

Bioactive peptides from bovine cartilage and intestinal mucosa as a replacement for spray-dried bovine plasma in nursery piglet diets

Peptídeos bioativos da cartilagem e da mucosa intestinal de bovinos como substitutos do plasma bovino desidratado em dietas para leitões em fase de creche

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Highlights

Bovine cartilage and intestinal mucosa hydrolysates (Peptipro[®]) contain 71 bioactive peptides. Peptipro[®] improves piglet performance in the second week of nursery. Low occurrence of diarrhea is observed in piglets fed bovine plasma or Peptipro[®]. Peptipro[®] can replace bovine plasma in pre-starter diets of piglets.

Abstract

Soluble peptides derived from bovine cartilage and intestinal mucosa (Peptipro[®]) are obtained through enzymatic hydrolysis as byproducts of heparin and chondroitin extraction. This hydrolysis releases

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bioactive compounds and results in a highly digestible product. This study aimed to identify the bioactive peptides present in Peptipro® and evaluate the effects of its dietary inclusion on the growth performance and intestinal health of weaned piglets, compared with diets containing spray-dried bovine plasma (BP) used in nursery feeding. Peptipro® was subjected to peptide sequencing using mass spectrometry. The performance trial was conducted in a randomized block design with a 3 × 2 factorial arrangement and ten replicates. The factors included diet and sex of the piglets, while blocks were defined based on their initial body weight (BW). The experimental diets were as follows: BP: 6% and 3% spray-dried BP in Pre-initial I (first week) and II (second week) phases, respectively; Partial replacement of BP with Peptipro® (BP-Pep): 3% spray-dried BP and 3% Peptipro® in Pre-initial I phase, and 1.5% of each in Pre-initial II phase; and Peptipro®: 3% and 1.5% Peptipro® in Pre-initial I and II phases, respectively. During Initial I (third week) and Initial II (last 18 days) phases, all experimental groups received the same diets. A total of 120 piglets (6.92 ± 0.77 kg BW), comprising 60 immunocastrated males and 60 females, were distributed in 30 pens with four animals per pen and fed *ad libitum* for 39 days. In total, 176 peptides were identified in Peptipro®, of which 71 exhibited bioactive functions related to energy metabolism regulation (74.6%), blood pressure regulation (63.4%), and antioxidative activity (8.4%), immunomodulation (2.8%), and other metabolic processes (4.2%). Intestinal permeability, assessed using fluorescein isothiocyanate-dextran (FITC-dextran), was not affected by diet ($P = 0.308$). In the Pre-initial II phase, Peptipro® diet increased the average daily gain by 12.8% compared with the BP-Pep diet (0.397 vs. 0.352 kg animal⁻¹ day⁻¹; $P = 0.044$), without affecting feed intake (0.444 kg animal⁻¹ day⁻¹, on average; $P = 0.198$), resulting in a better feed conversion ratio (1.08 vs. 1.25 kg feed kg⁻¹ gain; $P = 0.001$). Fecal score was not influenced by diet, sex, or their interactions ($P > 0.05$), with mean relative frequencies of 51.5, 35.5, and 13.0% for normal, pasty, and liquid feces, respectively. Owing to its favorable amino acid profile, the presence of a broad spectrum of bioactive peptides, and its positive effects on animal performance, Peptipro® can replace spray-dried BP in pre-starter diets for nursery piglets.

Key words: Bovine plasma replacement. Feed efficiency. Functional peptides profile. Post-weaning performance. Weaned piglets.

Resumo

Peptídeos solúveis da mucosa intestinal e da cartilagem de bovinos (Peptipro®) são obtidos por hidrólise enzimática, sendo coprodutos da extração de heparina e condroitina. Esta hidrólise libera componentes bioativos e resulta em um produto de alta digestibilidade. O objetivo deste estudo foi identificar os peptídeos bioativos contidos no Peptipro®, e avaliar o efeito da sua inclusão na dieta sobre o desempenho e a saúde intestinal de leitões desmamados, comparado com dietas contendo plasma bovino desidratado fornecidas na creche. Uma amostra de Peptipro® foi submetida a sequenciamento de peptídeos por espectrometria de massa. O experimento de desempenho foi realizado em delineamento de blocos ao acaso em arranjo fatorial 3 x 2 com dez repetições. Os fatores foram a dieta e o sexo dos leitões, e os blocos foram definidos com base no peso corporal (PC) inicial. As dietas experimentais foram Plasma bovino (PBov): 6% e 3% de plasma bovino desidratado nas fases Pré-inicial I (primeira semana) e II (segunda semana); Substituição parcial do PBov por Peptipro® (PBov-Pep): 3% de PBov desidratado e 3% de Peptipro® na fase Pré-inicial I, e 1,5% de cada ingrediente na

fase Pré-inicial II; e Peptipro®: 3% e 1.5% de Peptipro® nas fases Pré-inicial I e II, respectivamente. Nas fases Inicial I (terceira semana) e Inicial II (últimos 18 dias), todos os grupos experimentais receberam as mesmas dietas. O total de 120 leitões ($6,92 \pm 0,77$ kg PC), sendo 60 machos imunocastrados e 60 fêmeas, foram distribuídos em 30 baias com quatro animais por baia e alimentados à vontade por 39 dias. Um total de 176 peptídeos foram identificados no Peptipro®, dos quais 71 possuem funções bioativas relacionadas à regulação do metabolismo energético (74,6%), regulação da pressão arterial (63,4%), ação antioxidante (8,4%), imunomodulação (2,8%) e outros processos metabólicos (4,2%). A permeabilidade intestinal, avaliada com o marcador isotiocianato de fluoresceína-dextran (FITC-dextran), não foi influenciada pela dieta ($P = 0,308$). Na fase Pré-inicial II, a dieta Peptipro® aumentou o ganho médio diário em 12,8% em relação a dieta P_{Bov}-Pep ($0,397$ vs. $0,352$ kg animal⁻¹ dia⁻¹; $P = 0,044$) sem afetar o consumo de ração (média de $0,444$ kg animal⁻¹ dia⁻¹; $P = 0,198$), resultando em melhor conversão alimentar ($1,08$ vs. $1,25$ kg de ração kg⁻¹ ganho de peso; $P = 0,001$). O escore de fezes não foi influenciado pela dieta, pelo sexo ou pela interação entre ambos ($P > 0,05$), apresentando valores médios de 51,5; 35,5 e 13,0% para frequência relativa de fezes normais, pastosas e aquosas. Devido ao bom perfil de aminoácidos, ao amplo espectro de peptídeos bioativos e seu efeito positivo no desempenho animal, o Peptipro® pode substituir o P_{bov} desidratado em dietas pré-iniciais de leitões na creche.

Palavras-chave: Desempenho pós-desmame. Eficiência alimentar. Leitões desmamados. Perfil de peptídeos funcionais. Substituição do plasma bovino.

