



## A functional carrot-based food product in the prevention of post-weaning stress in piglets

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### ABSTRACT

**Background:** Post-weaning stress in piglets is characterized by the development of diarrhea, intestinal dysbiosis, systemic inflammation, and significant economic losses in pig farming. Limiting the use of feed antibiotics stimulates the search for safe functional products capable of modulating intestinal homeostasis. The historical protocol of E. Moreau (1908) for long-boiled carrots has demonstrated anti-adhesive and prebiotic potential but has not been considered as an element of systemic prevention in industrial pig farming.

**Objectives:** To develop and scientifically substantiate the use of a functional carrot-based food product (Moro soup) in the prevention of post-weaning stress and associated diarrhea in piglets.

**Methods:** The experiment was conducted on 60 piglets of a large white breed (28-35 days old, live weight  $7.2 \pm 0.5$  kg). The animals are divided into 4 groups: 1st – control (standard diet); 2nd – antibiotic (colistin); 3rd – probiotic (*Bacillus subtilis*); 4th – experimental (Moro soup at a dose of 5-7 ml/kg 3 times a day, 5 days before, and 7 days after weaning). The Moro soup's main ingredients of 1 kg carrots, 2 liters of water, and 1.5 g of salt were cooked for 2 hours, after which

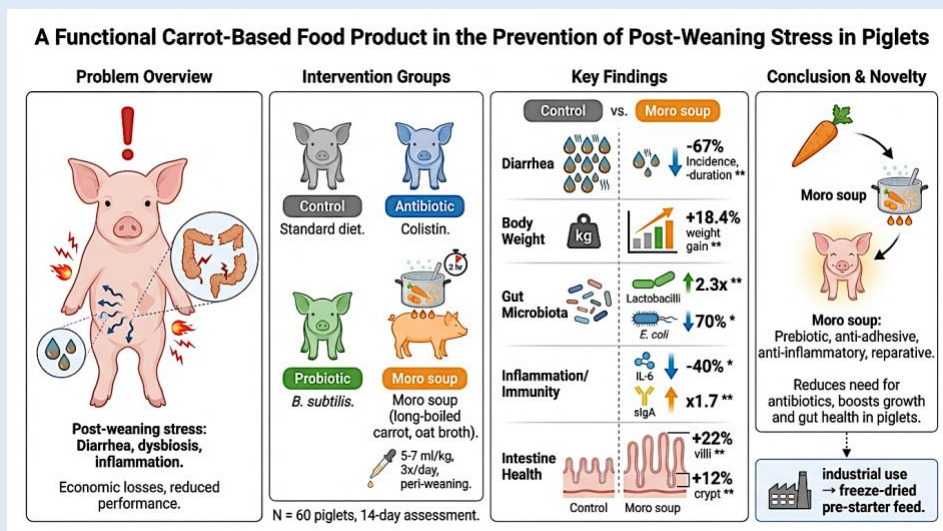
5% oat broth was added. The frequency and severity of diarrhea, body weight gain, large intestinal microbiota (PCR-RV), IL-6, secretory IgA (ELISA) levels, and jejunum histology were evaluated.

**Results:** Moro soup supplementation resulted in a 67% decrease in the incidence of diarrhea ( $p < 0.01$ ) compared with the control group and reduced the duration of diarrhea syndrome to  $1.6 \pm 0.3$  days. The average daily weight gain during the 14 days after weaning in the experimental group exceeded that of the control group by 18.4% ( $p < 0.05$ ) and was comparable to the antibiotic group. Microbiological analysis revealed a 2.3-fold increase in *Lactobacillus* spp. and a 70% decrease in enteropathogenic *E. coli*. The level of the pro-inflammatory cytokine IL-6 decreased by 40% ( $p < 0.05$ ), and the concentration of sIgA in the intestinal contents increased by 1.7 times. Morphometry of jejunum villi showed an increase in villi height of 22% and crypt depth by 12%, indicating a regenerative effect.

**Conclusion:** Moro soup is an effective functional food product that provides systemic prevention of post-weaning stress in piglets due to its combined anti-adhesive, prebiotic, anti-inflammatory, and reparative effects. Including the product in standard feeding protocols reduces reliance on antibiotics, increases livestock safety, and improves productivity. Next steps should focus on developing a free-dried form of Moro soup for industrial production and on incorporating it into commercial pre-starter feeds.

**Novelty of study:** This is the first study to develop a functional carrot-based food product (Moro soup) and examine efficacy in the prevention of post-weaning stress in piglets. Additionally, this study is the first to obtain comprehensive data on the effects of Moro soup on the microbiota, immune response, and morphofunctional state of piglets' intestines, compared with antibiotics and probiotics.

**Keywords:** post-weaning stress, functional animal food product, Moro soup, oligogalacturonides, intestinal microbiota, alternatives to antibiotics.



**Graphical abstract:** A functional carrot-based food product in the prevention of post-weaning stress in piglets.

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## INTRODUCTION

Modern industrial pig farming is facing a complex of challenges, with post-weaning stress being a main issue. During a piglet's weaning stage, abrupt changes in feed ration, type of feed, and the transportation and regrouping of the piglets all contribute as major stress factors on the animal's premature digestive system. Weaning stress syndrome in piglets seriously endangers the healthy development of the breeding industry. The physiological immaturity of the intestinal barrier, low activity of pancreatic enzymes, and an unformed immune response determine the high susceptibility of weaned piglets to gastrointestinal diseases. Diarrheal syndrome is reported in 30-60% of livestock during this period, and mortality can reach 15-25%, resulting in significant economic loss for pig farms. Thus, it is necessary to find ways to alleviate weaning stress, improve immunity, and promote intestinal health in animal nutrition [1-6].

