SSN: 2663-2187c

https://doi.org/10.48047/AFJBS.6.7.2024.2775-2787

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

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

Nguyen Xuan Huy[1,3], Kieu Thi Huyen[3], Tran Vinh Phuong[1], Tran Nguyen Ngoc[3], Tran Quang Khanh Van[3], Nguyen Quang Linh[2*],

[1] *Hue University, No.3 le Loi St., Hue City, Vietnam 490000;*

[2] *Faculty of Animal Sciences and Veterinary Medicine, University of Agriculture and Forestry, No.102 Phung Hung St. Hue City, Vietnam, 490000;*

[3] *Faculty of Biology, University of Education, No.22 Le Loi St. Hue City, 490000;*

[4]*faculty of Fisheries, University of Agriculture and Forestry, No.102 Phung Hung St. Hue City, Vietnam, 490000.*

To cite this article: Nguyen Xuan Huy[1,3], Kieu Thi Huyen[3], Tran Nguyen Ngoc[3], Nguyen Quang Linh[2,4] (2024). Collection conditions and processing of pig blood plasma to improve piglets' growth performance and immune response.

Nguyen Xuan Huy, nguyenxuanhuy@hueuni.edu.vn ORCID: https://orcid.org/0000-0002-8744-0927

Tran Vinh Phuong, tvphuong@hueuni.edu.vn, ORCID: https://orcid.org/0000-0002-7616-2482

Kieu Thi Huyen, kieuthihuyen@huaf.edu.vn, ORCID: https://orcid.org/0000-0002-7395-2462

Tran Nguyen Ngoc, trannguyennggoc@huaf.edu.vn,

*Corresponding authors: Nguyen Quang Linh: nguyenquanglinh@hueuni.edu.vn, ORCID: https://orcid.org/0000-0002-8424-1148

Article History
Volume 6, Issue 7, 2024
Received: 25 Mar 2024
Accepted: 25 Apr 2024
doi: 10.48047/AFJBS.6.7.2024.2275-2287

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. 216 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, showed 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-dried 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 dried- 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. 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. It has been documented and extended to other feed rations, such as finishing pigs and sows. 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 aquaculture products 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 also responds to a certain extent. Contributing to effective farming technology, we used plasma spray dried 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: 1) 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 slaughterhouseand; 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 pig slaughterhouses, as outlined in Circular No. 08/VBHN-BNNPTNT dated October 5, 2022, include hiring qualified animal staff to oversee

the pre-slaughter process, ensuring that measurements taken five minutes after blood draw are used as a reference, with the mean difference, relative difference (%), and 95% confidence interval recorded for each value. It is necessary to maintain detailed records documenting the origin of animals brought to the slaughterhouse and to ensure compliance with hygiene regulations for slaughter participants, including the use of protective clothing. Only healthy animals that meet veterinary hygiene standards should be selected for slaughter, while injured or exhausted animals, provided they do not exhibit clinical symptoms of infectious diseases, may be slaughtered first after being cleaned and kept in a designated waiting area to recover to a normal state and undergo a clinical examination before slaughter. Additionally, it is essential to maintain clear and organized areas for managing these animals.

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 was 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 was expected to be used at 0.2 - 0.4% in fresh whole blood. Blood was mainly composed of ≥ 80% water. Therefore, spray-dried plasma powder was expected to contain no more than 0.1 % sodium citrate.

Iron was removed by complexation with citrate and phosphate buffer solution. After this process, the blood from the pigs was boiled, filtered, and dried to produce blood powder. The finished blood powder products were then evaluated according to physicochemical and microbiological standards, including protein content, iron content, carbohydrate content, total microorganisms, and mould. These evaluations were conducted in a laboratory at the University of Agriculture and Forestry, University of Hue.

