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Research Article

Behavior of Hematologic Indicators in Pre-Fattening Pigs Fed with Multipurpose Autochthonous Microorganisms' Fermented Concentrates

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Abstract

The objective of this research was to evaluate the behavior of hematological indicators in pre-fattening pigs supplemented with multipurpose autochthonous microorganism's (MAM) fermented concentrate. Sixty pigs (York Land x CC21) weaned at 30 days of birth and an average weight of 8.1 kg, randomly selected from a larger total, were used. They were divided into three groups of 20 animals each ones: 1) consumed starter feed and were treated with antibiotics and antiparasitics as prophylactics; functioned as a control group. 2) Received the same concentrate but fermented during 12 hours with MAM. 3) Consumed fermented concentrate for 24 hours. Bought fermented variants were offered as first ration in the morning; the rest of the day the animals consumed dry concentrate. All animals were kept in Flat-Deck systems. Only the 24-hour fermented feedstuffs reached the suggested protein content for pre-fattening. Exclusively, animals in group 3 reached normal hematocrit and hemoglobin values. The quantities of leukocytes, lymphocytes and eosinophil were similar in the three investigated groups. Fermentation of starter concentrates with MAM for 24 hours positively influences hematocrit and hemoglobin values of pre-fattening swine. Its prophylactic effect is compatible with that achieved with antibiotics and antiparasitics without its adverse side effects..

Keywords: *Fermented Feed; Growth Promoters; Hematological Indicators; Multipurpose Autochthonous Microorganisms; Pre-Fattening Swine; Weaned Pigs*

Introduction

The post-weaning period in swine production is a turning point where the zootechnical-veterinary and epidemiological measures adopted have a positive or negative impact on the final product. Stress impacts at the intestinal level and, among its immediate negative effects, the morphological and physiological alteration of enterocytes stands out, reducing nutrient absorption. This damage increases with the ingestion of dry concentrates [1].

The previous digestion of feed with lactic acid bacteria (LAB) and yeasts is a variant to antibiotics as growth promoters. Without their negative effects, they contribute to repair the intestinal microvilli damage and guarantee the establishment of *Lactobacillus* spp., *Streptococcus* spp. and other bacterial species with probiotic action. Due to the reduction in pH, lactic acid and alcohol produced in fermentation,

gastrointestinal infections are reduced [2]. In addition to these advantages, the option promotes protein increases in the product; a more digestible and better absorbed protein, which gives it a greater nutritional value [3,4]. This variant, despite its virtues, is not feasible for some producers for whom the acquisition of these strains and their maintenance are beyond their resources and possibilities [5].

Efficient microorganisms (EM) have been widely accepted in animal production as growth promoters [6]. Their positive effect on both productive and health parameters is attributed to the probiotic potential of some of the microorganisms present in these natural products [7]. Multipurpose autochthonous microorganisms (MAM) are microbial mixtures obtained from leaf litter in areas with virgin soils in the province of Camagüey [8]. Like EMs, they are composed of lactic acid bacteria (*Lactobacillus plantarum*, *L. casei* and *Streptococcus lactis*), phototrophs (*Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*), yeasts (*Saccharomyces cerevisiae* and *Candida utilis*), actinomycetes (*Streptomyces albus* and *S. griseus*) and filamentous fungi (*Aspergillus oryzae*, *Penicillium* spp. and *Mucor hiemalis*). They develop a joint feeding system in which they depend on each other, achieving a synergy that makes possible the exclusion of pathogens [4,5,8].

After so many years of getting productive increases in swine breeding systems through the use of antibiotics, copper salts and hormones, at the expense of negative collateral effects on health and the environment [9], it is necessary to joint efforts on less aggressive ecological variants. In this sense, the evaluation of the behavior of hematological values in animals treated with them can be a simple and reliable option of their innocuousness in the triad of nutritional efficiency, physiological conditions and general health status of the animals [10].

Research Objective

The objective of this research was to evaluate the behavior of hematological indicators in pre-fattening swine supplemented with multipurpose autochthonous microorganism's fermented concentrate.

Materials and Methods

Study area

The research was carried out at a Multipurpose Base State Unit (MBSU) for pig production in Camagüey. 60 weaned pigs, approximately 30 days old with an average weight of 8.1 kg, were used. They were randomly selected from the total gestated by 10 York Land breeders (in their highest breeding stage) and CC21 stallions. From these, three groups of 20 animals each were made.

Experiment

Group 1: Consumed starter feed in the proportions suggested for this stage (Table 1) and without mixing with the MAM, acting as a control. As part of the MBSU's parasitic and bacterial disease prevention plan, in the second week, Levamisole and Shotapen® L.A. (Penicillin G procaine 100,000 IU, Penicillin G benzathine 100,000 IU, Dihydrostreptomycin base 200 mg.) were applied; then, in the fourth week, Fortius® L.A. (each ml contains 100 mg of Enrofloxacin), all by parenteral way.

