Probiotic Effect of *Saccharomyces cerevisiae* in Hematic and Metabolic Parameters of Grazing Calves

Randolph Delgado Fernández, Herlinda de la Caridad Rodríguez**, Guillermo Barreto Argilagos*** and Roberto Vázquez Montes de Oca***

***University of Medical Sciences, Ciego de Ávila, Cuba**

****Faculty of Agricultural Sciences, University of Camagüey, Cuba**

*****Faculty of Chemistry, University of Camagüey, Cuba**

ABSTRACT

The probiotic effect of *Saccharomyces cerevisiae* was studied in certain hematic and metabolic parameters of grazing calves. Forty specimens aged 180 days on average, were included after selection (Cuban Siboney), with a live weight of 80 kg . Two groups (control and experimental) were made of 20 animals each, all receiving Norgold. In the experimental group, it was mixed with 100 ml of live culture of *S. cerevisiae*. The hematological studies were performed bimonthly. Blood was drawn from each animal through venipuncture in the jugular vein, to set up hemoglobin, hematocrit, and complete blood count with differential values; glycemia tests were made for blood glucose. The hemoglobin and hematocrit values had a significant difference in favor of the experimental group; similar values were observed for blood glucose. *Saccharomyces cerevisiae* used as nutritional supplement for grazing calves is a sustainable alternative with a probiotic effect observed in increased hematic and metabolic parameters.

Key Words: *hematology, blood chemistry, supplement, yeast, Cuban Siboney*

INTRODUCTION

Although the usefulness of probiotics dates back to thousands of years ago, as shown in the Sumerian charts (370 B.C), recording the use of fermented milk to treat gastrointestinal infections (Jans, 2005), its rational use in that direction took place only in the late Nineteenth Century, when Elie Metchnikoff suggested yogurt consumption as a way to face outbreaks of dysentery that scourged France in 1889 (Pelczar and Reid, 1966). However, in none of the examples presented the term "probiotic" was used; several years would go by for its acceptance, and several more, to link it to the field of veterinary (Delgado *et al.*, 2014).

Parker (1974) proposed the term probiotic (Greek, meaning for life) to name all organisms and substances that contributed to intestinal microflora balance. Five years later, Fuller (1979) used it for foodstuffs with microorganisms that provided benefits to the host animal, with effects on intestinal microbial balance. In such a way, the biological products began to penetrate the field of veterinary medicine (Barreto and Rodríguez, 2010). This new perspective explained the use of probiotics in ruminants, a variant that mainly made possible increases in animal productive parameters without the adverse collateral effects of conventional growth promoters (Barreto and Rodríguez, 2010).

Once the veterinary alternative was accepted, two main areas were addressed: animal health and production. The current trend to sustain the beneficial effect of microbiota through the use of probiotics (maybe by using immunostimulants in the near future) will bring new and promising hopes in the fields of animal science and health (Donovan *et al.*, 2012). However, most research developed nowadays relies on studies in favor of using diverse probiotics to increase the productive parameters in animal areas (Doležal *et al.*, 2012). The influence of this alternative on health parameters has undoubtedly been the most controversial, and also the least studied (Barreto and Rodríguez, 2010).

Saccharomyces cerevisiae has been used in a wide range of *in vitro* and *in vivo* experiments in calves, in order to assess their probiotic effects, together with high concentrate doses for productive parameters (Cakiroglu *et al.*, 2010). Nevertheless, in spite of the wide variety of consulted information (Moallem *et al.*, 2009; Cakiroglu *et al.*, 2010 and Doležal *et al.*, 2012), no probiotic effect reference was found for *S. cerevisiae* in grazing Siboney calves.

The purpose was to set up the possible probiotic effect of *Saccharomyces cerevisiae* in hematic

and metabolic parameters in grazing Siboney calves.

MATERIALS AND METHODS

To evaluate the possible probiotic effect of *Saccharomyces cerevisiae* on certain hematic and metabolic parameters in grazing calves, forty 180 day-old specimens (Cuban Siboney, 5/8 H and 3/8 C), with mean live weight of 80 kg, were selected. They were divided into two groups (control and experimental) of twenty animals each. Two treatments were assessed, following a completely randomized experimental design.

