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Production of a Symbiotic Biopreparation (Mixture of *Agave fourcroydes* Lem. Pulp and PROBIOLACTIL®) for Use in Calves

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Abstract

Context: Artificial calf breeding may lead to gastric disorders in animals, which are produced due to inappropriate nutritional practices.
Objective: Production of a Symbiotic Biopreparate (Mixture of Agave fourcroydes Lem. Pulp and PROBIOLACTIL®) in Calves.
Methods: The design and optimization of this biopreparation was based on the response surface method, with a rotating central composite experimental design 2^2, and two replications. Henequen pulp was used with the addition of molasses and yeast hydrolyzate, as sources of sugar and nitrogen, respectively, and PROBIOLACTIL® for supplementation of *Lactobacillus salivarius*. The experiment lasted 28 days, with a completely randomized design, to evaluate the effect of this additive on the productive and health indicators of Mambi de Cuba calves, at weaning and breeding.
Results: Optimum values were achieved for the components of the biopreparation, which increased the production of *Lactobacillus*. The application of the additive improved live weight, mean daily gain, and weight gain, from the 21st day on, and its influence on health was seen through a reduction in the occurrence of diarrhea.
Conclusions: The biopreparation was designed from a probiotic culture of agroindustrial residues, then enriched with highly available national components. This symbiotic biopreparation may be used as a nutritional additive in weaned calves.

Key words: probiotics, zootechnical additives, Lactobacillus salivarius.

Introduction

Artificial calf breeding makes animals more prone to gastric disorders, many of which are produced due to inappropriate nutritional practices, respiratory, and parasitological problems, which affect their healthy development (Calzadilla et al., 1999; Malacari, 2016). Over the years, antibiotics have been used to fight these conditions; however, new alternatives are being explored in the world today to replace these antimicrobials, including biotherapeutical agents (probiotics, prebiotics, and symbiotics), which are considered natural, with active biological properties, and preventive and curative capacities (Corzo & Gilliland, 1999; Uyeno Shigemori & Shimosato, 2015).

These additives can be made from microorganisms or substances that help stabilize, maintain, reproduce, and enhance a favorable balance of microbial ecology in the intestine, along with proper functioning of the immune system (Alzahal et al., 2014; MacPherson et al., 2014; Pandey, Suresh & Babu, 2015).

Probiotics are defined as live microorganisms with beneficial effects on the health of hosts, through administration in adequate amounts (FAO/WHO, 2001; Castañeda, 2018). However, prebiotics are non-digestible food ingredients that affect animals positively, by selective stimulation of growth and metabolic activity of a limited number of colonic bacteria (Olagnero et al., 2007). Symbiotics combine prebiotic and probiotic principles that act in synergy (Abreu, 2014).

Koteswara Reddy et al. (2013) refer that there is a global tendency to use stalks as animal food. They are enriched through biological treatments, like microbial fermentation, which contributes to higher nutritional value of stalks. In the province of Matanzas, thousands of henequen (*Agave fourcroydes* Lem.) stalks are generated every year, which are used for cropland fertilization and animal nutrition. The pulp is a derivative obtained by extraction from the plant fibers. It has low dry matter contents, therefore requires the addition of other components to enhance its usefulness. Some studies say that henequen pulp has a low nutritional value; however, it is highly digestible and rich in inulin, one of the most important prebiotic substances (García et al., 2015).

Several research projects have been conducted at the University of Matanzas in order to develop probiotics such as PROBIOLACTIL®, a biopreparate made from *Lactobacillus salivarius* cultures, which was evaluated in birds and pigs with remarkable results in terms of higher productive and health indicators. Accordingly, the aim of this paper was to produce a symbiotic biopreparate based on a mixture of henequen pulp (Agave fourcroydes Lem. Pulp) and PROBIOLACTIL®) for application in Calves.

Materials and Methods

To produce the probiotic biopreparate, each kilogram of henequen pulp (produced at Eladio Hernandez henequen company in Matanzas) required molasses (carbon source), *Saccharomyces serevisiae* yeast hydrolyzate (total nitrogen source), and PROBIOLACTIL® as inoculate. The was pH=6.5, and the incubation temperature was 30 ºC.

