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Effect of Feeds Fermented with Multipurpose Autochthonous Microorganisms on the Productive Parameters of Pre-Fattening Pigs

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ABSTRACT

Background: Weaning causes physiological changes and the transformation of the intestinal microbiota, which can affect the productive parameters of pre-fattening pigs. **Aim.** To evaluate the effect of feeds fermented (12 and 24 hours) on the productive parameters of post-weaned pre-fattening pigs, using multipurpose autochthonous microorganisms. **Method:** A total of 60 pigs (York Land x CC21) were selected at 30.9 days of weaning, weighing 8.1 kg on average. Three groups of twenty animals each were created: G1 (control): consuming the starter feeds, and receiving Levamisole, Shotapen ®, and Fortius to prevent parasitic and respiratory diseases. Experimental groups G2 and G3 received 1/3 of the ration in the form of fermented liquid feed (12 and 24 hours, respectively), as the first choice in the morning, along with starter feedstuff the rest of the day, and received no medication. The crude protein and microbiological quality were determined in each feeding alternative. After 45 days of treatment, the mean daily gain, feed conversion, and post-weaned weight were evaluated. **Results:** Only the feed fermented for 24 hours showed adequate protein contents for pre-fattening animals, and caused a highly significant increase ($P < 0.001$) in mean daily gain and post-weaned weight, with a more efficient feed conversion at the end of the experiment. **Conclusions:** The inclusion of liquid feedstuffs upon 24h-fermentation with multipurpose autochthonous microorganisms was a sustainable alternative to feed pre-fattening pigs.

Keywords: liquid feeds, pigs, protein increase, prebiotics, probiotics, crude protein (*Source: AIMS*)

Citations (APA)

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INTRODUCTION

Post-weaning in swine production is a pivotal point in which the effectiveness of zoo-hygienic actions and decisions made influence the final stage of pre-fattening pig development. This stage-related stress has a negative impact on the animal's initial homeostasis. Among the immediate effects are morphological and physiological changes in microvilli, which lead to a remarkable reduction in nutrient uptake. The ingestion of dry feedstuffs worsens this condition, compromising up to 60% of these structures (Barreto, Rodríguez, and Campal, 2020).

Feed fermentation using lactic acid bacteria (LAB), in addition to being the simplest alternative to growth-promoting antibiotics, contributes to the repair of damage in the microvilli and helps settle *Lactobacillus* spp., *Streptococcus* spp, and other bacterial species with probiotic action. The ensuing reduction of pH, and the production of lactic acid with lower damage caused by enteropathies (Missotten *et al.*, 2015; Rodríguez *et al.*, 2021). The fermented feed includes an increase in protein levels. This represents a qualitative increase, as the animals' intake of microbial proteins generated can be easier (Polyorach *et al.* 2018).

The EM-type microorganisms (efficient microorganisms), IH-plus (Indio Hatuey-plus), and MAM (multipurpose autochthonous microorganisms), depending on their use, could restrain acid lactic bacteria (*Lactobacillus plantarum*, *L. casei*, and *Streptococcus lactis*), phototrophic bacteria (*Rhodopseudomonas palustris* and *Rhodobacter spaeroides*), actinomycetes (*Streptomyces albus* and *Streptomyces griseus*), yeasts (*Saccharomyces cerevisiae* and *Candida utilis*), and filamentous fungi (*Aspergillus oryzae*, *Penicillium* sp. and *Mucor hiemalis*). They work in partnership that can adapt to the damaged environment. Hence, they create synergic dependencies that ensure their existence and the exclusion of transient pathogen exclusion (Barreto *et al.*, 2021; Rodríguez *et al.*, 2021).

Both EM and MAM can be administered to pigs directly or indirectly, or in the drinking water, to stimulate the production parameters and health indicators (Rodríguez *et al.*, 2013; Rodríguez *et al.*, 2021; Barreto *et al.*, 2015. Barreto *et al.*, 2021). Meanwhile, the pre-fattening animals receive it in the diet, along with the feeds (Valdés *et al.*, 2019). There are no precedents to feed fermentation using these microbial mixes, or the optimal process time.

