracho tree not only alleviates PWD but also improves growth performance of piglets [13]. The results showed that adding a 0.3% concentration of MGM-P demonstrated greater efficacy in alleviating post-weaning diarrhea than 0.2% concentration. A

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comparison of MGM-P at concentrations of 0.5% and 1.0% revealed that the 0.5% concentration group was more effective in improving growth performance [14]. However, the 1.0% concentration group exhibited several anti-nutritional effects, resulting in reduced feed conversion efficiency and daily weight gain. An in vitro study by Serena Reggi et al demonstrated that quebracho tannins and chestnut tannins, whether used alone or in combination, exhibited significant antioxidant activity and inhibited the growth of F4+ and F18+ strains in E. coli [15]. Valentina Caprarulo et al, when they fed the composite tannin of chestnut and quebracho continuously for 40 days at 1.25% to weaned piglets (n = 120; 28 ± 2 days of age) [16]. No adverse effects of the mixed tannin on feed intake or growth performance were observed, confirming that dietary inclusion of Chestnut/Quebracho at a concentration of 1.25% did not impair animal performance on the 28 days weaned piglets. Nevertheless, P B Disler. et al. reported that tea, a kind of tannin-containing beverages, was inhibited on iron absorption in human volunteers. Regardless of the presence or absence of ascorbic acid, iron absorption from $FeCl_3$ and $FeSO_4$ solutions, bread, rice served with potato and onion soup, and uncooked hemoglobin was inhibited [17].

To identify more effective plant tannins to prevent PWD and enhance growth performance in piglets, the present study conducted trials on early-weaned piglets, administering either hydrolysable chestnut tannin alone or in combination with MGM-P. Furthermore, fecal moisture content, bacterial load and hematological parameters were also analyzed.

2. Methods and Materials

2.1. Materials

2.1.1. Tannins

Commercial MGM-P supplementation (Kawamura Ltd., Tokyo, Japan) contained more than 50% of CTs extracted from the quebracho tree. Commercial Chestnut extract supplementation (Tanin Sevnica, Slovenia) contained 75% of tannin extracted from the sweet chestnuts (Castanea sativa mill).

2.1.2. Animals, Treatment and Experimental Design

The experiment was conducted at the Animal Resource Science Center of the University of Tokyo (Kasama, Japan) and approved by the Animal Care and Use Committee of the Faculty of Agriculture, the University of Tokyo (P22-027). All procedures followed the guidelines for animal testing of The University of Tokyo. Four pregnant specific-pathogen-free sows were purchased from Nakamura Chikusan (Ibaraki, Japan) two weeks before delivery. Thirty-six piglets (Duroc × Landrace × Yorkshire) were born and survived within three days, including twenty-five males and eleven females. All piglets were measured for birth weight and numbered. The male piglets were castrated at 10 days of age. The lactation period was 21 days. After weaning, all piglets were weighed and divided into three groups (n = 12 per group, 4 repetitions) using the Experimental Animal Allotment Program (version 1.1) based on body weight (BW), gender, and maternal factor (Tables 1 and 2). Each group comprised four pens, with 3 piglets per pen. The control group were fed fodder basal diet without any antibiotic or plan extracts. The 0.75N group received basal diet with 7.5g/kg (0.75%) Chestnut tannin and the MN group received basal diet with 7.5g/kg (0.75%) Chestnut and 5g/kg (0.5%) MGM-P together. The experiment period was four weeks after weaning (from 3 to 7 weeks of age).

Table 1. Experimental animal allotment (Each group).

Group	BW (kg)	*n*	SEM	*p*-Value	CV (%)
C	5.68	12	0.45		
0.75N	5.67	12	0.49	>0.99	0.04
MN	5.68	12	0.43		

Abbreviations: n, number of piglets; SEM, standard error of the mean; CV, coefficient of Variation; C, control; 0.75N, 0.75% Chestnut; MN, 0.75% Chestnut and 0.5% MGM-P.

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Table 2. Experimental animal allotment (Each repetition).

Group	Repetition				Mean
	1	2	3	4	
C	7.05	5.8	5.15	4.08	5.68
0.75N	7.77	5.95	5.00	4.12	5.67
MN	7.63	6.18	4.97	3.77	5.68
Mean	7.69	5.98	5.04	3.99	5.67
CV	0.9	3.23	1.94	4.84	0.04

Abbreviations: CV, coefficient of Variation; C, control group; 0.75N, 0.75% Chestnut; MN, 0.75% Chestnut and 0.5% MGM-P.