The compositional analysis of fruit, vegetables, and their by-products showed that identified phytochemicals could act as bioactive compounds to modulate weaning-induced disturbances in piglets. Plant-Derived Polysaccharides Benefit Weaned Piglets by Regulating Intestinal Microbiota. Antibacterial plant combinations prevent postweaning diarrhea in organically raised piglets challenged with enterotoxigenic *Escherichia coli* F18 [7-9].

The main etiological agents of post-weaning diarrhea are enterotoxigenic strains of *Escherichia coli*, producing adhesins F4 and F18, as well as *Salmonella* spp., *Clostridium perfringens*, and rotaviruses. The pathogenesis of infections includes the adhesion of microorganisms to the brush border of enterocytes, colonization of the mucosa, and secretion of enterotoxins, which leads to hypersecretion of fluid and electrolytes, and damage to the villous epithelium and malabsorption. The traditional control strategy includes the use of feed antibiotics, but the long-term practice of subtherapeutic doses has led to a global spread of antibiotic resistance [10].

When the digestive system of piglets is affected, various food additives are used-mineral elements. Dietary fatty acids have a positive effect on intestinal health and diarrhea of weaned piglets, which is associated with the regulation of the intestinal barrier and microbial community function [11-12]. Overall, plant-derived bioactive substances offer significant advantages for strengthening the endogenous intestinal defense capacity of weaned piglets and demonstrate great potential as alternatives to in-feed antibiotics and zinc oxide to achieve sustainable and healthy pig production [13].

According to WHO data, the level of resistance of *E. coli* to tetracyclines and ampicillin in the farm animal population exceeds 60%. prompting the need to develop and implement alternative strategies for the prevention and treatment of intestinal infections [14].

The extraction of functional components from fruit and vegetable waste has the potential to generate new value-added products for various sectors (e.g., food) [15]. Carrot is a versatile crop that the food industry should further exploit due to its functional and nutraceutical properties, which enable the generation of novel food ingredients and the formulation of innovative healthy foods. Papaya and carrot root extracts are increasingly recognized for their ability to modulate inflammation and alter immunological functions due to their richness in bioactive compounds [16-17].

A promising direction is the use of functional food products (FFP) – ingredients or ready-made diets with nutritional value and physiological and metabolic effects on the body, that modulate key homeostatic processes. In pig breeding, FFPs include probiotics, prebiotics, organic acids, phytobiotics, and enzymes, but their effectiveness varies and often requires combined use of FFPs that can suppress pathogen adhesion, restore microbiocenosis, and exert anti-inflammatory properties are of particular interest. While traditionally established as a pediatric remedy, Moro soup attracts attention as a

multifunctional food matrix with a unique profile of bioactive properties [18].

Moro soup was developed in 1908 by the Austrian pediatrician Ernst Moro, who proposed using carrot broth obtained by long-term boiling to treat severe diarrhea in infants. His proposal decreased the mortality rate of the children's hospital at that time by 50%. The mechanism underlying the soup's properties was unknown until the beginning of the 21<sup>st</sup> century, when Italian researchers found that carrot pectins depolymerize during heat treatment to form acidic oligohalacturonides, which bind to enterobacteria fimbriae receptors, preventing their adhesion to enterocyte glycocalyx. Subsequent studies also found that carrot oligosaccharides have a prebiotic effect by selectively promoting the growth of bifidobacteria and lactobacilli and exert anti-inflammatory properties through the presence of beta-carotene and phenolic compounds [19]. Probiotics may facilitate weight loss by increasing microbiome quantity and variety, regulating immune responses, and improving metabolic rates [20].

Carrots are exceptional sources of bioactive compounds with potential health benefits. Carrot (*Daucus carota* L.) is a globally consumed root vegetable, enjoyed both raw and after thermal processing [21-22]. From an agricultural perspective, carrots are a significant tap root vegetable crop in the *Apiaceae* family because of their nutritional value, health advantages, and economic importance. The edible part of a carrot, known as the storage root, contains various beneficial compounds, such as carotenoids, anthocyanins, dietary fiber, vitamins, and other nutrients. It has a crucial role in human nutrition as a significant vegetable and raw material in the nutraceutical, food, and pharmaceutical industries. Carrot cultivation is susceptible to a wide range of biotic and abiotic stresses, which can significantly compromise the plant's health and decrease yield and quality. Scientific research mostly focuses on important biotic stressors, including pests, such as

nematodes and carrot flies, as well as diseases, such as cavity spots, crown or cottony rot, black rot, and leaf blight, caused by bacteria, fungi, and oomycetes. The emerging challenges in the field include gaining a comprehensive understanding of the interaction between hosts and pathogens in the carrot-pathogen system, identifying the elements that contribute to disease development, expanding knowledge of systemic treatments, exploring host resistance mechanisms, developing integrated control programs, and enhancing resistance through breeding approaches. In fact, the primary carrot-growing regions in tropical and subtropical climates are experiencing abiotic pressures, such as drought, salinity, and heat stress, which limit carrot production. This review provides an extensive, up-to-date overview of the literature on biotic and abiotic factors for enhanced and sustainable carrot production, considering the use of different technologies to extend carrot shelf life. Therefore, it addresses current issues in the carrot production chain, opening new perspectives for exploring carrots as both a food commodity and as a source of natural compounds [23].