The aim of this paper was to evaluate the effect of fermented feeds (12 and 24 hours), with multipurpose autochthonous microorganisms on certain production parameters of post-weaned pre-fattening pigs.

MATERIALS AND METHODS

The experiment was conducted at the breeding and growth facility of a basic state facility (UEB) Swine Productions, in Camaguey. The experimental design included a 45-day post-weaning evaluation period of the effect of fermented feeds using multipurpose autochthonous microorganisms (MAM), on the main production parameters of growing pre-fattening pigs.

Animals

A total of 60 weaned pigs (30 days old/8.1 kg) were included in the experiment. The animals were chosen randomly from the weaned pre-fattening pigs of 10 York Land breeding animals (between the second and third farrowing), and CC21 breeding males. Overall, three groups of animals were made, using a completely randomized design.

- Group 1 (G1) was given starter feed (Table 1) according to the preset quantities (Table 2), without MAM, so it was the control. During the second week of pre-fattening, the animals were given Levamisol and Shotapen ® L.A. (commercial brand made of Procaine G penicillin, 100 000 IU, benzathine G penicillin 100 000 IU, and dihydrostreptomycin, 200 mg). Then, it was used as prophylaxis in the fourth week, treated with Fortius ® L.A. (each mL contains 100 mg enrofloxacin). These products were administered parentally, following the set standards of the program for the prevention of respiratory and parasitic processes at the UEB, as described by Rodríguez *et al.* (2021).
- Group 2 (G2) was given 1/3 of the starter feed ration mixed thoroughly with MAM, then covered with water to enhance fermentation. The volume was calculated according to the following proportion: 120 mL of the biopreparation/pig/day. The procedure was made in a 20 L container with a lid (loose) to prevent contact with insects and rodents. Then it was kept in a fresh place for 12 hours until use. The operation was repeated daily until the experiment was over.
- Group 3 (G3) was under a diet of starter feeds at similar proportions to the previously described conditions, but with a 24h fermentation).

Table 1. Feedstuff concentration for pre-fattening animals, as described by the manufacturer

Components	Percentage	Components	Percentage
Corn	54.55	Methionine	0.10
Soybean	37.50	Lysine	0.40
Calcium	0.80	Choline	0.15
Phosphate	0.90	Biotonic	0.10
Salt	0.30	Widened glucosyl	0.20
Swine nucleus	2.50	Sugar	2.50

Source: Rodríguez *et al.* (2021).

Table 2. Pre-fattening feeding technology

Technological week	Animal/day/per capita (kg)	Group/week/consumption (kg)
Week 1	0.228	31.92
Week 2	0.44	61.6
Week 3	0.68	95.2
Week 4	0.98	137.2
Week 5	1.32	184.8
Week 6	1.6	224.0
Week 7	1.97	118.2
Overall intake	7.218	852.92

Source: Rodríguez *et al.* (2021).

No medication was administered in the last two experimental variants. The fermented feeds (12 and 24h) were administered first thing in the morning, and throughout the day, the animals ingested starter feed (2/3 of the ration), like the animals in the control group. The same technician assisted the three groups, on Flat-Deck, and water was given *ad libitum* through nipples.

Feed sample collection and processing

It was done using the quarter method. Accordingly, the container was split into four parts, and the portions from opposite quarters were collected. The procedure was repeated successively to ensure a quantity corresponding to three replicas. The samples were transported to the Agroenvironmental Laboratory (LABCA) at the University of Camaguey, in 1 kg translucent polyethylene labeled and tied bags to prevent the loss of humidity. The samples were processed at the moment to comply with MAM's time of action.

Determination of crude protein (CP) percentages

The crude protein percentages were determined using the Kjeldahl method, with a Kjeltex I system. Each feed portion had three replicas. The contents were expressed as $CP = N \times 6.25$, according to the recommendations of the *Association of Official Analytical Chemist* (AOAC, 1995).

Microbiological analysis of feeds

The microbiological analysis was performed at the Territorial Laboratory located in Camaguey, from the National State Verification Office (ONIE), the Ministry of Food Processing Industry. The feeds were transported in compliance with the previously described measures. The determinations were made according to NC 605:08, for the detection of *Salmonella*, and NC 1004: 2016, for yeast numbering in non-lactic products (cfu/g).