Step 2. Approximately 4-5% of a live animal's weight was collectable blood, containing about 10% animal protein. When fresh blood was extracted from animals, fibrinogen was converted into fibrin. Fibrin catalyzed the formation of a fibrous network that encased blood cells and other blood components into a clot. Fresh whole blood was stirred well with sodium citrate at 0.2 - 0.4% to prevent blood clotting.

Step 3. Raw blood from the steel pans was 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 was 200 - 400 litres of raw blood. Crude blood was collected to produce a sufficient volume of product.

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

Determined by drying method at 105 ºC 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 in the research section of the Faculty of Fisheries, University of Agriculture and Forestry, Hue University of Vietnam.

Data processing methods

Blood collection batches were repeated 03 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 reduced fibrin's gelation (coagulation) in plasma. We chosed 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 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 improved growth performance, survival rate, feed utilization, immune response and reduced 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 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 was relatively high; at pH 5.5, it was lower; and at pH 6.5, the amount of residue was the largest, before ultimately decreasing at pH 7.5. The pH value of 4.5 was close to the isoelectric point of albumin, so the precipitate at this pH was mainly albumin. Since albumin accounts for about 60% of total plasma protein, and plasma makes up 60% of blood, the residue obtained was relatively high. At pH 5.5, near the isoelectric point of globulin, the precipitate volume was mainly globulin, which constitutes about 35% of total plasma protein, resulting in a lower precipitate mass. At pH 6.5, close to the isoelectric point of haemoglobin, the residue was predominantly haemoglobin, which accounts

for 96% of visible and 40% of the tangible substances in the blood, making the precipitate volume the largest at this pH. The precipitate mass was low at pH 7.5, far from the isoelectric point of blood proteins. These results indicated that pH 6.5 was optimal for collecting coagulated blood products. The citrate buffer was prepared from two solutions of 0.2 M disodium citrate and 0.2 M monosodium citrate at pH 6.5. Research showed that the iron content in the blood powder was lowest when the citrate buffer added to the blood fluid was at a 10:90 ratio (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.

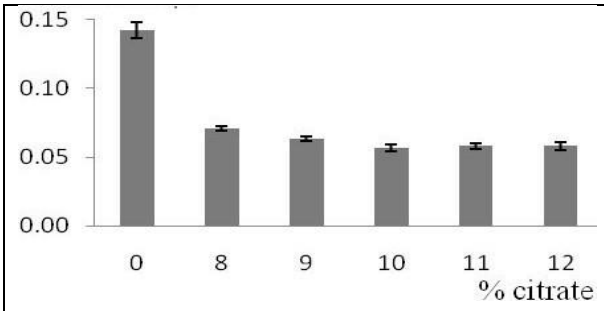

Figure 1. Effect of citrate buffer on Iron% remaining in plasma powder

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*

To investigate the effects of temperature and drying time on the moisture content of blood meal, it is important to consider their inverse relationship. Table 4 shows that as the drying temperature decreases, the exposure time to air increases. Drying at temperatures between 35-40 °C is conducive to microbial growth, which negatively affects the quality of the finished blood powder. In the temperature range of 0 to 45°C, proteins in the blood powder do not denature; however, from 45 to 75°C, most proteins lose their colloidal properties and become

irreversibly denatured, especially above 65°C, leading to a loss of protein solubility, Polo J. et al. (2015). Therefore, a drying temperature of 45°C and a drying time of 15 hours are optimal to achieve a moisture content of 18% in blood meal.

Tests comparing iron-depleted blood meal on mice with 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) showed that and given the necessary amount of food, gained weight. However, weight gain varied due to differences in food ingredients. The weight gain in all experimental samples was higher than in the negative control group. The experimental group outperformed the positive control group at the same concentration for piglets fed with food supplemented with iron-depleted blood meal exhibited more significant weight gain, better protein absorption, and reduced fishy smell, making the food more palatable by Jiravanichpaisal P. et al. (2006); Junkunlo K. et al. (2012). At a 5% blood meal concentration, mice had the highest weight gain of 11.23g, indicating that the optimal blood meal supplement concentration is 5%.

