Combined Therapy of Probiotic Supplementation and Rehydration Improves Blood Dehydration Parameters and Decreases Mortality of Neonatal Piglets Naturally Infected with Porcine Epidemic Diarrhea Virus: A Clinical Trial

Takio Inatomi 1,2, Takamitsu Tsukahara 3,*, Gustavo A. Romero-Pérez 3 and Ryo Inoue 4

Abstract: Although rehydration therapy (RT) has been used to treat animals suffering from viral diarrhea, mortality among farm animals still remains high, as RT alone neither significantly minimizes the duration of the illness nor reduces the looseness of stools. As porcine epidemic diarrhea (PED) is a viral disease and PED treatments are still very limited, vaccination is the common strategy to prevent it. Thus, the aim of the present study was to test whether a combination of RT and probiotics supplementation could help to improve the mortality of suckling piglets kept in a commercial farm and naturally infected with PED virus. Piglets receiving a combination of probiotic supplementation and RT showed improved (p < 0.01) blood parameters such as base excess and bicarbonate ion concentration when compared with untreated control piglets and piglets administered with RT alone. When compared with that of control piglets, mortality during the suckling period was the lowest (p < 0.05) in piglets receiving the combined therapy, but statistically unchanged between piglets receiving either RT or RT and probiotics. Our preliminary results should motivate further research on the use of a combined rehydration and probiotics therapy to reduce mortality in piglets suffering from acute diarrhea.

Keywords: porcine epidemic diarrhea; rehydration therapy; probiotics; neonatal piglets

1. Introduction

Porcine epidemic diarrhea (PED) is an enteric disease that severely affects the pig industry worldwide [1]. Like many others across Asia, farms in Japan have been stricken by PED epidemics, including one that caused 1000 outbreaks in 2013 alone [2]. PED virus belongs to the group 1 of the genus Coronavirus and can be described as an enveloped, single-stranded ribonucleic acid (RNA) virus [3]. As watery diarrhea, vomiting and dehydration are among its clinical signs, acute PED can cause a high mortality in piglets [4]. For example, mortality caused by PED in newborn piglets is nearly 100% [5,6], and in suckling piglets, it can be as high as 80% [7].

Rehydration therapies (RT) have been used to treat acute diarrhea and reduce vomiting in children [8]. The most common RT is the oral rehydration therapy, in which to prevent dehydration by loss of fluids, an individual is orally hydrated with a solution [9]. In farm animals, similar forms of RT have been utilized. For example, oral RT has been especially effective in neonatal piglets against diarrheic symptoms, which are caused by transmissible gastroenteritis infections [10]. Silanikove found no differences in the proportions of fluids retained in goats receiving either oral or intraperitoneal RT [11,12].
However, Basu et al. reported the effect of RT is limited, as it does not significantly reduce diarrhea or the looseness of stools [13].

Probiotics is a collective term to refer to those “live microorganisms, which when administered in adequate amounts, confer a health benefit on the host” [14,15]. Oral probiotic supplementation to infants has been proven effective in improving acute infectious diarrhea [16]. In farm animals, lactic acid bacteria supplementation has also been used to treat certain infections. For example, supplementing neonatal human rotavirus (HRV)-infected gnotobiotic pigs infected with \textit{Lactocaseibacillus} (formerly known as \textit{Lactobacillus}) \textit{rhamnosus} strain GG and \textit{Bifidobacterium animalis} ssp. \textit{lactis} BB-12, significantly lowered fecal scores, reduced HRV shedding concentrations, and increased intestinal IgA HRV antibody concentrations and HRV-specific IgA antibody-releasing cell numbers [17]. Similarly, at these premises, we used a heat-killed cell preparation of \textit{Enterococcus faecalis} strain EC-12 to treat rotavirus infection in weaning piglets [18] and to stimulate luminal IgA secretion in young calves [19] and chicks [20].

