Probiotic effect of a strain of *Wickerhamomyces anomalus* on fattening broilers

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In order to evaluate the probiotic type effect of a strain of *Wickerhamomyces anomalus* on some physiological and productive indicators of fattening broilers, a completely randomized design was used, and two groups of animals were established, with a hundred of broilers each. The first group had a basal diet of corn and soy, and the second had a basal diet with the addition of a strain of *W. anomalus* LV-6, with a concentration of 108 cfu/g of food. By including this yeast into the diet, an increase of the relative weight of the caeca (4.38 vs. 5.19 g/kg of liveweight, $P < 0.05$) and of the carcass (53.81 vs 56.12 %) ($P < 0.01$) and breast yield (24.86 vs 27.05 %, $P < 0.001$) was confirmed, as well as the good health of the animals and the improvement of the response to vaccines ($P < 0.05$). It can be concluded that the addition of *W. anomalus* LV-6 to the diet of broilers provokes a probiotic response on the behavior of some physiological and productive indicators of the animals.

Key words: *yeast, probiotic activity, microbial additive, animal production*

Lactic acid bacteria and yeasts are the microorganisms most commonly used as probiotics in animal production. (Anadon *et al*. 2010). These microbial additives can contribute to the stability on the gastrointestinal ecosystem and influence of the digestive and absorptive processes, which determines the good functioning of the gastrointestinal tract and, therefore, the good health of the animal, which is reflected on the increase of the productive yields (Collins and Gibson 1999 and Rondón *et al*. 2013). According to FAO/WHO (2002), besides the *in vitro* characterization of the probiotic candidates, checking the biological respond of the animals before these microorganisms is essential.

The yeast *Wickerhamomyces anomalus*, LV-6 strain, was isolated from the excretions of fattening broilers and it demonstrated its potential as probiotic candidate in *in vitro* studies (García-Hernández *et al*. 2012). Therefore, the possible probiotic response in animals has to be evaluated because the functional effect of these microorganisms depends on the specie or strain used for these purposes (FAO/WHO 2002 and Angelakis *et al*. 2013). Therefore, the objective of this study was to evaluate the effect of the addition of a strain of *Wickerhamomyces anomalus* on some physiological and productive indicators of fattening broilers.

Materials and Methods

Animals and basal diet. The experimental work was developed within the facilities of the Institute of Animal Science (Mayabeque, Cuba). For this study, 200 animals (females, with an average liveweight of 40 g) from the EB34 hybrid were used, distributed in 100 broilers per mtreatment. They received water *ad-libitum* and a diet based on corn and soy, which composition was different for the beginning, growth and ending, according to the requirements for fattening broilers (Anon 2003).

Microorganisms. The *W. anomalus* LV-6 strain, from the bank of microorganisms from the Institute of Animal Science was used, cultivated in a YPG medium (yeast extract at 1.0 %, peptone at 2 %, and glucose at 2 %, w/v) at 30 ºC during 24 h in the orbital shaker, with temperature control and 100 rpm. After the incubation period, the absorbency (600 nm) of the culture was adjusted to 1, equivalent to 10^8 cfu/mL. The inoculum was prepared weekly and it was kept in sterile flasks at 4 ºC until its usage. It was mixed manually every day, with a basal diet of 1/1(v/w) in order to obtain a concentration of 10^8 cfu/g of food, which was checked through the sowing in dishes of Sabouraud Dextrose agar (Biocen, Cuba).

Experimental treatments. Two groups of animals were established. Group I had a basal diet (control) and group II had a basal diet with the addition of the strain of *W. anomalus* (LV-6). The broilers from the last group were treated since the first day of stay in the poultry unit until they were 42 d old.

Experimental conditions and management system of the animals. The study was carried out from July to August of 2010. According to data from the Meteorological Station from the Institute of Animal Science, the mean temperature was 27 ºC, the minimum was 23.5 ºC and the maximum was 31.1 ºC. The mean relative humidity was 71 %. Before beginning the study, the health control of the building for the broilers was applied, according to the established in the Technical Instructive for the management of fattening broilers (Anon 2003). The broilers were located in metal cages and a density of 25 broilers/m^2 was used until they were 18 d old. Later, there was a relocation with 12-13 broilers/m^2. In every cage, the water was offered in nipple drinkers, which were adjusted to the size of the broilers during the rearing. The basal diet was offered in tubular troughs,

which were adjusted to the height of the wings of the broilers, while they were growing. The animal vaccination system consisted on a dose of Marek, for preventing the fowlpox and the infectious bronchitis, the day after the pullets were born, and a dose of Gumboro at 1, 7 and 21 d. The Newcastle vaccine was also administered at 14 d old.

Experimental procedure for taking and analyzing the samples. At 42 d old, eight animals were randomly chosen from each treatment and they were slaughter by bleeding of the jugular vein (Sánchez 1990). In order to determine the probiotic activity of the microorganism, the methodology of Rondón (2009) was used and some physiological (relative weight of the gastrointestinal tract, small intestine, large intestine, caeca, gizzard, liver and abdominal fat), immunological (hemoaglutination inhibiting (HI) tests for the Newcastle vaccine and relative weight of the spleen and the Fabricius bourse) and hematological (hemoglobin (Hb), hematocrit (Hto) and total proteins) indicators were determined. The broilers were used to determine the behavior of some productive indicators during the biological evaluation of the yeast in the animals (liveweight, food intake, weight gain, food conversion, carcass yield, breast yield and viability).

