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Collection conditions and processing of pig blood plasma to improve the growth performance and immune response of piglets

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Abstract

Our research on using plasma as a supplementary food source in livestock and aquaculture was conducted with the utmost rigour. Swine blood samples were collected from abattoirs and meticulously analysed for protein compositions and other factors. 180 samples were collected and analysed for Albumin, Globulin, Hemoglobin, and immune responses. The study also determined the influence of NaCl, pH, and citrate phosphate on Iron Fe++ for processing the plasma to store and apply for animal feed supplements. The results, which were obtained through a comprehensive and systematic process, show that NaCl, pH, citrate and phosphate were all the optimal anticoagulation times and temperatures, ultrasonic violet ray (UV) for blood to make plasma and mean differences, relative difference (%) and 95% confidence interval for the mean difference are given for each value. Using citrate and phosphate buffers for conservation and storing the plasma in power also helped Fe to remain well. Temperature and ultraviolet irradiation influenced spray dry plasma's storage and quality (SDP). Suckling piglet diets contained 2% SDP to improve immune response to resistance by IgM, $P < 0.05$ from 14 – 21 days old.

Keywords: Spray Dry plasma, anticoagulants, Ultrasonic Violet, blood draw and processing, and immune responses

Introduction

Plasma is considered a supplementary food source in livestock and aquaculture. The effectiveness of plasma has been confirmed by leading scientists, who have shown that for many years, pig farmers have struggled with pigs being treated for diseases immediately after weaning, leading to the animal's ability to handle stress as pets, Anna Pérez-Bosque et al. (2016); Bosi P., L. et al. (2004); Chuchird, N. et al. (2021); Elena Blázquez et al. (2019). Premature separation of milk from herds can easily cause diseases and negatively affect the health of pigs. APC worked with researchers at Iowa State University, USA, to create It's OK. That's right; I'm sorry; the result is that weaned pigs fed fresh plasma in their diets from the weaning stage are less sick, eat more and grow better than the group fed feed with similar diets but no FP (frozen plasma), Anna Pérez-Bosque, et al. 2016), Balan, P., et al. (2021); Bosi P. et al. (2004); Campbell, J.M. et al. (2019). FP is so effective that most export pig weaning diets contain the function. That's right, it's okay, but I don't know what to do. It has been documented and extended to other feed rations, such as finishing pigs and sows. If you don't know what to do, don't. 'Do not worry about that. Let's talk about Vietnam; I know a lot about it. Large livestock and shrimp farming potential in the Southern and Western provinces. That is where a lot of food is produced that does not meet domestic demand but is still exported. However, the industry wants to have exported products. It is required to ensure safety and no antibiotics to proceed with the Food and Agriculture Organization (FAO) to reduce and eventually end the phenomenon of antibiotic resistance in products. aquaculture products (Aquaculture) and livestock, Gatnau R. (1990); Guo, J. et al. (2021); Hamburger M.K.H. (1991); Harborne J.B. (1973). The situation in shrimp farming and piglet farming that contains antibiotics is unavoidable, so replacing antibiotics with herbal products or using antagonistic microorganisms (VSV) also responds to a certain extent. determined. Contributing to effective farming technology, we research plasma spray drying technology to make supplementary food for shrimp. Knowing that plasma plays a unique role in the body's growth and development, immune function includes and complements involved in the body's protection process, increases phagocytosis, and specific reactions to antigens, bacteria, and viruses. Maintaining colloidal pressure - blood viscosity: due to albumin, while shrimp blood pigmentation is entirely different from land animal blood; 2) Blood clotting function: includes anticoagulants and anticoagulants (antithrombin III, protein C, protein S); 3) transporting substances: transporting nutrients to the organisation and transporting waste products excreted through the kidneys, lungs, sweat, and digestion such as Transferrin transports iron, transcobalamin transports b12, and haptoglobin transports haemoglobin element of freedom. Transport proteins are necessary for cell synthesis and organisation. Hormones and cytokines exist in tiny amounts but are essential in all body activities. Plasma plays a vital role in the body, so when there is a deficiency of a specific component in plasma or loss of plasma, it causes disorders in the body. Our research aims to collect blood from pigs after the slaughter at the slaughterhouse; 2) develop a collection process to ensure safety for people and the environment and supplement the health of pigs. Also, the study aims to discover the influence of SDP on the immune response to the resistance of piglets.