Recently, we also showed that supplementing pregnant sows with \textit{BIO-THREE} helped increase body weights at birth and survival rates of their suckling piglets born in a PED-infected farm [21,22]. \textit{BIO-THREE} is a probiotic formulation containing \textit{Bacillus subtilis} strain TO-A, \textit{Clostridium butyricum} strain TO-A and \textit{Enterococcus faecium} strain T-110. \textit{BIO-THREE} has been used in Japan by government health agencies to prevent and/or treat diarrheal and constipation-related symptoms in humans (Ministry of Health, Labor and Welfare, approval number: 2316017B1041) and livestock (Ministry of Agriculture, Forestry and Fisheries, approval number: 14Seichiku1477). Moreover, several studies reported that \textit{BIO-THREE} supplementation improved diarrheic symptoms caused by pathogenic infections not only in infants [23,24], but also in young livestock [25,26].

The aim of the present study was to test whether a combination of RT and probiotic supplementation could help to improve diarrheal symptoms and to reduce mortality caused by acute PED in suckling piglets, in a more enhanced manner than if those treatments were given separately.

2. Materials and Methods

2.1. Farm

The present work was conducted in a commercial pig farm in the Kyushu region of Japan. The farm was the same as that described in a previous study [21,22]. The farm is a farrow-to-finish type and has approximately a stock of 900 sows (Landrace × Large white). For the present work, all sows were impregnated by Duroc boars.

A preliminary enteropathogenic survey of the sows and the suckling piglets in the farm has been previously described [22]. On December 2013, an infection outbreak of acute PED took place in the region where the farm was located. An infection screening conducted by an independent livestock hygiene center confirmed that the piglets used in the present study were naturally infected with PED virus.

2.2. Rehydration Solutions and Probiotics

Calf Light-S (Zenoaq, Fukushima, Japan) was used as the oral rehydration solution. Calf Light-S was suspended in warm water (48 g/2000 mL) and 50 mL/day was given orally to individual piglets. A commercial lactated Ringer’s solution (Solulact; TERUMO Corp., Tokyo, Japan) was used for the abdominal rehydration therapy. The dosage of Ringer’s solution was prescribed by a veterinarian, which was based on his assessment of the body weight of each piglet and the individual severity of PED.

A mixture of \textit{BIO-THREE} Plus (TOA Pharmaceutical Co. Ltd., Tokyo, Japan) containing \(1 \times 10^8\) CFU/g of \textit{Bacillus subtilis} TO-A, \(1 \times 10^8\) CFU/g of \textit{Clostridium butyricum} TO-A and \(1 \times 10^9\) CFU/g of \textit{Enterococcus faecium} T-110, with digestive enzymes (cellulase, protease and pectinase; 100 mg/g each) was used in the present study. The \textit{BIO-THREE} Plus mixture was suspended in the oral rehydration solution so that each piglet received 5 g/day.
2.3. Animals and Experimental Design

The present experiment was carried out between December 2013 and February 2014 and approved by the ethical committee of the Inatomi Animal Hospital (approval number: 201404).

A total of 109 3-day-old piglets from 19 PED-infected sows were initially selected for the present work. None of the piglets had diarrhea at the time of group allocation. The piglets were randomly allocated into untreated (control; C), oral and abdominal rehydration therapy (OA) and oral and abdominal rehydration therapy with probiotics (probiotics in oral rehydration solution; OAP) groups. Allocation of piglets to experimental groups was carried out by randomization, which was based on random numbers generated by rolling a die. For instance, when a die showed 1 or 2, piglets were allocated to group C, 3 or 4 to group OA and 5 or 6 to group OAP. Treatments were given once daily for five consecutive days.

One hundred and three 4-to-7-day-old (mean piglet age: 5.6-day-old) piglets defecated watery, yellowish, diarrheic stools and hence, progressed to the experimental stage. The new number of piglets in each experimental group was: 23 in group C (mean body weight at day post treatment (dpt) 0: 1.1 kg), 31 in group OA (mean body weight at dpt 0: 1.1 kg) and 49 in group OAP (mean body weight at dpt 0: 1.1 kg). The experimental procedure is shown in Figure 1.

