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Effect of Indigenous Phytase-Producing Yeast Cultures on Growth Performance, Digestion and Health of Rabbits (*Oryctolagus cuniculus*)

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Abstract

This study investigated the effects of dietary supplementation of indigenous phytase producing live yeast cultures on growth performance, phytate degradation and blood biochemical parameters of weanling rabbits. Fifty-six rabbits (28 males and 28 females) were allotted randomly into four groups containing a control and three groups administrated each with the yeasts *Saccharomyces cerevisiae* ADR1B1, *Hanseniaspora jakobsenii* ADR3E1, *Hanseniaspora guilliermondii* RD31 during 8 weeks. The results showed that the administration of the three yeast strains accelerated the weight gain and improved rabbits growth performance in comparison to the control group, but did not affect the serum biochemical and hematological parameters. The highest weight gain (1715.95 ± 236 g), and phytate degradation rate (73.82% ± 0.92%) and the lowest biochemical parameters (triglycerides, cholesterol, HDL and LDL) were observed on rabbits fed with the yeast *Hanseniaspora jakobsenii* ADR3E1. This indigenous yeast species, which is used for the first time in animal feeding provided the best beneficial effects in rabbit breeding. Therefore, based on this finding, *Hanseniaspora jakobsenii* ADR3E1 can be recommended to supplement rabbit diets for growth performance and profitability enhancement.

Keywords

Feed, Growth Performance, Indigenous Yeasts, Phytate Digestion, Rabbit

1. Introduction

In Côte d'Ivoire as well as in most African countries, there is a growing increase in the demand for animal proteins. To solve this increasing shortage of meat production problem, [1] recommended as solution among many others, the use of small species such as rabbits, which is reported to have the ability in improving meat supply and food security [2]. Indeed, rabbit's performance is favored by several factors, such as small body size, short generation interval, rapid growth rate, high productive capacity and genetic diversity, which make this animal breeding suitable as meat producing small livestock in developing countries [3]. In addition, rabbits can convert 20% of the protein they eat into edible meat, which is higher than beef (8% - 12%) [4].

However, balanced feeding of rabbits is one of the most important challenges faced by breeding and production programs due to the complex digestion and nature of the diet [5] [6]. Improving feed quality and nutrition could contribute to enhance the rabbit production profitability, as feed has the largest part of the total production cost.

Indeed, rabbits are monogastric mammals that feed on forages, cereal seeds, and compound feeds [7]. Plant and grain-based diets naturally contain phytic acid and several minerals (iron, zinc, calcium, magnesium…). Phytic acid is naturally present in plant-based foods as phytate where it serves as a storage form for phosphorus [8]. However, it is negatively charged at acidic, neutral and basic pH conditions. In consequence, this compound can bind to positively charged molecules in the diet and in endogenous secretions of the gastrointestinal tract, such as digestive enzymes and mucins, thus reducing nutrients digestibility and increasing endogenous nutrient secretion [9]. Indeed, the phytic acid present in the food ingredients acts as an antinutritional factor and can cause mineral deficiency due to efficient chelation of metal ions such as Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+}, which forms complexes with proteins, thus affecting their digestion and also inhibits some digestive enzymes, such as a-amylase, trypsin, acid phosphatase and tyrosinase [10]. This results in important nutritional deficiencies and real problems of nutrition in a large part of the population in these monogastric animals. Moreover, due to the lack of adequate degradation of phytates in monogastric animals, these compounds are excreted in faeces, which is degraded by the activity of soil microbes, releasing phosphorus in the soil. This phosphorus leads to eutrophication after entering into aquatic bodies. According to various existing methods, notably physical (such as boiling, cooking and autoclaving) and chemical (acid hydrolysis and ion exchange chromatography) to remove phytates decreases the food nutritional value [11]. Therefore, enzymatic hydrolysis using phytases to reduce phytic acid is desirable as it improves the nutritional quality of the food. Indeed, phytases are a great deal to food and feed of non-ruminant animals to make bioavailable P; this clearly translates to recent market trends towards the importance of phytase in the food and feed sectors.

Phytases have a wide distribution in plants, microorganisms, and in some animal tissues [12] [13]. Previous researches have shown that microbial phytases are

most promising for a biotechnological application [13] [14]. However, although several scientific reports discussed phytase produced by a diverse range of microorganisms, many of them may not be useful in process development due to the lack of enzyme yields and pH stability at drastic gastrointestinal tract (GIT) conditions, as reported by [15]. Thus, the isolation of new phytase-producing microorganisms, effectively capable of releasing dietary phosphate in the gastrointestinal tract and remaining stable during food processing and storage is a challenge.

In our recent studies on the characterization of the microbial communities of palm wines, traditional spontaneously fermented drinks, we discovered that yeasts populating these drinks produced acid phosphatases [16], most of which after testing for specific substrates have been identified as phytases. Additionally, these yeast strains, containing *Saccharomyces cerevisiae* and non-Saccharomyces yeasts were able to tolerate and withstand the harsh environment of the gastrointestinal transit and possessed the functional attributes of autoaggregation, coaggregation, hydrophobicity and bile salt hydrolase activity while tested *in vitro*. Considering that these drinks are consumed with their living microbial content without any toxicity being observed and the limited number of reports on yeast with probiotic and phytase properties used for animal nutrition, we hypothesized that microorganisms of these drinks, particularly the phytase-producing yeasts, could contribute to the reduction *in vivo* of phytate in phytase deficient organisms such as rabbits and improve the health status.