In summary, foods supplemented with iron-depleted blood meals are superior to those without and those with non-iron blood meals. The appropriate supplementation level is 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 blood plasma, 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 post-weaning diets. UV irradiation is a recognized technology capable of inactivating various germs. The disease includes a variety of bacteria and viruses, including porcine parvovirus, a model virus that is highly resistant to heat and chemicals, Muller L.K.F. et al. (2017); Polo J. Rodríguez C. et al. (2015); Quigley J.D.

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. pneumoniae***		
	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			

M = Mean; 90% Confidence interval (CI)

Evaluate the energy value and microbiological criteria of the product.

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 noted that the dose of oxidants and Lectin C in creep feed for piglets would be completed and can reduce the colonization of pathogens in the intestine, leading to a reduction in post-weaning diarrhea and increased 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 collected under anticoagulant conditions, temperatures up to 105 ^{o}C by spray drying, different times and UV rays, and 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 45^{o}C 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

Grant information: DTNN/ DL: ĐTĐL.CN-56/22.

Acknowledgements: This paper was also supported by colleagues from the University of Agriculture and Forestry, Hue University, and laboratories for participating in this research.

References

Anna Pérez-Bosque, Javier Polo, David Torrallardona (2016) Spray-dried plasma as an alternative to antibiotics in piglet feeds, mode of action and biosafety. *Porcine Health Management* 2:16. https://doi.org/10.1186/s40813-016-0034-1

Balan, P., Staincliffe, M., Moughan,. PJ. (2021). Effects of spray-dried animal plasma on the growth performance of weaned piglets-A review. *J Anim Physiol Anim Nutr*. 105(4):699–714 http://dx.doi.org/10.1371/journal.pone.0257792

Bosi P., L. Casini, A Finamore, C. Cremokolini, G. Merialdi, P. Trevisi, F. Nobili, E. Mengheri (2004) Spray-dried plasma improves growth performance and reduces inflammation status of weaned pigs challenged with enterotoxigenic *Escherichia coli* K88. *Journal of Animal Science* 82(6):1764-72. https://doi.org/10.2527/2004.8261764x

Campbell, J.M., Crenshaw, J.D., González-Esquerra. R., Polo, J. (2019) Impact of spray-dried plasma on intestinal health and broiler performance. *Microorganisms*. 7(8):219. pmid:31357672. DOI: 10.3390/microorganisms7080219

Cottingim, K.M., Johnston, L.J., Hilbrands, A.M., Shurson, G.C., Urriola, P.E.(2017). Ultraviolet irradiation of spray-dried porcine plasma does not affect the growth performance of nursery pigs when compared with nonirradiated bovine plasma. *Journal of Animal Science* 95(7):3120:3128. https://dx.doi.org/10.2527%2Fjas.2017.1486.

Chuchird, N., Rairat, T., Keetanon, A., Phansawat, P., Chou, C.C., Campbell, J. (2021). Effects of spray-dried animal plasma on growth performance, survival, feed utilisation, immune responses, and resistance to *Vibrio parahaemolyticus* infection of Pacific white shrimp (*Litopenaeus vannamei*). *PLoS ONE* 16(9): e0257792. https://doi.org/10.1371/journal.pone.0257792

Duffy, M.A., Chen, Q., Zhang, J., Halbur, P. G., Opriessnig, T. (2018) Impact of dietary spray-dried bovine plasma addition on pigs infected with porcine epidemic diarrhoea virus. *Translational Animal Science* 2(4):349-57. https://doi.org/10.1093/tas/txy088

Ejikeme, C.M., and Eboatu, A.N. (2014). Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. *European Scientific Journal* **10**(18)**:** 247–270. http://eprints.gouni.edu.ng/1312/

El-Sayed, A.F.M. (1998) Total replacement of fish meal with animal protein sources in Nile tilapia *Oreochromis niloticus* (L.) feeds. *Aquaculture research* 29(4):275-80. https://doi.org/10.1046/j.1365-2109.1998.00199.x