Materials and methods

Blood was collected using slaughterhouse procedures and factors affecting plasma biosafety. A mixture of raw blood from pigs was collected to maintain neutralizing antibodies, and the blood was sterilized to ensure the biosafety of the final product. 216 samples were surveyed at different times at the slaughterhouse to collect plasma from pigs within 5 minutes to collect blood for determination of platelet count (PLT), mean platelet volume (MPV) and platelet fraction (PCT) based on anticoagulants.

Blood collection process at slaughterhouses

Pre-slaughter inspection: Requirements for slaughter control and veterinary hygiene inspection of pig slaughterhouses under Circular No. 08/VBHN-BNNPTNT dated October 5, 2022. Hiring Animal staff Check the process before slaughtering pigs using the following steps. Measurements five minutes after the blood draw are used as a reference. The mean difference, relative difference (%) and 95% confidence interval for the mean difference are given for each value. Collect records and books to record the origin of animals brought to slaughter at the slaughterhouse. Collect records on implementing hygiene regulations for slaughter participants and protective clothing while working. Only collect animal samples that must be healthy and ensure veterinary hygiene requirements. For animals that are injured, exhausted due to transportation, or unable to recover but do not have clinical symptoms of infectious diseases, slaughter is allowed first; clean and kept in the slaughter waiting area to ensure that cattle return to their normal state and have been clinically examined before slaughter; there are clear corners.

Experiments on trial for plasma as supplementation to suckling piglets

Plasma powder was used as a supplement for piglets and as creep feed after 07 days of farrowing; growth performance and immune response were also examined by blood sampling from piglets. Therefore, a new alternative matrix is needed to assess the early immune status of piglets effectively. The present study aimed to evaluate the usefulness of the treatment liquid for determining the immune parameters of selected piglets on 30 suckling piglets, 15 females and 15 males, in each group of 6 individuals, control and test-1 with 0.5; test-2, 1; test-3, 2%. Piglets from commercial farrowing to weaning; 30 serum samples were collected. Serum was collected from all piglets. ELISA assays evaluated the concentrations of various immunoglobulins, cytokines and acute phase proteins in each matrix under the supplementation of 0.5-2% DSP mixed with creep feed for suckling piglets from 7-21 days after birth. Statistical analysis was used to determine the differences in measured concentrations of the indicators between piglet serum and treatment fluid and the correlations in tested concentrations of the indicators between specific matrices.

Implementation and plan for collection

Method to develop blood collection process at sample slaughter facility:

Step 1. Blood is collected in a stainless steel pan with sodium citrate or sodium tripolyphosphate, anticoagulants commonly used to produce SDP, added to prevent blood clotting. Sodium citrate is expected to be used at 0.2 - 0.4% in fresh whole blood. Blood is mainly composed of $\geq$ 80% water. Therefore, spray-dried plasma powder is expected to contain no more than 0.1 % sodium citrate.

- Iron removal by complexation with citrate and phosphate buffer solution. Next to the pigs, the blood fluid is boiled, filtered and dried to obtain blood powder. Finished blood powder products were evaluated according to physicochemical and microbiological standards: protein content, iron content, carbohydrate content, total microorganisms, mould, etc., carried out in a laboratory at the University of Agriculture and Forestry, University of Hue.

Step 2. About 4-5% of the live animal's weight is collectable blood, which contains about 10% animal protein. When fresh blood is extracted from animals, fibrinogen is converted into fibrin. Fibrin catalyses the formation of a fibrous network that encases blood cells and other blood components into a clot. Sodium citrate with a 0.2 - 0.4% concentration in fresh whole blood should be stirred well to prevent blood clotting.

Step 3. Raw blood from the steel pans is put into an enormous container, stored in a refrigerated truck at 2 - 4 ºC, and brought to the factory for production.

Step 4. Each batch collected from slaughterhouses is 200 - 400 litres of raw blood. Crude blood is collected to produce a sufficient volume of product.

Step 5. After being anticoagulated with NaCl, the blood continues to be adjusted to the appropriate pH to precipitate and determine blood meal moisture content

Determined by drying method at 105 0C to constant weight in multi-function drying oven SHELLAB, Model: CE3F-2 (TCVN 4326:2001).