Therefore, the aims of the present study were to investigate the effects of three phytase producing yeasts on *in vivo* dephytinization and growth performance in breeds of weanling rabbits. Furthermore, the effects on some blood biochemical parameters and changes in caecal volatiles fatty acids were also studied.

2. Materials and Methods

2.1. Yeast Strains, Animals, Management and Experimental Design

All procedures applied in this study were implemented according to the local experimental animal care committee and approved by the ethics institutional committee of University Nangui Abrogoua of Côte d'Ivoire. The experiment was carried out in a private farm located in Abobo municipality from January to march 2021, with fifty-six (56) weaned rabbits (28 males and 28 females) of 35-day-old and 595 ± 80 g body weight. The young rabbits were divided into four groups of fourteen (14) rabbits (3 treated groups and 1 control). Each of the four groups was then randomly divided in subgroups of 7 animals to conduct experiments in duplicate. Each rabbit subgroup was raised in a well aerated cage of 1.40 m^2 (0.7 m width and 2.0 m length), equipped with galvanized steel feeding hoppers and drinkers. Each rabbit was identified by a number between 1 and 56, continuously provided with fresh water and daily fed on a standard pelleted ration at 8 am and 5 pm. The cages were regularly cleaned and disinfected every day. No medication was administered to the rabbits during the 90-day of experiment.

Rabbits of the control group were fed with a conventional diet obtained from

an Ivorian company (IVOGRAIN), which composition is stated in Table 1. For the three treated groups, rabbits were fed with the conventional diet, followed an hour after for each group by the oral administration of 1 ml of a physiological solution containing about 5×10^8 CFU/ml of a probiotic yeast strain. Three probiotic yeast strains namely *Saccharomyces cerevisiae* ADR1B1, *Hanseniaspora jakobsenii* ADR3E1 and *Hanseniaspora guilliermondii* RD3 1 as presented in Table 2 were tested. These strains isolated from palm wines [16] are in the culture collection of the Laboratory of Biotechnology and Food Microbiology of the University of Nangui Abrogoua (Côte d'Ivoire).

2.2. Data Collection and Measurements

The live weight of each rabbit was individually recorded on a weekly basis and weight gain was calculated during the whole period. Weighing was done early in the morning before giving any feed. By the last week of the feeding trial, 4 rabbits from each treatment and 4 from the control were randomly selected, housed individually in cages and fed in the same conditions. At the end of experiment (12 weeks of age), faeces (free of feed particles and other contaminants) were collected from each cage for the determination of the undigested phytates at 490 nm using a spectrophotometer after extraction following to the method of [17].

Rabbits were fasted for approximately 6 hours and then, their venous blood were collected from the neck in different colored cap tubes for the determination of hematological and biochemical serum parameters.

Table 1. List of yeasts strains tested in this study, their accession numbers and isolation sources.

Code	species	Accession numbers	Sources of isolation
ADR1B1	*Saccharomyces cerevisiae*	MG833312	*Raffia wine*
ADR3E1	*Hanseniaspora jakobsenii*	MG833303	*Raffia wine*
RD3 1	*Hanseniaspora guilliermondii*	CLIB 3085	*Ron wine*

Table 2. Composition of the conventional diet used to feed the rabbits.

Constituents	**Quantity**
Metabolizable energy	0 kcal/kg
Crude protein (%)	15
Mat. Crude fat (%)	3.54
Crude ash (%)	7.94
Crude cellulose (%)	14.57
Calcium (g/kg)	10.73
Total phosphorus (g/kg)	6.5
Sodium (%)	0.5
Vitamin A	9000 UI/Kg
Vitamin D3	1800 UI/Kg
Vitamin E	1800 UI/Kg

Hematological analysis [number of red and white globules, hemoglobin (Hb) and hematocrit (HCT) levels, mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and the number of lymphocytes], were performed using an automatic hematology analyzer (Sysmex KX-21, Canada) according to the manufacturer's instruction manual with specific standard assay kits for the different hematological parameters.

Concerning the serum biochemical parameters, blood glucose level was determined by using a glucometer with reactive strips (One Touche Ultra) [18], triglycerides and cholesterol were determined following an enzymatic triglyceride colorimetric method (Glycerol Phosphate Oxidase) using the Cholesterol Reagent Kit [19], HDL (High Density Lipoprotein) cholesterol was performed after precipitation using the phosphotungstic reagent associated with magnesium chloride while LDL (Low Density Lipoprotein) cholesterol was deducted following the Friedewald formula [20]. Total proteins were determined according to the method described by [21], serum aspartate amino-transferase (ASAT) and alanine amino-transferase (ALAT) according to [22], urea by [19] and blood creatinine by the method of [23].

After blood collection, rabbits were slaughtered by severing the neck with a sharp knife. Carcass was eviscerated and the caecal contents collected for the volatile fatty acids (VFA) assays, mainly propionic and butyric acids, using a gas chromatograph (Shimadzu 20 A) with a double column, equipped with two flame ionization detectors after VFA extraction. The concentration of each volatile compound was determined from the regression lines established using standard solutions according to the method [24].