Research underscores the potential of carrot-based products to address the evolving needs of the pet food market, offering a nutritious and sustainable solution that leverages agricultural by-products effectively and opens avenues for product diversification within the carrot value chain [24].

The knowledge of effective feed additives that can prevent or cure problems and their consequences is important in modern animal production systems [25].

Despite its well-documented history in pediatrics and established molecular mechanisms, the application of the Moro method in veterinary practice remains inconsistent and limited. There are reports on the efficacy of carrot soup in treating enteritis in dogs and cats; however, there are no systematic studies regarding productive animals, particularly in weaned pigs. Beyond their significance in veterinary use, pigs also serve as an

optimal biological model for studying intestinal immunity and the microbiome; their gastrointestinal anatomy and physiology are comparable to those of humans, providing translational value.

**The study's current challenges:** Currently, there are inconsistencies between the efficacy of Moro soup in vitro and in clinical pediatrics; there is also a lack of standardized protocols, prescription regulations, and evidence of efficacy in pig farming. Additionally, the inclusion of Moro soup in the prevention of post-weaning stress has not been studied; optimal dosages, as well as the frequency and administration schedule for piglets of different age groups, have not been established. Furthermore, there are no comparative studies on the effectiveness of Moro soup in comparison with traditional antibiotics and probiotics. Criteria for assessing the systemic physiological response to the introduction of this functional product have not been developed.

The inclusion of modified Moro soup in the diet of piglets during the weaning period may reduce the frequency and severity of diarrheal syndrome, improve growth and preservation due to the combined action of oligogalacturonides (anti-adhesive and prebiotic effect), carotenoids (anti-inflammatory and antioxidant effect), and dietary fiber (detoxification and trophic effect); positioning Moro soup as a potential alternative to antibiotic therapy in preventative measures.

**Purpose and objectives of the study:** The purpose of this work was to develop and scientifically substantiate the use of a functional carrot-based food product (Moro soup) in the prevention of post-weaning stress and associated diarrhea in piglets. To achieve this goal, the following tasks were solved: 1) optimize the formulation and technology of Moro soup preparation in relation to the physiological characteristics of weaned piglets; 2) evaluate the product's effects on clinical and physiological status, particularly incidence of diarrhea

and productive indicators; 3) to study the dynamics of the large intestine's microbiocenosis; 4) to determine systemic and local immunological effects; 5) to conduct a morphometric assessment of regenerative processes in the small intestine; 6) to compare the effectiveness of Moro soup with an antibiotic and a probiotic in a controlled experiment.

## MATERIALS AND METHODS

The study was conducted from February 2025 to March 2026 at the National Agrarian University of Armenia's veterinary research laboratory and veterinary sanitary expertise, as well as some pig breeding complexes of the Republic of Armenia. All experimental procedures were carried out in accordance with the principles of the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes.

The study included 60 clinically healthy large white breed of piglets, aged 28-35 days, with an average weight of  $7.2 \pm 0.5$  kg; these animals were obtained from sows in their second or third farrowing. Weaning from sows was carried out simultaneously on the first day of the experiment. The animals were kept in a controlled microclimate in boxes for group rearing (5 piglets per pen) on slotted floors. The air temperature was maintained at 26-28° C, relative humidity – 60-65%, light mode – 12 hours day/12 hours night. Access to water was available through nipple drinkers.

Standard balanced compound feed for weaned piglets (PK-51) was used. Dry food was fed twice a day, and the specified feeds and residues were accounted for and carried out daily.

Animal groups were formed using the method of analog pairs (considering sex, body weight, and origin); piglets were divided into 4 groups of 15 animals each (3 repetitions of 5 animals):

- Group 1 (negative control, K-) – received the basic diet (RR) without any additives.
- Group 2 (positive control, AB) – received the main diet (RR) with the addition of the antibiotic colistin

sulfate at a dose of 1.5 mg/kg of body weight orally once a day for 7 days after weaning (preventive regimen).

- Group 3 (probiotic, PB) – Basal Diet (BD) + probiotic based on *Bacillus subtilis* B-2998D (Vetom 2, NPF Research Center LLC, Russia) at a dose of  $5 \times 10^9$  CFU/head /day, mixed with a small amount of feed.

- Group 4 (experimental, CM) – BD + functional product "Moro Soup" (modified formulation), which was drunk individually through a dispenser at a dose of 5-7 ml/kg of live weight 3 times a day, 5 days before weaning and for 7 days after weaning. The experiment lasted 21 days (7 days of adaptation before weaning + 14 days after weaning).

Throughout the entire period, the clinical condition, feed and water intake were assessed daily, and diarrhea cases were recorded. Individual weighing was performed on days 0, 7, 14, and 21 of the study.

The modified Moro soup was prepared daily according to the original formulation Fresh table carrots (*Daucus carota* L.) were purchased in a single batch, peeled, and ground on a grater with a 5 mm hole diameter. 1 kg of chopped carrots was placed in an enameled container, and 2 liters of water and 1.5 g of table salt (NaCl) were added. The mixture was brought to a boil and low-boiled for 120 minutes without a lid until the volume was reduced to 2 liters. 20 minutes before the end of cooking, 50 ml of oat broth was added (10 g of oat flakes "Hercules" brewed with 100 ml of boiling water for 15 minutes), which improved the taste and provided additional energy value.