Step 6. The SST-10-UV-A130-G385-00 Luminus Device and drying machine were installed in a room of the research section, Faculty of Fisheries, University of Agriculture and Forestry, Hue University of Vietnam.

Data processing methods

Blood collection batches were repeated 3 times. Results are processed on STATGRAPHICS Centurion software. Statistical difference when P-value < 0.05.

Results and Discussions

Effect of NaCl, hirudin, and citrate concentration on blood clotting time

Table 1. Influence of the time between blood draw for collection and (PLT, *10^9/L) Platelet count, (MPV, fL) mean platelet volume, plateletcrit (PCT, %) measurement for each anticoagulant

	5 min	30 min (vs. 5 min)		60 min (vs. 5 min)		120 min (vs. 5 min)		180 min (vs. 5 min)	
(PLT, *10^9/L) platelet count									
EDTA	0	487 ± 112	***	252 ± 93	***	206 ± 55	***	106 ± 76	***
Hirudin	0	502 ± 85	***	290 ± 112	***	306 ± 56	***	206 ± 67	***
Citrate	0	526 ± 91	***	313 ± 122	***	322 ± 77	***	256 ± 66	***
(MPV, fL) mean platelet volume									
EDTA		9.71 ±0.82	***	9.51 ±0.71	***	9.55 ±0.72	***	9.56 ±0.67	***
Hirudin		8.77 ±0.99	***	8.71 ±0.77	***	8.23 ±0.87	***	7.78 ±0.87	***
Citrate		6.74 ±0.91	***	7.21 ±0.92	***	6.79 ±0.88	***	6.34 ±0.56	***
(PCT, %) plateletcrit									
EDTA		0.21±0.05	***	0.21±0.05	***	0.21±0.05	***	0.21±0.05	***
Hirudin		0.15± 0.03	***	0.15± 0.03	***	0.15± 0.03	***	0.15± 0.03	***
Citrate		0.39±0.09	***	0.39±0.09	***	0.39±0.09	***	0.39±0.09	***

$p < 0.05$ (*), < 0.01(**), < 0.001 (***). EDTA: ethylenediaminetetraacetic acid.

When increasing the NaCl concentration from 1 to 3% (g/100ml of blood), the anticoagulation time increased, but after that, there was no difference between the levels of 3%, 4% and 5%. As the NaCl concentration increases, the polarity of the solvent increases, so the interaction between the polar groups of the protein and the solvent increases, so the protein is stabilized.

Our research results are also consistent with the publication of Cottingim K.M. et al. (2017); stating that 0.9% NaCl solution will reduce fibrin's gelation (coagulation) in plasma. We chose a 4% NaCl concentration for the anticoagulation process from the above results. The study's results showed that the survival rate of shrimp fed with spray-dried plasma (SDP) at the end of experiments 1 and 2 was significantly higher than that of shrimp in the control group, Chuchird N. et al., (2021); Duffy M.A. et al. (2018). Blood was collected in potassium-ethylenediamine tetra-acid (EDTA) and sodium-citrate tubes. Measurements five minutes after the blood draw are used as a reference. The mean difference, relative difference (%) and 95% confidence interval for the mean difference are given for each value. Shrimp feed supplemented with SDP from pigs at 3 to 6% of the diet can improve growth performance, survival rate, feed utilization, immune response and reduce mortality. when infected with E.coli. The dose of SDP was effective in improving the overall health status of Pacific white shrimp, and this study was consistent with what was reported in previous studies on pigs (5 to 8% SDP). Various immune parameters (haemoglobin count and phagocytic activities, phenoloxidase and superoxide dismutase) of shrimp fed 3 to 6% SDP also showed significant improvement compared to the control group, Zhang, Y. et al. (2016) and Zimmerman D.R. (1987).

Table 2. Mean differences in MEA results at the five-time points (30 min is the reference) for both activators and under the three anticoagulant conditions.