Introduction

Weaning is a highly stressful event for piglets due to the drastic environmental and nutritional changes (Everaert et al., 2017). The transition from milk to a solid and less digestible, plant-based diet containing unfamiliar ingredients represents a major challenge, combined with the transition phase between passive and active immunity, which increases the susceptibility to infections (Sun & Kim, 2017). In addition, the change in the environment, combined with the need to adapt to new facilities and a new group, means that piglets do not feed properly during this period, which increases the likelihood of developing diseases (Duarte & Kim, 2024; Jayaraman & Nyachoti, 2017).

Maintaining adequate nutrition after weaning is essential to maintain the balance

and diversity of the intestinal microbiota. Nutritional strategies should be adopted to maintain intestinal mucosal integrity, preserve villus height and crypt depth, and promote adequate nutrient absorption (Wijtten et al., 2011). In this context, special attention must be given to the diet provided during this period, prioritizing highly palatable and digestible ingredients to reduce the negative effects of weaning (Brownlee, 2011).

One approach to reduce intestinal disorders in piglets during this period is the inclusion of high-quality animal proteins in their diet (Figueroa et al., 2016). Blood plasma has been considered a source of animal protein for post-weaning piglets, especially in the first week, because it contains highly digestible protein and offers several benefits, including the absence of antinutritional

factors and high solubility (Kazimierska & Biel, 2023). Plasma from cattle, pigs, or other animal sources that is spray-dried is often used as a main ingredient in the diet of weaned piglets to improve growth performance and welfare (Van Dijk et al., 2001). Bovine plasma (BP) is obtained through a process involving blood collection, anticoagulation, and centrifugation. Anticoagulants, such as sodium citrate or heparin, are added to prevent clotting. The blood is then centrifuged to separate the plasma from red blood cells and other components. Further purification steps, such as filtration, may be employed to obtain sterile plasma (Balan et al., 2021).

Animal plasma mainly comprises proteins, minerals, and water, and the protein fraction is largely composed of albumins and globulins (Pujols et al., 2023). It acts as a promoter of digestive function, maintaining integrity and reducing intestinal inflammation (Ruckman et al., 2020). In addition, this ingredient can increase the performance of the piglet's immune system in the post-weaning phase, especially in the first week (Balan et al., 2021).

Spray-dried bovine soluble peptides (BSPeps) are byproducts of the heparin industry and are obtained by enzymatic hydrolysis of bovine intestinal mucosa (Huting et al., 2021). In addition, spray-dried BSPeps can be considered byproducts of the food and meat processing industries. They are derived from bovine blood plasma or other byproducts, such as bones and skin. These peptides can be extracted and processed through spray drying to create a powder, making them suitable for use as functional ingredients in food, animal feed, or other applications (Huting et al., 2021; Zhu et

al., 2022). Bioactive peptides have promising therapeutic, functional, and/or nutritional applications (Vidal et al., 2022).

One of the spray-dried BSPeps is Peptipro[®], which is obtained through enzymatic hydrolysis of bovine cartilage and intestinal mucosa as a heparin and chondroitin extraction byproduct. Hydrolysis releases bioactive compounds and results in a product with high digestibility and a characteristic flavor. Peptipro[®] has a high crude protein (CP) content (58.5%), which is highly digestible, rich in essential amino acids (methionine 1.11%, lysine 3.06%, threonine 1.91%, tryptophan 0.18%, valine 2.22%, leucine 3.18%, isoleucine 1.64%, arginine 3.15%, glycine 8.02%, cysteine 0.56%, and methionine + cysteine 1.67%), and is abundant in glutamine. This amino acid is responsible for ensuring the integrity of muscle and intestinal tissue, participates in energy metabolism, and strengthens the immune system (Lange et al., 2010).

The identification of peptides in protein hydrolysates has been widely performed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), a technique that combines high sensitivity and selectivity in the detection of small peptide chains. This approach enables detailed characterization of peptide sequences, comparison with databases, and inference of potential biological activities. The use of specific acquisition modes, such as multiple reaction monitoring (MRM), ensures greater accuracy in quantification. In this context, the methodology developed by Poliseli et al. (2020) has proven to be a robust and reliable tool for hydrolysate evaluation, whereas the BIOPEP-UWM database (Minkiewicz et al., 2019) allows the

prediction and classification of the biological activities associated with identified peptides. Subsequent studies have demonstrated the applicability of this approach in different protein matrices, reinforcing its versatility and potential in the food, nutraceutical, and agricultural industries (Crozatti et al., 2023).

The nutritional effect has a substantial impact on animal health status and subsequent performance, especially during periods of stress such as weaning (Torrallardona, 2010). Besides that, the bioactive peptides present in Peptipro® can contribute to immunomodulatory, antimicrobial, and anti-inflammatory effects, which may improve performance outcomes (González-Solé et al., 2020; Xu et al., 2021). The hypothesis of this study is that peptides derived from bovine sources (Peptipro®) will support intestinal health, maintain gut barrier function, and promote equal or superior performance compared to animals fed diets without Peptipro®.

This study aimed to identify the bioactive peptides in Peptipro® and evaluate the effect of including this amino acid source in the diet on the performance and intestinal health of weaned piglets, compared to diets containing spray-dried BP provided during the nursery period.

Material and Methods

Peptide sequencing of Peptipro® by LC-MS/MS

A sample of Peptipro® was subjected to peptide sequencing, as described by Poliseli et al. (2020). Analysis was performed using a Quattro Premier XE triple-quadrupole mass

spectrometer (Waters Corporation, Milford, MA, USA) equipped with an electrospray ionization (ESI) source, a Waters 515 pump, and an XBridge C18 column (4.6 × 50 mm, 3.5 μm, Waters). For sample preparation, 0.1 g of freeze-dried material was dissolved in 1 mL of 50 mM ammonium bicarbonate solution. The solution was homogenized, and the first dilution was performed by mixing 100 μL of this solution with 900 μL of the mobile phase (acetonitrile:water:formic acid, 70:30:0.1, v/v/v), followed by centrifugation at 3000 × g for 10 min. The supernatant was maintained at 4 °C for 60 min. A second dilution was then performed by mixing 100 μL of the supernatant with 900 μL of the mobile phase, followed by vortexing for 1 min. A 5 μL aliquot was injected into the LC-MS/MS system, with each run lasting 5 min.

LC-MS/MS analyses (neutral loss at 46 Da and fragmentation) were conducted using a conventional ESI source in positive ion mode (ESI+). Desolvation and source gas temperatures were set at 350 and 110 °C, respectively. The electrospray capillary voltage was 4.0 kV, while the cone voltage, collision energy, and collision gas pressure (argon) were 20 V, 15 V, and 3.0 × 10⁻³ bar, respectively. The resulting spectra were interpreted according to Cantú et al. (2008), generating di- and tripeptide sequences that were subsequently used for database searches and computational analysis.

Animal performance and health status evaluation

This study complied with the ethical principles adopted by the Brazilian College of Animal Experimentation (*Colégio Brasileiro*

de Experimentação Animal – COBEA) and was approved by the Animal Ethics Committee (*Comitê de Ética no Uso de Animais – CEUA*/ protocol number 13/2022) of the Federal University of Paraná (*Universidade Federal do Paraná – UFPR*), Palotina Campus, Brazil.