The response surface method (Box et al., 1978) was used for design and optimization of the biopreparate, with a rotating central composite experimental design 2^2, and two replications in the center of the plan. The independent variables were total reductive sugars (TRS-X_1) and total nitrogen (TN-X_2) supplied by molasses and the enzymatic hydrolyzate of yeast, respectively. The other variable (X_3) was the bacterial inoculate (PROBIOLACTIL®), and the response variable (Y) was counting of colony forming units per gram (UFC.g^{-1}).

Statgraphics Plus, 5.1 (2002) was used following definition of the levels of independent variables, to design the codified matrix, showing the combinations to be applied. The minimum, mid, and maximum levels were used for TRS (10, 15, 20 g), TN (1, 2, 3 g), and the inoculate (5, 10, 15 mL). The program also develops multiple regression analysis to obtain the polynomial equation: $Y= b_o+ b_1X_1 + b_2X_2 + b_3X_{3+} b_{11}X_1^2 + b_{22}X_2^2 + b_{23}X_2X_3+b_3X_3^2$.

Each experimental run required Erlenmeyers (250 mL effective volume), containing 50 g of pulp from henequen, but the concentrations of the independent variables were changed (X_1, X_2 y X_3), according to the matrix codified. The initial pH of the fermentation was adjusted (6.5) with NaOH 1N, and it was sterilized (15 min-1.5 atm). Then, PROBIOLACTIL® was added according to the experimental design; after 24 h at 30 ºC, the samples of the biopreparates were collected to count CFU. The runs were made by triplicate.

A 28-day experiment was conducted at Breeding Ares No. 306 (Genetic Company of Matanzas), to evaluate the effects of the symbiotic biopreparation on calves. This work was performed in June-July, 2018, during the rainy season. Overall, 30 Mambí de Cuba animals aged 7 weeks (50 days) were included.

A completely randomized experimental design was used in three experimental groups. 1. Control group (control animals), basal diet, 2. Group based on henequen pulp administration, 3. Group based on the symbiotic biopreparation. The 30 calves (aged 49-50 days) were chosen at random, and their average live weight was 48.2 kg. The animals were given whole lactating/lacto replacing feeds (Raltec®) at 50 days, or milk substitute, and complementary lactating feed (Raltec®). At 84 days, the animals received complementary lactating feed (Raltec®) and forage (*Pennisetum purpureum*).

The productive and health indicators, such as live weight, weight increase, and mean daily gain were raised; the occurrence of diarrhea was recorded daily.

The CFU.mL^{-1} count were converted to LN, in order to perform the statistical analysis and decoding of variables during the design and optimization of the biopreparation. The significance of each model parameter was evaluated, and the response surface was determined using Statgraphics Plus version 5.1 (2002). The optimum values of the independent variables were defined from the model used. The data from the *in vivo* experiment were processed using INFOSTAT, version 2012 (Di Rienzo et al., 2012). In cases when the data met the requirements, they were processed through one-way ANOVA; Duncan's Multiple Range Test (1955) was used for comparison of means. To evaluate the occurrence of diarrhea, CompaProp (Castillo & Miranda, 2014), 95% confidence, was used.

Results and discussion

Table 1 shows the coded matrix, with all the combinations and results achieved for CFU.g^{-1} (expressed in LN CFU.g^{-1}) of *Lactobacillus salivarius* in the biopreparate. The statistical processing of the design and data adjustment produced the following model: LN UFC.g^{-1} = 5,38319 + 0,649132 * X_1 + 2,26854 * X_2 + 0,976018 * X_3 - 0,0297623 * X_1^2 - 0,0206667 * X_1 * X_2 + 0,0285667 * X_1 * X_3 - 0,83952 * X_2^2 + 0,111167*X_2*X_3 - 0,0775626*X_3^2.

According to the experimental data, this model estimated the maximum value of CFU.g^{-1} when X_1, X_2, and X_3 had the optimum conditions (Table 1). The factor with the greatest influence on the response variable was TN concentration, indicating that the nitrogen levels used affected viable count.

