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MICROBIOLOGICAL AND CHEMICAL CHARACTERISTICS OF A ZOOTECHNICAL ADDITIVE OBTAINED IN ECUADOR FOR ITS USE IN ANIMAL FEEDING

CARACTERÍSTICAS MICROBIOLÓGICAS Y QUÍMICAS DE UN ADITIVO ZOOTÉCNICO OBTENIDO EN ECUADOR PARA SU USO EN LA ALIMENTACIÓN ANIMAL

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Microbiological and chemical characteristics of a zootechnical additive were determined for its use in animal feeding. The production process consisted of batch submerged fermentation at 18 ± 2 °C for seven days, mixed with an absorbent material, and then dried naturally. Two batches were formed, and the concentrations of mesophilic aerobes, fungi and yeasts, enterobacteria, total coliforms, *Escherichia coli*, *Salmonella*, and *Listeria* were calculated in duplicate. Humidity percentage, crude protein, ash, fat, crude fiber, total carbohydrates, pH, and total solids were also determined. The solid additive presented $1.4x10^7$ cfu/g of mesophilic aerobes, 10^6 cfu/g of yeast, absence of pathogens, 14.2 % of humidity, 14 % of protein and crude fiber, 11.8 % of ash, 0.21 % of fat, pH of 6.72, and 3.0° Brix. Results indicate that the product has potential for its use as a zootechnical additive.

Keywords: *bacteria, chemical composition, fermentation, yeasts*

Se determinaron las características microbiológicas y químicas de un aditivo zootécnico para su uso en la alimentación animal. El procedimiento de obtención consistió en una fermentación sumergida discontinua, a 18 ± 2 °C durante siete días, mezclada con un material absorbente y secado de forma natural. Se conformaron dos lotes y se calculó por duplicado la concentración de aerobios mesófilos, hongos y levaduras, enterobacterias, coliformes totales, *Escherichia coli*, *Salmonella* y *Listeria*. Se determinó además, porcentaje de humedad, proteína bruta, cenizas, grasa, fibra cruda, carbohidratos totales, pH y sólidos totales. El aditivo sólido presentó $1.4x10^7$ ufc/g de aerobios mesófilos, 10^6 ufc/g de levaduras, ausencia de patógenos, 14.2 % de humedad, 14 % de proteína y fibra bruta, 11.8 % de cenizas, 0.21 % de grasa, pH de 6.72 y 3.0 º Brix. Los resultados indican que el producto obtenido posee potencialidades para su utilización como aditivo zootécnico.

Palabras clave: *bacterias, composición química, fermentación, levaduras*

In animal production, there is an increasing interest in the use of zootechnical additives that would produce beneficial effects on animal health and, at the same time, to be considered as alternatives to antibiotic growth promoters (Kholif *et al.* 2024). These additives have an influence on the digestive and absorptive processes of dietary nutrients, modulate the immune system, improve intestinal and host health, and, therefore, can improve production yields (Anee *et al.* 2021). Consequently, the use of zootechnical additives can contribute to increasing the availability and quality of animal products, free of antibiotic residues, intended for humans.

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In Ecuador, some educational and research studies address the topic of obtaining and applying additives for animal feeding, as well as their advantages (Flores-Mancheno *et al*. 2016). However, the available scientific information is scarce. The objective of this research was to determine the microbiological and chemical characteristics of a new zootechnical additive produced in Ecuador for its use in animal feeding.

The study was conducted at an Experimental Poultry Farm of the Faculty of Agricultural Sciences, Technical University of Ambato, located in the Querochaca sector, Cevallos Canton, Tungurahua Province, Republic of Ecuador. The weather features of the site are 2,855 m a. s. l., with a mean annual rainfall of 442.4 mm, and a mean temperature of 16 °C.

The additive production process consisted of: 1) batch submerged fermentation, 2) mixing the culture with a solid support, and 3) natural drying by sun exposure. These steps and the procedure for microbiological and chemical analysis of the additive samples are described below.