2.3. Statistical Analysis

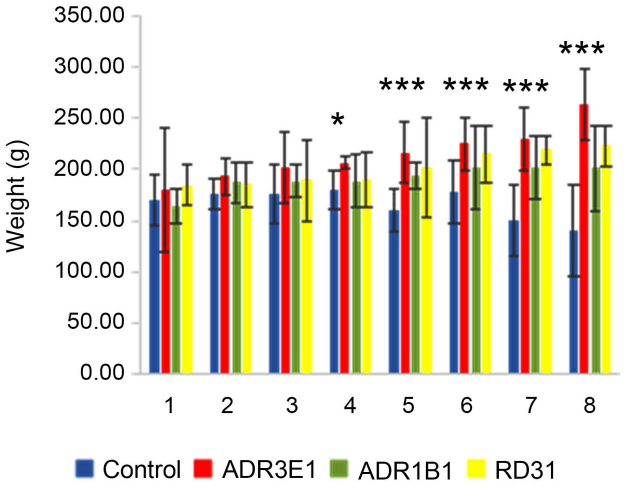
Semiquantitative values were expressed as mean values ± standard deviations. One-way ANOVA followed by Tukey-Kramer (HSD) test to determine the difference at p-value < 0.05 between mean values, using R software (R 3.6.1). (R 3.6.1, 2019) with the agricolae package. Multivariate analyses, notably principal component analysis (PCA) were performed with R software using the factoextra library to assess dissimilarities between the various groups of treated rabbits and the control group.

3. Results

3.1. Growth Performance

The administration effect of the indigenous phytase producing yeasts on rabbit's growth is shown in **Figure 1**. In general, whether they are fed only with the conventional feed or in combination with the indigenous yeast strains, a gradual growth of the rabbits was observed from the start to the end of the experiment (**Figure 1(a)**). However, this growth was more accentuated in rabbits having received the yeast strains, particularly ADR3E1 and RD31, where from the 5th

week of experience their growth differed significantly from those of the others. At the end of experiment, the animals reached total weights of 1927.7, 2097.3, 2248.9, and 2300.2 g respectively for the control group and the groups treated with strains ADR1B1, RD31, ADR3E1. As a result, average weekly weight gains steadily increased in the yeast fed rabbits. This was not the case in the groups of control rabbits. The highest averages weight gains were 262.88 ± 34.04 and 223.26 ± 20.06 g obtained at the end of the experiment respectively in rabbits groups fed with the ADR3E1 and RD31 strains, 202.99 ± 41.42 g with the ADR1B1 strain at the 6th week and 179.69 ± 18.56 g with the control groups obtained at the 4th week (Figure 1(b)). As it can be seen on Figure 1(c), the ANOVA pair-wise comparison of the weekly weight gains showed significant differences among the various groups of rabbit tested. Indeed, all treated rabbit groups displayed weight gains higher than the control group, with the strain ADR3E1 most affecting the rabbit's growth performance. This phytase-producing yeast strain also outperforms the others strains in improving growth performance.



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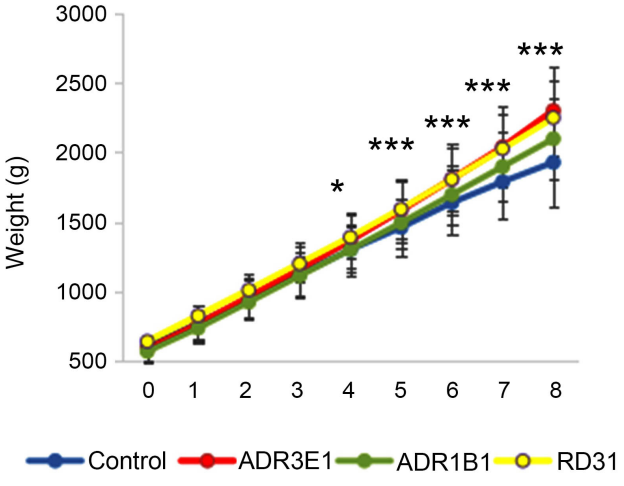

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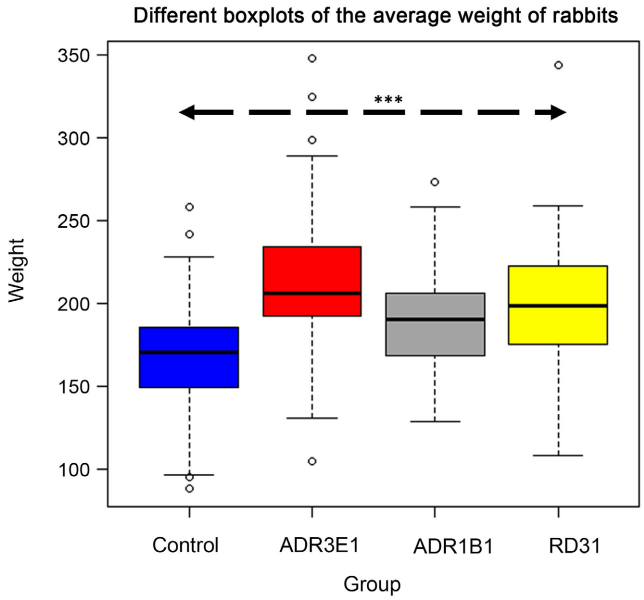


(c)

Figure 1. Effect of indigenous strains of phytase producing yeasts administration on the growth performance of rabbits. (a) weekly weight gains; (b) growth curve, (c) Boxplot showing the differential weight gains of the four groups of rabbit. The significance in difference was calculated by Tukey-Kramer honestly significant difference (HSD) test and indicated as at *p < 0.05 and ***p < 0.001.