		5 min (vs. 30 min)	**60 min (vs. 30 min)**	**120 min (vs. 30 min)**	**180 min (vs. 30 min)**
Phosphate	Citrate + NaCl	−9** (−19.9%) (−15; −3)	1 (3.0%) (−5; 7)	−0.2 (0.1%) (−6; 6)	−9 ** (−17.1%) (−15; −3)
	Citrate + $CaCl_2$	−24 *** (−35.5%) (−30; −18)	1 (2.6%) (−5; 7)	4 (7.5%) (−2; 10)	−2 (1.0%) (−8; 4)
	Hirudin	−7* (−10.0%) (−14; −1)	4 (7.0%) (−2; 10)	7 (11.6%)* (1; 13)	6 (10.0%) (−0.01; 12)

$p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).
MEA values are expressed in arbitrary units. The mean difference, relative difference (%) and 95% confidence interval for the mean difference are given for each value.

When the environmental pH equals its isoelectric point (pI), a protein with a corresponding isoelectric point value will lose its charge, leading to coagulation. The main protein components in the blood include three proteins: Albumin, Globulin, and Hemoglobin, with corresponding pH values of 4.9, 5.4 and 6.8. Elena Blázquez et al. (2019), so we investigated the pH range from 4 to 7. At pH = 4.5, the amount of sediment obtained is relatively high; At pH = 5.5, it is lower; at pH 6.5, the amount of residue obtained is the largest, then decreases ultimately at pH = 7.5. The pH value of 4.5 is close to the isoelectric point of albumin, so the blood precipitate at pH = 4.5 is mainly albumin. Albumin accounts for about 60% of total plasma protein, and plasma accounts for 60% of blood, so the amount of residue obtained is relatively high. At pH = 5.5, near the isoelectric point of globulin, the precipitate volume here is mainly globulin precipitate. Globulin is about 35% of total plasma protein, so the precipitate mass is lower. At pH = 6.5 near the isoelectric point of haemoglobin, the precipitate mass here is mainly

haemoglobin and accounts for 96% of the visible and 40% of the blood's tangible substances, so at pH = 6.5, the precipitate volume is the largest, El-Sayed A.F.M. (1998); Gatnau R (1990); Henrichs B.S. et al. 2019; Torrallardona D. (2010); Zimmerman D.R. (1987). The precipitate mass is low at pH 7.5, far from the isoelectric point of blood proteins. The above results show that the pH value = 6.5 is suitable for collecting coagulated blood products, Ejikeme C.M., and Eboatu A.N. (2014); El-Sayed A.F.M. (1998); Elena Blázquez et al. (2020); Elena Blázquez (2019); Elena Blázquez et al. (2021). Citrate buffer was prepared from two solutions of 0.2 M disodium citrate and 0.2 M monosodium citrate at pH value = 6.5. Research results show that the remaining iron content in blood powder is lowest when the citrate buffer added to the blood fluid is 10:90 (10% volume). Figure 1 shows that when citrate buffer is not added, the iron in the blood powder obtained is relatively high (0.14%). Still, when the amount of citrate buffer is 10%, the iron remaining in the blood powder is only 0.057%. The significant decrease in iron content is due to forming a soluble complex between iron and citrate ion, which is removed at the precipitation filtration stage. This theory confirmed the suggestion that citrate can bind with Fe (II) at the aconitase active site and form a low molecular weight form of cytosolic iron, Ellis A. E. (1990) and Ezeonu C.S. & Ejikeme C.M. (2016). However, the complex formation mechanism between Fe (III) and citrate is quite complex and inconsistent in the literature. Still, most studies suggest that when pH is low, the molar ratio between Fe (III) and citrate is greater than 1:6. However when the citrate content increased from 10 to 12%, there was no statistically significant difference between the samples (P-value > 0.05). Based on the results of this study, we chose a citrate buffer content of 10% to remove iron from the blood. Fe remains in the mixed plasma, and phosphate buffer is on the remaining iron content.