The experiment was set up in a randomized complete block design in a 3 × 2 factorial arrangement with ten replicates. The factors included diet and sex, and the blocks were defined based on the initial body weight (BW) of the piglets. The experimental diets were spray-dried BP: 6% and 3% in Pre-initial I (first week) and II (second week) phases, respectively; partial replacement of BP with Peptipro® (BP–Pep): 3% spray-dried BP and 3% Peptipro® in the Pre-initial I phase, and 1.5% of each in the Pre-initial II phase; and Peptipro®: 3% and 1.5% Peptipro® in the Pre-initial I and II phases, respectively. Each

cage containing four piglets served as the experimental unit (replicate). The blocks were organized by cage rows (three rows with 10 cages per row) distributed inside the barn. The distribution of piglets in each cage was based on sex, and in each row on their initial BW (light, medium, and heavy groups). The treatments were evenly distributed in each row of cages.

A total of 120 piglets (60 immunocastrated males and 60 females) from Danbred sows were used (Table 1). After weaning, at 21 days of age, the animals were transferred to the nursery, weighed, identified with ear tags, separated by sex and BW, and distributed into the experimental groups. Piglets showed 6.92 ± 0.77 kg BW (mean ± standard deviation [SD]) at the beginning of the experiment.

Table 1
Distribution of piglets in the experimental groups

Diet ¹	Phase		Sex		Total piglets (n)
	Pre-Initial I	Pre-Initial II	Male (n)	Female (n)	
Bovine plasma	6% Bovine plasma	3% Bovine plasma	20	20	40
BP–Pep	3% Bovine plasma 3% Peptipro®	1.5% Bovine plasma 1.5% Peptipro®	20	20	40
Peptipro®	3% Peptipro®	1.5% Peptipro®	20	20	40

¹BP–Pep: diet with partial replacement of bovine plasma with Peptipro®.

The experimental area consisted of suspended cages with perforated plastic floors, with dimensions of 1 × 2 m and capacity for four piglets, on average. Each cage had a stainless-steel trough-type feeder and a nipple drinker. The cages were distributed in three rows along the barn.

The temperature inside the barn was recorded twice a day using a maximum and minimum thermometer (Digital Thermometer with External Sensor and Alarm Incoterm 7427.02.0.00 - Instrusul) to maintain it within the desired standards for each week of housing (Dias et al., 2014). As needed,

the temperature of the barn was controlled by opening or closing the side curtains, allowing air renewal, and preventing the direct incidence of cold air currents on the piglets. The temperature was maintained within the thermal comfort range to promote maximum animal performance. The average temperature inside the barn varied from 23.84 °C (min) to 26.91 °C (max) in the morning and from 23.98 °C (min) to 28.36 °C (max) in the afternoon. The animals' behavior was constantly observed to assess whether they were subjected to thermal comfort conditions.

The animals were fed *ad libitum* manually approximately ten times a day (according to the visual assessment of feed levels in the feeders) with small amounts to avoid feed wastage and maintain feed quality in the feeders. In addition, the animals were subjected to a high-frequency feeding strategy because of the composition of the diets, which contained many unstable ingredients, such as BP and Peptipro[®], to avoid accumulation in the feeders and wastage. The animals were constantly encouraged to stand to stimulate the ingestion of water and feed. Each pen had a 30 kg storage bucket identified at the front, where the experimental feed for each group was stored to ensure a consistent supply of the correct feed to the experimental group.

The animals had access to water through a nipple drinker, which was checked daily to ensure adequate water flow. The area was cleaned twice a day to remove feces accumulated on the suspended floor and to remove feces and feed residues from underneath the cages.

The experimental diets were produced in the experimental unit and formulated based on the nutritional requirements of the animals in the nursery phase (Rostagno et al., 2017), covering four phases: Pre-initial I (first week of housing), Pre-initial II (second week of housing), Initial I (third week of housing), and Initial II (fourth week until the animals left the nursery). Peptipro[®] was added to the diets provided in the Pre-initial I and II phases at a maximum inclusion of 3%, to ensure that the dietary sodium level remained within the established limits of the animals' diets. BP was added at a maximum inclusion of 6% of the diet (Table 2). The CP level was adjusted using soybean meal. The Initial I and Initial II diets were identical for all experimental groups. The CP and metabolizable energy levels of these diets were 19.7% and 3415 kcal kg⁻¹ for Initial I, and 20.2% and 3380 kcal kg⁻¹ for Initial II.

Table 2
Ingredient composition and crude protein and metabolizable energy levels of the experimental diets

Phase	Ingredient (%)	Experimental diet ^{IV}			
		Bovine plasma	BP-Pep	Peptipro®	
Pre-Initial I	Commercial concentrate ^I	60.00	60.00	60.00	
	Ground corn	29.93	28.76	26.30	
	Soybean meal	2.09	3.27	8.35	
	Bovine blood plasma	6.00	3.00	0.00	
	Peptipro®	0.00	3.00	3.00	
	Soybean oil	0.68	0.43	0.59	
	Zinc oxide	0.365	0.365	0.365	
	L-lysine	0.362	0.452	0.539	
	DL-methionine	0.225	0.255	0.296	
	L-threonine	0.144	0.185	0.227	
	L-valine	0.059	0.115	0.167	
	L-tryptophane	0.044	0.066	0.074	
	Mycofix® FUM ^{III}	0.100	0.100	0.100	
	Crude protein (%)	20.03	20.04	19.97	
	Metabolizable energy (kcal kg ⁻¹)	3496.4	3479.8	3458.6	
	Pre-Initial II	Commercial concentrate ^{II}	40.00	40.00	40.00
		Ground corn	32.98	32.39	30.17
Soybean meal		22.03	22.62	26.19	
Soybean oil		0.50	0.38	0.49	
Bovine blood plasma		3.00	1.50	0.00	
Peptipro®		0.00	1.50	1.50	
Zinc oxide		0.301	0.301	0.301	
L-lysine		0.463	0.508	0.521	
DL-methionine		0.256	0.271	0.284	
L-threonine		0.216	0.236	0.244	
L-valine		0.092	0.120	0.129	
L-tryptophane		0.059	0.070	0.069	
Mycofix® FUM ^{III}		0.100	0.100	0.100	
Crude protein (%)		19.41	19.42	19.71	
Metabolizable energy (kcal kg ⁻¹)		3401.6	3393.3	3384.8	

^IConcentrate containing 18.65% of crude protein and 3570.1 kcal kg⁻¹ of metabolizable energy.

^{II}Concentrate containing 8.59% of crude protein and 3596.2 kcal kg⁻¹ of metabolizable energy.

^{III}Anti-mycotoxin enzymatic feed additive (DSM-Firmenich).

^{IV}BP-Pep: diet with partial replacement of bovine plasma with Peptipro®.

The experimental diets were adjusted to be isocaloric and isonitrogenous (Table 2) and prepared without the addition of antimicrobials for most of the experimental period. Drug therapy was administered individually, and when the need for collective therapy via feed was identified, antimicrobials were added in accordance with the recommendations of the integrator to which the animals were linked, and all treatments were subjected to the same therapy.