3.2. Serum Biochemical Parameters

The mean values of serum biochemical parameters notably blood glucose, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, total proteins, creatinine, and uremia, obtained after eight (8) weeks of the various rabbit groups feeding with indigenous phytase producing yeasts are shown in **Table 3**. Values are in the ranges of 1.12 ± 0.16 and 1.36 ± 0.23 g/l for glucose, 0.33 ± 0.13 and 0.72 ± 0.41 g/l for triglycerides, 0.42 ± 0.25 and 0.53 ± 0.28 g/l for total cholesterol, 0.30 ± 0.09 and 0.36 ± 0.08 g/l for HDL (high density lipoprotein), 0.17 ± 0.11 and 0.32 ± 0.06 g/l for LDL (low density lipoprotein), 44.36 ± 8.25 and 48.62 ± 8.48 g/l for total proteins, 11.48 ± 0.65 and 15.08 ± 3.36 mg/l for creatinine and 0.18 ± 0.04 and 0.25 ± 0.05 g/l for Concerning the serum enzymatic activity, the average values of transaminases were between 41.60 ± 6.11 IU/l and 63.20 ± 20.32 IU/l for ASAT and between 67.20 ± 11.71 and 77.80 ± 24.50 IU/l for ALAT. Supplementing the diet with the yeast strain ADRE3E1 seems to slightly decreases the values of these parameters but the difference with the other rabbit groups were not statistically significant.

3.3. Hematological Parameters

The mean values of red blood cells, hemoglobin, hematocrit of the different groups of fed rabbits were respectively between (5.65 ± 0.51) × 10^6/mm^3 and (5.91 ± 0.47) × 10^6/mm^3, 11.00 ± 0.28 g/dl and 11.75 ± 0.21 g/dl and 36.80% ± 1.13% and 38.35% ± 1.63% (**Table 4**). No significant differences were observed

Table 3. Serum biochemical indices of rabbits in Control and experimental Groups.

Parameters	Groups of rabbits				Standards*
	Control	ADR3E1	ADR1B1	RD31	
Blood Glucose g/l	1.13 ± 0.14[a]	1.12 ± 0.16[a]	1.36 ± 0.23[a]	1.19 ± 0.21[a]	0.75 - 1.4
Triglycerides g/l	0.72 ± 0.41[a]	0.33 ± 0.13[a]	0.47 ± 0.26[a]	0.63 ± 0.52[a]	1.2 - 1.6
Cholesterol g/l	0.47 ± 0.14[a]	0.42 ± 0.25[a]	0.45 ± 0.19[a]	0.53 ± 0.28[a]	0.1 - 0.8
HDL g/l	0.32 ± 0.14[a]	0.30 ± 0.09[a]	0.36 ± 0.08[a]	0.32 ± 0.02[a]	
LDL g/l	0.24 ± 0.08[a]	0.17 ± 0.21[a]	0.17 ± 0.11[a]	0.32 ± 0.06[a]	
Total proteins g/l	48.62 ± 8.48[a]	44.48 ± 6.56[a]	46.08 ± 5.73[a]	44.36 ± 8.25[a]	50 - 75
Urea g/l	0.21 ± 0.04[ab]	0.20 ± 0.02[ab]	0.18 ± 0.04 [b]	0.25 ± 0.05[a]	0.5 - 1.5
Creatinine mg/l	15.08 ± 3.36[a]	11.48 ± 0.65[a]	15.00 ± 1.29[a]	14.26 ± 2.25[a]	5 - 26
ASAT (UI/l)	51.20 ± 15.40[a]	41.60 ± 6.11[a]	63.20 ± 20.32[a]	54.60 ± 29.44[a]	10 - 98
ALAT (UI/l)	72.20 ± 17.92[a]	67.20 ± 11.71[a]	77.20 ± 18.19[a]	77.80 ± 24.50[a]	55 - 260

*[61]; HDL high density lipoprotein; LDL low density lipoprotein; ASAT Aspartate Amino-Transferase; ALAT Alanine Amino-Transferase.

Table 4. Hematological parameters of rabbits in control and experimental Groups.

	Groups of Rabbits				
Parameters	Control	ADR3E1	ADR1B1	RD31	Standards*
RG/mm^3	5.6 ± 0.5 × 10^{6a}	5.8 ± 0.4 × 10^{6a}	5.9 ± 0.5 × 10^{6a}	5.7 ± 0.7 × 10^{6a}	3.8 - 7.9 × 10^6
WG/mm^3	5.3 ± 1.4 × 10^{3a}	7.5 ± 2.5 × 10^{3a}	5.0 ± 0.7 × 10^{3a}	7.4 ± 3.3 × 10^{3a}	5 - 13 × 10^3
Hemoglobin g/dl	11.6 ± 0.3[a]	11.7 ± 0.2[a]	11.4 ± 0.8[a]	11.0 ± 0.28[a]	9.4 - 17.4
Hematocrit %	37.7 ± 1.5[a]	37.9 ± 1.2[a]	38.3 ± 1.6[a]	36.8 ± 1.13[a]	33 - 50
Lymphocyte/mm^3	3.5 ± 1.1 × 10^{3a}	3.0 ± 0.4 × 10^{3a}	2.4 ± 0.1 × 10^{3a}	3.8 ± 1.3 × 10^{3a}	3 - 9 × 10^3
MCV mm^3	66.8 ± 3.2[a]	65.9 ± 2.0[a]	65.0 ± 2.5[a]	64.3 ± 6.3[a]	50 - 75
MCH pg/cellule	20.6 ± 1.3[a]	20.6 ± 1.3[a]	19.3 ± 0.1[a]	19.2 ± 2.9[a]	18 - 24
MCHC %	30.9 ± 0.6[a]	31.2 ± 1.0[a]	29.7 ± 1.0[a]	29.9 ± 1.5[a]	27 - 34