Statistical analysis and design. A completely randomized design was used, with eight repetitions for the physiological indicators, where each animal was considered as an experimental unit. Four repetitions were used for the productive indicators, where two cages were considered as the experimental unit. The results were analyzed with the statistical package InfoStat version 1.0 (Balzarini *et al*. 2001). It was confirmed the data to fulfill the normality and homogeneity of variance and the ANOVA was performed. The t-student test was carried out in necessary cases to state the differences of the means. The values of viability were analyzed through the proportions comparison with the ComparPro 1.0 program (Font *et al*. 2007).

Results and Discussion

Table 1 shows the effect of the addition of *W. anomalus* LV-6 in some morphometric indicators of fattening broilers. There were only differences among the treatments for the relative weight of the empty caeca, with an increase in the group where the yeast was added. This effect could be related to the interaction of this yeast with the microbiota of the caecal ecosystem, which could favor the increase of the activity of the organ and, consequently, its relative weight. According to Álvarez-Martín *et al*. (2008), the yeasts, through the production of carbon dioxide, piruvate, propionate and succinate or by the vitamins or amino acids they produce, can stimulate the growth of the populations of lactic bacteria. These microorganisms, for their part, can colonize the surface of the caecal epithelium and produce organic acids in variable amounts during their metabolism, which influence on the increase of the size of the organ (Fooks and Gibson 2002 and Chichlowski *et al*. 2007). The mechanisms previously mentioned are not known with accuracy, which is why they have to be checked in further studies.

Table 1. Effect of the addition of *W. anomalus* LV-6 on some morphometric indicators of the gastrointestinal tract of fattening broilers, at 42 d old

Relative weight, g/kg liveweight	Treatments		SE± Sign
	Control	LV-6	
Empty gastrointestinal tract	78.25	78.52	2.01
Empty small intestine	38.71	36.16	0.86
Empty large intestine	6.94	7.69	0.25
Empty caecum	4.38	5.19	0.21*
Empty gizzard	24.36	25.09	0.63
Liver	25.93	25.29	0.53
Abdominal fat	14.82	13.01	0.81

*P < 0.05

There were variations in the hematological and immunological indicators determined, except for the total proteins and the Fabricius bourse (table 2). The low relative weight of this last organ, at 42 d old (moment in which the determinations were carried out), can be related to the process of involution that occurs when the animals get to maturity and the immune response depends then on the peripheral system (Mateos *et al*. 2002 and Perozo-Marín *et al*. 2004).

As for the hemoglobin and hematocrit, there were inferior values in the animals of the group treated with the yeast, although they were within the normal ranges (7.0-18.6 g•dL^{-1} of Hb and 23-55 % of Hto) for fattening broilers, according to Anon (1993). The HI test for the Newcastle vaccine increased when the strain LV-6 was added, regarding the control (table 2). These results indicate an improvement of the response to the vaccine and, therefore, stimulation to the immune response, which agrees with the stated by Yang *et al*. (2009) and Quigley (2011) about the immune-regulating action that probiotics can exert. Besides, the values of the hematological and immunological indicators showed a good health of the animals, which was confirmed through the viability percentages (94.74 vs. 94.68 for the control and LV-6, respectively).

Table 3 shows the behavior of some productive indicators during the biological evaluation of *W. anomalus* LV-6 in the animals. This microorganism produced no effect on the animal liveweight, intake, weight gain and food conversion. However, there were increases in the yield of the carcass and of the breast, which, apparently, are closely related. It is possible that the evaluated yeast works on the metabolism of the animal, either energetic or protein, when obtaining an eubiosic state in the intestine. This action favors

Table 2. Effect of the addition of *W. anomalus* LV-6 on health indicators of fattening broilers at 42 d old

Indicators	Treatments		SE±Sign
	Control	LV-6	
Fabricius bourse, g/kg liveweight	0.78	0.75	0.07
Spleen, g/kg liveweight	2.24	1.91	0.09*
HI for Newcastle vaccine [1]	5.83	8.20	0.63*
Hemoglobin, g/dL	9.47	8.50	0.15***
Hematocrit, %	31.50	28.33	0.47***
Total protein, g/dL	3.21	3.21	0.12
Mortality[2], %	5.26 ± 2.02	5.32 ± 2.03	

P < 0.05. *P < 0.05 ***P < 0.001

[1]The values are the mean of the logarithms of the titer

[2]Mean ±SE

Tabla 3. Effect of the addition of *W. anomalus* LV-6 on productive indicators of fattening broilers at 42 d old

Indicators	Treatments		SE±Sign
	Control	LV-6	
Liveweight, g	1592.25	1594.50	30.23
Intake, g	3329.25	3378.75	73.61
Weight gain, g	1552.25	1554.50	30.23
Conversion, g DM/g liveweight	2.15	2.12	0.07
Carcass yield, %	53.81	56.12	0.58**
Breast yield, %	24.86	27.05	0.39***

**P < 0.01 ***P < 0.001

the increase of the turnover time of the intestinal cells, which provokes a substantial saving of the nutrients digested by the animal and makes possible their use on the production of muscular mass, among other functions. These aspects should also be checked in further studies with a higher number of animals.

As a general view, the results of this study allow to conclude that the addition of the strain *W. anomalus* LV-6 to the diet of fattening broilers provokes a probiotic response on the behavior of some physiological and productive indicators of the animals.

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