To assess intestinal permeability, fluorescein isothiocyanate-dextran (FITC-dextran, 4000 kDa), a nonabsorbable fluorescent marker, was administered orally to 10 piglets per treatment at 14 days after weaning. Prior to administration, the animals were fasted for 6 h. A dose of 1 mL of FITC-dextran solution was administered per animal. Six hours post-administration, blood samples (10 mL per piglet) were collected via jugular venipuncture using vacuum tubes without an anticoagulant. Immediately after sampling, the samples were homogenized five to eight times to prevent coagulation. The samples were stored at a temperature of 2 to 8 °C and sent to laboratory analysis.

During the performance trial, the variables analyzed included total weight gain (TWG), average daily gain (ADG), and feed conversion ratio (FCR). Piglets were weighed individually at 0, 7, 14, 21, and 39 days of the trial. Based on these measurements, TWG and ADG were calculated for each phase. In cases of piglet mortality, feed intake from housing until death was estimated for the affected animal and subtracted from the pen feed intake to ensure accurate adjustment and calculation of FCR. Fecal consistency score was determined as follows: 1 = normal;

2 = pasty; and 3 = liquid (diarrheic feces). Scores were evaluated every day at 8:00 am and 4:00 pm, always by the same person, as recommended by Sobestiansky and Barcellos (2007).

Statistical analysis

Data on peptide profiles and their biological activities were analyzed descriptively. Data on intestinal permeability, animal performance, and health status were analyzed using the Statistical Analysis System (SAS) program, version 9.0. In all analyses, probability values lower than 0.05 were considered significant.

Intestinal permeability data were subjected to analysis of variance (ANOVA) among dietary treatments. The analysis was performed using a completely randomized design with three treatments and ten replicates, using an individual piglet as the experimental unit.

Data on animal performance and health status were analyzed for the presence of outliers (intra- and inter-cages) and tested using the Shapiro–Wilk normality test. Outliers were removed from the database, and variables that did not fit the normal distribution were normalized using rank transformation. Subsequently, ANOVA was performed for each nursery phase (Pre-initial I, Pre-initial II, Initial I, and Initial II) and for the total nursery period following a randomized block design in a 3 × 2 factorial arrangement, with three diets and two sexes. The blocks were defined based on the BW of piglets at the start of the experiment. The independent effects of the factors and their interactions were tested. When the diet effect was

significant, the means were compared using Tukey's test; when the sex effect was significant, the means were considered distinct based on the F test of the ANOVA.

Results

Peptide sequencing analysis allowed the identification of 176 peptides in Peptipro® (163 derived from cartilage, 6 from intestinal mucosa, and 7 found in both sources), of which 71 had bioactive functions described in the literature (Table 3). Among these, 71.8% (51/71) act as dipeptidyl peptidase (DPP)-IV inhibitors; 53.5% (38/71) act as angiotensin-converting enzyme (ACE) inhibitors; 15.5% (11/71) act as DPP-III inhibitors; 7% (5/71) act as renin inhibitors or exhibit antioxidant activity; 4.2% (3/71) act as α -glucosidase

inhibitors; 2.8% (2/71) function as hypotensive peptides, glucose uptake stimulators, or immunomodulators; and 12.7% (9/71) are associated with the regulation of other metabolic processes (Figure 1). Specific activities, such as chemotactic and antibacterial effects, were classified as immunomodulatory functions, whereas stimulation of vasoactive substance release, hypotensive and antithrombotic effects; inhibition of insulin secretion, stimulation of glucagon-like peptide-1 (GLP-1), and phosphoglycerate kinase (PGK) activity, regulation of stomach mucosal membrane activity, activation of ubiquitin-mediated proteolysis, and prolyl endopeptidase (PEP) inhibition and neuropeptide-related functions were categorized as metabolic regulatory processes.

Table 3
Peptide amino acid sequences identified by LC-MS/MS and biological activity in Peptipro®

N	Source	Amino acid sequence	Biological activity ¹	Reference
1	Cartilage	(I/L)A	a, b) ACE inhibitor; c) DPP-III inhibitor; d, e) DPP-IV inhibitor; f) Ubiquitin-mediated proteolysis activating peptide	a) Wang & Mejia (2006) b) Cushman et al. (1981) c) Dhanda et al. (2008) d) Hikida et al. (2013) e) Bella et al. (1982) f) Turner et al. (2000)
2	Cartilage	(I/L)A(Q/K)	a, b) ACE inhibitor; c) Antibacterial peptide; d) Hypotensive peptide	a) J. Wu et al. (2006) b) Gómez-Ruiz et al. (2007) c) Sedaghati et al. (2014) d) Miguel et al. (2010)
3	Cartilage	(I/L)H	a) DPP-III inhibitor; b) DPP-IV inhibitor	a) Dhanda et al. (2008) b) Lan et al. (2015)
4	Cartilage	(I/L)NP	ACE inhibitor	Miyoshi et al. (1991)
5	Cartilage	(I/L)T	DPP-IV inhibitor	Lan et al. (2015)
6	Cartilage	(I/L)V	a) DPP-IV inhibitor; b) Glucose uptake stimulating peptide	a) Lan et al. (2015) b) Morifuji et al. (2009)

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7	Cartilage	(I/L)VR	a, b) ACE inhibitor	a) Rawendra et al. (2014) b) Maruyama et al. (1989)
8	Cartilage	(K/Q)V	DPP-IV inhibitor	Lan et al. (2015)
9	Cartilage	(Q/K)A(I/L)	a) ACE inhibitor; b) Antioxidative peptide	a) Kohmura et al. (1990) b) Suetsuna (1999)
10	Cartilage	(Q/K)PS	Neuropeptide	Caliendo et al. (1996)
11	Cartilage	(Q/K)PT	Stimulating GLP-1 release	Theysgeur et al. (2021)
12	Cartilage	A(Q/K)(I/L)	ACE inhibitor	J. Wu et al. (2006)
13	CIM	AD	a) DPP-IV inhibitor; b) α -glucosidase inhibitor	a) Lan et al. (2015) b) Mora et al. (2020)
14	Cartilage	AE	DPP-IV inhibitor	Lan et al. (2015)
15	Cartilage	AF	a) ACE inhibitor; b) DPP-IV inhibitor	a) Cheung et al. (1980) b) Lan et al. (2015)
16	Cartilage	AG	a) ACE inhibitor; b) DPP-IV inhibitor	a) Cheung et al. (1980) b) Lan et al. (2015)
17	Cartilage	AH	a) ACE inhibitor; b) DPP-IV inhibitor; c) Antioxidative peptide	a) Van Platerink et al. (2008) b) Lan et al. (2015) c) Chen et al. (1995)
18	Cartilage	AR	ACE inhibitor	Sentandreu & Toldrá (2007)
19	Cartilage	AY	a) ACE inhibitor; b) DPP-IV inhibitor; c) Antioxidative peptide	a) Yano et al. (1996) b) Lan et al. (2015) c) Yokomizo et al. (2002)
20	Cartilage	CF	ACE inhibitor	H. Wu et al. (2008)
21	CIM	DA	a) ACE inhibitor; b) DPP-III inhibitor	a) Cushman et al. (1981) b) Dhanda et al. (2008)
22	Cartilage	DP	DPP-IV inhibitor	Lan et al. (2015)
23	Cartilage	ES	DPP-IV inhibitor	Lan et al. (2015)
24	Cartilage	EV	a) ACE inhibitor; b) DPP-IV inhibitor	a) Van Platerink et al. (2008) b) Lan et al. (2015)
25	Cartilage	FA	a) DPP-III inhibitor; b) DPP-IV inhibitor	a) Dhanda et al. (2008) b) Bella et al. (1982)
26	Cartilage	FT	Renin inhibitor	Udenigwe et al. (2012)
27	Cartilage	GA	a) ACE inhibitor; b) DPP-IV inhibitor	a) Cheung et al. (1980) b) Hikida et al. (2013)
28	CIM	GE	a) ACE inhibitor; b) DPP-III inhibitor; c) DPP-IV inhibitor	a) Cheung et al. (1980) b) Dhanda et al. (2008) c) Lan et al. (2015)
29	Cartilage	GF	a) ACE inhibitor; b) DPP-III inhibitor; c) DPP-IV inhibitor	a) Cheung et al. (1980) b) Dhanda et al. (2008) c) Lan et al. (2015)
30	IM	GG	a) ACE inhibitor; b) DPP-IV inhibitor	a) Cushman et al. (1981) b) Lan et al. (2015)