Figure 1. Schematic representation of the experimental design of the study. Group C, untreated control; group OA, piglets receiving oral and abdominal rehydration therapy; group OAP, piglets receiving oral and abdominal rehydration therapy. dpt, days post-treatment.
2.4. Blood Collection and Analysis, and Monitoring of Mortality

Blood was collected from the jugular vein of each piglet immediately prior to and 24 h post-administration of the first therapy. As soon as their blood was collected, dehydration parameters, such as base excess, pH and bicarbonate ion (HCO$_3^-$) concentration were analyzed in the samples of all piglets using a portable blood analyzer (i-STAT system, Abbott Japan, Tokyo, Japan). Mortality of piglets was monitored during the suckling period.

2.5. Statistical Analysis

Depending on the results of the Bartlett test, either completely randomized design 1-way analysis of variance or the Kruskal–Wallis test was used to analyze differences between the means of the blood parameters at each sampling point. Tukey–Kramer post hoc comparison (parametric or non-parametric) was used for multiple comparisons, as needed. The Kruskal–Wallis test was used to analyze differences in mortality of piglets. Again, Tukey–Kramer post hoc comparison (non-parametric) was used for multiple comparisons, as needed. Furthermore, differences between blood parameters at pre- and post-treatment days were evaluated by the paired t-test.

Values are shown as the means ± standard errors. In all statistical analyses, differences between means were considered significant when $p < 0.05$. All calculations were made using Statcel3 (OMS, Tokyo, Japan) as an add-in application for Microsoft Excel® (Microsoft, Seattle, WA, USA).

3. Results

3.1. Blood Dehydration Parameters

The mean base excess in the blood of piglets greatly ($p < 0.01$; paired t-test) improved after receiving the combined oral rehydration and probiotic supplementation therapy (OAP), remained fairly unchanged after the administration of the oral and abdominal rehydration therapy (OA) and worsened ($p < 0.01$; paired t-test) in control piglets, as time passed (Figure 2a).

The concentration of HCO$_3^-$ was greater after administering both OA ($p < 0.05$; paired t-test) and OAP ($p < 0.01$; paired t-test), while it decreased in control piglets, as time passed (Figure 2b).

The pH of blood of OAP piglets remained almost the same after receiving the treatment. However, the pH of blood significantly decreased in OA piglets ($p < 0.01$; paired t-test) even after receiving the treatment, as well as in control piglets ($p < 0.01$; paired t-test) as the experiment progressed (Figure 2c).

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**Figure 2.** Blood parameters pre- (dpt 0) and post- (dpt 1) rehydration therapy with or without probiotic supplementation. Black bars, untreated control (C; $n = 23$); gray bars, piglets receiving oral and abdominal rehydration therapy (OA; $n = 31$); white bars, piglets receiving oral and abdominal rehydration therapy (probiotics added to the oral solution; OAP; $n = 49$). (a) Base excess of blood; (b) bicarbonate ion (HCO$_3^-$) concentration in blood; (c) pH of blood. Bars with different letters are significantly different ($p < 0.05$) at each time point. Data are shown as the means ± standard errors.
3.2. Mortality of PED-Infected Piglets

When compared with that of control piglets (91%), mortality considerably ($p < 0.05$) decreased when OAP and OA were administered to piglets (41% and 55%, respectively) (Figure 3).

![Figure 3. Mortality of porcine epidemic diarrhea-infected piglets. Bars represent mortality of piglets. Black bar, untreated control ($C; n = 23$). Gray bar, piglets receiving oral and abdominal rehydration therapy only ($OA; n = 31$). White bar, piglets receiving oral and abdominal rehydration therapy and oral probiotic supplementation ($OAP; n = 49$). Bars with different letters are significantly different ($p < 0.05$).](image)

4. Discussion

As porcine epidemic diarrhea is a viral disease, prevention is the best tool to control it. Among the prevention strategies, vaccination is the most common and the gold standard [4]. Administration of an antibody against PED also seems effective to prevent PED virus infection [27,28]. In addition, we recently demonstrated that the combination of vaccination with supplementation of probiotics prevented PED infection more effectively than vaccination alone [21]. As treatments for PED infections are still limited, in the present study, our aim was to test whether a combination of RT and supplementation of probiotics could substantially reduce the mortality of suckling piglets kept in a farm and naturally infected with PED.