Table 1. Coded matrix and results from CFU.mL^{-1} count (changed into LN) for growth of *Lactobacillus salivarius*, according to the composite central rotating design

TRS	TN	Inoculate	LN CFU.g^{-1}	
10	1	5	13.13	13.85
20	3	5	11.51	11.51
20	1	15	13.82	14.91
15	0.318207	10	17.03	15.42
15	2	10	18.46	19.74
15	2	1.59104	11.61	12.21
10	3	5	15.61	13.82
10	1	15	13.82	14.91
15	2	18.409	11.51	11.51
20	1	5	14.51	14.51
10	3	15	13.82	13.82
20	3	15	17.77	16.86
15	2	10	15.69	16.01
23.409	2	10	14.91	13.82
6.59104	2	10	15.42	15.76
15	3.68179	10	13.30	14.15

TRS Total reductive sugars; TN. Total nitrogen

Variable decoding allowed for calculation of optimum TRS (15.26), TN (1.85), and the inoculate (10.43) values of variable evaluated response (Table 2). These results indicated that the symbiotic biopreparation must contain these levels supplied by molasses and the enzymatic hrydolyzate of yeast, respectively, to achieve maximum response of viable count. The graphic of the model's response surface shows the presence of optimal TRS (X_1), TN (X_2), and inoculate (X_3) for the response variable (LN CFU.g.$^{-1}$), as well as a defined concave area, which is commonly observed in the maximal.

Variable decoding allowed for calculation of optimum TRS (15.26), TN (1.85), and the inoculate (10.43) values of variable count response of CFU.g^{-1} (Table 2).

Table 2. Composition of the symbiotic biopreparation

Composition	Optimum value	Final composition
Henequen pulp, kg	1	1
Molasses, g (58% TRS)	15.26	25.86
Enzymatic hydrolyzate of yeast (17% TN)	1.85 g	11,17 mL
Inoculate, mL PROBIOLACTIL®	10.43	10.43

The response surface approach is known for its effective optimization of culture media, since microbial activity is not only affected by the components of the biopreparate and its concentrations, but also by their interactions (Rodríguez Bernal et al., 2014).

Lactobacilli require complex media containing several amino acids, vitamins, growth factors, fermentable carbohydrates, etc., that stimulate their growth (Liew et al., 2005). The formulation of the new biopreparation was made chiefly to use the high-inulin concentration henequen pulp, along with carbohydrate and protein sources supplied by molasses and the enzymatic hydrolyzate. This contribution is made to increase the number of probiotic microorganisms (*Lactobacillus*), which require these nutrients for fast growth and colonization.

Molasses contains sucrose, glucose, and fructose (Cabello, 1980). These carbs contribute with high concentrations of TRS that can be used by microorganisms as sources of energy. Other authors, such as Sosa et al. (2018) noted that molasses is used today in the composition of culture media for the growth of microorganisms with probiotic purposes, because it increases the microbial population and growth speed.

Pérez et al. (2006) established the methodology to obtain an enzymatic hydrolyzate using cream from yeast *Saccharomyces cerevisiae* resulting from the residues of national distilleries. The composition of this product is between 16 and 20% of TN, so it is an alternative to the inclusion of nitrogen sources in the biopreparates.

Table 2 shows live weight behavior of animals treated in relation to the control, during the experiment. After 28 days of experiment, live weight increased (P≤ 0.05) in the group receiving the symbiotic biopreparation, compared to the control and the treatment where the pulp of henequen was applied.

Table 3. Behavior of productive indicators at the end of the experiment.

Indicator	G-1	G-2	G-3	±SE P
LW	57.4[a]	62.3[b]	66.2[c]	1.61 0.012
WI	8.80[a]	12.10[b]	18.40c	0.52 0.001
MDG	0.31[a]	0.43[b]	0.65[c]	0.02 0.001

LW Live weight, WI Weight increase, MDG. Mean daily gain.

The live weight increase in animals that consumed the symbiotic biopreparation may be associated to the fact that after consumption, probiotics and prebiotics induce numerous mechanisms in the gastrointestinal tract (GIT), which favor the balance of intestinal microorganisms, and provide a better response of digestive processes in the host (Flores, 2015; Adjei-Fremah et al., 2018).

These results demonstrate the importance of including native microorganisms in the diet of these animals in order to keep the microbial balance. Sánchez et al. (2015) said that probiotics can withstand specific conditions in the GIT, like proteolytic enzymes for over 4 hours, low pH values (1.8-3.2) prevailing in the stomach, and the concentration of bile, pancreatic acids, and mucus present in the small intestine, so that the colonizing microorganisms can make it live in sufficient numbers when the acidic and biliary barriers are surpassed within the digestive tract.