Weeks	Per capita/animal/day (kg)	Consumption/group/week (kg)
1	0.228	31.92
2	0.44	61.60
3	0.68	95.20
4	0.98	137.20
5	1.32	184.80
6	1.6	224.00
7	1.97	118.20
Total consumption	7.218	852.92

Table 1: *Proposed feeding scheme for pre-fattening in swine units in Cuba.*

Group 2: Received starter feed in the proportions suggested but fermented with activated MAM. For this purpose, 120 ml of the active microbial mixture was added to the per capita feed/animal/day (kg). Then, the homogenized mixture was completely covered with water and fermented during 12 hours.

Group 3: Consumed starter feed as described above, but fermented during 24 hours.

In the last two variants no medication was applied and the described diets were given as the first offer of feed consumption in the morning. During the rest of the day the animals consumed starter concentrate the same as those in the control group. All three groups were provided with water *ad libitum*, were placed in Flat-Deck systems and were attended by the same operator. During the experiment (45 days) all animals were clinically inspected daily by the same specialist. To preserve the health of the animals, an optimal handling and compliance with the biosecurity measures established in the unit was guaranteed.

Multipurpose autochthonous microorganisms (MAM)

The elaboration of the multipurpose autochthonous microorganism's mixture, and its activation, prior to its use was released according to the suggested purpose [8]. Any mention made about the effects of this natural product throughout the article refers to this activated form of MAM (MAM-A).

Determination of crude protein values

Collection and processing of feed samples

Dry feed as well as feed fermented with MAM for 12 and 24 hours was sampled by the quarter method. For this purpose, the corresponding tank was divided into four parts to take portions from two opposite quarters. The procedure was repeated successively to guarantee in each case the quantity equivalent to three replicates. They were transported to the laboratory in 1 kg polyethylene bags duly identified and sealed to avoid loss of humidity. They were worked at the time so as not to violate the action time of the MAMs.

Determination of the percentage of crude protein (CP)

It was established by the Kjeldahl method, using a Kjeltec I system. The CP contents were expressed as CP = N x 6.25 as established [11]. Each type of feed had three replicates. From these, statistical evaluation was carried out by means of a simple ANOVA. The HSD Tukey multiple comparison test was used to compare the means for each type of feed.

Hematological studies

At the end of the experiment (45 days) whole blood was drawn from each animal at the optic vertex, which was mixed with anticoagulant (EDTA). The hematic values were established according to Suardíaz., *et al* [12].

Results and Discussion

As can be seen, only the concentrate treated with MAM for 24 hours reached the protein content required by the pre-fattening (Table 2).

Types of feed	CP	
	%/Replications	Average (%)
Dry concentrate (control)	16,4	16,2[a]
	16,5	
	15,6	
Fermented concentrate 12 hours	21,8	20,7[b]
	20,2	
	20,0	
Fermented concentrate 24 hours	25,8	26,5[c]
	27,0	
	26,7	

Table 2: *Percentage values of crude protein (CP) in the types of feed evaluated. a, b, c different letters differ significantly (HSD Tukey, P < 0.05).*

Nutritional requirements have been established under conditions of feeding, crossbreeding, and ownership specific to each country, so they may differ at the time of comparison. In the case of Cuba, it has been established that pre-fattening weighing between 5 and 10 kg should be fed concentrates with 23.70% crude protein, a value that will decrease as the animals increase their weight [13,14].

In two independent investigations aimed at improving crude protein contents in foods based on *Manihot esculenta* Crantz, through fermentation with *Saccharomyces cerevisiae*, values between 30.4 and 47.0%, respectively, were reached. These results were remarkable because, before the treatments, protein values ranged between 2 and 3% [15,16]. The protein increase was related to the high yeast growth (3.0 x 1011 cells/ml) in all cases. Regarding the adequate impact of this liquid artisanal feed on animal conversion, in addition to the above mentioned, the capacity of *Saccharomyces cerevisiae* to secrete extracellular enzymes (amylases, linamarase and cellulase) in the cassava mass and to degrade starches and other polymers, making them more digestible for the animal [17] stand out.

In another experiment, from fresh pulp and ground dry matter of *Manihot esculenta* Crantz (with CP contents equal to 3.1% and 3.5%, respectively), significant increases in crude protein were obtained by fermentations with yeasts (Y), efficient microorganisms (EM) and a mixture of both (EMY). The results, after three days of fermentation in the shade, followed by drying in the sun for 48 hours, are impressive. The CP contents from fresh pulp amounted to: 28.7% (Y), 30.4% (EM) and 31.8% (EMY). Those obtained from ground dry matter were higher: 42.1% (Y), 44.2% (EM) and 45.3% (EMY). They attributed such high increases to the growth of yeasts, as well as to the bacterial complex implicit in EM, which behave as single cell proteins [4].