Diarrheic animals experience metabolic acidosis; hence, they are commonly treated with oral rehydration therapies [29]. In the present work, 94% (103/109 piglets) piglets delivered from naturally PED virus-infected sows at a commercial farm started to show PED clinical signs, e.g., diarrheic incidence, in the first 4 to 7 days of life. Severe dehydration [30] was confirmed after measuring blood parameters of piglets. For example, the mean base excess of all piglets was found to be $<-17$ mmol/L (Figure 2a), due to the fact that severe diarrhea was causing loss of $\text{HCO}_3^-$ (Figure 2b), as observed in the blood samples. Moreover, a significantly lower pH observed in blood (normal: 7.4 [31]) (Figure 2c) confirmed that PED virus-infected piglets were undergoing metabolic acidosis, as their body likely failed to generate sufficient $\text{HCO}_3^-$ [32] during the course of the disease.

The mean base and the $\text{HCO}_3^-$ and pH levels markedly improved in OAP piglets when compared with OA and C piglets, especially in the latter (Figure 2). The likely healing effects of probiotics may explain the improvement in blood parameters that was not detected in the other piglets. Probiotics have shown health-conferring properties when given to pigs, especially neonatal piglets, suffering from acute diarrhea [26,33]. Although it remains unclear the mechanisms by which probiotics exert such effects, evidence has shown that supplementation with probiotics seemingly inhibits pathogens by stimulating the immune system, competing for binding sites on the intestinal epithelial cells and producing bacteriocins [34,35]. The probiotic formulation used in the present study contained not only live bacteria but also digestive enzymes, such as cellulase, protease and pectinase. As sow milk contains neither cellulose nor pectin, it was very unlikely that cellulase or pectinase were involved in the effect observed in the OAP group. Nonetheless, sow milk
does contain protease inhibitors, especially during the first 7 days post farrowing [36], which would have prevented protease, contained in the probiotic formulation used in the present work, from being activated.

In addition to the improved blood parameters, compared with control piglets, mortality decreased in OAP and OA piglets. Although, no significance differences were found between groups OAP and OA, mortality of piglets also receiving the probiotics was the lowest. A possible explanation for this may lie in the fact that blood dehydration parameters improved the greatest in OAP piglets (Figure 2). The combination of probiotics and RT has been receiving increasing attention for the treatment of diarrheic diseases in humans [37]. For instance, Ali [37] demonstrated that infants given a combination of probiotics with RT fended off acute diarrhea more effectively, although this phenomenon was observed more markedly in children older than 1-year-olds, and less so in younger children. This difference in effectiveness may at least partly be due to differences in the developmental stages of the immune systems of infants, as the existing evidence shows that the effect of probiotics depends on the status of the immune system. Due to the fact that the porcine immune system develops intensively after 7 days post-birth, and especially during the suckling period, it is likely that differences in treatment effects between OA and OAP piglets would have been clearer had the treatment period lasted longer than 5 days [38].

A major limitation of the present study was the unbalanced number of piglets in the experimental groups. Due to the fact that personnel working in the experiment were limited to better control the PED epidemic, piglets were allocated to the experimental groups by randomization, using the die rolling method [39]. Hence, the number of piglets allocated to each group was unbalanced. The unbalanced number of piglets per group likely affected the robustness of statistical analyses to a certain degree, although it was confirmed that the actual statistical power at day 1 post-treatment was greater than 0.9.

5. Conclusions

When compared with those of untreated control piglets and piglets receiving only RT, piglets receiving a combination of probiotic supplementation and RT markedly showed improved blood parameters. Although mortality was the lowest in piglets receiving the probiotics in addition to the RT, the difference was non-significant when compared with piglets receiving only the rehydration therapy. The preliminary results reported in the present work should motivate further research on the use of a combination of RT with probiotics supplementation to reduce mortality in piglets suffering from acute diarrhea.