Table 3. Mean differences in MEA results between the anticoagulants at the five-time points. MEA values are expressed phosphate and citrate

		5 min	30 min	60 min	120 min	180 min
Phosphate	Citrate + $CaCl_2$ (vs. Hirudin)	−20*** (−30.3%) (−26; −14)	−4 (−3.0%) (−10; 2)	−7 * (−6.3%) (−13; −1)	−6* (−6.7%) (−12; −0.4)	−11*** (−8.3%) (−17; −5)
	Citrate + $CaCl_2$ (vs. + NaCl)	3 (12.0%) (−3; 9)	19*** (41.5%) (13; 25)	19*** (41.9%) (13; 25)	23*** (52.1%) (17; 29)	26*** (76.7%) (20; 32)
	Citrate + NaCl (vs. Hirudin)	−24*** (35.5%) (−30; −18)	−22*** (−29.2%) (−28; −16)	−26*** (−32.3%) (−32; −20)	−29*** (−36.0%) (−36; −23)	−37*** (−44.6%) (−43; −31)

$p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

For each value, the mean difference, relative difference (%), and 95% confidence interval for the mean difference are given.

Total 40% in globulin including α-globulin, β-globulin and γ-globulin, control without plasma 3.90 ± 0.6***, 0.80 ±·0.2; *1.30 ± 0.4; 0.95 ± -0.2; 0.90 ± 0.2; 120 ± 25**; 9.6 ± 29; 62 ± 219*; test 1 (0.5% plasma), 5.50 ± 0.3 2.14 ± 0.6 1.20 ± -0.3 1.05 ± 0.1 1.15 ± 0.1; test 2 (plasma 1%), 4.90±0.9* 1.95±0.7**; 1.10±-0.3; 0.80±0.2; 1.00±0.2; 96±24; 106±26; 86±34** (negative autopsy) test 3 (plasma 2%) 5.90±0.5; 2.90±0.2; 1.10±0.1; 0.95±0.2; 1.00±0.1; 78±18; 118±13; 134 ± 17. Using 10% citrate buffer removed iron from pig blood most effectively (remaining 0.057%, less than 0.083% corresponding to 12% phosphate buffer). Citrate is also a

preservative, inhibiting the deterioration of blood powder, so the remaining buffer after complexation will be suitable for later preservation. Figure 1 shows that the volume of the precipitate increases gradually with heating time, but at the 45th and 60th minutes, the volume of the precipitate increases slightly (the volume difference is insignificant); the exact amount of residue obtained is 23, conducted on 300 ml of pig blood 49 and 24.75 g (d = 1.05 g/ml). The results of testing the filtrate with TCA (trichloroacetic) showed that the protein in the blood precipitated almost wholly (the color of the TCA test solution of these 03 samples was almost the same). The amount of residue obtained increases as the heating time increases because the temperature breaks down the hydrate layer surrounding the protein molecules.

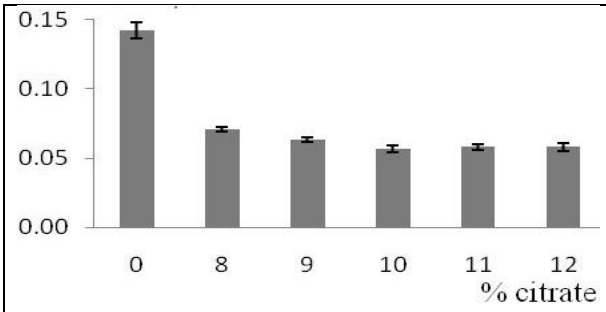
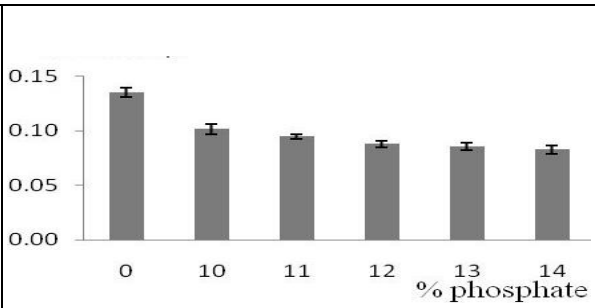