2.1.3. Diets and Animal Management

The basic diet consisted of two custom-made fodder basal diets customized by KIMURA NOSAN SHOJI CO., LTD (Tokyo, Japan) without any antibiotics or plant extracts added. Which meets the nutritional requirements of piglets at different stages of growth after weaning. The composition of basal diets meets the National Research Council (NRC) standards of pigs. The ingredient and chemical composition of the diets for the pre-phase for 3w-4w of age and the post-phase for 4w-7w of age are summarized in Table 3 and Table 4, respectively. The feeds for the Control group and two Tannin-treated groups were individually mixed using an industrial blender for 30 minutes. All piglets were placed in four high-bed nursery houses, equipped with mechanical ventilation, molded plastic pen floors, a feed hopper, and a SUEVIA water cup for ad libitum access to food and water.

Table 3. Ingredient and Chemical composition of basal diet in the pre-phase [1].

Ingredient	Content (%)	Chemical Composition	Content
Corn	34.3	CP,%	23.23
Defatted milk powder	18	DE, Mcal/kg	3.69
Fatty powder	6.2	TDN, %	84.7
Sugar	10	Ca, %g	0.81
Soybean meal	25	NpP, %	0.45
Fish meal, CP 65%	4.5	Na, %	0.26
Calcium diphosphate	0.2	Cl, %	0.36
Calcium carbonate	0.65	K, %	0.99
Salt	0.2	Mg, %	0.14
B vitamins	0.20	Fe, mg/kg	182.12
Vitamins A, D and E	0.20	Zn, mg/kg	105.29
Trace minerals	0.15	Mn, mg/kg	87.5
L-Lysine hydrochloride	0.06	Cu, mg/kg	755.28
DL-Methionine	0.09	I, mg/kg	1.95
L-Threonine	0.03	Se, mg/kg	0.30
Copper sulphate	0.21	Vitamin A, IU/kg	200051.62
Vitamin in K3	0.01	Vitamin D, IU/kg	4000.0
Total	100	Vitamin E, IU/kg	30.01
		Vitamin K, IU/kg	0.57
		Thiamine, mg/kg	5.65
		Riboflavin, mg/kg	18.88
		Pantothenic acid, mg/kg	33.27
		Nicotinic acid, mg/kg	28.6
		Vitamin B_6, mg/kg	6.17
		Choline, mg/kg	1232.83
		Vitamin B_{12}, mg/kg	26.88
		Biotin, mg/kg	0.16

		Folic acid, mg/kg	0.36

[1] The other pre-phase diets were based on this diet, to which 0.75N and MN tannin treated groups. Abbreviations: CP, crude protein; DE, digestible extract; TDN, total digestible nutrients; NpP, non-phytate phosphorus.

Table 4. Ingredient and Chemical composition of basal diet in the post-phase [2].

Ingredient	Content(%)	Chemical Composition	Content
Corn	60.89	CP,%	19.14
Defatted milk powder	8.00	DE, Mcal/kg	3.42
Fatty powder	0.50	TDN, %	77.9
Sugar	4.00	Ca, %g	0.88
Soybean meal	20.00	NpP, %	0.5
Fish meal, CP 65%	3.5	Na, %	0.18
Calcium diphosphate	1.2	Cl, %	0.24
Calcium carbonate	0.745	K, %	0.79
Salt	0.2	Mg, %	0.14
B vitamins	0.20	Fe, mg/kg	202.95
Vitamins A, D and E	0.1	Zn, mg/kg	103.6
Trace minerals	0.15	Mn, mg/kg	88.32
L-Lysine hydrochloride	0.32	Cu, mg/kg	125.08
DL-Methionine	0.05	I, mg/kg	1.85
L-Threonine	0.08	Se, mg/kg	0.27
Copper sulphate	0.03	Vitamin A, IU/kg	100022.94
Vitamin in K3	0.01	Vitamin D, IU/kg	2000.0
Total	100	Vitamin E, IU/kg	24.83
		Vitamin K, IU/kg	0.57
		Thiamine, mg/kg	6.04
		Riboflavin, mg/kg	16.97
		Pantothenic acid, mg/kg	30.44
		Nicotinic acid, mg/kg	32.27
		Vitamin B_6, mg/kg	7.28
		Choline, mg/kg	1089.13
		Vitamin B_{12}, mg/kg	23.06
		Biotin, mg/kg	0.13
		Folic acid, mg/kg	0.31

[2] The other post-phase diets were based on this diet, to which 0.75N and MN tannin treated groups. Abbreviations: CP, crude protein; DE, digestible extract; TDN, total digestible nutrients; NpP, non-phytate phosphorus.