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**Conflicts of Interest:** T.I. is the president of a commercial animal hospital namely “Inatomi Animal Hospital”. Further, T.I. is employed by TOA Pharmaceutical Co., Ltd. Other authors have no conflicts of interest. T.T. and G.A.R.-P. are employees of Kyoto Institute of Nutrition & Pathology, Inc. R.I. declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
References

1. Sasaki, Y.; Alvarez, J.; Sekiguchi, S.; Sueyoshi, M.; Otake, S.; Perez, A. Epidemiological factors associated to spread of porcine epidemic diarrhea in Japan. *Prev. Vet. Med.* 2016, 123, 161–167. [CrossRef] [PubMed]

2. Suzuki, T.; Murakami, S.; Takahashi, O.; Kodera, A.; Masuda, T.; Itoh, S.; Miyazaki, A.; Ohashi, S.; Tsutsui, T. Molecular characterization of pig epidemic diarrhoea viruses isolated in Japan from 2013 to 2014. *Infect. Genet. Evol.* 2015, 36, 363–368. [CrossRef] [PubMed]

3. Murphy, F.; Gibbs, E.; Horzinek, M.; Studdert, M. Coronaviridae. In *Veterinary Virology*, 3rd ed.; Murphy, F., Gibbs, E., Horzinek, M., Studdert, M., Eds.; Academic Press: San Diego, CA, USA, 1999; pp. 495–508.

4. Pensaat, M.B.; de Bouch, P. A new coronavirus-like particle associated with diarrhea in swine. *Arch. Virol.* 1978, 58, 243–247. [CrossRef]

5. Jung, K.; Saif, L.J. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunophrophylaxis. *Vet. J.* 2015, 204, 134–143. [CrossRef]

6. Sueyoshi, M.; Tsuda, T.; Yamazaki, K.; Yoshida, K.; Nakazawa, M.; Sato, K.; Minami, T.; Iwashita, K.; Watanabe, M.; Suzuki, Y.; et al. An immunohistochemical investigation of porcine epidemic diarrhoea. *J. Comp. Pathol.* 1995, 113, 59–67. [CrossRef]

7. Shibata, I.; Tsuda, T.; Mori, M.; Ono, M.; Sueyoshi, M.; Uruno, K. Isolation of porcine epidemic diarrhoea virus in porcine cell cultures and experimental infection of pigs of different ages. *Vet. Microbiol.* 2000, 72, 173–182. [CrossRef]

8. Khin Maung, U.; Greenough, W.B., 3rd. Cereal-based oral rehydration therapy. I. Clinical studies. *J. Pediatr.* 1985, 109, 93–97. [CrossRef]

9. Lexmond, W.S.; Rufo, P.A.; Fiebiger, E.; Lencer, W.I. Electrophysiological studies into the safety of the anti-diarrheal drug clotrimazole during oral rehydration. *PLoS Negl. Trop. Dis.* 2015, 9, e0004098. [CrossRef]

10. Drolet, R.; Morin, M.; Fontaine, M. Fluid therapy trials in neonatal piglets infected with transmissible gastroenteritis virus. *Can. J. Comp. Med.* 1985, 49, 357–360.

11. Silanikove, N. Effects of oral, intraperitoneal and intrajugular rehydrations on water retention, rumen volume, kidney function and thirst satiation in goats. *Comp. Biochem. Physiol. A Comp. Physiol.* 1991, 98, 253–258. [CrossRef]

12. Silanikove, N. Comparison among the effects of oral, intraperitoneal and intravenous fluid-loading on kidney function and drinking in goats. *Comp. Biochem. Physiol. Part A Physiol.* 1994, 109, 749–754. [CrossRef]

13. Basu, S.; Paul, D.K.; Ganguly, S.; Chatterjee, M.; Chandra, P.K. Efficacy of high-dose Lactobacillus rhamnosus GG in controlling with salmonella and rotavirus gastroenteritis. *J. Clin. Gastroenterol.* 2014, 58, 135–138. [CrossRef] [PubMed]