Figure 1. Effect of citrate buffer on Iron% remaining in plasma powder



F i g u r e 2 . Effect of phosphate buffer in iron remaining in blood power

The purpose of this research is to examine the ability of iron to form complexes with phosphate buffer, thereby comparing with the results of complexation with citrate buffer. Phosphate buffer is mixed with pH = 6.5. Figure 2 shows that phosphate buffer also forms soluble complexes with iron because phosphate ions form complexes with Fe_{2+} and Fe_{3+} ions, which are then removed during filtration to collect the residue. The above results show that the lowest remaining iron content is 0.083%, corresponding to a buffer content of 14%, and there is no significant difference at phosphate concentrations of 12, 13 or 14%. From the above research results, we chose the iron removal method using citrate buffer because Shen H.G. et al. (2011), Torrallardona D. (2010), and Touchette K.J. et al. (2001). Table 5 shows the piglet serum analyses by immune responses to different bacteria (*S. aureus, P. aeruginosa, E. coli, S typhimurium and K. pneumoniae* and values significantly differing from normals are noticed as mean and SD. The strength of the hydrophobic interaction is highest when the temperature is 60-70^{0}C, He S. et al. (2015); Henrichs B.S. et al. (2021); Hussain S.M. et al. (2011). Therefore, prolonged heating at 105^{o}C causes the protein to denature and clump. From the above results, we choose a cooking time of 45 minutes.

Table 4. Effect of drying temperature, drying time and UV ray on plasma humidity function

Drying time (hours)	Temperature (^{O}C)		
	35 (5/10/15)	40 (5/10/15)	45 (5/10/15)
5	46.37 ± 1.08	40.70** ± 1.47	35.42*** ± 1.67
10	36.02 ± 1.13	30.32** ± 2.51	24.51*** ± 1.76
15	29.17 ± 1.94	25.14** ± 1.96	18.73*** ± 0.74

****P < 0.001; ** P <0.01 with different significantly*

Investigate the effects of temperature and drying time on the moisture content of blood meal. Table 5 shows that temperature and UV drying time are inversely proportional. When drying at low temperatures, the time the raw materials are exposed to air is longer. When drying at

temperatures between 35-40oC, it will be favourable for microorganisms to grow, which will therefore be unfavourable and hurt the quality. amount of finished blood powder. In addition, when drying blood powder in the temperature range from 0 to 45oC, the protein is not denatured, but in the temperature range from 45 to 75^{o}C, most proteins lose their colloidal properties and are irreversibly denatured, especially at high temperatures. temperature above 65^{o}C, and this denaturation process causes the protein to lose solubility, Polo J. Et al. (2015). The above reasons show that a drying temperature of 45^{o}C is appropriate, and a drying time of 15 hours will result in blood meal reaching a moisture content of 18%. Test of iron-depleted blood meal on mice compared to food without blood meal supplementation and food supplemented with blood meal from another manufacturer, Muller, L.K.F, et al., (2017); Polo, J., Rodríguez C. et al. (2015); Quigley J.D. et al. (2002); and Russell L. (2000). In general, mice were fed the necessary amount of food, so they all showed signs of weight gain; however, due to differences in food ingredients, the level of weight gain was different. Table 2 shows that the weight gain of mice in all experimental samples is always higher than in the negative control group. The experimental group is still higher than the positive control group at the same concentration. Mice using food supplemented with iron-separated blood meal provided more protein and reduced the fishy smell of iron, so mice liked to eat more and absorbed better. Solve the disadvantages mentioned, Jiravanichpaisal P. et al. (2006); Junkunlo K. et al. (2012). At 5% supplemented blood meal concentration, mice had the highest weight gain of 11.23g. This shows that the best blood meal concentration supplement is 5%. The above reasons show that foods supplemented with iron-type blood meals are better than those without added blood meals and better than those supplemented with non-iron blood meals, and the amount of iron-type blood meal supplements is appropriate. at least 5%.

Table 5. Protein, Albumin, Globulin và Fibrinogen in pig fresh blood

No	(n)	Protein g/L		Albumin, g/L		Globulin, g/L		Fibrinogen, g/L	
		M ± m	%	M ± m	%	M ± m	%	M ± m	%
1	2	63.22 ± 7.33	63	37.74 ± 5.13	38	25.33 ± 7.12	25	14.4 ± 2.26	14
2	12	64.45 ± 6.25	64	36.23 ± 8.25	36	28.22 ± 6.26	28	13.98 ± 1.98	14
3	12	65.54 ± 5.28	66	38.12 ± 6.15	38	27.42 ± 8.43	27	13.21 ± 2.34	13