The positive effects of probiotics and prebiotics in the GIT are also seen in the productive performance of animals (Bartkiene et al., 2018). The inclusion of the symbiotic product in the rations consumed by this species also had a positive influence on the productive indicators. Various researchers have stated that these additives may improve live weight, daily gain, and food conversion (Zhang et al., 2016).

These results are linked to the functions developed by probiotics, since they change the intestinal microbial population, stimulate the immunological system, take place in metabolic processes, prevent pathogenic colonization, increase volatile fatty acids (VFA), reduce the absorption of toxic substances like NH_3, amines, indol, mercaptanes, and sulfites, and reduce blood cholesterol, synthesized vitamins (especially vitamin K and the B complex vitamins), and enhance mineral absorption (Simmering & Blaut, 2001).

Short chain fatty acids (SCFAs) produced in the GIT are metabolized in the mucosa; when probiotics are used there is an improvement in the microbial balance, thus increasing the number of beneficial microorganisms. Accordingly, an increase of SCFAs is observed in the intestine, and there will be greater bioavailability of these substances as sources of energy (Rondón & Laurencio, 2008).

Lactobacilli release enzymes that enhance the digestive capacity of animals, deactivate the toxic metabolites from the harmful biota effectively, and increase the absorption process due to a better cellular state of villi and greater synthesis of vitamins (Segura & De Bloss, 2000).

The evidence says that the use of probiotic microorganisms (*Lactobacillus* spp.) in the form of monoculture or mixtures increases the retention of the nutrients included in the diet. Apparent nutrient retention (the amount of nutrients consumed minus the amount of excreted nutrients) is favored by the use of probiotics, especially due to the retention of N, P, and Ca (Ángel et al., 2005).

These results match the reports of Zhang et al. (2016), who studied the effect of probiotic microorganisms *Lactobacillus plantarum* GF103 and *Bacillus subtilis* B27. These authors noted that an improvement was observed in nutrient digestibility and the productive yields.

Flores (2015) evaluated the effect of a probiotic on productive and health indicators in lactating Mambi de Cuba calves. The additive (PROBIOLACTIL®) was made using strain *Lactobacillus salivarius* C-65, and included 24 calves between the ages of 7 and 9 days, and 12 calves distributed in each treatment. Accordingly, the calves that consumed the biopreparation showed a lower occurrence of diarrhea, and there were differences in live weight increase ($P \leq 0.05$), compared to the control group.

Similarly, Malik & Bandla (2010), demonstrated that the administration of probiotic *Lactobacillus acidophilus* raised mean daily weight increase (MDW) and fodder efficiency. Meanwhile, Zapata (2011) evaluated the probiotic effect of Vitafert® on pre-weaned calves, with better results ($P<0.05$) in live weight at the end of the experiment.

Table 4 shows the behavior of diarrhea in the animals that consumed the symbiotic biopreparation, compared to the control group.

Table 4 Occurrence of diarrhea in the animals studied

Weeks	Treatments	Proportion	SE
1	(1)	0.30	0.14
	(2)	0.20	0.14
	(3)	0.10	0.13
2	(1)	0.20	0.11
	(2)	0.10	0.11
	(3)	0.00	0.13
3	(1)	0.10	0.07
	(2)	0.00	0.07
	(3)	0.00	0.09
4	(1)	0.00	0.00
	(2)	0.00	0.00
	(3)	0.00	0.00

1. Control group, 2 Animals treated with henequen pulp Animals treated with the symbiotic biopreparate

Despite the absence of statistical biological differences, there was a slight increase in the occurrence of diarrhea in the control group in relation to the group treated. Moreover, as weeks passed, this condition tended to decrease, which demonstrated the occurrence of colonization of bacteria present in the symbiotic product.

The results achieved in decreasing the incidence of diarrhea in the animals treated with the symbiotic biopreparation may have occurred thanks to the native intestinal bacteria, which developed different pathogenic mechanisms causing diarrhea, such as competition over colonization and nutrient sites, the production of toxic compounds, and the stimulation of the immune system. These processes are not mutually exclusive, and inhibition can include one, several, or all these mechanisms (Saalfeld et al., 2016).