The finished product was cooled to 38-40 °C, immersion-blended to a puree state, and filtered through a metal sieve to remove large fibers. The resulting homogenate had a total dry matter concentration of  $6.8 \pm 0.3\%$ , an oligohalacturonide content of  $2.1 \pm 0.2$  mg/ml, and a pH of  $5.4 \pm 0.1$ . The product was evaporated and freshly prepared within 1 hour after manufacturing.

Fecal consistency was assessed three times a day (morning, afternoon, and evening) on a 4-point scale.

0 – normal, decorated.

1 – soft, mushy.

2 – liquid, unformulated.

3 – watery with an admixture of mucus or blood.

A piglet was considered to have diarrhea if it had a score of  $\geq 2$  in at least one bowel movement per day. The following parameters were evaluated:

- Incidence (incidence) – the proportion of animals (%) with at least one episode of diarrhea during the period.

- Duration of diarrhea (days) – the average number of days with a score  $\geq 2$ .

- Diarrhea severity index – the sum of the scores for the period/number of days of follow-up.

The live weight of piglets was determined by individual weighing on an electronic scale VM-150 (accuracy  $\pm 10$  g). The following parameters were evaluated:

Average daily increase (CP, g/day) =  $(M_k - M_n) / t$ .

Feed consumption (g/day) – by the group method based on 1 head.

Feed conversion (KK, kg/kg) = feed intake / live weight gain.

On the 14th day after weaning, 6 animals per group (2 from each replicate) were randomly selected and humanely euthanized for microbiological analysis of the colon contents. The colon's contents were collected in sterile containers and immediately cooled to +4 °C and were delivered to the laboratory within 2 hours.

Quantitative accounting of *Lactobacillus spp.* and *Escherichia coli* was carried out by serial dilution with seeding on selective media: MRS-agar (HiMedia, India) for lactobacilli (anaerobically, 48 h, 37 °C) and Endo agar (GBUN SSC PMB, Russia) for enterobacteria (aerobically, 24 h, 37 °C).

The results were expressed in lg CFU/g. Additionally, PCR-RV was performed to identify enterotoxigenic *E. coli* by detecting the estA, eltB, faeG, and fedA genes using a commercial AmpliPrime kit

(NextBio LLC, Russia) on a CFX96 Touch amplifier (Bio-Rad, USA).

Blood samples were taken from the cranial vena cava of 6 piglets from each group on the 14th day of the experiment into vacuum tubes with a coagulation activator. The serum was obtained by centrifugation (1500 g, 15 min) and stored at -80 °C.

The concentration of the pro-inflammatory cytokine IL-6 was determined by solid-phase ELISA using the Pig IL-6 ELISA Kit (Invitrogen, USA, sensitivity 2 pg/ml) on an Infinite F50 tablet reader (Tecan, Austria).

The content of secretory IgA (sIgA) in the contents of the jejunum (flushes of 10 cm of the proximal part of 5 ml of PBS) was assessed with the Pig IgA ELISA Kit (Bethyl Laboratories, USA). The results were normalized to total protein (biuretic method) and expressed in mg sIgA/mg protein.

For histological studies, jejunum fragments (the middle third) were fixed in 10% neutral buffered formalin (24 hours), dehydrated in a series of alcohols, and embedded in paraffin. Sections with a thickness of 5 microns were stained with hematoxylin and eosin. Morphometry was performed using a Leica DM2000 microscope (Germany) and Leica Application Suite v4.12 software. The height of 10 well-oriented villi and the

depth of 10 associated crypts were measured in each preparation. The ratio of villus height to the crypt depth (V/C) was calculated].

Statistical data processing was performed using IBM SPSS Statistics v.26 software package (IBM Corp., USA). The distribution's normality was checked by the Shapiro–Wilk criterion. The data are presented as the arithmetic mean and the standard error of the mean (M±SEM). Intergroup differences were assessed using univariate analysis of variance (ANOVA) followed by Tukey's post hoc test. Fischer's exact criterion was used to compare frequency indicators (morbidity). The differences were considered statistically significant at p<0.05. The graphs are made in GraphPad Prism 9.0 (GraphPad Software, USA).

No unplanned mortality was recorded during the entire experiment period (7 days before weaning through 14 days after weaning), and all piglets completed the study according to the protocol. The clinical condition of all animals prior to weaning was assessed as normal, without diarrhea or systemic disorders. The introduction of Moro soup 5 days before weaning did not cause food refusal, vomiting, or allergic reactions; the product was well-tolerated and readily consumed via individual dispensers.