****P < 0.001; ** P <0.01 with different significantly*

Protein plays an essential role in building all cells and tissues. Protein is vital for body growth, development and health protection. They are a structural component of most parts of the body. They are also enzymes and hormones that regulate the body's activities. This test measures total amounts of all types. Protein is in the plasma in your blood, and two main types of Protein, Albumin and Globulin, are found in the blood**,** Kirimi J.G. et al. (2016) and Lee C. et al. (2018). Albumin is a protein containing many amino acids and has a small molecular weight; its primary role is to keep fluid (water) from leaking out of blood vessels through osmotic pressure. Globulin is a class of proteins that includes enzymes, antibodies, and more than 500 other proteins. The Albumin/Globulin ratio (A/G ratio) was calculated from values obtained by directly measuring Total Protein and Albumin. The ratio represents the relative amounts of Albumin and Globulin. Plasma fibrinogen participates in the final stages of this process, converting it to insoluble fibrin. Table 5 shows that the protein content (Albumin and Globulin) is at a typical ratio in pig blood. The leading manufacturer of spray-dried plasma recently introduced a new technology based on ultraviolet (UV) irradiation of liquid plasma before condensation and spray drying, which will be considered a safe step. redundant. UV irradiation of liquid plasma has been shown not to affect the function of SDP when supplemented in suckling piglet diets. UV irradiation is a recognized technology capable of inactivating various germs. The disease includes a variety of bacteria and infections, including porcine parvovirus,

a model virus that is highly resistant to heat and chemicals reported by Muller L.K.F. et al. (2017); Polo J. Rodríguez C. et al. (2015); Quigley J.D. et al. (2002); and Russell L. (2000). While, the experiments were carried with tested on different bacteria, as *S. aureus. P.aeruninosa, E.coli, S. typhinurium and K. pneunomiae* showed in Table 6. The differences in the mean $\log_{10}$ reductions between each of the bacteria in one pair and each of those in the other pair were almost all the time statistically significant (Table 2): only at position 1, at 2 m directly under a lamp (Fig. 3), was the mean $\log_{10}$ reduction of *S. typhimurium* not significantly different from that of *S. aureus* and *P. aeruginosa* (ANOVA, $p > 0.05$. *Escherichia coli* showed average $\log_{10}$ reductions comparable to those of *S. aureus* and *P. aeruginosa* at position 1, and intermediate values at positions 2 and 3, which was statistically different from S. *aureus, P. aeruginosa* and *S. typhimurium* at position 3 and from K. pneumoniae at position 1. No statistically significant differences were found in all comparisons with the other bacteria at position 2 for *E. coli* (ANOVA, $p>0.05$). It can also be noted that the two lowest log10 reductions are for *K. pneumoniae* in position 2 (0.80) and S. typhimurium in position 3 (0.66). Their 95% CIs are very large, with lower bounds very close to 0. Although these confidence intervals may decrease as the number of replicates increases, this could also indicate that too low log10 reduction mean values tend not to be statistically significant. The highest microbial reduction rates were observed for all bacterial species at an irradiance value of 957 µW/cm^2 (position 1). The lowest $\log_{10}$ reduction was observed for 4 out of 5 bacterial species at an irradiance of 477 µW/cm^2 (position 2). For *S. typhimurium*, the lowest bacterial reduction was observed at an irradiance of 497 µW/cm^2 (position 3). We observed a constant decrease in airborne microorganisms from the different trail tests after 12 hours of exposure. We also observed that consecutive exposure to the ceiling lights for more days reduced environmental contamination. The average bacterial count at T0 (1 h after start) over the five days of the experiment was 61.8 CFU/m^3. In contrast, the average value collected at T1 (12 h later) over the five days was 13 CFU/m^3.

et al. (2002); and Russell L. (2000).

Table 6. Microbes $\log_{10}$ reductio after 15h exposure to UV with BL405nm

No	*S. aureus*		*P. aeruginosa*		*E. coli*		*S.typhimurium*		*K.pneumonia*	
	M	95%	M	95	M	95%	M	95%	M	95%
1	3.98	3.78-4.17	3.86	3.23-4.49	3.84	3.17–4.50	2.93	2.45–3.41	2.31	2.15–2.46
2	2.84	2.77-2.91	2.53	2.12–2.94	1.76	1.30–2.23	0.89	0.77–1.02	0.80	0.01–1.58
3	3.49	3.33-3.65	3.79	3.11–4.48	2.10	1.77–2.43	0.66	0.01–1.31	3.49	

M = Mean; 90% Confidence interval (CI)

Evaluate the energy value and microbiological criteria of the product.