MAM preparation and activation

The liquid mother acquired from the Indio Hatuey Experimental Station of Pastures and Forages was propagated and activated, as suggested by Barreto *et al.* (2021). A product of the same color as molasses and the particular smell of lactic fermentations was obtained a couple of weeks later, having a pH lower than 3.5. This form of activated MAM was used to ferment feeds.

Productive performance

The mean daily gain (MDG), feed conversion (FC), and post-weaning weight (PWW) after 45 days, were determined to evaluate the effect of the three treatments on the productive parameters of the pre-fattening animals. The animals were weighed twice, at the beginning (30 days of age), and at the end of the experiment (75 days of age). The two procedures were performed in the morning before the animals were fed. A 50 kg Selter scale (± 0.01 kg precision) was used in all cases.

Statistical analysis

The CP values achieved in the three feed replicas were assessed through one-way ANOVA, while the minimum significant difference method was used for mean comparison. The study of independent variables: mean daily gain (MDG), feed conversion (FC), and post-weaning weight (PWW) at 45 days consisted of an analysis of variance to determine their behavior in the groups studied and their relevance. The independent variable was the treatment applied with the starter feed with MAM, at 12 and 24 hours, which permitted the evaluation of the inter-group treatment through the Multiple Comparison Test. Previously, other models were used (age and weight at weaning as co-variables), which were dismissed due to their lack of significance, except the PWW, which had been adjusted to 8.1 kg at weaning. IBM SPSS, 23 (2015) was used for all statistical determinations.

RESULTS AND DISCUSSION

Percentages of crude protein in the feeds used

The three variants of the feeds studied were approved for consumption, according to microbiological analysis (NC 605:08), but only the fermented feed for 24 hr with the MAM had the crude protein percentage suggested in the Manual of Technical Procedures for the Nutrition of Pre-fattening Pigs (Macías *et al.*, 2015) (Table 3).

Table 3. Percentages of crude protein in the feeds determined in the three treatments

Groups in the study	CP contents (%) Replications	CP average (%)
Dry feed (control)	16.4	16.1 ^a
	16.5	
	15.6	
Feed fermented (12 hr).	21.8	20.6 ^b
	20.2	
	20.0	
Feed fermented (24 hr).	25.8	26.5 ^c
	27.0	
	26.7	

Legend: CP: crude protein. Unequal scripts differ significantly (p< 0.05)

The nutritional requirements criteria by pig category have been set up under feeding conditions, race crossing, and ownership in every country, so they differ upon comparison. The Cuban standard rules that the growing pigs between 5 and 10 kg should receive feeds containing 23.70% crude protein. This value will lower gradually, as the animals increase their weight (Macías *et al.*, 2015). The one supplied by the manufacturer did not meet this requirement. This often occurs in feeds included in this stage, as will be seen below.

Boonnop *et al.* (2009) and Polyorach *et al.* (2013), in separate studies to enhance the crude protein contents in cassava-based feeds (*Manihot esculenta* Crantz) through fermenting with *Saccharomyces cerevisiae*, were able to achieve 30.4% and 47.0%, respectively. These results are outstanding, considering that the previous results were between 2% and 3%. Polyorach *et al.* (2013) claimed it resulted in the high growth of yeast (3.0×10^{11} cells/mL). It was associated with the capacity of *Saccharomyces cerevisiae* of segregating extracellular enzymes (amylases, lymamarase, and cellulase) in the cassava mass, and degrading starch and other polymers that contribute to such growth.

With a view of detailing the microbial role in the previous results, Polyorach *et al.* (2018) conducted an experiment with fresh pulp and grated cassava (CP = 3.1^f% and 3.5^f%, respectively), and fermented them with yeasts (Y), efficient microorganisms (EM), and a mix of them (EMY). After three days of microorganism-substrate interactions in the shade, then drying for 48 h, the CP contents of the freshly milled substrate were, 28.7^e% (Y), 30.4^d% (EM), and 31.8^c% (EMY). Meanwhile, the values for the grated dry cassava were, 42.1^b% (Y), 44.2^a% (EM), and 45.3^a% (EMY).