Figure 1: Moro's carrot soup as a functional food for piglet diarrhea

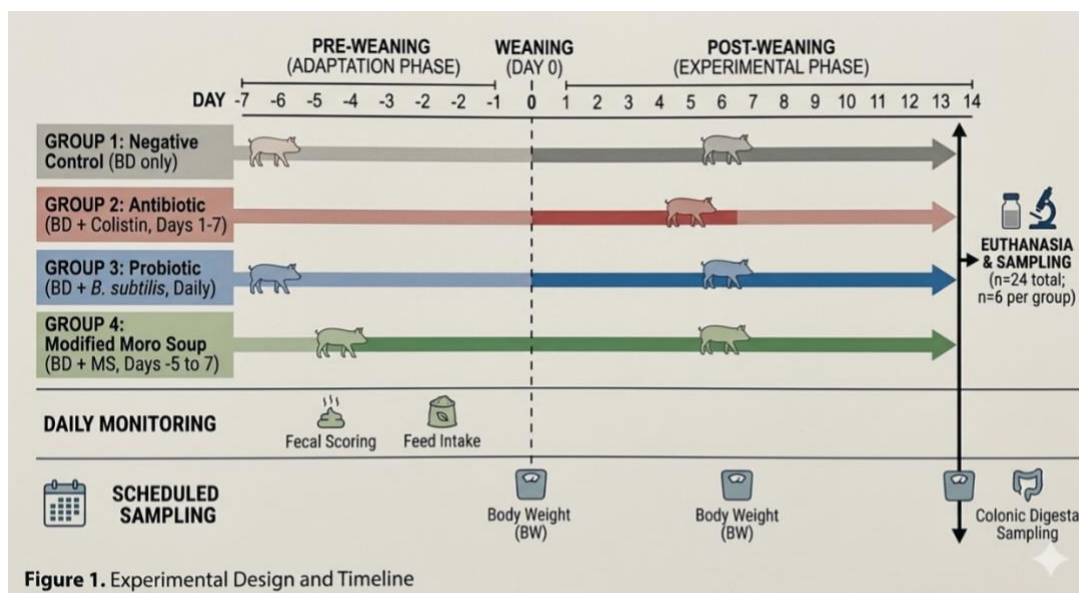


Figure 1. Experimental Design and Timeline

**Table 1.** Clinical parameters of diarrhea in weaned piglets under various prevention regimens (M±SEM)

Indicator	Control (K-)	Antibiotic (AB)	Probiotic (PB)	Moro soup (CM)
Incidence of diarrhea, %	86.7 (13/15)	20.0 (3/15)***	60.0 (9/15)*	26.7 (4/15)***
Average duration of diarrhea, days	4.8 ± 0.4	1.3 ± 0.2***	2.9 ± 0.3**	1.6 ± 0.2***
Diarrhea severity index, points	2.4 ± 0.2	0.6 ± 0.1***	1.4±0.2*	0.7 ± 0.1***
Age of the first episode, days after weaning	2.1 ± 0.2	4.8 ± 0.5**	3.2 ± 0.3	4.3 ± 0.4*

Diarrhea Incidence (n/N), % 86.7 (13/15); 20.0 (3/15)\*\*\*; 60.0 (9/15)\*; 26.7 (4/15)\*\*\* Statistically significant differences with the control: \* p<0.05; \*\* p<0.01; \*\*\* p<0.001.

## RESULTS AND DISCUSSION

The incidence and severity of diarrhea during the post-weaning period are summarized in Table 1.

In the control group, diarrhea was registered in 86.7% of the animals, with the first cases occurring as early as the 2nd day after weaning. In the antibiotic group, the incidence decreased to 20.0% (p<0.001), and in the probiotic group, to 60.0% (p<0.05). The use of Moro soup reduced the incidence to 26.7% (p<0.001), showing no significant difference compared to the antibiotic group (20.0%; p=0.672). Furthermore, the soup

was significantly more effective than the probiotic treatment (p<0.05).

The duration of diarrheal syndrome in the experimental group was 1.6 ± 0.2 days, which was 3 times shorter than that in the control group but did not differ from the antibiotic group. The severity index of diarrhea in the CM group was reduced by 71% compared with the control group.

The body weight and performance indicators are presented in Table 2.

**Table 2.** Productive indicators of weaned piglets for 14 days after weaning (M±SEM)

Indicator	Control (K-)	Antibiotic (AB)	Probiotic (PB)	Moro soup (CM)
Body weight per day of weaning, kg	7.21 ± 0.18	7.19 ± 0.20	7.24 ± 0.16	7.18 ± 0.19
Live weight on day 14, kg	10.42 ± 0.25	11.58 ± 0.28**	10.96 ± 0.24	11.67 ± 0.26**
Gross weight gain, kg	3.21 ± 0.15	4.39 ± 0.18**	3.72 ± 0.16	4.49 ± 0.17**
Average daily increase, g	229 ± 11	314 ± 13***	266 ± 12*	321 ± 12***
Feed consumption, g/day	412 ± 18	468 ± 15*	439 ± 16	477 ± 14*
Feed conversion, kg/kg	1.80 ± 0.06	1.49 ± 0.04**	1.65 ± 0.05	1.49 ± 0.03**

The initial live weight of animals in all groups was similar (p>0.05). By day 14 post-weaning, piglets in the CM group significantly outperformed the control in live weight by 12.0% (p<0.01) and were statistically equivalent to the antibiotic group (p=0.814). The average daily increase in the CM group was 321 ± 12 g/day, which was 40.2% higher than in the control (p<0.001) and comparable to that in the antibiotic group (314 ± 13 g/day, p=0.691). The probiotic group had an average daily increase of 266 ± 12 g/day, which is 16.2% higher than

the control (p<0.05), but significantly lower than the CM group (p<0.05).

Feed intake in the AB and CM groups was higher than that of the control by 13.6% and 15.8%, respectively (p<0.05). The feed conversion in the experimental group (1.49 ± 0.03 kg/kg) improved by 17.2% relative to the control and reached values comparable to the antibiotic.

The results of a quantitative microbiological analysis of the colon contents on the 14th day after weaning are presented in Table 3.