The microorganisms used as probiotics usually produce different substances that inhibit pathogenic microorganisms. These microorganisms have the capacity to adhere to the intestinal mucosa of animals and cause enteric diseases (Bajagai et al., 2016). Probiotics also have the capacity to stimulate the immune system of animals and to produce organic acids that reduce the pH of the intestinal lumen, which curtails the proliferation of pathogenic bacteria (Zapata, 2011; Fernández et al., 2018).

Signorini et al. (2012) achieved similar results to this study. They defined that the occurrence of diarrhea is in correspondence with the LAB proportion: coliform. It means that diarrhea occurs when the coliform population is greater than LAB. Therefore, if *Lactobacillus* cultures are often supplied during that stage, the population of those bacteria in the GIT will increase, and diarrhea will diminish (Liepa & Viduža, 2018). Other authors, like Thomas & Elliott (2013), and Bertin et al. (2017) also used probiotics in calves, reducing the population of *E. coli* O157:H7, which demonstrated the efficacy of these biopreparations against this bacterium, which causes diarrhea in animals.

Mycotoxins and enterotoxins are known to decrease due to the action of additives (Bi et al., 2017). Baines et al. (2013) applied a mixture of prebiotic/probiotic, which eliminated morbidity and mortality-related losses, caused by *E.coli* infections of the gastrointestinal tract.

When *Lactobacillus*-based probiotics are administered, the incidence of diarrhea is lower during the first weeks of a calf's life. In that sense, Satık & Günal (2017) studied the effects of kefir as a probiotic on calf's performance and health. As a result, the animals were more inclined to have a positive effect of lactic acid bacteria in the stools at 14 days, and a reduction of diarrheal diseases.

Conclusions

The new biopreparation designed from a probiotic culture of agroindustrial residues enriched with highly available national components, is a symbiotic additive that may be used as a nutritional additive in calves during weaning and post-weaning. The animals that consumed the biopreparation underwent improvements in live weight, weight increase, and mean daily gain.

Author contribution

Ana Julia Rondón Castillo: Design, research planning, analysis of results, manuscript redaction, final review.

Arianne del Valle Pérez: Development of the experimental part, analysis of results, and redaction of the manuscript.

Grethel Milián Florido: analysis of results, manuscript redaction, manuscript review.

Fátima Arteaga Chávez: Critical review of the manuscripts, analysis of results.

Marlen Rodríguez Oliva: analysis of results, manuscript redaction, manuscript review.

Marlene Martínez Mora: Development of the experimental part, analysis of results, and redaction of the manuscript.

Conflicts of interest

Conflicts of interest: no conflict of interest has been declared

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References

Abreu y Abreu, A.T. (2014). Probióticos, prebióticos y simbióticos. *Revista de Gastroenterología de México, 79*(Supl.1), 17-18. Retrieved on March 12, 2018, from: http://www.revistagastroenterologiamexico.org/index.php?p=revista&tipo=pdf-simple&pii=X0375090614691212

Adjei-Fremah, S., Ekwemalor, K., Asiamah, E. K, Ismail, H., Ibrahim, S., & Worku, M. (2018) Effect of probiotic supplementation on growth and global gene expression in dairy cows. *Journal of Applied Animal Research*, 46:1, 257-263, doi: https://doi.org/10.1080/09712119.2017.1292913

Alzahal, O., McGill, H., Kleinberg, A., Holliday, J. I., Hindrichsen, I. K., Duffield, T. F., & McBride, B.W. (2014). Use of a direct-fed microbial product as a supplement during the transition period in dairy cattle. *J Dairy Sci.*, *97* (11), 7102–7114, doi: https://doi.org/10.3168/jds.2014-8248

Ángel, R., Dalloul, R.A., & Doerr, J. (2005). Metabolism and nutrition: Performance of broiler chickens fed diets supplemented with a direct-fed microbial. *Poult. Sci.*, *84*, 1222–1231. Retrieved on March 12, 2018, from: https://primalac.com/wp-content/uploads/2015/08/Angel-Reduced-Nutrient.pdf