*[61]; RG: Red Globules; WG: White Globules; MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration.

regarding the type of probiotic strains administrated to rabbit groups. However, the highest concentration of red blood cells (5.91 ± 0.47) × 10^6/mm^3) and hematocrit (38.35% ± 1.63%) was observed in rabbits that received ADR1B1 strain. On the other hand, the high hemoglobin concentration (11.75 ± 0.21 g/dl) was recorded in those fed with the yeast strain ADR3E1. Also, no difference in the means was observed between the control and treated groups for MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration). However, the values vary from 64.35 ± 6.29 to 66.80 ± 3.25 mm^3 for MCV, from 19.25 ± 2.90 pg/cell to 20.65 ± 1.34

pg/cell for MCH, and from 29.70% ± 0.99% to 31.25% ± 1.06% for MCHC. In the same way, white blood cells and lymphocytes contents did not vary significantly between treated rabbits and the control groups. Their concentrations ranged from (5.30 ± 1.41) × 10^3/mm^3 to (7.55 ± 2.47) × 10^3/mm^3, from (2.40 ± 0.14) × 10^3/mm^3 to (3.85 ± 1.34) × 10^3/mm^3 respectively. The highest concentration in white blood cells (7.55 ± 2.47) × 10^3/mm^3 was observed in rabbit groups fed with the yeast strain ADR3E1 while lymphocytes highest content (3.85 ± 1.34) × 10^3/mm^3 appeared in those fed with the yeast strain ADR1B1.

3.4. Ceacal Volatile Fatty acid (VFA) Content

The concentration of volatile fatty acids in the ceacal content of the rabbit groups fed with the phytase producing yeasts and those of the control groups were quantified by GC and results are presented in Table 5. Fed with the same conventional diet, for all groups of rabbits, the volatile fatty acids (VFA) content namely propionic and butyric acids varied significantly from one group to another. The average values of propionic acid (105.74 ± 3.67 mM) and butyric acid (137.14 ± 8.17 mM) were the lowest in rabbit fed with the yeast strain ADR3E1 while these VFA were most abundant in those fed with the yeast strain RD31.

3.5. Phytate Concentration in Faecal Material

Table 6 shows the residual phytate contents in the faeces of the various rabbit groups after 8 weeks of experiment. The concentration of phytate in the basic diet was 214.64 ± 0.71 µg/ml. After treatment, the phytate concentrations in the droppings vary from 56.06 ± 1.97 µg/ml to 202.65 ± 1.48 µg/ml. The lowest concentration (56.06 ± 1.97 µg/ml) was recorded in the group of rabbits fed with the strain ADR3E1, while the highest concentration (202.65 ± 1.48 µg/ml) was measured in the group of control rabbits. Thus, the degradation of phytate was most accentuated in rabbit groups administrated with phytase producing yeasts, with in decreasing order ADR3E1 (73.82% ± 0.92%), RD31 (66.49% ± 0.21%) and ADR1B1 (50.75% ± 3.01%). In the control groups, the reduction rate was only 5.37% ± 0.69%.

Table 5. Volatile fatty acids content in ceacal materials of rabbits in control and experimental Groups.

	Volatile fatty acids (mM)	
Groups of rabbits	Propionic acid	Butyric acid
CONTROL	160.41 ± 5.08^b	205.43 ± 12.52^a
ADR3E1	105.74 ± 3.67^c	137.14 ± 8.17^b
ADR1B1	156.14 ± 3.59^b	213.75 ± 18.81^a
RD31	177.74 ± 8.94^a	202.97 ± 11.52^a

Table 6. Phytate content in the conventional diet and rabbit droppings after treatments.

	Phytate Content (µg/ml)	Percentage (%) of degradation
Conventional Diet	214.64 ± 0.71[a]	-
Groups of rabbit		
Control	202.65 ± 1.48[a]	5.37 ± 0.69[c]
ADR3E1	56.06 ± 1.97[c]	73.82 ± 0.92[a]
ADR1B1	105.45 ± 6.99[b]	50.75 ± 3.01[b]
RD31	71.76 ± 0.44[c]	66.49 ± 0.21[a]