The previous values, besides corroborating the previously found contents (Polyorach *et al.*, 2013), and regardless of the yeast contents, demonstrated the participation of other microorganisms in EM-formulation mixes (Valdés Suarez *et al.*, 2022). In such mixes, the bacterial presence is high, and like the yeasts, they behave as single-cell proteins (Akwu Omede *et al.*, 2018). There is also the contribution of filamentous fungi, in terms of cell protein contents and abundant production of extracellular enzymes capable of degrading polymer complexes present in the substrate, and turning them into bacteria and yeasts nutrients. This microbial diversity, stable as a partnership, is also present in MAM (Rodríguez *et al.*, 2021; Barreto *et al.*, 2021).

In this particular case, the yeast contents were 1.1×10^3 , 2.2×10^3 , and 2.9×10^3 cfu/g in the unfermented feeds, and the same feed pre-digested with MAM at 12 and 24 hr, respectively (NC 1004:2016). It shows that the yeast concentration in fermented feeds increases in comparison to the dry feeds despite the short time of fermentation. Unfortunately, the Territorial Laboratory of Camaguey does not perform microbiological analysis of *Lactobacillus* spp., actinomycetes, or filamentous fungi. Though they may be present in EM and MAM (Barreto *et al.*, 2021; Valdés Suárez *et al.*, 2022), their contents were not quantified in the three variables studied. However, there are reports about their contribution to CP increases in fermented feeds (Polyorach *et al.* (2018).

Effect of fermented feeds with MAM on the productive parameters of pre-fattening pigs

When evaluating the significance of the treatment in the study variables (MDW, FC, and PWW), the greatest positive effect corresponded to the animals that consumed the fermented feed with MAM for 24 hours. The highest values of mean daily gain and post-weaning weight were observed in this group, along with more efficient feed conversion. The value of the partial determination coefficient (ETA^2) corroborates the effect of the treatment on the findings. The potency observed in all the cases was broadly acceptable, over 93% (Table 4).

Table 4 Behavior of variables by group studied and their significance

Variables in the study	Effect data			Treatments in the study	Values observed	Significance
	ETA ² (Treatment)	Potency observed	FD			
MDG (g)	,336	,998	57	Control	345.2	***
				12 h	318.3	
				24 h	413.3	
FC (kg)	,211	,936	57	Control	2.8	***
				12 h	3.3	
				24 h	2.3	
PWW (kg)	,336	,998	56	Control	23.9	***
				12 h	22.4	
				24 h	26.6	

Legend: MDG: mean daily gain; FC: feed conversion; PWW: post-weaning weight; FD: error freedom degree.

The pre-fattening area comprises pigs, between weaning and no more than 96 days of age, on average. At this point, they must weigh over 35.0 kg. In some commercial programs and raising systems, this stage lasts 75 days, and the animals must weigh at least 25.0 kg (Macías *et al.*, 2015). The discussion proposal is more related to the latter variant.

Only the animals treated with feeds fermented for 24 hr adjusted to the nutritional requirements set for pre-fattening animals, with higher values of MDW and PWW, as well as satisfactory FC in this stage. Proper interpretation of the above can only be possible when the results extrapolate to the site responsible for such responses: the intestine of the post-weaned animals. These organs host dynamic microbiota in permanent symbiosis with the host, which is key to the adequate functional, physiological, and immunological functioning of the animal (Álvarez *et al.*, 2021). These microbial populations differ between efficient animals and inefficient animals in terms of production. The former has a greater presence of *Lactobacillus* spp. (Fouhse *et al.*, 2016).

Lactobacillus spp. is part of a cluster of bacterial species, which has been named lactic acid bacteria (LAB), as all of them, to a greater or lesser extent, produce this important organic acid. Although this is not the only trait that relates to them, all are abundant in the soil, and they can settle down with certain stability as part of the intestinal microbiota of mammals, being one of the predominant genera found in the EM, Hi-plus, and MAM microbial mixes.