Baines, D., Sumarah, M., Kuldau, G., Juba, J., Mazza, A., & Masson, L. (2013). Aflatoxin, fumonisin and Shiga toxin-producing *Escherichia coli* infections in calves and the effectiveness of Celmanax®/Dairyman's Choice™ applications to eliminate morbidity and mortality losses. *Toxins*, *5*, 1872-1895, doi: https://doi.org/10.3390/toxins5101872

Bajagai, Y. S., Klieve, A. V, Dart. P. J., & Bryden, W.L. (2016). Probiotics in ruminant Nutrition. En: H.P.S. Makkar (Ed.), FAO *Animal Production and Health Paper*, (No. 179, pp. 37-48). Roma, Italia: FAO. Retrieved on March 12, 2018, from: http://www.fao.org/3/a-i5933e.pdf

Bartkiene, E., Bartkevics, V., Ikkere, L. E., Pugajeva, I. Zavistanaviciute, P., Lele, V.,… Juodeikiene, G. (2018). The effects of ultrasonication, fermentation with *Lactobacillus* sp., and dehydration on the chemical composition and microbial contamination of bovine colostrum. *J. Dairy Sci.*, *101*(8), 6787–6798, doi: https://doi.org/10.3168/jds.2018-14692

Bertin, Y., Habouzit, C., Dunière, L., Laurier, M., Durand, L., Duchez, D.,… Forano, E. (2017). *Lactobacillus reuteri* suppresses *E. coli* O157:H7 in bovine ruminal fluid: Toward a pre-slaughter strategy to improve food safety? PLoS ONE, *12*(11), e0187229, doi: https://doi.org/10.1371/journal.pone.0187229

Bi, Y., Yang, Ch., Diao, Q., & Tu, Y. (2017). Effects of dietary supplementation with two alternatives to antibiotics on intestinal microbiota of preweaned calves challenged with *Escherichia coli* K99. *Scientific Reports*, *7* (5439). Retrieved on March 15, 2018, from: https://www.nature.com/articles/s41598-017-05376-z

Box, G. E. P., Hunter, W. G., & Hunter, J. S. (1978). *Statistics for experimenters: an introduction to design, data analysis, and model building.* New York, USA: John Wiley y Sons.

Cabello, A. (1980). Utilización de los subproductos de la industria azucarera en la alimentación animal. Derivados de la caña de azúcar. *ICIDCA*: 393-419.

Calzadilla Dodd, D., Castro, A., Soto Márquez, E. Hernández Rodríguez, M., & Andrial, P. (1999). *Ganadería tropical.* La Habana, Cuba: Editorial Félix Varela.

Castañeda Guillot, C. (2018). Probióticos, puesta al día. *Revista Cubana de Pediatría*, *90*(2), 286 – 298. Retrieved on March 15, 2018, from: http://scielo.sld.cu/pdf/ped/v90n2/ped09218.pdf

Castillo Duvergel, Y., & Miranda, I. (2014). COMPAPROP: Sistema para comparación de proporciones múltiples. *Rev. Protección Veg.*, *29*(3), 231-234. Retrieved on March 15, 2018, from: http://scielo.sld.cu/pdf/rpv/v29n3/rpv13314.pdf

Corzo, G., & Gilliland, S.E. (1999). Bile salt hydrolase activity of three strains of *Lactobacillus acidophilus*. *J. Dairy Sci.*, *82*, 472-480, doi: https://doi.org/10.3168/jds.S0022-0302(99)75256-2

Duncan, B. (1955). Multiple ranges and multiple F. Test. *Biometrics*, *11*, 1-42.

FAO/OMS. (1-4 de octubre, 2001). Informe de la Consulta de Expertos FAO/OMS sobre Evaluación de las propiedades saludables y nutricionales de los probióticos en los alimentos, incluida la leche en polvo con bacterias vivas del ácido láctico. En *Probióticos en los alimentos. Propiedades saludables y nutricionales y directrices para la evaluación.* (No. 85). Córdoba, Argentina: Autor. Retrieved on March 15, 2018, from: http://www.fao.org/3/a-a0512s.pdf

Fernández, S., Fraga, M., Silveyra, E., Trombert, A. N., Rabaza, A., Pla, M., & Zunino, P. (2018). Probiotic properties of native *Lactobacillus* spp. strains for dairy calves. *Beneficial Microbes*, 9 (4), 613 - 624, doi: https://doi.org/10.3920/BM2017.0131

Flores, O. (2015). Efecto del PROBIOLACTIL® en indicadores productivos y de salud en terneros lactantes. (Trabajo Científico Técnico Salud y producción bovina). Universidad Agraria de La Habana, Cuba.