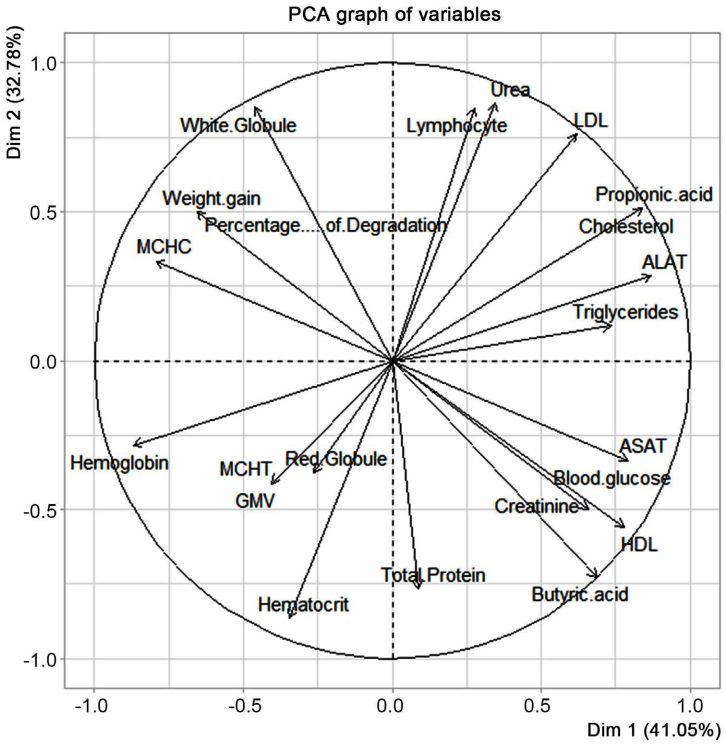
3.6. Multivariate Analysis of Characteristics Related Each Group of Tested Rabbits

In order to evaluate the differences among the three potential probiotic and phytase producing yeasts isolated from palm wine, among the overall characteristics investigated, we carried out a principal component analysis (PCA) using 22 parameters as input variables (**Figure 2**). The first two components (PCA) account for 73.83% of the total variance. Based on the loadings, PC1 (which explains 41.05% of the variance) displayed the higher contributions on some hematological parameters, VFA, total proteins, HDL, LDL, triglycerides…The second principal component (PC2, variance 32.78%) represents the growth performance, most of the serum biochemical parameters. Particularly, three clusters were formed while plotting the different groups of rabbits tested. The cluster 1 included rabbits administrated with the strains ADR3E1 which displayed a high growth performance and phytate degradation rate. The cluster 2 included rabbits of the control group and those fed with the yeast strain ADR1B1 which displayed highest total proteins, LDL, creatinine, glucose and ASAT contents. And finally, the third cluster containing rabbits fed with the yeast strain RD31 was characterized by high triglycerides, ALAT, propionic acid, cholesterol, LDL and urea contents.

4. Discussion

Over the last three decades, the research and development of strategies to improve livestock production, to reduce production cost and alleviate the negative environmental impacts of livestock production was of utmost importance for scientists particularly, microbiologists, nutritionists and biochemists. Feed supplementation with either antibiotics or a number of prebiotics and/or probiotics has been exploited to achieve the above objectives [25]. Historically, beneficial microorganisms have been used by humans in agriculture and nutrition. Yeasts are an important source for obtaining products with probiotic activity, either live strains or derivatives of their cell walls. These preparations demonstrated a proven immunosuppressant activity in livestock as well as improvements in gastrointestinal physiology, which contributed to improved production results [26]. In this study, three indigenous yeasts namely *S. cerevisiae* ADR1B1, *Hanseniapora jakobsenii* ADR3E1 and *Hanseniaspora guillermondii* RD3 1, all isolated from palm wines and displaying phytase and probiotic abilities, were tested

in rabbit breeding with a purpose of improving conventional feed digestion, growth performance and animal health.



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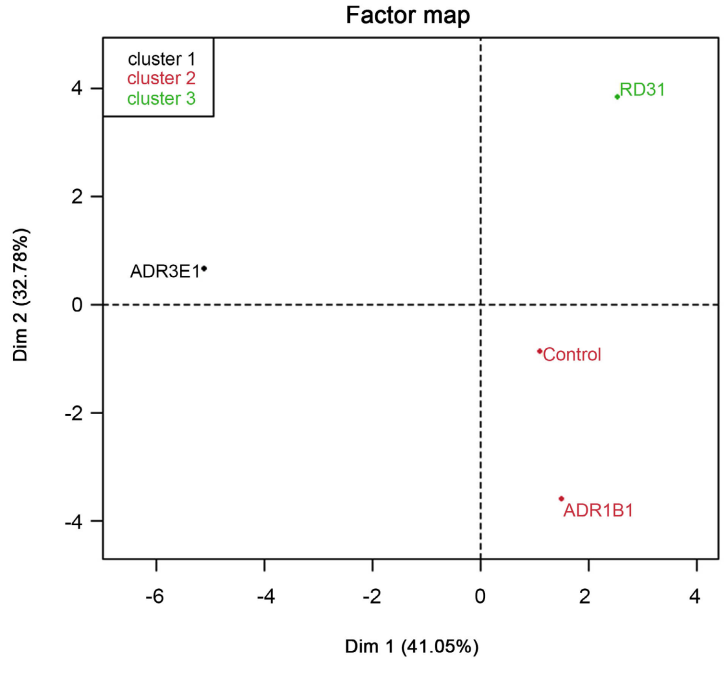

(b)

Figure 2. Principal component analysis based on growth performance, digestion, serum biochemical and hematological parameters shows differences between the four groups of rabbits tested in this study.