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31	Cartilage	GH	a) ACE inhibitor; b) DPP-IV inhibitor	a) Cheung et al. (1980) b) Lan et al. (2015)
32	Cartilage	GHG	ACE inhibitor	Balti et al. (2010)
33	Cartilage	GPP	a) ACE inhibitor; b) Antioxidative peptide	a) Murray & FitzGerald (2007) b) Chi et al. (2015)
34	Cartilage	GV	a) ACE inhibitor; b) DPP-IV inhibitor	a) Cheung et al. (1980) b) Lan et al. (2015)
35	Cartilage	GVR	ACE inhibitor; Hypotensive peptide	Manoharan et al. (2017)
36	Cartilage	H(I/L)	a) ACE inhibitor; b) DPP-III inhibitor; c, d) DPP-IV inhibitor	a) Cushman et al. (1981) b) Dhanda et al. (2008) c) Lan et al. (2015) d) Nongonierma et al. (2013)
37	Cartilage	HS	DPP-IV inhibitor	Lan et al. (2015)
38	IM	LP	a) ACE inhibitor; b) DPP-IV inhibitor	a) Wei et al. (2021) b) Hatanaka et al. (2012)
39	Cartilage	MA	DPP-IV inhibitor	Bella et al. (1982)
40	Cartilage	MP	DPP-IV inhibitor	Hatanaka et al. (2012)
41	Cartilage	NH	DPP-IV inhibitor	Lan et al. (2015)
42	Cartilage	NN	DPP-IV inhibitor	Lan et al. (2015)
43	IM	NR	a) DPP-IV inhibitor; b) Renin inhibitor	a) Lan et al. (2015) b) Udenigwe et al. (2012)
44	Cartilage	PGP	a) Peptide regulating the stomach mucosal membrane activity; b) Inhibitor of insulin secretion; c) Antithrombotic peptide; d) PEP inhibitor; e) Chemotactic peptide	a, b, c, d) Ashmarin et al. (1998) e) O'Reilly et al. (2009)
45	Cartilage	PH	a) ACE inhibitor; b) DPP-IV inhibitor	a) Van Platerink et al. (2008) b) Lan et al. (2015)
46	Cartilage	PP	a) ACE inhibitor; b) DPP-IV inhibitor; c) α -glucosidase inhibitor	a) Van Platerink et al. (2008) b) Hatanaka et al. (2012) c) Mora et al. (2020)
47	Cartilage	PPG	DPP-IV inhibitor	Lafarga et al. (2014)
48	Cartilage	PS	DPP-IV inhibitor	Lan et al. (2015)
49	Cartilage	RR	a) ACE inhibitor; b) DPP-III inhibitor; c) DPP-IV inhibitor	a) Sentandreu & Toldrá (2007) b) Lee & Snyder (1982) c) Lan et al. (2015)
50	Cartilage	S(I/L)	a, b) DPP-IV inhibitor; c) Regulator of PGK activity	a) Lan et al. (2015) b) Nongonierma et al. (2013) c) Luzarowski et al. (2021)
51	Cartilage	SE	Stimulating vasoactive substance release	Ringseis et al. (2005)

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52	Cartilage	SF	a) ACE inhibitor; b) DPP-IV inhibitor; c) Renin inhibitor	a) Suetsuna (1998) b) Lan et al. (2015) c) Udenigwe et al. (2012)
53	Cartilage	SH	DPP-IV inhibitor	Lan et al. (2015)
54	Cartilage	SM	DPP-III inhibitor	Dhanda et al. (2008)
55	Cartilage	SP	DPP-IV inhibitor	Hatanaka et al. (2012)
56	CIM	SV	DPP-IV inhibitor	Lan et al. (2015)
57	Cartilage	SY	a) ACE inhibitor; b) DPP-IV inhibitor	a) Meisel et al. (2016) b) Lan et al. (2015)
58	Cartilage	T(I/L)	DPP-IV inhibitor	Lan et al. (2015)
59	Cartilage	TF	a) ACE inhibitor; b) DPP-III inhibitor; c) DPP-IV inhibitor; d) Renin inhibitor	a) Nogata et al. (2009) b) Lee & Snyder (1982) c) Lan et al. (2015) d) He et al. (2013)
60	Cartilage	TNP	ACE inhibitor	Arihara et al. (2001)
61	Cartilage	TT	DPP-IV inhibitor	Lan et al. (2015)
62	Cartilage	TV	DPP-IV inhibitor	Lan et al. (2015)
63	Cartilage	V(I/L)	a) DPP-IV inhibitor; b) Glucose uptake stimulating peptide	a) Lan et al. (2015) b) Morifuji et al. (2009)
64	Cartilage	V(Q/K)	a) ACE inhibitor; b) DPP-IV inhibitor	a) C.-H. Li et al. (2002) b) Lan et al. (2015)
65	Cartilage	V(Q/K)P	ACE inhibitor and antioxidative peptide	J. Li et al. (2014)
66	Cartilage	VE	a) ACE inhibitor; b) DPP-IV inhibitor; c) α -glucosidase inhibitor	a) Van Platerink et al. (2008) b) Lan et al. (2015) c) Mora et al. (2020)
67	Cartilage	VP(Q/K)	ACE inhibitor	Pihlanto-Leppälä et al. (2000)
68	CIM	VS	DPP-IV inhibitor	Lan et al. (2015)
69	Cartilage	VT	DPP-IV inhibitor	Lan et al. (2015)
70	Cartilage	YA	a) ACE inhibitor; b) DPP-IV inhibitor; c) Renin inhibitor	a) Cushman et al. (1981) b) Lan et al. (2015) c) Udenigwe et al. (2012)
71	Cartilage	YY	a) ACE inhibitor; b) DPP-III inhibitor; c) DPP-IV inhibitor	a) Lafarga et al. (2014) b) Lee & Snyder (1982) c) Lan et al. (2015)

¹IM: intestinal mucosa; CIM: cartilage and intestinal mucosa.