Culture medium and inoculum: A medium composed of sugarcane molasses (20%, v/v), unsalted cow milk whey (34%, v/v), corn meal (1%, w/v), soybean cake meal (1%, w/v), urea (1%, w/v), Pecutrin® mineral-vitamin supplement (1%, w/v), and 100% water q.s. was used. Toni natural yogurt (2%, w/v), containing the *Lactobacillus* GG strain (Industrias Lácteas Toni S.A., Ecuador), was used as the inoculum.

Fermentation and drying process: Two batches of batch fermentation were carried out in duplicate in 50-L plastic tanks. The raw materials were added and weighed on a MOCCO SF-400D technical scale with a precision of ±0.01 units. Later, natural yogurt and drinking water were added to complete the fermentation volume corresponding to 45 L. The components were mixed for 10 min and incubated at room temperature (18 ± 2 °C) for seven days. After this time, the culture in each tank was homogenized, and an aliquot was taken to form a 1 L sample, measure its pH, and check the microbial concentration. The remaining culture was mixed (40:60, v/w) for 20 min with wheat bran and barley meal (50:50 w/w) and, finally, spread on an asphalt and roofing plate, forming a bed approximately 10 cm high. The material was turned every 3 h until dry. Samples of the dried material were taken from the ends and center of the drying plate until a homogeneous 1 kg sample was formed, which was then subjected to microbiological and chemical analyses. The remaining product was packaged in polyethylene bags and stored at room temperature for preservation.

Microbiological and chemical analysis: The additive samples were analyzed in duplicate, according to AOAC procedures (2019) at the Food Control and Analysis Laboratory of the Faculty of Food Sciences and Engineering and Biotechnology of the Technical University of Ambato (Ecuador), with accreditation No. SAE LEN 10-008. Concentrations of mesophilic aerobes, fungi and yeasts, enterobacteria, *Salmonella* and *Listeria* were determined in the samples of the additive, which were previously diluted in a serial and decimal manner, using the 3M Petrifilm plate technique. In addition, total coliforms and *Escherichia coli* were determined using CompactDry®. Chemical analysis included percentages of humidity, crude protein, ash, fat, crude fiber, total carbohydrates, and pH.

After seven days of fermentation, the microbial culture had a concentration of 10^7 and 10^6 cfu/mL of mesophilic aerobic bacteria and yeasts, respectively. Furthermore, it did not contain pathogenic microorganisms, and its pH was 4.10. These results are due to the fact that the microbial population increases its concentration with the fermentation process, metabolizing the nutrients from the substrates used in the mixture and producing organic acids, carbon dioxide, and other metabolites. High concentrations of acids, in turn, contribute to decrease the pH of the medium (García *et al.* 2020), which affects the cells of microorganisms such as *Salmonella* and *Escherichia coli*, which are sensitive to acidic pH.

Upon mixing the culture with the absorbent material and drying it in the sun, a brown solid with a fermented odor was obtained, the microbiological and chemical composition of which is shown in Tables 1 and 2, respectively. Similar to the culture, the presence of mesophilic aerobic bacteria (10^7 cfu/g) and yeasts (10^6 PU/g) were found at concentrations appropriate for their action as an additive and to guarantee its efficacy, as recommended by the FAO/WHO (2002). Likewise, its adequate hygienic and sanitary quality was verified, with no *Salmonella* or *Listeria* found, and minimal and harmless concentrations of *Enterobacteriaceae* and *E. coli*.

The bran meal, mixed with the microbial culture and naturally dried, had a humidity content of 14.2 %. This value is lower than the maximum established for dehydrated foods, which facilitates their handling and transportation, as well as extends their shelf life by preventing the proliferation of contaminating microorganisms. This is due to the fact that dehydration decreases water content and activity, which, consequently, reduces or inhibits microbial growth and the speed of various spoilage reactions (Toledo 2007). However, Bustamante *et al.* (2021) suggested that, when products are air-dried and sun-dried, fermentation may continue for a short period and affect protein percentage. This is believed to depend on the proportions in which mixtures are made and the absorption capacity of the material used.