Bifidobacterium, *Lactobacillus*, *Bacillus* genera, and other bacterial genera with the capacity of colonizing the animal intestine, have a prolonged probiotic action or activate thanks to the stimuli of prebiotic and postbiotic compounds. However, yeasts and *saccharomyces cerevisiae* are the best examples, lacking that capacity. Therefore, their probiotic effect is transient, according to their presence in the intestines. Hence, there is a need for a more systematic supply of the diet. Their main role is associated with the feed to be fermented. When they are used correctly, they enrich the protein levels qualitatively and quantitatively and stimulate the activity of enzymes in the microvilli, improving nutrient rupture and uptake (Hancox *et al.*, 2015; Barreto *et al.*, 2021).

Saccharomyces cerevisiae, in swine production, has a probiotic contribution as a live microorganism due to its high concentration of β -glucans in its cell wall; it acts as a probiotic (Song *et al.*, 2014) or post-biotic (Tsilingiri and Rescigno, 2013). Both actions are complemented and have a positive effect on intestinal morphology, the concentration of amino acids (leucine, phenylalanine, and arginine), and the reduction of oxidative stress which is commonly found in weaned pigs (Liu *et al.*, 2017).

The Multiple Comparison Test, as an independent variable treatment applied, proved the absence of significant differences between the animals in the control group and the ones that ingested fermented feeds with MAM for 12 hours (G2). However, there are differences in the two variants with the pre-fattening animals that ingested the fermented feed for 24 hours (G3) (Table 5).

Two aspects must be stressed in this sense:

1. The pre-fattening animals that ingested 1/3 of the fermented feed for 12 h with MAM ration daily were found to reach adequate values (MDG, FC, PWW), similar to the ones observed in the control group. The latter were treated preventively with various antibiotics. Besides being costly, these antibiotics (Rodríguez Torrens, Barreto Argilagos, and Hernández Casado, 2022) create residuals that pose a risk both for animals and their surroundings, and the end consumers (Zamora, Ortiz, and Utria, 2020; Barreto *et al.*, 2021). This result confirms the said benefits of liquid feeds, particularly, fermented feeds containing probiotic microorganisms, following the weaning (Missotten *et al.*, 2015; Polyorach *et al.*, 2018).

Table 5. Results of the Multiple Comparison Test of treatments (minimum significant difference)

Effect data Inter-group comparison (treatments)		Variables in the study								
		MDG (g)			FC (kg)			PWW (kg)		
		Sig	CI (95%)		Sig	CI (95%)		Sig	CI (95%)	
			UL	LL		UL	LL		UL	LL
Control (G1)	12 hours (G2)	Ns	63.0	12.4	Ns	,039	,976	Ns	3.01	,438
	24 hours (G3)	***	34.4	109.9	*	1.02	,013	***	1.46	4.92
12 hours (G2)	Control (G1)	NS	12.4	63.0	NS	,976	0.39	NS	,438	3.01
	24 hours (G3)	***	59.8	135.2	***	1.49	,481	***	2.75	6.20
24 hours (G3)	Control (G1)	***	109.9	34.4	*	,013	1.02	***	4.92	1.46
	12 hours (G2)	***	135.2	59.8	***	,481	1.49	***	6.20	2.75

Legend: G1= Control group; the pre-fattening animals only ingested feed and received medication for prevention; G2 = ingested 1/3 of fermented ration with MAM 12 h and received no medication; G3 = ingested 1/3 of their ration fermented with MAM 24 h, and received no medication; FC = feed conversion; Mean daily gain = mean daily gain; CI = confidence interval; LL = lower limit; UL = upper limit; Ns = non-significant differences; PWW = post-weaning weight; *** = (P< 0.001); * = (P< 0.05).

2. The best response from the pre-fattening animals to the fermented feeds (24 h) may have been caused by three elements: a) a higher percentage of CP depending on the microbial growth since the feed was fermented twice as much the regular time. b) A qualitative increase of more digestible protein contents, previously discussed. c) A higher prebiotic, post-biotic, and probiotic effect, according to the greater presence of LAB and yeasts (Salminen *et al.*, 2021).

Blanco *et al.* (2017) in a similar study using IH-plus®, developed from a mother culture similar to the one in this research, found significantly higher production parameters (live weight increase, mean daily gain, and feed conversion) than the ones in the control group. Three rations were supplied daily, while the feed mix with IH-plus® was made under dry conditions before supplying it to the animals.