²ACE: angiotensin-converting enzyme; DPP: dipeptidyl peptidase; GLP: glucagon-like peptide; PEP: prolyl endopeptidase; PGK: phosphoglycerate kinase.

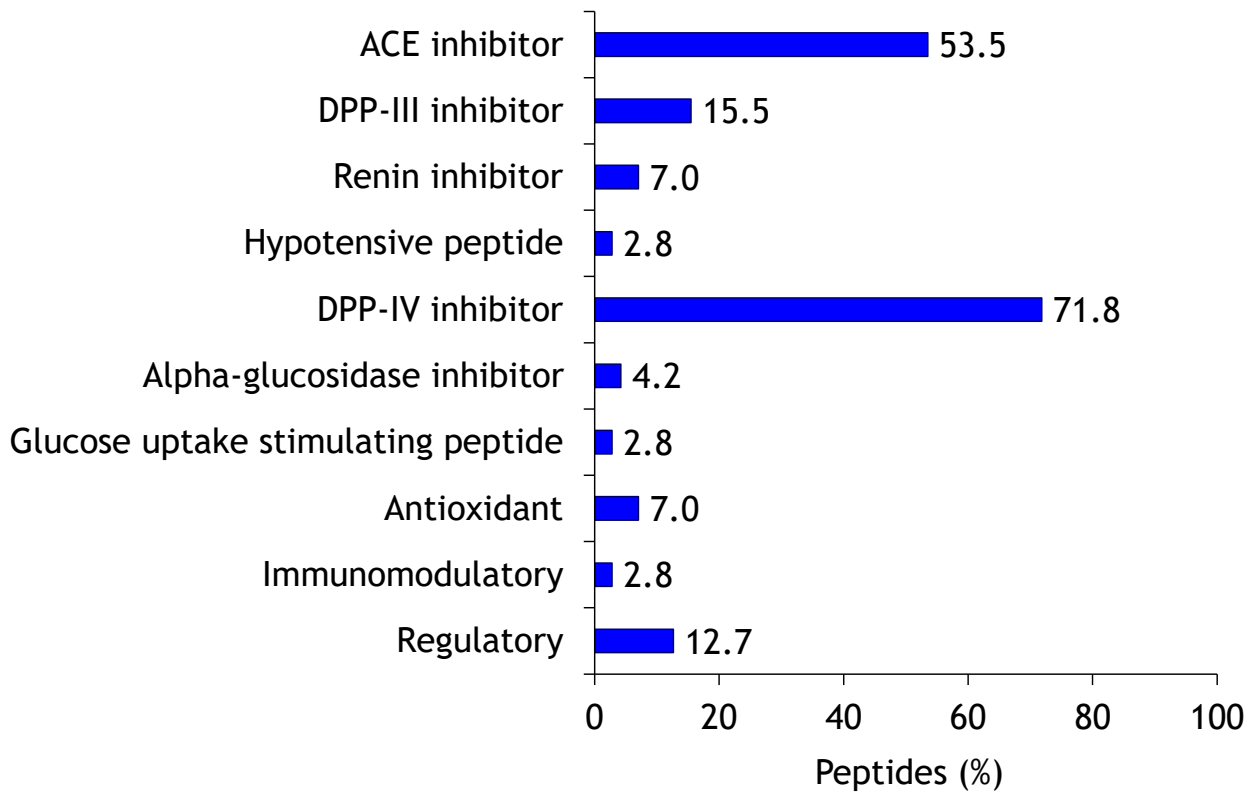


Figure 1. Proportion of peptides (%) associated with each biological activity identified in Peptipro®.

ACE: angiotensin-converting enzyme; DPP: dipeptidyl peptidase.

Regarding intestinal permeability assessed using FITC-dextran, the administered marker was identified in the plasma of all sampled animals. No differences

between dietary treatments ($P = 0.308$) were detected (Figure 2), and the serum level of the marker averaged $0.41 \mu\text{g mL}^{-1}$.

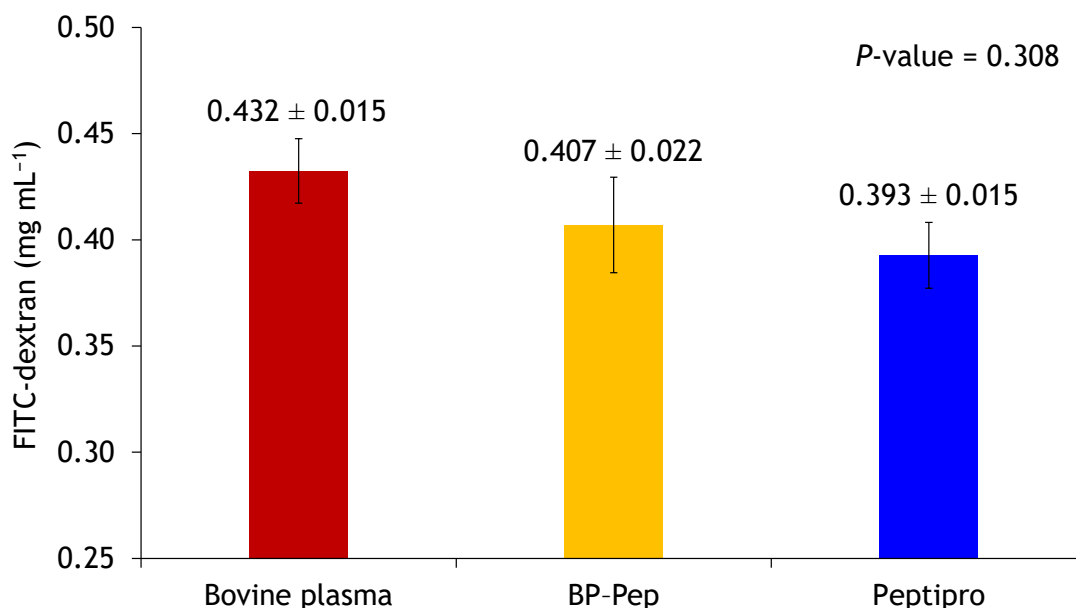


Figure 2. Plasma concentration of fluorescein isothiocyanate-dextran (FITC-dextran) in weaned piglets fed diets containing bovine plasma and Peptipro® during the nursery phase. Values are expressed as mean ± standard error (error bar). BP-Pep: diet supplemented with partial replacement of bovine plasma with Peptipro®.

The performance results showed that the initial BW of piglets was similar among treatments ($P = 0.355$). Furthermore, BW at the end of each nursery phase was not affected ($P > 0.05$) by diet, sex, or their interactions (Table 4). The average BW of piglets at the end of the experiment was 25.12 kg. In addition, feed intake in each phase and during the total nursery period was not influenced ($P > 0.05$) by diet, sex, or their interactions. Mean values of 0.198, 0.444, 0.624, and 0.931 kg animal⁻¹ day⁻¹, and 0.656 kg animal⁻¹ day⁻¹ were found for feed intake during Pre-initial I, Pre-initial II, Initial I and Initial II phases, and for the total nursery period, respectively (Table 4).