Only the values corresponding to hematocrit and hemoglobin, in the animals that consumed fermented feed for 24 hours, coincided with the ranges assumed as normal. The rest of the blood tests corresponded to acceptable values, regardless of the group of animals analyzed (Table 3).

Essays	Average values			
	Normal range*	Group 1	Group 2	Group 3
Hematocrit (%)	32.0 - 50.0	26.0	29.0	35.0
Hemoglobin (g/dL)	10.0 - 16.0	8.8	9.8	11.7
Leucocytes (10^9/L)	11.0 - 22.0	11.8	12.5	13.5
Eosinophils (10^9/L)	5.0 - 13.0	4.0	1.0	3.0
Lymphocytes (%)	39.0 - 62.0	56.0	57.0	58.0

Table 3: *Pre-fattening hematic values determined at the end of the experience.*

**Serem., et al. [18]. Group 1 (Control): Consumed only dry start concentrate; antibiotics were applied prophylactically.*

Group 2: Received starter feed fermented with MAM for 12 hours. Group 3: Consumed starter feed fermented with MAM for 24 hours.

The results allow a descriptive analysis by comparing each assay with the ranges of values assumed as normal [18]. The fact that hematocrit and hemoglobin determinations only reached normal values in pigs that consumed feed fermented with MAM for a period of 24 hours may be associated with their ability to increase the protein content, its quality and the digestibility of these concentrates. Phenomenon influenced by the microbial growth time [4,19]. Hematological indicators, in general, constitute tests that allows knowing the relationship between health disorders and nutritional deficiencies [20].

There are reports of the positive impact of probiotic microorganisms, individually or in combination, on the hematological indicators analyzed [20,21]. Meanwhile, other researchers report not having found significant differences when comparing hemoglobin and hematocrit values in animals supplemented with prebiotics and probiotics, although those corresponding to neutrophils and basophils did correspond to a better state of health [22,23].

The remaining values testify to the absence of infectious processes during the evaluated period. Even those corresponding to eosinophils, lower than those referred to in the scale used [18], evidence of the aforementioned, are normal for other specialists [24]. Several factors may have played a role in the flattering results, some of them general, others more specific. Among the first ones, a correct zootechnical-veterinary management in the unit could have influenced [14]. In the latter, the application of antibiotics and antiparasitics for prophylactic purposes in the control group and of MAM in the experimental ones. Variants aimed at avoiding or minimizing the establishment of pathogens, especially enteric pathogens, in pigs at weaning, as is the case here [6,25,26].

Blood cells are excellent indicators of health status and essential for innate and adaptive immune responses to pathogens. Leukocytes are prominent in primary responses that generally neutralize invading agents. They also play a role in adaptive responses, some in a nonspecific manner, others, as in the case of lymphocytes (T and B) in a specific immune response. Eosinophils, included in this group, regulate hypersensitivity responses and act against microorganisms that escape phagocytosis due to their size. The count of these white cells is a reliable indicator of infectious processes. Erythrocytes (red cells) transport oxygen, carbon dioxide and remove the antigen-antibody complexes for their final elimination; any alteration in their values denotes a lack of control of the organism, and high risks of anemia, hypertension, etc [27,28].

As mentioned above, a microbiota develops at the intestinal level in dynamic interaction with its host. This microbiota is decisive for the optimal utilization of nutrients and the correct activity of both the animal physiology and the responses of its immune system [29]. These microbial populations include potential pathogens. Such is the case of several pathotypes of *Escherichia coli*, *Salmonella* serovars and *Clostridium* species, to cite some bacterial examples [30,31]. This was the justification for the prophylactic use of antibiotics in livestock production [6]. However, their bactericidal action, added to the changes due to stress generated during weaning, cause an alteration in the intestinal microbial barrier (dysbiosis) that dramatically increases the risks of diseases caused by anti-bioresistant pathogens [5,30,31], including those present in the microbiota itself [29].

In contrast to this questionable proposal, strategies based on the use of prebiotics and probiotics that, free of this limitation, reinforce the intestinal biota, potentiate the antagonistic action against pathogens and stimulate protective immune responses at that level, are gaining more and more followers [28,29,31]. EM, as well as MAM, due to their microbial composition, have prebiotic and probiotic action that justify their use as stimulators of productive and health indicators in porcine pre-fattening [4,8,19,26].

Conclusion

The fermentation of starter concentrates with multipurpose autochthonous microorganisms during 24 hours has a positive effect on the hematocrit and hemoglobin values of pig pre-fattening. Its prophylactic effect is compatible with that achieved with antibiotics and antiparasitics, without their adverse side effects.

Conflict of Interest Declaration

The authors have declared that there is no conflict of interests.

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