A) Experimental group: *Ad libitum* chopped sugar cane and 100 ml of liquid culture of *Saccharomyces cerevisiae* var C-40 (1.3×10^8 ufc/g) mixed / kg of Norgold / animal.

B) Control group: *Ad libitum* chopped sugar cane and 1 kg of Norgold / animal. To evaluate the effect of treatments on the parameters studied, measurements were made during four months (two in the rainy season, and other two in the dry season).

C) To evaluate the probiotic effect on hematology and blood chemistry values, the following measurements were made, hematological studies: bimonthly. Blood was drawn from each animal through venipuncture of jugular vein, with anticoagulant (EDTA).

Hematology

Total cell count (hematocrit) was determined through microhematocrit; hemoglobin, through the Drabkin method; total and differential leukocyte counts (percentage of neutrophils, eosinophils, basophils, lymphocytes and monocytes), according to Suardíaz *et al*. (2004).

Blood chemistry

Blood glucose was determined according to Suardíaz *et al*. (2004).

Statistical analysis

For comparison of means, non-parametric test, the Mann-Whitney test was performed, with $P < 0.05$ significance (Machado Sampaio, 2002). SSPS (2006), version 15.0 for Windows was used.

RESULTS AND DISCUSSION

A comparison of hemoglobin and hematocrit values produced a significant difference in favor of the values obtained in the experimental group (Tabla 1).

Sandoval *et al*. (2007) claimed that the hematological indicators are a para-clinical test that allows researchers to gain more knowledge about the relationship between health disorders and nutritional deficiencies; they are expressions of wellbeing of animals in livestock systems.

Every measurement of hematology and blood chemistry showed lower values in the first measurement made during the dry season (April), in comparison to the second one, made in the rainy season (June). This occurred because of a progressive recovery of animals with more grass availability as a primary source of nutrition. The better nutritional use observed in the experimental group, compared to the control group may be the cause for higher hemoglobin and hematocrit values (Paulus *et al*. 2010).

Several studies, including Paulus *et al*. (2010), report better absorption of minerals like, Ca, Fe, Cu, Zn, Mn and Se, by Holstein calves fed with beer yeast. Furthermore, Hossain *et al*. (2012) note that total protein, albumin and globulin values were higher in the yeast-supplemented groups.

In that sense, Garg (2008) concludes that this kind of yeast, used as a supplement, leads to a significantly higher nutrient digestibility, and increases the production of methyl carboxyl cellulase in the rumen. This assessment coincides with reports by Hossain *et al*. (2012), who found a digestion coefficient for organic matter (MO), crude protein (CP), crude fiber (CF), neutro detergent fiber (NDF) and acid-detergent fiber (ADF), significantly higher ($P < 0.05$) in two instances where the effect of different levels of yeast were compared to a control group.

Hindi Kumar and Ramana (2008) considered supplement the cause of increased microbial protein flow that exits the rumen; as well as an increase of aminoacids coming into the small intestine.

All the previous have an influence on the results of the blood variable. In that sense, Wittwer and Contreras (1988) argue that blood concentration of a metabolite is an indicator of nutritional balance.

The comparative hematocrit values between the experimental and control groups in the first and second measurements show differences. The bi-

monthly analyses performed are higher for the experimental group (Table 2).

The hematological indicators achieved behaved within the permissible range for this animal category, according to the reference values given by Figueredo *et al*. (2010).

The blood metabolic profile has a high correlation with the productive state and season; as well as with the kind of diet and herd handling, so it is a useful tool to diagnose the metabolic and nutritional state of cattle (Ayala *et al*., 2001).

In the particular case of hemoglobin, the values were higher for the experimental group, with a sustained value of 120 g/l as average on both measurements; whereas for the control group, it was 110 g/l. Studies conducted by Marín *et al*. (2010) have similar values for hemoglobin and hematocrit in the experimental group supplemented with probiotics, in comparison to the control group, without the supplement.