**Table 3.** Abundance of the main microbiota taxa of piglets, lg CFU/g (M±SEM)

Indicator	Control (K-)	Antibiotic (AB)	Probiotic (PB)	Moro soup (CM)
<i>Lactobacillus spp.</i>	6.84 ± 0.21	5.12 ± 0.18***	8.03 ± 0.24**	8.47 ± 0.23***
<i>Escherichia coli</i> (common)	8.23 ± 0.25	6.45 ± 0.20***	7.56 ± 0.22	6.92 ± 0.19**
ETEC (F4/F18-positive)	5.76 ± 0.18	3.89 ± 0.14***	4.92 ± 0.16*	4.08 ± 0.15***
<i>Lactobacillus/E. coli</i> ratio	0.83 ± 0.04	0.79 ± 0.05	1.06 ± 0.05*	1.22 ± 0.06**

The highest increase in *lactobacilli* was observed in the CM group, reaching 8.47±0.23 lg CFU/g, which is 23 times higher than in the control (p<0.001) and significantly higher than in the antibiotic group (p<0.001). The total number of *E. coli* in the experimental group decreased by 1.31 lg (20 times) compared to the control (p<0.01). The content of enterotoxigenic ETEC (F4/F18) strains in the CM group was reduced by 1.68 lg (48 times) relative to the control (p<0.001) and did not differ from the antibiotic group (p=0.342).

The most significant improvement in the *lactobacilli*-to-*E. coli* was observed in the CM group (1.22 ± 0.06), indicating a shift in the microbiocenosis towards the symbiont microflora.

The concentration of IL-6 in the blood serum of piglets on the 14th day after weaning is shown in Table 5.

In the control group, IL-6 levels were 84.6 ± 5.2 pg/ml, reflecting a systemic inflammatory response associated with post-exertion stress and diarrhea. The use of antibiotics reduced cytokine concentration to 52.3

± 4.1 pg/mL (a 38.2% reduction, p<0.01). In the probiotic group, IL-6 levels were 67.8 ± 4.7 pg/mL (a decrease of 19.9%, p<0.05). The most notable decrease in IL-6 was observed in the Moreau soup group, reaching 48.7 ± 3.8 pg/mL, which is 42.5% lower than the control (p<0.001) and does not differ from the antibiotic (p=0.517).

The concentration of sIgA in the jejunum flushes in the control was 12.4 ± 0.9 mcg/mg protein. No significant change was observed in the antibiotic group (13.1 ± 1.0 mcg/mg, p>0.05). However, the probiotic treatment significantly increased sIgA levels to 18.7 ± 1.3 mcg/mg (a 50.8% increase, p<0.01). The maximum sIgA concentration was recorded in the CM group (21.6 ± 1.5 mcg/mg, which is a 74.2% increase over the control (p<0.001) and a significantly higher induction than the probiotic group (p<0.05).

The results of histological analysis of the jejunum of piglets on the 14th day after weaning are presented in Table 4.

**Table 4.** Morphometric parameters of jejunum mucosa (M±SEM)

Indicator	Control (K-)	Antibiotic (AB)	Probiotic (PB)	Moro soup (CM)
Villi height, microns	312 ± 15	358 ± 14*	341 ± 13	396 ± 12***
Crypt depth, microns	178 ± 8	169 ± 7	172 ± 7	159 ± 6*
The V/C ratio	1.75 ± 0.09	2.12 ± 0.10*	1.98 ± 0.09	2.49 ± 0.11***

Serum IL-6 concentrations in piglets on day 14 post-weaning are presented in Table 5. In the control group.

**Table 5.** Serum IL-6 levels in piglets on day 14 post-weaning (M±SEM)

Group	N	IL-6, pg/ml
Control (C-)	6	84.6 ± 5.2
Antibiotic (AB)	6	52.3 ± 4.1 ***
Probiotic (PB)	6	67.8 ± 4.7 *
Moro Soup (MS)	6	48.7 ± 3.8 ***

Note. Statistically significant differences vs. control:

\* p < 0.05; \*\*\* p < 0.001. V/C is the height of the villi/depth of the crypts.

The highest villi height was recorded in the Moro soup group ( $396 \pm 12$  microns), which is 26.9% higher than the control ( $p < 0.001$ ) and significantly higher than the antibiotic ( $p < 0.05$ ) and probiotic ( $p < 0.01$ ) groups. Crypt depth in the experimental group decreased by 10.7% ( $p < 0.05$ ). The ratio of villi height to crypt depth (V/C), which is an integral indicator of the functional activity and regenerative potential of the mucous membrane, reached  $2.49 \pm 0.11$  in the CM group, which is 42.3% higher than the control ( $p < 0.001$ ), 17.5% higher than the antibiotic group ( $p < 0.05$ ), and 25.8% higher than the probiotics ( $p < 0.01$ ).

The histological picture of the control group of piglets showed shortening and deformation of the villi, swelling of the lamina, and moderate lymphohistiocytic infiltration. The CM group had a well-developed villous structure with a clear brush border of enterocytes and minimal signs of inflammation.