2.2. Methods

2.2.1. Growth Performance

During the experimental period, the body weight (BW) and feed intake of piglets were recorded from 3 to 7 week of age. The average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were then calculated.

2.2.2. Diarrhea Incidence

To determine the diarrhea incidence after weaning, piglet feces were observed twice daily (9:00 a.m. and 4:00 p.m.) during experiment period. The standard of PWD was the presence of loose feces in piglets lasting for more than two days. The diarrhea incidence was calculated as the proportion of piglets with diarrhea to the total number of piglets in each group.

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2.2.3. Blood Sampling and Hematology Analysis

Blood was collected from the jugular vein of 36 piglets at 3 to 7 weeks of age. A 21-gauge needle (VENOJECT II; Terumo, Tokyo, Japan) was used to harvest blood for storage in 5 mL collection tubes containing EDTA-Na. Hematology analyses, including white blood cell (WBC) count, red blood cell (RBC) count and hemoglobin were performed by using a pocH-100iV Diff hematology analyzer (Sysmex Corp., Kobe, Japan)

2.2.4. Fecal Sampling

Fecal samples from each piglet were individually collected at 4 to 7 weeks of age by piglet rectal ampoule stimulation using sterility swab. Taking a 2g sample of each piglet for moisture analysis. The remaining samples were immediately frozen and stored at −20 °C until analysis.

2.2.5. Fecal Moisture Content Analysis

The moisture content of fecal samples was analyzed from 5 to 7 weeks of age. Two grams of the fecal sample were immediately placed in an aluminum foil container and dried at 110 °C for 22 hours. After drying, the samples were placed in the desiccator for 30 minutes to cool. Recording weight of the dried fecal sample. The moisture content was calculated using the following formula.

$$Moisture\ content=(Weight_{before\ drying}-Weight_{after\ drying})/Weight\ of\ sample*100\%$$

2.2.6. DNA Extraction and Real-Time PCR Amplification

Total DNA from 200mg of fecal samples was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. Real-time PCR testing was performed using the MyGo Mini S real-time PCR instrument (IT-IS Life Science Ltd., Ireland) to determine the levels of Escherichia coli and Salmonella related genes in DNA extracted from faecal samples. The sequence of primer is showed in Table 5. The mix qPCR solution(10ul) contained 1ul of template, 3ul of water, 1uL each of 6 uM forward and reverse primers, and 5 uL of TB Green Fast qPCR Mix (TAKARA Ltd., Tokyo, Japan). PCR was carried out for 95 °C for 30s and 40 cycles of 95 °C for 5s and 60 °C for 15 s.

Table 5. Primers used for qPCR analysis.

Primers	Sequencing	Reference
E.coli F	5′-CATGCCGCGTGTATGAAGAA-3′	[18]
E.coli R	5′-CGGGTAACGTCAATGAGCAAA-3′	
Inva F	5′-GTGAAATTATCGCCACGTTCGGGCAA-3′	[19]
Inva R	5′-TCATCGCACCGTCAAAGGAACC-3′	

2.2.7. Statistical Analysis

GraphPad Prism (Version 9.5.1) was used for all figure generation, and the data were analyzed using two-way ANOVA followed by Tukey's multiple comparison test. *p*-values < 0.05 were considered to indicate statistical significance. Results are presented as the means ± standard errors of the mean.

3. Results

3.1. Growth Performance

As shown in Table 6, the final BW in two tannin treated groups were lower than that in the control group, but no statistical difference. The ADG in 0.75N group was significantly lower than that in the C group. The ADG of the MN group was much lower than that of the C group, but the difference was not statistically significant. Although the ADFI and FCR among the three groups did not show significant differences, the 0.75N and MN groups were showed tendency lower compared to the C group.

Table 6. Effects of Tannin supplementation on growth performance of weaned piglets.