14. Jung, K.; Saif, L.J. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. *Vet. J.* 2015, 204, 134–143. [CrossRef]

15. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 2014, 11, 506–514. [CrossRef]

16. Szajewska, H.; Mrukowicz, J.Z. Probiotics in the treatment and prevention of acute infectious diarrhea in infants and children: A systematic review of published randomized, double-blind, placebo-controlled trials. *J. Pediatr. Gastroenterol. Nutr.* 2001, 33 (Suppl. 2), S17–S25. [CrossRef]

17. Kandasamy, S.; Chattaha, K.S.; Vlasova, A.N.; Rajashekar, G.; Saif, L.J. Lactobacilli and Bifidobacteria enhance mucosal B cell responses and differentially modulate systemic antibody responses to an oral human rotavirus vaccine in a neonatal gnotobiotic pig model. *Gut Microbes* 2014, 5, 639–651. [CrossRef]

18. Tsukahara, T.; Nakanishi, N.; Matsubara, N.; Itoh, M.; Ushida, K. The effect of Enterococcus faecalis cell preparation (EC-12) against the diarrhea in the nursing and weaning piglets under the clinical condition. *Proc. Ipn. Pig Vet. Soc.* 2006, 48, 19–23.

19. Tsuruta, T.; Inoue, R.; Tsukahara, T.; Matsubara, N.; Hamasaki, M.; Ushida, K. A cell preparation of Enterococcus faecalis strain EC-12 stimulates the luminal immunoglobulin A secretion in juvenile calves. *Anim. Sci. J.* 2009, 80, 206–211. [CrossRef]

20. Sakai, Y.; Tsukahara, T.; Bukawa, W.; Matsubara, N.; Ushida, K. Cell preparation of Enterococcus faecalis strain EC-12 prevents vancomycin-resistant enterococci colonization in the cecum of newly hatched chicks. *Poult. Sci.* 2006, 85, 273–277. [CrossRef]

21. Inatomi, T.; Amatatsu, M.; Romero-Perez, G.A.; Inoue, R.; Tsukahara, T. Dietary probiotic compound improves reproductive performance of porcine epidemic diarrhea virus-infected sows reared in a Japanese commercial swine farm under vaccine control condition. *Front. Immunol.* 2018, 8, 1877. [CrossRef]

22. Tsukahara, T.; Inatomi, T.; Otomaru, K.; Amatatsu, M.; Romero-Perez, G.A.; Inoue, R. Probiotic supplementation improves reproductive performance of unvaccinated farmed sows infected with porcine epidemic diarrhea virus. *Anim. Sci. J.* 2018, 89, 1144–1151. [CrossRef] [PubMed]

23. Chen, C.C.; Kong, M.S.; Lai, M.W.; Chao, H.C.; Chang, K.W.; Chen, S.Y.; Huang, Y.C.; Chiu, C.H.; Li, W.C.; Lin, P.Y.; et al. Probiotics have clinical, microbiologic, and immunologic efficacy in acute infectious diarrhea. *Pediatr. Infect. Dis. J.* 2010, 29, 135–138. [CrossRef] [PubMed]

24. Huang, Y.F.; Liu, P.Y.; Chen, Y.Y.; Nong, B.R.; Huang, I.F.; Hsieh, K.S.; Chen, K.T. Three-combination probiotics therapy in children with salmonella and rotavirus gastroenteritis. *J. Clin. Gastroenterol.* 2014, 48, 37–42. [CrossRef] [PubMed]

25. Fukuda, T.; Otsuka, M.; Nishi, K.; Nishi, Y.; Tsukano, K.; Noda, J.; Higuchi, H.; Suzuki, K. Evaluation of probiotic therapy for calf diarrhea with serum diamine oxidase activity as an indicator. *Jpn. J. Vet. Res.* 2019, 67, 305–311. [CrossRef]
26. Hayakawa, T.; Masuda, T.; Kurosawa, D.; Tsukahara, T. Dietary administration of probiotics to sows and/or their neonates improves the reproductive performance, incidence of post-weaning diarrhea and histopathological parameters in the intestine of weaned piglets. *Anim. Sci. J.* **2016**, *11*, 1058–1065. [CrossRef]