The following comments are made using the table comparing the above content. indicators: Carbohydrate content is below the detection threshold due to sugar being removed during the protein precipitation filtration stage. The protein content in the blood meal sample and lipid content were 78.3% and 0.4%, respectively. This high protein content is suitable. for adding to animal feed to increase the protein content of the feed.

The following comments are made through the table comparing the content of the above indicators: Carbohydrate content is below the detection threshold due to sugar being removed during the protein precipitation filtration stage. The protein content in the blood meal sample

and lipid content were 78.3% and 0.4%, respectively. This high protein content is very suitable for adding to animal feed to increase the protein content of the feed.

Table 7. Variables of plasma in the trails, n = 6

Variables	Experimental plasma	Commercial plasma
Carbohydrate (g/100 g)	ND	ND
Protein (g/100 g)	78.3 ± 5.34	72.43 ± 6.87
Lipid (g/100 g)	0.4 ± 0.02	0.42 ± 0.05
E.coli (cfu/g)	10 ± 2.21	12 ± 2.26
Salmonella (25g)	ND	ND
Fe (mg/kg)	1672 ± 142	1567 ± 145

Protein, antibody and coagulation ratio

A recent study showed that early-weaned piglets fed diets without ZnO and AGP supplementation were protected from intestinal health problems, Kirimi J.G. et al. (2016) and Lee C. et al. (2018). The authors reported an increased incidence of diarrhea during the weaning transition. However, growth performance was reduced, causing long-term animal welfare problems and significant economic losses, the authors wrote in the December 2021 issue of Animal Nutrition. In pig production, antibiotic growth promoters (AGPs) have been used as an effective tool to reduce diarrhea and improve growth in piglets for decades. Recently, the government has encouraged using plant medicines or enzymes that are better for animals. However, many manufacturers also note that the dose of oxidants and Lectin C in creep feed for piglets will be completed and can reduce the colonization and colonization of pathogens in the intestine, reducing post-weaning diarrhoea and increasing growth performance. Therefore, LvCTL3/LvCTL4, UV-treated plasma, Elena Blázquez et al. (2019) and pharmacological levels of phytopharmaceuticals are widely applied in weaned piglet diets to reduce post-weaning diarrhea and promote growth performance.

Table 8. Effect of spay dried with plasma on growing and immune response of piglets

Trails	Days old	ADG (g/day)	IgA (µg/mL)	IgG (µg/mL)	IgM (µg/mL)
Control	7 - 21	155.23 ± 11.50**	25.56^{a}	25.56^{a}	33.33^{Aab}
Trail 1	7 - 21	260.82 ± 14.32**	24.30^{a}	24.30^{a}	32.00^{AB}
Trail 2	7 - 21	319.28 ± 20.30**	24.71^{a}	24.71^{a}	28.26^{B}
Trail 3	7 - 21	427.33 ± 55.42**	23.75^{b}	23.75^{a}	28.66^{B}
SEM			1.05	9.71	1.37
p values			0.667	0.769	0.030

***, a,b,c,Aab, AB, and B are significant differences in the same column.*

Plasma results were analyzed at the Quality Measurement and Standards Technical Center 3 (QUATEST 3). According to the test method, the results were expressed as < 10 CFU/g when no colonies developed on the plate. According to our results, 10 liters of pig blood was collected under anticoagulant conditions, temperatures up to 105 degrees Celsius by spray drying,

different times and UV rays, analysis of albumin, globulin and fibrinogen components, no bacteria were detected in the blood meal sample, keeping the pH around 6.5. The reason is that the blood meal is dried to a moisture content of 18% to prolong the storage time. At the same time, sodium nitrate is added to maintain the red color of the product and limit the growth of microorganisms, Kirimi J.G. et al. (2016) and Lee C. et al. (2018). After collection, fresh blood is immediately supplemented with 4% table salt to prevent blood clotting. Prepare the raw materials for spray drying at 45oC for 15 hours and package the SDP product. Supplement SDP for piglets from 7 to 21 days after birth to improve growth performance, and the immune system responds more strongly to resistance.

Declarations

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper

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