Elena Blázquez, Carmen Rodríguez, Jesús Ródenas, Joaquim Segalés, Joan Pujols, Javier Polo (2020) Biosafety steps in the manufacturing process of spray-dried plasma: a review with emphasis on the use of ultraviolet irradiation as a redundant biosafety procedure. *Porcine Health Management* 6:16. https://doi.org/10.1186/s40813-020-00155-1

Elena Blázquez, C. Rodríguez, J. Ródenas, N Navarro, R Rosell, S Pina-Pedrero, J. M. Campbell, M Sibila, J. Sega lés, J. Pujols, J. Polo (2019) UV-C irradiation can inactivate pathogens found in commercially collected porcine plasma as demonstrated by swine bioassay. *Veterinary Microbiology* 239:108450. https://doi.org/10.1016/j.vetmic.2019.108450

Elena Blázquez, Carmen Rodríguez, Jesús Ródenas, Joaquim Segalés, Joan Pujols, Javier Polo (2021) Effect of spray-drying and ultraviolet C radiation as biosafety steps for CSFV and ASFV inactivation in porcine plasma. *PLoS* 16(4). https://doi.org/10.1371/journal.pone.0249935

Ellis, A. E. (1990). Lysozyme Assays. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S. and Van Muiswinkel, W.B., Eds., *Techniques in Fish mmunology*, SOS Publications, Fair Haven, 101-103.

https://books.google.com.vn/books/about/Techniques_in_Fish_Immunology_Fish_Immun.html

Ezeonu, C.S., & Ejikeme, C.M. (2016). Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. *New Journal of Science* 2016: 1-9. https://downloads.hindawi.com/archive/2016/5601327.pdf

Gatnau R (1990) Spray dried porcine plasma as a source of protein and immunoglobulin for weanling pigs. Master Thesis Iowwa State University Department of Animal Science. https://lib.dr.iastate.edu/cgi/viewcontent.cgi?referer=https://www.google.com/&httpsredir=1&article=17876&context=rtd.

Guo, J., Davis, R., Starkey, C., Davis, D.A. (2021). Efficacy of various coated materials to prevent nutrient leaching for Pacific white shrimp *Litopenaeus vannamei* commercial diets. *J World Aquacult Soc.* 52:195–203. https://doi.org/10.1111/jwas.12732

Hamburger, M.K.H. (1991). Bioactivity in plants. The link between phytochemistry and medicine. *Phytochem Anal* 30(12): 3864-3874. https://doi.org/10.1016/0031-9422(91)83425-Kb

Harborne, J.B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. *Chapman and Hall, London, UK.* https://www.springer.com/gp/book/9780412572609

He, S., Song, L., Qian, Z., Hou, F., Liu, Y., Wang, X., Peng, Z., Sun, C., Liu, X. (2015), Molecular characterization of LvAV in response to white spot syndrome virus infection in the Pacific white shrimp (*Litopenaeus vannamei*), *Developmental & Comparative Immunology*, 51(1), 48-55. https://doi.org/10.1016/j.dci.2015.02.020

Henrichs, B.S., Brost, K.N., Hayes, C.A., Campbell, J.M., Drackley J.K. (2021), Effects of spray-dried bovine plasma protein in milk replacers fed at high plane of nutrition on performance, intestinal permeability, and morbidity of Holstein calves. *Journal of Diary Science* 104 (7):7856-7870. https://doi.org/10.3168/jds.2020-20104

Hussain, S.M., Afzal, M., Salim, M., Javid, A., Khichi, T.A.A., Hussain M., Raza, S.A. (2011) Apparent digestability of fish meal, blood meal and meat meal for *Labeo rohita* fingerlings. *The journal of animal and plant sciences* 21(4):807-11. https://www.cabdirect.org/cabdirect/abstract/20123206395