27. Kweon, C.H.; Kwon, B.J.; Woo, S.R.; Kim, J.M.; Woo, G.H.; Son, D.H.; Hur, W.; Lee, Y.S. Immunoprophylactic effect of chicken egg yolk immunoglobulin (Ig Y) against porcine epidemic diarrhea virus (PEDV) in piglets. *J. Vet. Med. Sci.* **2000**, *62*, 961–964. [CrossRef]

28. Shibata, I.; Ono, M.; Mori, M. Passive protection against porcine epidemic diarrhea (PED) virus in piglets by colostrum from immunized cows. *J. Vet. Med. Sci.* **2001**, *63*, 655–658. [CrossRef]

29. Schwedhelm, L.; Kirchner, D.; Klaus, B.; Bachmann, L. Experimentally induced hyperchloremic and DL-lactic acidosis in calves: An attempt to study the effects of oral rehydration on acid-base status. *J. Dairy Sci.* **2013**, *96*, 2464–2475. [CrossRef]

30. Shaoul, R.; Okev, N.; Tamir, A.; Lanir, A.; Jaffe, M. Value of laboratory studies in assessment of dehydration in children. *Ann. Clin. Biochem.* **2004**, *41*, 192–196. [CrossRef]

31. Martini, W.Z.; Pusateri, A.E.; Usolowicz, J.M.; Delgado, A.V.; Holcomb, J.B. Independent contributions of hypothermia and acidosis to coagulopathy in swine. *J. Trauma Acute Care Surg.* **2005**, *58*, 1002–1010. [CrossRef]

32. Cieza, J.A.; Hinostroza, J.; Huapaya, J.A.; León, C.P. Sodium chloride 0.9% versus Lactated Ringer in the management of severely dehydrated patients with choleriform diarrhoea. *J. Infect. Dev. Ctries.* **2013**, *7*, 528–532. [CrossRef]

33. Schroeder, B.; Duncker, S.; Barth, S.; Bauerfeind, R.; Gruber, A.D.; Deppenmeier, S.; Breves, G. Preventive effects of the probiotic Escherichia coli strain Nissle 1917 on acute secretory diarrhea in a pig model of intestinal infection. *Dig. Dis. Sci.* **2006**, *51*, 724–731. [CrossRef]

34. Bernardreau, M.; Lehtinen, M.J.; Forststen, S.D.; Nurminen, P. Importance of the gastrointestinal life cycle of Bacillus for probiotic functionality. *J. Food Sci. Technol.* **2017**, *54*, 2570–2584. [CrossRef]

35. Yang, F.; Hou, C.; Zeng, X.; Qiao, S. The use of lactic Acid bacteria as a probiotic in Swine diets. *Pathogens* **2015**, *4*, 34–45. [CrossRef]

36. Weström, B.R.; Svendsen, J.; Karlsson, B.W. Protease inhibitor levels in porcine mammary secretions. *Biol. Neonate* **1982**, *42*, 185–194. [CrossRef]

37. Ali, R. The Use of Probiotic with ORS and ORS Only in Children with Acute Diarrhea. *J. Coll. Physicians Surg. Pak.* **2019**, *29*, 1179–1182. [CrossRef]

38. Inoue, R.; Tsukahara, T.; Nakatani, M.; Okutani, M.; Nishibayashi, R.; Ogawa, S.; Harayama, T.; Nagino, T.; Hatanaka, H.; Fukuta, K.; et al. Weaning Markedly Affects Transcriptome Profiles and Peyer’s Patch Development in Piglet Ileum. *Front. Immunol.* **2015**, *6*, 630. [CrossRef]

39. Suresh, K.P. An overview of randomization techniques: An unbiased assessment of outcome in clinical research. *J. Hum. Reprod. Sci.* **2011**, *4*, 8–11. [CrossRef]