García Curbelo, Y., Bocourt, R., Savón, L. L., García Vieyra, M. I., & López, M. G. (2015). Prebiotic effect of Agave fourcroydes fructans: an animal model. *Food Funct.* *6*(9), 3177–3182, doi: 10.1039/c5fo00653h

Koteswara Reddy, G., Mohana Lakshmi, S., Ashok Kumar, C. K., Satheesh Kumar, D., & Lakshmi Srinivas, T. (2013). Evaluation of anti-inflammatoy and antioxidant activity of methanolic extract of *Agave*. *Journal of Global Trends in Pharmaceutical Sciences*, *4*(4), 1300-1309. Retrieved on May 13, 2018, from: https://www.jgtps.com/admin/uploads/fvtXsn.pdf

Liepa, L., & Viduža, M. (2018). The effect of peroral administration of *Lactobacillus fermentum* culture on dairy cows health indices. *Macedonian Veterinary Review*, *41*(2), 143-151. Retrieved on January 5, 2019, from https://www.macvetrev.mk/2018-2/macvetrev-2018-0017.pdf

Liew, S. L., Ariff, A. B., Raha, A. R., & Ho, Y. W. (2005). Optimization of medium composition for the production of a probiotic microorganism, *Lactobacillus rhamnosus*, using response surface methodology. *International J. of Food Microbiol.*, *102*, 137-142, doi: https://doi.org/10.1016/j.ijfoodmicro.2004.12.009

MacPherson, C., Audy, J., Mathieu, O., & Tompkins, T. A. (2014). Multistrain probiotic modulation of intestinal epithelial cells' immune response to a double stranded RNA ligand, poly (I·). *Appl Environ Microb.*, *80*(5), 1692–1700, Retrieved on March 16, 2018, from: https://aem.asm.org/content/aem/80/5/1692.full.pdf

Malacari, D. A. (2016). *Guía para la crianza y mantenimiento de terneros privados de calostro para su utilización como modelo animal.* Buenos Aires: INTA. Retrieved on March 16, 2018, from: https://inta.gob.ar/sites/default/files/inta_-_guia_para_la_crianza_y_mantenimeinto_de_terneros_privados_de_calostro_0.pdf

Malik, R., & Bandla, S. (2010). Effect of source and dose of probiotics and exogenous fibrolytic enzymes (EFE) on intake, feed efficiency, and growth of male buffalo (Bubalus bubalis) calves. *Tropical Animal Health and Production*, *42*(6), 1263-1269, doi: https://doi.org/10.1007/s11250-010-9559-5

Olagnero, G., Abad, A., Bendersky, S., Genevois, C., Granzella, L., & Montonati, M. (2007). Alimentos funcionales: fibra, prebióticos, probióticos y simbióticos. *Diaeta*, *25*(121), 20- 33. Retrieved on March 15, 2018, from: http://andeguat.org.gt/wp-content/uploads/2015/03/Alimentos-funcionales-fibra-prebi%C3%B3ticos-probi%C3%B3ticos-y-simbi%C3%B3ticos1.pdf

Pandey, R., Suresh, R. N., & Babu, V. (2015). Probiotics, prebiotics and synbiotics - a review. *Association of Food Scientists y Technologists*, *52*(12), 7577-7587. Retrieved on January 8, 2019, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4648921/

Pérez, M. Q., Milián, G., Piad, R. B., González, R.C., Bocourt, R. S., & Savón, V. (2006). Hidrolizado de fondaje de cubetas de destilerías de alcohol con un crudo enzimático de la cepa de *Bacillus licheniformis* E-44 y su procedimiento de obtención. Patente No. 23179. (Int.cl.8) A 23 J 1/00,3/30, C 12N 9/56.