Dietary treatment influenced TWG and ADG in the Pre-initial II phase ($P = 0.044$). During this period, piglets fed the Peptipro®

diet had higher weight gain (2.78 kg animal⁻¹ and 0.397 kg animal⁻¹ day⁻¹) than those fed the BP-Pep diet (2.46 kg animal⁻¹ and 0.352 kg animal⁻¹ day⁻¹) (Table 4). Piglets fed a BP diet had similar weight gain to the other groups (2.66 kg animal⁻¹ and 0.379 kg animal⁻¹ day⁻¹). For the other phases and total nursery period, no effects ($P > 0.05$) of diet, sex, or their interactions were observed. Mean values of 0.93, 2.15, 12.56, and 18.20 kg animal⁻¹ for TWG, and 0.133, 0.307, 0.698, and 0.467 kg animal⁻¹ day⁻¹ for ADG were found for the Pre-initial I, Initial I, and Initial II phases, and for the total nursery period, respectively.

FCR was influenced by diet during the Pre-initial II phase ($P = 0.001$), by diet ($P = 0.018$) and sex ($P = 0.001$) during the Initial

II phase, and also by diet ($P = 0.006$) and sex ($P = 0.001$) for overall nursery period (Table 4). In the Pre-initial II phase, piglets fed the Peptipro® diet had better FCR (1.08 kg feed kg^{-1} gain) than those fed BP and BP-Pep diets (1.25 kg feed kg^{-1} gain, on average). In the Initial II phase, piglets fed BP diet had better FCR (1.31 kg feed kg^{-1} gain) than those fed BP-Pep diet (1.36 kg feed kg^{-1} gain), whereas the FCR of piglets fed Peptipro® diet (1.33 kg feed kg^{-1} gain) was similar to both groups. For the total nursery phase, piglets fed BP and Peptipro® diets had similar FCR (1.39 kg feed kg^{-1} gain), which were better than piglets fed BP-Pep diet (1.43 kg feed kg^{-1} gain). Regarding the sex effect, males exhibited improved FCR in Initial II phase (1.31 kg feed kg^{-1} gain) and during the total nursery period (1.38 kg feed kg^{-1} gain) than females (1.36 and 1.43 kg feed kg^{-1} gain, respectively). No interaction effect between diet and sex on FCR was observed in any phase and the total nursery period, and no independent effects of diet and sex on

FCR in the Pre-initial I and Initial I phases were observed. For both phases, mean values of 1.57 and 2.06 kg feed kg^{-1} gain were found for FCR, respectively.

Diet, sex, and their interactions in the nursery did not influence fecal scores ($P > 0.05$) (Table 5). Mean values of 51.5, 35.5, and 13.0% were recorded for the relative frequency of normal, pasty, and liquid feces, respectively. This represented an absolute frequency of 21, 14, and 5 piglets per treatment for each fecal score, respectively. Regarding medical interventions, seven piglets fed a BP diet were treated for diarrhea; 15 piglets fed a BP-Pep diet were medicated, 10 for diarrhea, and five for lethargy; and 11 piglets fed a Peptipro® diet were medicated, 10 for diarrhea, and one for lethargy. The mortality rate during the experiment was 1.66% (2/120), and the piglets that died were fed a BP diet ($n = 1$) and Peptipro® diet ($n = 1$).

Table 4
Performance of weaned piglets fed diets containing bovine plasma and Peptipro® during the nursery phase

Variable	Phase	Diet ^I		Sex		Mean	SEM ^{II}	P-value ^{III}			
		BP	BP-Pep	Peptipro®	Male			Female	Diet	Sex	D × S
Body weight (kg)	Start of the experiment	6.91	6.92	6.91	6.95	6.88	6.92	0.14	0.993	0.820	0.355
	End of Pre-initial I	7.89	7.80	7.65	7.90	7.66	7.78	0.16	0.634	0.336	0.280
	End of Pre-initial II	10.56	10.26	10.45	10.54	10.30	10.42	0.21	0.660	0.444	0.576
	End of Initial I	12.67	12.33	12.70	12.80	12.33	12.56	0.24	0.663	0.244	0.528
	End of Initial II	25.59	24.54	25.25	25.55	24.70	25.12	0.38	0.362	0.184	0.732
Feed intake (kg animal ⁻¹ day ⁻¹)	Pre-initial I	0.205	0.202	0.187	0.205	0.191	0.198	0.007	0.659	0.330	0.949
	Pre-initial II	0.468	0.439	0.426	0.444	0.444	0.444	0.010	0.198	0.977	0.543
	Initial I	0.624	0.605	0.643	0.632	0.616	0.624	0.013	0.454	0.540	0.675
	Initial II	0.942	0.923	0.930	0.928	0.934	0.931	0.012	0.800	0.800	0.779
Total nursery period	0.667	0.648	0.652	0.657	0.655	0.656	0.010	0.702	0.702	0.909	0.774
Total weight gain (kg animal ⁻¹)	Pre-initial I	0.99	0.95	0.85	1.02	0.84	0.93	0.06	0.633	0.140	0.890
	Pre-initial II	2.66 ab	2.46 b	2.78 a	2.65	2.61	2.63	0.06	0.044	0.709	0.767
	Initial I	2.14	2.07	2.25	2.26	2.04	2.15	0.06	0.534	0.120	0.602
	Initial II	12.92	12.21	12.55	12.75	12.37	12.56	0.17	0.156	0.199	0.548
Total nursery period	18.67	17.61	18.34	18.59	17.82	18.20	0.29	0.278	0.149	0.698	
Average daily gain (kg animal ⁻¹ day ⁻¹)	Pre-initial I	0.141	0.136	0.122	0.146	0.120	0.133	0.008	0.633	0.144	0.898
	Pre-initial II	0.379 ab	0.352 b	0.397 a	0.378	0.373	0.376	0.009	0.044	0.715	0.762
	Initial I	0.305	0.295	0.321	0.322	0.292	0.307	0.009	0.539	0.124	0.606
	Initial II	0.718	0.678	0.697	0.708	0.688	0.698	0.009	0.156	0.203	0.552
Total nursery period	0.479	0.452	0.470	0.477	0.457	0.467	0.008	0.276	0.150	0.698	
Feed conversion ratio (kg feed kg ⁻¹ gain)	Pre-initial I	1.53	1.54	1.65	1.45	1.69	1.57	0.06	0.635	0.058	0.579
	Pre-initial II	1.24 a	1.25 a	1.08 b	1.19	1.19	1.19	0.03	0.001	0.799	0.057
	Initial I	2.06	2.08	2.04	1.99	2.13	2.06	0.04	0.895	0.087	0.478
	Initial II	1.31 b	1.36 a	1.33 ab	1.31 B	1.36 A	1.34	0.01	0.018	0.001	0.390
Total nursery period	1.39 b	1.43 a	1.39 b	1.38 B	1.43 A	1.41	0.01	0.006	0.001	0.199	

^IBP: bovine plasma; BP-Pep: diet with partial replacement of bovine plasma with Peptipro®. ^{II}SEM: standard error of the mean. ^{III}D × S: interaction between dietary treatment and sex. Lowercase letters compare means among dietary treatments by Tukey's test ($P < 0.05$), and uppercase letters compare means between sexes by F test of ANOVA ($P < 0.05$).