Item	Initial BW (kg)	Final BW (kg)	ADG	ADFI	FCR
C	5.68±1.49	16.26±3.89	0.41±0.10^{a}	0.63±0.08	1.55±0.08
0.75N	5.67±1.61	12.81±5.49	0.27±0.17^{b}	0.47±0.15	1.77±0.18
MN	5.68±1.41	13.29±5.24	0.29±0.16^{ab}	0.50±0.15	1.76±0.14

Abbreviations: BW—body weight; ADG—average daily gain; ADFI—average daily feed intake; FCR—feed conversion ratio. Data are expressed as the mean ± SEM (n = 12). a, b Mean values within a row with dissimilar superscript letters are significantly different ($p < 0.05$).

3.2. Diarrhea Incidence

As shown in Figure 1, the diarrhea incidence in C group was 41.7%, and 25% in MN group. However, none of the piglets in the 0.75N group developed diarrhea, which was significantly lower than that in C group. All piglets with diarrhea recovered without any medical treatment.

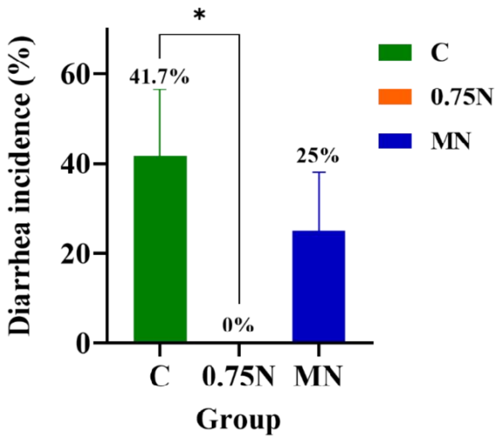

Figure 1. Effects of Tannin supplementation on diarrhea incidence in weaned piglets. Values are expressed as mean ± SEM (n = 12). *Asterisk indicates statistical differences between columns at $p < 0.05$.

3.3. Fecal Moisture Content Analysis

As shown in Figure 2, there is no difference among the groups in fecal moisture content in the 5 weeks of age, which were 69.9%±2.5, 69.9±4.4 and 69.9±4.9 separately. Besides, from the 6 week of age, the moisture content in the 0.75N and MN group became lower than that in the C group, although the differences were not significant, at 73.1% ± 4.0, 71.3% ± 4.9 and 71.7% ± 3.9 separately in the 6 weeks of age and 75.1%±3.6, 72.9±3.9 and 72.3±4.6 separately in the 7 weeks of age. In the present study that was recognized as diarrhea sloppy feces the water content is 88.01%±2.2%.

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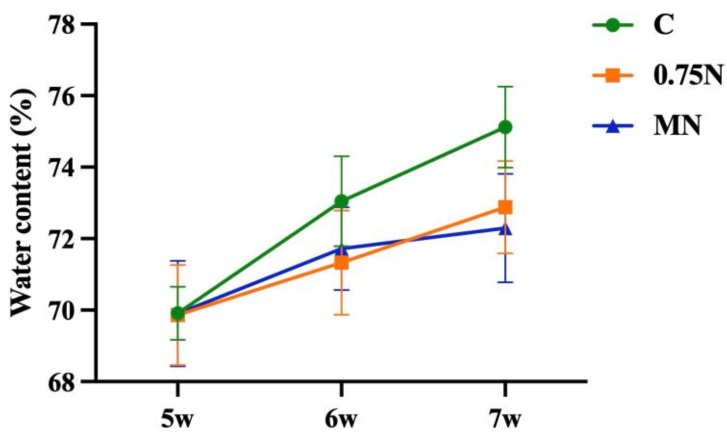

Figure 2. The fecal water content changes analyzed. Everly line represent each treatment, green line is control group, orange line is 0.75N group and blue line is MN group.

3.4. Cq Value of E.coli and Salmonella in Feces

As shown in Figure 3. At the end of the experiment The Cq value of E.coli in the 0.75N group was lower compared with the C group (p=0.03). Meanwhile, the Cq value of Salmonella did not show different among those three groups.