Rodríguez Bernal, J. M., Serna Jiménez, J. A., Uribe Bohórquez, M. A, Klotz, B., & Quintanilla-Carvajal, M. X. (2014). Aplicación de la metodología de superficie de respuesta para evaluar el efecto de la concentración de azúcar y de cultivos iniciadores comerciales sobre la cinética de fermentación del yogurt. *Revista Mexicana de Ingeniería Química*, *13*(1), 213-225. Retrieved on June 16, 2018, from: www.scielo.org.mx/pdf/rmiq/v13n1/v13n1a17.pdf

Rondón, A. J., & Laurencio, M. (2008). Utilización de las mezclas de Exclusión Competitiva en la avicultura moderna. *Rev. Cienc. Agríc.*, *42*(1), 3-11. Retrieved on June 16, 2018, from: https://www.redalyc.org/html/1930/193015413001/

Saalfeld, M. H., Brayer Pereira, D. I., Silveira Valente, J. S., Lopes Borchardt, J. L., Weissheimer, C. F., Arocha Gularte, M., & Pereira Leivas Leite, F. (2016). Effect of anaerobic bovine colostrum fermentation on bacteria growth inhibition. *Ciênc. Rural*, *46*(12), 2152–2157, doi: http://dx.doi.org/10.1590/0103-8478cr20160393

Sánchez, L., Omura, M., Lucas, A., Pérez, T., Llanes, M., & Ferreira, C. de L. (2015). Strains of *Lactobacillus* spp. with probiotic capacity isolated from the intestinal tract of calves. *Rev Salud Anim.*, *37*(2), 94-104.

Satık, S., & Günal, M. (2017). Effects of kefir as a probiotic source on the performance and health of young dairy calves. *Turkish Journal of Agriculture - Food Science and Technology*, *5*(2), 139-143. Retrieved on June 16, 2018, from: http://agrifoodscience.com/index.php/TURJAF/article/download/978/467

Segura, A., & De Bloss, M. (2000). La alternativa a los promotores del crecimiento. En *III Congreso Nacional de Avicultura. Memorias.* (pp. 37-44). Varadero, Cuba: Centro de Convenciones Plaza América.

Signorini, M. L., Soto, L. P., Zbrun, M. V., Sequeira, G. J., Rosmini, M. R., & Frizzo, L.S. (2012). Impact of probiotic administration on the health and fecal microbiota of young calves: a meta-analysis of randomized controlled trials of lactic acid bacteria. *Res Vet Sci*., *93*(1), 250-258, doi: https://doi.org/10.1016/j.rvsc.2011.05.001

Simmering, R., & Blaut, M. (2001). Pro- and prebiotics-the tasty guardian angles? *Appl. Microbiol. Biotech*., *55*(1), 19-28, doi: https://doi.org/10.1007/s002530000512

Sosa Cossio, D., García Hernández, Y., & Mendoza Dustet, J. C. (2018). Development of probiotics for animal production. experiences in Cuba. *Cuban Journal of Agricultural Science*, 52(4), 357-373. Retrieved on January 16, 2019, from: http://www.cjascience.com/index.php/CJAS/article/download/836/868

Statgraphics. (2002). *Statgraphics Plus version 5.1*. USA: Statgraphic Technical Support Center. Manugistics, Inc., Rockville, Maryland.

Thomas, D., & Elliott, E. (2013). Interventions for preventing diarrhea-associated hemolytic uremic syndrome: systematic review. *BMC Public Health*, *3*(13), 799, doi: https://doi.org/10.1186/1471-2458-13-799

Uyeno, Y., Shigemori, S., & Shimosato, T. (2015). Effect of Probiotics/Prebiotics on cattle health and productivity. *Microbes Environ*, *30*(2), 126–132, doi: 10.1264/jsme2.ME14176

Zapata, C. (2011). *Valoración de los efectos del cultivo de lactobacilos (Vitafert) en la cría de terneros en Tabasco*. (Tesis Maestría en Ciencias, especialista en Producción Agroalimentaria en el Trópico). Colegio de Postgraduados, México. Retrieved on May 16, 2017, from: http://hdl.handle.net/10521/581

Zhang, R., Zhou, M., Tu, Y., Zhang, N. F., Deng, K. D., Ma, T., & Diao, Q. Y. (2016). Effect of oral administration of probiotics on growth performance, apparent nutrient digestibility and stress-related indicators in Holstein calves. *J Anim Physiol Anim Nutr* (Berl) 100 (1), 33-80, doi: 10.1111/jpn.12338