Table 5

Relative frequency (%) of each fecal score in weaned piglets fed diets containing bovine plasma and Peptipro® during the nursery phase

Phase	Diet ^I			Sex		Mean	SEM ^{II}	P-value ^{III}		
	BP	BP-Pep	Peptipro®	Male	Female			Diet	Sex	D × S
Normal (score = 1)	48.99	49.49	56.17	48.23	54.87	51.55	2.38	0.388	0.177	0.524
Pasty (score = 2)	39.75	37.71	28.98	36.76	34.20	35.48	1.93	0.076	0.516	0.935
Liquid (score = 3)	11.29	12.82	14.87	15.05	10.93	12.99	1.77	0.988	0.248	0.113

^IBP: bovine plasma; BP-Pep: diet with partial replacement of bovine plasma with Peptipro®.

^{II}SEM: standard error of the mean.

^{III}D × S: interaction between dietary treatment and sex.

Discussion

No dietary treatment effect on intestinal permeability, as indicated by the FITC-dextran marker (Figure 2), was observed. The detection of this marker occurs proportionally to the levels of injury in the intestinal epithelial monolayer (Vicuña et al., 2015). Higher plasma concentrations of FITC-dextran indicate a greater degree of paracellular permeability and mucosal injury. Therefore, the results suggest that BP, Peptipro®, and its association in the diet led to a similar nutrient absorption potential by the intestinal epithelium.

The Peptipro® diet improved weight gain without affecting feed intake, resulting in better feed efficiency (FCR; Table 4) of piglets during the second week (7–14 days) of the nursery phase. Balan et al. (2021) demonstrated that piglet ADG increased during the first week post-weaning with the inclusion of 4%–10% BP. However, these authors did not verify the effect of BP levels on piglet performance up to 14 days post-weaning. In the current study, the Peptipro® inclusion levels (1.5%–3%) were half those of

BP (3%–6%) in the diets containing both as exclusive amino acid sources of animal origin and were the same as that of BP in the BP-Pep diet. Compared with this diet, the Peptipro® diet increased TWG by 13% (0.320 kg animal⁻¹) and ADG by 12.8% (0.045 kg animal⁻¹ day⁻¹). The improved piglet performance during the Pre-initial II phase demonstrates that the use of Peptipro® in the diet may help overcome the stress conditions of the first 14 days of the nursery phase, which is a consequence of weaning and changes in the environment, pen mates, and feeding management.

In addition to its high CP content and favorable amino acid profile, Peptipro® contains a broader spectrum of bioactive peptides than BP (Table 3). A total of 71 bioactive peptides were identified in Peptipro® (Figure 1). By grouping the peptides according to their main biological functions, 74.6% are related to energy metabolism regulation (DPP-IV inhibitors, insulin secretion inhibitor, α -glucosidase inhibitors, GLP-1 release stimulator, glucose uptake stimulators, and PGK regulator). Additionally, 63.4% are related to blood pressure regulation (ACE inhibitors, DPP-

III inhibitors, renin inhibitors, hypotensive peptides, and vasoactive substance release stimulator), 7% act as antioxidative peptides, 2.8% act in immunomodulatory processes (chemotactic and antibacterial functions), and 4.2% are associated with various metabolic pathways (anticoagulant function [antithrombotic peptide], neuropeptide function and neuropeptide regulation [PEP inhibitor], stomach mucosal membrane activity regulation, and cell metabolism [ubiquitin-mediated proteolysis activating peptide]). It is important to note that each peptide may have more than one bioactivity, as demonstrated in Table 3.

Previous studies have identified 24 bioactive peptides in bovine blood and plasma, of which 15 peptides have an immune function (Bah et al., 2016) and 9 peptides have an antioxidant function (Boonkong et al., 2024). In both Peptipro® and BP, bioactive peptides may affect intestinal mucosa maturation and exert anti-inflammatory, antimicrobial, and immunostimulant effects (Canibe et al., 2022; Sharma et al., 2011; Vidal et al., 2022). These compounds can interfere with the gene expression of enterocytes in the intestine, thus promoting better intestinal health, contributing to better digestion and absorption of dietary nutrients, and resulting in improved performance (González-Solé et al., 2020; Wylensek et al., 2020; Xiong et al., 2019).

Peptipro®, which contains peptides with potential to modulate glucose metabolism, blood pressure, and oxidative balance, has potential applications in animal nutrition. These effects could be especially relevant for piglets, where the supplementation with protein hydrolysates enriched in bioactive peptides supports

metabolic regulation, enhances growth, and contributes to overall animal welfare. The presence of peptides associated with energy metabolism and immunomodulatory function may explain the better performance results in piglets fed a Peptipro® diet during the Pre-initial II phase of the nursery. Increased availability of immunostimulant compounds in the small intestine may reduce pathogen activity, improving intestinal health and performance during the nursery period (Balan et al., 2021; Huting et al., 2021). Furthermore, a residual effect of Peptipro® intake was noted in the Initial II phase and for the total nursery period, with piglets expressing FCR similar to those fed BP. Given that animal plasma is among the most expensive ingredients in post-weaning diets, Peptipro® represents a technically and economically more viable alternative.

Regarding sanitary conditions, piglets presented with post-weaning diarrhea (fecal score = 3; 13%) within the expected range for the nursery period. Post-weaning diarrhea is a multifactorial disorder related to changes in the environment and diet, succession of gut microbiota from pre- to post-weaning, and immune competence (Canibe et al., 2022; Silva et al., 2023). When a fecal score of 3 is identified in $\geq 20\%$ of the piglets, the incidence of diarrhea is classified as high (Sobestiansky & Barcellos, 2007). The frequency of diarrhea was approximately half of the reference limit, which may be explained by the antimicrobial activity and immunostimulatory effects promoted by some bioactive peptides contained in BP and Peptipro®.

Piglets with diarrhea were individually subjected to drug therapy for up to 14 days of the nursery phase. To avoid interference

with the intestinal permeability test, no organic acids or mass antibiotic therapies were administered. If necessary, individual medication interventions related or not to diarrhea were performed up to 21 days of the nursery period. The two piglets that died during the experiment presented lateral recumbency, lethargy, pedaling movements, opisthotonos, and death, even after drug intervention. Post-mortem examination revealed lesions suggestive of streptococcal meningitis.

Conclusions

Peptipro® contains a broad spectrum of bioactive peptides that may modulate glucose metabolism, blood pressure, oxidative balance, and immune function. Regarding animal performance, this ingredient increases weight gain without affecting feed intake, thereby improving feed efficiency in piglets during the second week of the nursery phase. Owing to its favorable amino acid profile, the presence of diverse bioactive peptides, and its positive effects on animal performance, Peptipro® can effectively replace spray-dried BP in pre-starter diets for nursery piglets.

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