Indeed, phytates represent the major form of phosphorus storage in plants and mainly in cereal grains which are also the main ingredients used in animal feed. They are an organic form of phosphorus poorly utilized by non-ruminant animals such as rabbits, due to the absence of adequate level of phytase in their gastrointestinal tract. This is corroborated by the high rate of this compound present in the faeces of rabbits used as control on which no more than 6% were degraded. The undigested phytates found in faeces had been previously reported to cause eutrophication problems [27] [28] [29]. Contrarily to the control, significant reductions of phytates in the faeces of treated rabbits were observed, with reduction rates between 50% and 75%. Previous studies by [30], using a recombinant strain of Pichia pastoris reduced the phytate content of a wheat-based mixture and a soybean-maize flour mixture by 65.3% and 73.1% respectively. Thus, in addition to considerably enhance the bioavailability of phosphorus, thereby reducing the faecal phosphorus output, phytase producing yeasts are effective in protein digestibility and protein and amino acid utilization due to the breakdown of phytin-protein complexes, as reported by [31]. It is also reported elsewhere that phytase added to diets improves the bioavailability, absorption and concentration of minerals such as copper, zinc, iron, magnesium, phosphorus, calcium, manganese and zinc in plasma, bone and the whole body [31] [32]. Therefore, by improving digestion and nutrients absorption, phytase producing yeasts would exert a positive effect on growth performance and weight gain. This phenomenon was observed on rabbit fed with palm wine indigenous yeasts, on which the weights were significantly increased in comparison to the control. The obtained findings confirmed the previous results reported by studies of [24], who showed that supplementing the rabbit diet with prebiotics improved rabbit growth. The high performance of growing rabbits as a result of indigenous probiotic yeast dietary supplementation may be due to enhancing digestibility and absorption of feed nutrients, resulted in positive anabolic metabolism state, as reported by [33]. Moreover, analysis of the weekly weight gains shows significant differences from the fourth week after weaning of the rabbits, which is consistent with results of [34], showing improvement of rabbit growth between days 43 and 91. The positive effect of probiotics on food utilization and consequently resulting in growth increase was largely demonstrated [35] [36].

For an organism to be considered potentially probiotic, it must be non-invasive, non-carcinogenic and non-pathogenic [37]. Thus, the tested rabbit bloods were analyzed in comparison with the control group, in order to detect any effect of administrated indigenous yeasts on the animal's health. Serum biochemical parameters analyzed, notably blood glucose, triglycerides, total cholesterol, HDL (high density lipoprotein), LDL (low density lipoprotein), total protein, creatinine, ASAT and ALAT, showed no significant difference between treated rabbit groups and control one. Thus, probiotic yeast strains did not negatively impact rabbits' health with respect to serum biochemical parameters. Furthermore, the comparison of the recorded parameters averages to the European standards

shows no difference for most parameters except for triglycerides concentration. Indeed, this difference would be due to the difference in the composition of the diet offered to the rabbits in Africa and in Europe. However, these results are close to those obtained by [38]. According to some authors, serum biochemical parameters may show differences in animals. This difference would be related to the animals (age, species, strain and sex), environmental conditions, sampling method and analysis method [39] [40].

Like serum biochemical parameters, hematological parameters analyzed were not significantly different regarding red blood cells, hemoglobin and hematocrit, excepted for the rabbits fed with *H. Jakobseni* ADR3E1, which hematological parameters were higher than the control. Indeed, according to [41], the level of red blood cells, hemoglobin and hematocrit would increase when yeast probiotics were added to the rabbit's diet. On the other hand, Chebab [42] studies showed a decrease in these blood parameters with the use of lactic acid bacteria probiotics. These recorded differences would be related either to the genetic and physiological characteristics of the rabbit, or to an effect exerted by the probiotic, or to a diet which is not balanced both qualitatively and quantitatively. Furthermore, the results obtained are consistent with the normal values referenced. Thus, the probiotic strains used would not cause anaemia in the treated rabbits. As for the leukocyte formula, the results obtained also showed no difference in the white blood cells and lymphocytes of treated rabbits compared to the control. Similar results were observed in the study of [41]. Furthermore, the results obtained were within the normal leukocyte range. Thus, the values obtained are not indicative of acute and chronic infection.

Microbial fermentation of the feed occurs in the caecum to ensure nutrient supply [43] [44]. The products of fermentation are important to the rabbit because the VFAs produced are an energy source for the host. The production of VFAs covers the maintenance energy requirements of rabbits [45] [46]. The concentration of VFAs produced by the different groups of treated rabbits varied significantly compared to the control group, and this finding is consistent with that of [47]. This variation can be related according to [48] to the animal age, the level of ingestion and the quality of the diet, especially the concentration of rapidly fermentable fiber [46] [49]. Gong *et al.* [50] observed a significant decrease in total VFA production in the rabbit group fed with a yeast-supplemented diet, which is in agreement with results obtained while rabbits were fed with *H. jakobseni* ADR3E1. This yeast strain can contribute to ferment in rabbit caecum foregut-undigested materials, volatile fatty acids, ammonia nitrogen, vitamins and other nutrients. Thus, the yeast strain ADR3E1 would promote the digestion of nutrients in the stomach before their arrival in the cecum. The volatile fatty acids can be rapidly absorbed in the intestine and can then provide 40% energy to maintain adult rabbits, in which butyric acid is a direct source of energy to hindgut, whereas acetic acid creates cholesterol and fat metabolism in the liver in metabolism, as reported by [51]. However, other researchers have reported an increase in VFA [52] [53], which can be associated with low amount of fer-

mentable sugars available in the cecum.