Nutrient concentration also occurs as a result of dehydration. Additionally, these values may increase due to the nutrient content of the material used as absorbent. This study used a mixture of wheat bran and barley meal, which fundamentally influenced on protein and fiber percentage. The aforementioned meals are widely available in Ecuador and contain a maximum of 23.5 % of protein, 16 % of fiber,

Table 1. Microbiological composition of the obtained zootechnical additive

Indicator/Technique	Method	Unit	Dry additive
Mesophilic aerobic bacteria, Petrifilm	PE-03-7.2-MB 990.12*	cfu/g	$1.4x10^7$
Molds, Petrifilm	PE-02-7.2-MB 997.02*	PU/g	$3.1x10^6$
Yeasts Petrifilm	PE-02-7.2-MB 997.02*	PU/g	$1.0x10^6$
Enterobacteria, Petrifilm	PE-04-5.4-MB 2003.01*	cfu /g	<10
Total Coliforms, CompactDry	PE-01-7.2-MB R.I: 110402*	cfu /g	<10
Escherichia coli, CompactDry	PE-01-7.2-MB R.I: 110402*	cfu /g	<10
Salmonella, Petrifilm	PE-08-7.2-MB 2014.01*	In 25 g	Not found
Listeria spp. Petrifilm	R.I: 081203* Petrifilm Listeria Dishes	In 25 g	Not found

*AOAC (2019)

Table 2. Chemical composition of the obtained zootechnical additive

Indicator/Technique	Method	Unit	Dry additive
Humidity, gravimetry	925.10*	%	14.2
Protein, Kjendhal	2001.11*	% (Nx6.25)	14.2
Ashes, gravimetry	923.03*	%	11.8
Fat (with hyrolisis), gravimetry	2003.06*	%	0.213
Crude fiber, gravimetry	NTE INEN 522:2013	%	14.3
Total carbohydrates, calculation	Calculation	%	45
pH, potentiometry	981.12*	pH units	6.72

*AOAC (2019)

8 % of ash, and 14.5 % of humidity (Molinos Miraflores S.A., Ecuador, https://www.molinosmiraflores.com and Prodal S.A., Ecuador, https://www.prodal.com.ec).

Some educational and research work, conducted in Ecuador, addresses the topic of obtaining zootechnical additives. Díaz *et al*. (2014) obtained a microbial preparation with lactic acid bacteria (10^6 cfu/mL), total aerobic bacteria (10^6 cfu/mL), and yeasts (10^5 cfu/mL), enzymes, organic acids, and a pH of 3.87 through submerged fermentation with agricultural and industrial byproducts. This preparation was applied as an inoculum to post-harvest agricultural residue silages intended for dairy cows, stimulating milk production and increasing milk fat and protein by 11 %. Subsequently, Flores-Mancheno *et al.* (2016) evaluated the same microbial consortium in growing-fattening pigs and confirmed its beneficial effect on animal health and increased productive yield. However, there was no information in the available scientific literature on additives developed in the country using the procedure proposed in this study.

An example of an additive, which production method is similar to the one used in this research, is Vitafert and its variants, and its beneficial effects have been demonstrated in monogastric animals and ruminants (Valiño *et al.* 2024). Dry Vitafert with corn meal showed a similar composition to the product under study, containing 90 % of dry matter, 14.58 % of crude protein, a pH of 5.41, a high concentration of lactic acid bacteria ($15x10^7$ cfu/g), an absence of pathogenic microorganisms, and a high content of non-essential amino acids (glutamic acid, proline, aspartic acid, alanine, serine, cystine, tyrosine, and glycine) and essential amino acids (leucine, arginine, valine, phenylalanine, lysine, and threonine).

The chemical and microbiological characteristics of the additive demonstrated that it contains bacteria, yeasts, and nutrients in adequate concentrations, as well as good hygienic and sanitary quality. Therefore, it is considered to have potential for use as a zootechnical additive in animal production. It is recommended that these properties may be considered for future research. It is also suggested to develop future studies directed toward determining the biological response of different categories and species of animals to its use.

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