The main effects of using Moro soup in the prevention of post-weaning stress in piglets can be summarized as follows:

1. Clinical efficacy: reduced the incidence of diarrhea by 69.1%, reduced the duration of diarrhea by 3 times, and reduced the severity index by 71% ( $p < 0.001$ ).
2. Productive indicators: increased the average daily increase by 40.2% and improved feed conversion by 17.2% ( $p < 0.001$ ).
3. Intestinal microbiota: resulted in a 23-fold increase in *Lactobacillus spp.*, a 48-fold decrease in ETEC, and improved the *lactobacillus/E. coli* ratio by 47% ( $p < 0.01-0.001$ ).
4. Immune response: a decrease in pro-inflammatory IL-6 by 42.5% and an increase in secretory IgA in the intestine by 74.2% ( $p < 0.001$ ).
5. Morphofunctional state of the intestine: an increase in the height of the villi by 26.9% and an increase in the V/C ratio by 42.3% ( $p < 0.001$ ).

**Practical significance and economic aspects:** The production cost of Moro soup (based on per piglet for a

7-day course) is approximately 0.45–0.60 rubles, which is 8-10 times lower than the cost of a standard antibiotic regimen and 15-20 times lower than a probiotic supplementation. Given that the average daily increase in the CM group was 92 g/day higher than the control, the projected additional profit per piglet over the 14 days is about 70-80 rubles (based on 2025 prices). While the economic efficiency of using Moro soup is superior, large-scale implementation requires industrial technology, such as freeze-drying or the production of a dry extract for inclusion in pre-starter diets. Pilot studies indicate that freeze-dried carrot soup powder maintains its anti-adhesive bioactivity and is highly palatable to weaning piglets.

**Research limitations and prospects:** The present study has several limitations. Firstly, the follow-up period was only 14 days after weaning, which does not assess the long-term effects of Moro soup at subsequent cultivation stages (rearing and fattening). Secondly, the sample size ( $n=60$ ) is sufficient to identify the main effects but limits the possibility of stratified analysis; for example, the influence of gender or initial body weight. Thirdly, we did not perform metagenomic sequencing of the microbiota, limiting ourselves to cultural methods and PCR-RV for target pathogens. A more detailed analysis using 16S rRNA would allow us to assess the effect of Moro soup on the entire microbiome, including Archaea and fungi.

**Further research should focus on:**

1. Optimizing the dosage regimen (study of dose-dependent effect)
2. Developing an industrial form of the product while preserving its biological activity
3. Evaluating clinical efficacy in the field on large livestock
4. Investigating the soup's effect on infection with zoonotic pathogens, specifically *Salmonella* and *Campylobacter spp.*

5. Exploring the possibility of combining Moro soup with organic acids and phytobiotics to further potentiate its protective effects.

The use of Moro soup reduced the incidence of diarrhea by 69.1% ( $p < 0.001$ ), the duration of diarrhea by 3 times, and the severity index by 71% ( $p < 0.001$ ). The effectiveness of Moro soup is comparable to that of an antibiotic ( $p = 0.672$ ) and significantly higher than that of a probiotic ( $p < 0.05$ ).

The average daily increase in the CM group was  $321 \pm 12$  g/day, which was 40.2% higher than the control ( $p < 0.001$ ) and comparable to the antibiotic ( $p = 0.691$ ). Feed conversion improved by 17.2% ( $p < 0.01$ ).

In the CM group, there was a 23-fold increase in *Lactobacillus spp.* ( $p < 0.001$ ), a 48-fold decrease in ETEC ( $p < 0.001$ ), and an increase in the L/E ratio to  $1.22 \pm 0.06$  ( $< 0.01$ ).

The level of IL-6 in the blood serum decreased by 42.5% ( $p < 0.001$ ), and the concentration of sIgA in the intestine increased by 74.2% ( $p < 0.001$ ).

The villi height increased by 26.9% ( $p < 0.001$ ), and the V/C ratio increased by 42.3% ( $p < 0.001$ ), both of which were significantly higher than the values of the antibiotic and probiotic groups.

The oligogalacturonides are behind the effect of Moro soup, where they competitively inhibit the binding of ETEC fimbriae to brush border glycoconjugates. The 48-fold decrease in ETEC abundance in our study is fully consistent with in vitro and animal-model studies.

Crucially, this mechanism must not create selective pressure, thereby avoiding the induction of antimicrobial resistance.

The increase in *lactobacilli* is 23 times higher than the effects of most commercial prebiotics. Carrot oligogalacturonides are fermented by representatives of *Bifidobacterium* and *Lactobacillus* to form short-chain fatty acids, which are confirmed by metagenomic studies. The L/E ratio  $> 1.0$  observed in the CM group is a

prognostic indicator of resistance to colonization by enteropathogens.

A 42.5% decrease in IL-6 indicates a systemic anti-inflammatory effect. Studies by scientists have shown that oligogalacturonides activate PPAR- $\gamma$ , suppressing NF- $\kappa$ B-mediated cytokine production. A 74.2% increase in sIgA may be due to both increased antigenic stimulation by normal microflora and the direct action of oligosaccharides on M cells.

Restoration of intestinal morphometry ( $V/C = 2.49$ ) indicates a pronounced reparative effect. Butyrate, produced during the fermentation of oligosaccharides, is the main energy substrate of colonocytes and stimulates epithelial proliferation. Beta-carotene of carrots additionally provides antioxidant protection of enterocyte membranes.

The clinical efficacy is similar to that of antibiotics, without side effects, and the low cost (0.45–0.60 rubles/head per course) makes Moro soup a cost-effective intervention.

The development of freeze-dried molds will allow the product to be integrated into industrial feeding technology. Limitations include a short follow-up period, a lack of metagenomic analysis of the complete microbiome, and a limited sample size. Future research should focus on synergistic interactions with organic acids, the assessment of the effect on zoonotic pathogens, and the development of industrial forms.