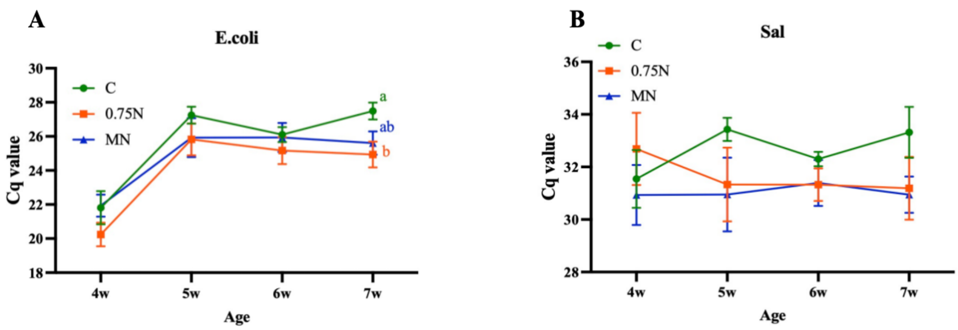

Figure 3. The analysis of the effect of tannin treatments in E.coli (A) and Salmonella (B). Green bars represent E.coli. level. The orange bars is chestnut tannin treatment group and blue bars is chestnut tannin combinate with MGM-P. Values are expressed as mean ± SEM (n = 12). Different lowercase letters on the right side of the nodes indicate significant differences ($p < 0.05$; Tukey HSD test).

3.5. Blood Hematology Analysis

The changes in WBC, RBC and HGB counts can be observed in Figure 4. The RBC levels both in the 0.75N and MN groups at w6 and w7 were significantly lower than those in the C group. HGB in the 0.75N group was significantly lower compared with MN group from 4 to 5 weeks of age and with C group from 4 to 7 weeks of age. While HGB in the MN group was significantly lower than those in the C group from 6 to 7 weeks of age.

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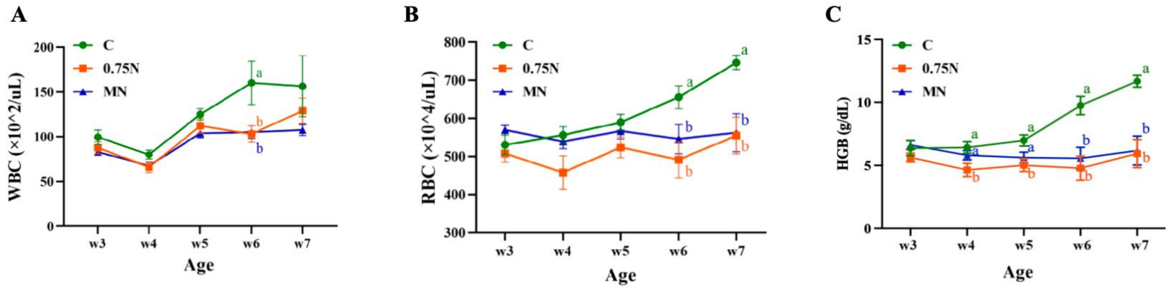

Figure 4. Effects of Tannin supplementation on white blood cell count (A), red blood cell counts (B) and hemoglobin (C) in weaned piglets. Everly line represent each treatment, green line is control group, orange 0.75N group and blue line MN group. Values are expressed as mean ± SEM (n = 12). a, b Mean values with dissimilar superscript letters indicate significantly differences between columns at $p < 0.05$.