In the leukogram test performed the figures were higher for the experimental group in comparison to the control concerning white cell count; whereas in the differential count of lymphocytes was also higher (Table 3).

The highest number of lymphocytes found in the experimental group during the differential count may be associated to probiotic action on the immune system. Sheih *et al*. (2006) insisted that the immunomodulating effects of probiotics stem from their capacity to increase phagocyte activity of intestinal leukocytes, facilitate higher B lymphocyte activity, and stimulate cytokine production (interleukine IL-10). Kekkonen *et al*. (2008) established that due to their location in the intestine and the possibility to interact directly with the epithelium of the mucosa, probiotics effect on specific and nonspecific intestinal immunity. *In vitro* y *ex vivo* studies have proven that probiotics also possess the capacity to modulate the immune system. In research done by Kirjavainen *et al*. (1999), increased *ex vivo* proliferation of lymphocytes in mouse spleen was observed.

Determination of blood glucose values as indicators of energy metabolism showed a significant difference in favor of the experimental group (Table 4).

In the experimental group, the results achieved from bimonthly measurements were 65.5 and 66.5 mg/dl. Lower values were observed in the control group, resulting in 47.5 mg/dl in the first measurement, and 48.5 mg/dl in the second.

The results obtained are similar to Hossain *et al*. (2012), who claimed that *S. cerevisiae* supplementation significantly increases ($P < 0.05$) glucose levels in the serum of growing calves.

Szucs *et al*. (2013) also claimed that an increase in yeast supplies brings about increases in glucose concentration in plasma.

Increased glucose concentration in calves may be explained by research from Lehloenya *et al*. (2008), who referred to *in vitro* and *in vivo* studies in bovines with a positive effect of yeast on nutrient digestion and propionate production increases. Cunningham (1994), in turn, argued that the latter increase up to ruminal levels helped increase blood glucose concentration. Studies demonstrate that greater availability of propionic acid favors glucose level increases through gluconeogenesis (Fahey and Berger, 1988).

The results previously discussed corroborate that the use of environmentally friendly *S. cerevisiae* as a nutritional supplement for grazing Cuban Siboney calves is an alternative to increase the health parameters evaluated.

CONCLUSIONS

The use of *Saccharomyces cerevisiae* var. C 40, as a nutritional supplement in grazing Cuban Siboney calves had a probiotic effect demonstrated by increased hematic parameters (hemoglobin, hematocrit, cell count and leukocyte differential); as well as metabolic parameters (glucose).

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Table 1. Mann-Whitney test for hemoglobin and hematocrit

Indicators	Group	Number of animals	Mean range	Sig. asymp. (bilateral)
Hemoglobin	Experimental group	20	107.50	.000
	Control group	20	53.50	
Hematocrit	Experimental group	20	111.53	.000
	Control group	20	49.48	

Table 2. Hematocrit and hemoglobin values for the control and experimental groups

	Hematocrit Control group (%)	Hematocrit Control group (%)	Hemoglobin Control group (g/l)	Hemoglobin Experimental group (g/l)
1st measurement	31	33	110	120
2nd measurement	32	34	110	120
Average	31.5	33.5	110	120

Table 3. Cell count and mean differential of leukocytes for the control and experimental groups

		Experimental group	Control group
Cell count	Leukocyte (white cells) 10^9/µL	11.00	9.00
Differential Leukocyte Count	Neutrohils (%)	0.20	0.31
	Eosinophils (%)	0.01	0.01
	Basophils (%)	0.06	0.06
	Lymphocytes (%)	0.71	0.60
	Monocytes (%)	0.02	0.02

Table 4. Mann-Whitney test for glucose concentration in blood and leukocyte cell count

Indicators	Group	Number of animals	Average range	Sig. asympt. (bilateral)
Blood glucose concentration	Experimental group	20	120.50	.000
	Control group	20	40.50	
Leukocyte cell count	Experimental group	20	40.70	.000
	Control group	20	120.30	