**Relevance and scientific novelty:** The global spread of antibiotic resistance and tightening legislation in veterinary medicine dictate the need to find safer alternatives to antimicrobial drugs in industrial pig farming. This is the first study to use and analyze a modified Moro soup, a functional product made from long-boiled carrots, to prevent post-weaning diarrhea and stress in piglets. The novelty of the study lies in a comprehensive assessment of the product's anti-adhesive, prebiotic, anti-inflammatory, reparative, and immunomodulatory effects, as well as in a direct

comparative analysis of its effectiveness with an antibiotic (colistin) and a probiotic.

**Scientific Innovation:** The innovation of the presented research lies in the following fundamental solutions:

1. A translational approach was employed to adapt a century-old pediatric protocol (Moro soup) for industrial pig production, incorporating a comprehensive re-evaluation of its biological activity.

2. Multi-target strategy - unlike existing alternatives to antibiotics (probiotics, organic acids, bacteriophages), Moro soup simultaneously addresses three key links in the pathogenesis of post-weaning diarrhea:

- Pathogen elimination (anti-adhesion)
- Restoration of normobiota (prebiotic)
- Tissue regeneration and activation of local immunity\

3. Import-substituting technology - the developed product utilizes domestically sourced raw materials (carrots and oat flakes), offering a reproducible, low-cost production technology.

4. Environmental friendliness and biosafety – a prevention system that eliminates the intake of antibiotics into the animal body and therefore into livestock products, ensuring the biosafety of livestock products and reducing the environmental impact of antibiotics.

5. Evidence-based - innovation is confirmed not only by clinical outcomes, but also by objective laboratory markers (microbiology, immunology, histology), which correspond to the principles of evidence-based veterinary medicine.

**Practical Application:** A scheme for the use of Moro soup (5-7 ml/kg 3 times a day for 5 days before and 7 days after weaning) has been developed and tested for veterinary specialists and pig breeding technologists, ready for introduction into the technological cycle, and available control methods (clinical assessment, weight gain, feed

conversion) have been proposed, allowing farms to independently evaluate the effectiveness. The use of Moro soup enables the reduction or elimination of feed antibiotics during the critical weaning period.

The possibility of creating a freeze-dried (lyophilized) form of Moro soup with preservation of the biological activity of oligogalacturonides has been substantiated by producers of feed and feed additives.

Functional products based on vegetable processing (carrots) for animal husbandry are an import-substituting, economically profitable niche. Further scientific research should focus on the synergistic effect of Moro soup with organic acids and probiotics, the product's effect on other farm animals (e.g., calves and broiler chickens), and the molecular mechanisms of PPAR- $\gamma$ -mediated anti-inflammatory activity of oligogalacturonides.

The development of Moro soup as a functional food product followed the 17-step protocol proposed by the Functional Food Center (FFC). According to this model, a functional food must: (1) be a conventional food (carrot soup); (2) contain identified bioactive compounds (oligogalacturonides 2.1 mg/mL,  $\beta$ -carotene); (3) have scientifically proven health benefits (reduction of diarrhea incidence by 69%, increase in average daily gain by 40%); (4) be consumed in physiologically relevant amounts (5–7 mL/kg body weight); and (5) be supported by peer-reviewed evidence (the present study). The FFC classification system assigns grades A, B, or C depending on the completion of epidemiological and post-marketing research. This work represents a controlled experimental trial (Level B according to FFC criteria); long-term field studies with larger herds would be required to achieve Level A [26-27].

## CONCLUSION

Modified Moro soup is an effective FFP for preventing post-weaning stress in piglets, reducing the incidence of diarrhea by 69%, increasing the average daily gain by 40%, and improving feed conversion by 17%.

Moro soup's efficacy is complex and includes anti-adhesive effect (decrease in ETEC by 48 times), prebiotic effect (increase in *lactobacilli* by 23 times), anti-inflammatory effect (decrease in IL-6 by 42.5%), stimulation of mucosal immunity (increase in sIgA by 74%), and regenerative effect (increase in V/C by 42%).

In terms of clinical effectiveness, Moro soup is similar to an antibiotic (colistin), but surpasses it in its effects on microbiocenosis, local immunity, and morphofunctional state of the intestine, making it an alternative to antibiotics in the system of preventive measures.

The potential of introducing Moro soup into industrial pig production depends on economic efficiency, technological simplicity, and the availability of raw materials. To increase the potential, the development of an industrial freeze-dried version of the soup should be considered.

**List of Abbreviations:** AB, positive control; ANOVA, univariate analysis of variance; BD, basal diet; CM, experimental; FFP, functional food product; K, negative control; PB, probiotic; PCR-RV, large intestinal microbiota; sIgA, secretory IgA; V/C, villus height to crypt depth; IL-6, interleukin 6; ELISA, enzyme linked immunosorbent assay; CFU, colony-forming units; PWD, post-weaning diarrhea; MSG, Moro soup group; ADG, average daily gain.

**Authors' Contributions:** Zh. M. is the main author and conductor of the research; D. M. critically reviewed and edited the article, and discussed novelty, scientific innovation, and practical implications. L. G., S. Y., V. G., and A. V. conducted a study on the dynamics of diarrhea in piglets; M. S. and H. B. conducted microbiological studies; R. G. and V. A. conducted histological and morphological studies; D.L. carefully reviewed the article, provided valuable comments, and contributed to editing the entire manuscript.

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