4. Discussion

The use of tannins as a feed additive in livestock production has long been a contentious issue. Numerous studies have shown that tannins have anti-nutritional effects due to their ability to bind proteins, thus reducing nutrient availability [20,21]. High concentrations of tannins are known to decrease nutrient utilization, particularly protein, and can negatively impact growth, palatability, and enzymatic activity [22]. But Ma, et al. [13,14] demonstrated that the appropriate type (MGM-P) and dosage of tannins not only prevent piglet from the early weaning diarrhea but also improve growth performance. The present study indicates that the addition of 0.75% Ch, whether individually or in combination with MGM-P, tends to have a negative impact on the growth performance of 21 days weaned piglets. The ADG in the 0.75N group showed significant and in the 0.75% Ch combined with 0.5% MGM-P group also showing tendency decrease, although it did not exhibit a significant decrease, which indicates that, for complete replacement of antibiotics, different tannin might have their own proper addition level. Valentina Caprarulo et al reported when they fed 1.25% composite tannin of chestnut and quebracho to 28 days weaned piglets no adverse effects of the mixed tannin on feed intake or growth performance were observed [16]. This suggest that 21 day weaned piglets might be more sensitivity to Ch addition level. The RBC levels and hemoglobin from the third week of the experiment, in the two tannin groups were significantly lower than those in the control group. Ma, et al. [13,14] demonstrated that no anemia occurred in animals when MGM-P was added at concentrations ranging from 0.1% to 1%, whereas this condition did occur in the present study. Thus, these results might be attributable to the addition of 0.75% chestnut tannin. Tannins, particularly hydrolysable tannins, have been demonstrated to interact with iron ions in the human blood, rendering them less available for biological processes that require iron, such as hemoglobin synthesis, thus reducing the RBC count [23]. Hemoglobin is a crucial protein in red blood cells responsible for transporting oxygen [24]. The reduction in available iron for hemoglobin synthesis could subsequently lead to a decreased RBC count [25]. Furthermore, tannins may limit dietary iron absorption in the intestine by forming insoluble complexes, leading to an overall decrease in iron bioavailability. This can have broader implications for the organism's overall health and metabolic functions [26,27]. Therefore, the interference of tannins with dietary iron metabolism and hemoglobin synthesis in vivo may explain the growth inhibition observed in the chestnut group at high concentrations, resulting in reduced growth performance and RBC levels. However, our previous research has shown that even the addition of 1% MGM-P did not lead to a reduction in red blood cells [14]. Delimont et al indicated that hydrolysable tannins and oligomeric catechin/epicatechin tannins generally reduce the bioavailability of iron, while long term long-term administration of condensed tannins did not affect iron status or bioavailability [28,29]. This suggests that different types of tannins have varying effects on hemoglobin and red blood cells. The WBC values in control group were higher, which may relate with diarrhea, as the incidence of diarrhea in the C group was higher compared to the MN group, and no diarrhea occurred in the 0.75N group, indicating 0.75% chestnut tannin significantly suppresses post-weaning diarrhea. Diarrhea is a major cause of mortality in early-weaned piglets, often resulting from stress-induced gut microbiota disruption and increased opportunistic disease incidence [30]. The present study analyzed the effect of tannin treatments on microbiota pathogens in the gut especially E. coli and Salmonella. The E. coli quantitative was a little bit higher than in the tannin treatment groups at the end of the experiment, but relatively low compare with the first week after weaning. The Salmonella levels showed no significant differences across the three groups throughout the experimental period. Those suggested that neither chestnut tannins nor the mixed tannins exhibited antimicrobial effects in the in vivo test. Since even though diarrhea occurred, there was no epidemic, and all piglet suffering from diarrhea recovered without any medical treatment. Thus, this study indicated that diarrhea might not be caused by E.coli and Salmonella. Our study showed tannins, as a primary component in anti-diarrheal drugs, are known for their astringent and anti-inflammatory properties. As an astringent, tannins can reduce excessive intestinal motility and decrease intestinal fluid secretion by inhibiting calcium-activated chloride channels (CACCs) and the cystic fibrosis transmembrane conductance regulator (CFTR) [31,32]. This is consistent with our findings that the tannin-treated groups, particularly the 0.75% chestnut group, had dark-colored and harder-textured feces, consistent with the fecal moisture measurements where the 0.75N group exhibited the lowest fecal moisture content. In contrast, the feces in the C group were softer and porridge-like. Therefore, the strong anti-diarrheal effect of 0.75% chestnut tannins in this study is likely due to its astringent properties.

5. Conclusions

In conclusion, the administration of 0.75% chestnut tannin to weaned piglets effectively alleviated post-weaning diarrhea and demonstrated an astringent effect by reducing fecal moisture content. However, it also had a negative impact on the growth performance and significantly reduced red blood cell counts. Therefore, the study indicates that addition of chestnut tannin to weaned piglet should be under the 0.75%. Furthermore, no synergistic effect was observed when combining 0.75% chestnut tannin with MGM-P.

Author Contributions: Methodology, Zhuoyu Yang and Min Ma; Software, Zhuoyu Yang; Formal analysis, Zhuoyu Yang and Min Ma; Investigation, Zhuoyu Yang, Peiwen Shi, Kaiting Wang, Yuriko Enomoto and Tomoko Suda; Resources, Peiwen Shi and Kaiting Wang; Data curation, Junyou Li; Writing – original draft, Zhuoyu Yang; Writing – review & editing, Zhuoyu Yang; Supervision, Junyou Li; Project administration, Junyou Li; Funding acquisition, Junyou Li. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The experiment was approved by the Animal Care and Use Committee of the Faculty of Agriculture, the University of Tokyo (P22-027). All procedures followed the guidelines for animal testing of University of Tokyo.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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