A similar result was reported by Montejo-Sierra-Sierra *et al.* (2017) upon evaluating the effect of non-conventional diets previously treated with natural microorganisms (NM) for pigs in different categories. After processing the results, they found significantly higher live weight gains in all the cases, in relation to the corresponding controls. The diets were supplied early in the morning and the afternoon and the feed was mixed with NM before supply.

Valdés *et al.* (2019) found positive results in the production parameters they included when adding EM to the diet of pre-fattening animals for 49 days. They supplied the pre-starter and starter feeds mixed with the bioproduct at the moment of administration. Another study conducted to evaluate the effectiveness of EM orally (1.0, 1.5, and 2.0 mL/kg live weight per day) in pre-fattening animals, found significant increases in the production parameters, in keeping with an increase in the doses of the microbial complex (Valdés *et al.*, 2020; Valdés Suarez *et al.*, 2022). This result corroborates the previous arguments, though a much harder effort is demanded from the technicians.

Liu *et al.* (2018), in a comprehensive review of the utilization of probiotic complexes in swine production, concluded that the benefit was observed right after weaning, as a result of dramatic changes in the intestine. It is an inevitable period, in which any strategy that leads to adaptation and the necessary re-adjustment for the reinstatement of this organ's functions, will be acceptable. Proper selection of the components of these probiotic mixes, their concentration, and dosage could help re-establish the normal levels more quickly, which was corroborated in the discussion, where the results from the experimental groups were compared.

These animals are similar races, with an average weaning weight of 8.1 kg, and the same technician in charge of caring and handling the three groups. Hence, and following the line of thought of Liu *et al.* (2018), the responses (Table 5) may have been influenced by the feed's composition in the three variants, along with the animals' health state. The apparent CP percentage increases had already been discussed in the former, regarding the feed variants observed. One element in favor of the control animal health state was the application of Levamisole and Shotapen® La, in the second pre-fattening week, and Fortius® La in the fourth. The former, in addition to the anthelmintic, demonstrated an immunomodulating action (Scheinfeld *et al.*, 2004). This effect could have contributed to the lack of statistical differences in the experimental animals treated with the feed fermented for 12 h, with MAM.

Furthermore, the control animals only ingested dry feeds, whereas the experimental animals were given a combination of fermented actions, using MAM as the first ration in the morning, while during the rest of the day, the animals ingested dry feeds. The inclusion of wet rations during the

first three weeks following weaning has a major influence on animal growth than when they are supplied as dry feeds. This behavior improves the functioning and wholeness of the digestive system, by 1) Offering proper conditions for the action of digestive enzymes. 2) Reducing alterations in the intestinal villi. The last two, between weaning-related stress and the action of dry feeds, reduce their size by up to 63%, which compromises nutrient digestion and intake (Rodríguez *et al.*, 2021).

These changes in the intestinal microbiota caused by weaning in the experimental animals are slowly reversed as the animals ingest MAM-fermented feeds. An insufficient concentration in the 12 h variant explains the differences observed in the parameters studied in the animals that ingested the feed fermented for 24 hours. Additionally, the transport of ingesta was observed to be faster in this animal category, which does not favor the adherence and later colonization of the intestine by probiotic bacteria (Armendáriz Tapia, 2015; Blanco *et al.*, 2017; Rodríguez *et al.*, 2021).

The previously studied results may be the starting point for new research that evaluate the behavior of the variables analyzed in the feeds fermented for 48 hours or more. This range might be extended to a moment in which there is a risk of acetic acid formation.

CONCLUSIONS

The inclusion of feeds fermented for 24 h, using multipurpose autochthonous microorganisms in post-weaned pre-fattening pigs is a positive alternative that can enhance the main production parameters in this stage.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: HCRT, GBA, ALC, RVMO, IMS, PJBR; data analysis and interpretation: HCRT, GBA, ALC, RVMO, IMS, PJBR; redaction of the manuscript: AVS, HCRT, GBA, ALC, RVMO, IMS, PJBR.

CONFLICT OF INTEREST STATEMENT

The authors state the are no conflicts of interest whatsoever.