Jiravanichpaisal P, Lee BL, Soderhall K (2006). Cell-mediated immunity in arthropods: hematopoiesis, coagulation, melanization and opsonization. *Immunobiology* 211: 213–236. https://doi.org/10.1016/j.imbio.2005.10.015

Kirimi, J.G., Musalia, L.M., Adiel Magana, Munguti, J.M. (2016) Performance of Nile Tilapia (*Oreochromis niloticus*) Fed Diets containing blood meal as a replacement of Fish Meal. *Journal of Agricultural Science* 8(8):79-87. https://doi.org/10.5539/jas.v8n8p79

Lee, C., Tebbe, A.W., Campbell, J.M., Weiss, W.P. (2018). Effects of spray-dried plasma protein product on early-lactation cows. *Journal of Diary Science* 101(7):6019-31. https://doi.org/10.3168/jds.2017-13795

Muller, L.K.F, da Silva, A.S., Baldissera, M.D., Santurio, J.M., Glombowsky, P., Gugel J., Campigotto, G., Gloria, E.M., Paiano, D., Machado G. (2017). Effects of supplementation with spray-dried porcine plasma on blood variables on piglets feed

with diet contaminated by mycotoxins. *Microbial Pathogenesis* 110:464-70. https://doi.org/10.1016/j.micpath.2017.07.028

Polo, J., Rodríguez, C., Jesús R.s, Louis E.R., Campbell, J.M., Crenshaw J.D., David T., Joan, P. (2015). Ultraviolet (UV) inactivation of porcine parvovirus in liquid plasma and effect of UV irradiated spray-dried porcine plasma on the performance of weaned pigs. *Plos 1*0(7). https://doi.org/10.1371/journal.pone.0133008

Quigley, J.D., Kost, C.J., Wolfe, T.A. (2002) Effects of spray-dried animal plasma in milk replacers or additives containing serum and oligosaccharides on growth and health of calves. *Journal of Diary Science* 85(2):413-21. https://www.journalofdairyscience.org/article/S0022-0302(02)74089-7/pdf

Russell, L. (2000). Trials show promise for spray-dried plasma protein in shrimp feeds. *Global Aquaculture Alliance*. https://www.aquaculturealliance.org/advocate/trials-show-promise-for-spray-dried-plasma-protein-in-shrimp-feeds

Shen, H.G., Schalk, S., Halbur, P.G., Campbell, J.M., Russell, L.E., Opriessnig, T. (2011) Commercially produced spray-dried porcine plasma contains increased concentrations of porcine circovirus type 2 DNA. Still, it does not transmit porcine circovirus type 2 when fed to naïve pigs. *Journal of Animal Science* 89(6):1930-38. https://doi.org/10.2527/jas.2010-3502

Torrallardona, D. (2010). Spray dried animal plasma as an alternative to antibiotics in weanling pigs—A review. *Asian-Aust J Anim Sci.* 23(1):131–48. *Asian-Australasian Journal of Animal Sciences* 2010;23(1): 131-148. https://doi.org/10.5713/ajas.2010.70630

Touchette, K.J., Carroll, J.A., Alle, G.L., Matteri, R.L., Dyer, C.J., Beausang, L.A., Zannelli M.E. (2001) Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs. *Journal of Animal Science* 80 (2) : 494-501. https://doi.org/10.2527/2002.802494x

Zhang, Y., Zheng, P., Yu, B., He, J., Yu, J., Mao, X.B., Wang, J.X., Luo, J.Q., Huang, Z.Q., Cheng, G.X., Chen, D.W. (2016) Dietary spray-dried chicken plasma improves intestinal barrier function and modulates immune status in weaning piglets. *Journal of Animal Science* 94(1):173-84. https://doi.org/10.2527/jas.2015-9530

Zimmerman, D.R. (1987) Porcine plasma proteins in diets of weaning pigs. *Swine Research Reports*. Ames, Iowa State University. https://cabidigitallibrary.org by 2402:800:6235:7b18:2c47:73f9:ca87:2052, on 05/13/24