The multivariate analysis achieved to classify the indigenous phytase producing yeasts tested regarding the various parameters analyzed led to three distinct clusters, with *Hanseniaspora jakobsenii* ADR3E1 displaying the best positive effect on rabbits growth performance and health. Usually, yeast strains, more well-known in their use as probiotics belonged to the genera *Saccharomyces, Kluyveromyces, Hansenula, Pichia* and *Candida* and within these genera, species *S. boulardii, S. cerevisiae, K. fragilis, K. lactis, C. saitoana* and *C. pintolopesii* [54] [55] [56] [57]. In addition, the most prominent yeast used as a feed additive in livestock is S. cerevisiae because of its richness in digestible proteins, vitamins (vitamin B6, thiamin, biotin, riboflavin, nicotinic acid and pantothenic acid), magnesium and zinc as reported by [58]. However, in this study, *H. jakobsenii*, which remains an almost unknown yeast, exhibits greater efficacy than *S. cerevisiae* from the same origin as it, following its supplementation to the rabbit's diet. This finding is of capital importance for the characterization of this strain and an important contribution in the study of its technological potential and its exploitation in the improvement of animal's diets in general. If some species of the genus *Hanseniaspora*, notably *H. osmophila, H. uvarum* [59] are well known for their probiotic potentials, this is not the case for the species *H. jakobsenii,* the isolation and identification of which are still recent [60]. The fact remains that this species has enormous potential that deserves to be elucidated. Indeed, its prowess in improving the growth performance and health of rabbits make it a good candidate to consider in the development of food supplements for animals.

5. Conclusion

The dietary supplementation with three palm wine indigenous phytase producing yeasts (*H. jakobsenii*, *H. guillermondii* and *S. cerevisiae*) increases weekly weight gain, growth performance and digestibility in rabbits. Serological data also showed that yeast supplementation did not alter the blood parameters assessed. Based on the findings, it is recommended to supplement rabbit diet with *H. jakobsenii* which displayed the best potentialities to enhance growth performance and health status.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Mahsoub, H. (2007) Some Factors Affecting Productive Traits in V-Line Rabbits

Raised under Egyptian Conditions. M.Sc. Thesis, Faculty of Agriculture, Alexandria University, Alexandria.

[2] El-Sheikh, E., Mahrose, K. and Ismail, E. (2015) Dietary Exposure Effect of Sublethal Doses of Methomyl on Growth Performance and Biochemical Changes in Rabbits and the Protective Role of Vitamin E plus Selenium. *Egyptian Journal of Rabbit Science*, **25**, 59-81. https://doi.org/10.21608/ejrs.2015.46698

[3] Anjeniwa, M. (2000) The Rabbit: Husbandry, Health and Production. FAO, Rome.

[4] Basavaraj, M., Nagabhushana, V., Prakash, N., Appannavar, M.M., Wagmare, P. and Mallikarjunappa, S. (2011) Effect of Dietary Supplementation of Curcuma Longa on the Biochemical Profile and Meat Characteristics of Broiler Rabbits under Summer Stress. *Veterinary World*, **4**, 15-18. https://doi.org/10.5455/vetworld.2011.15-18

[5] Kritas, S., Petridou, E., Fortomaris, P., Tzika, G. and Arsenos, K. (2008) The Effect of Probiotics on Microbiology, Health and Performance of Fattening Rabbits. *Asian-Australasian Journal of Animal Sciences*, **21**, 1312-1317. https://doi.org/10.5713/ajas.2008.70186

[6] Rabie, M., Sherif, K., Hussein, M. and El-Desouqi, A. (2011) Growth Performance of Rabbits as Affected by Dietary Fiber Level and Probiotic Addition during the Post Weaning Period. *Journal of Animal and Poultry Production*, **2**, 185-199. https://doi.org/10.21608/jappmu.2011.83360

[7] Kpodekon, M., Djago, Y., Yo T. and Adjanguidi J. (2018) Manuel Technique de l'éleveur de lapin au Bénin. Organisation des Nations Unies pour l'alimentation et l'agriculture.

[8] Bedford, M.R. (2000) Exogenous Enzymes in Monogastric Nutrition—Their Current Value and Future Benefits. *Animal Feed Science and Technology*, **86**, 1-13. https://doi.org/10.1016/S0377-8401(00)00155-3

[9] Woyengo, T.A. and Nyachoti, C.M. (2012) Review: Anti-Nutritional Effects of Phytic Acid in Diets for Pigs and Poultry Current Knowledge and Directions for Future Research. *Canadian Journal of Animal Science*, **93**, 9-21. https://doi.org/10.4141/cjas2012-017

[10] Vohra, A., Kaur, P. and Satyanarayana, T. (2011) Production Characteristics and Application of the Cell Bound Phytase of *Pichia anomala*. *Antonie van Leeuwenhoek*, **99**, 51-55. https://doi.org/10.1007/s10482-010-9498-1

[11] Singh, B., Kunze, G. and Satyanarayana, T. (2011) Developments in Biochemical Aspects and Biotechnological Applications of Microbial Phytases. *Biotechnology and Molecular Biology Reviews*, **6**, 69-87.

[12] Konietzny, U. and Greiner, R. (2002) Molecular and Catalytic Properties of Phytase-Degrading Enzymes (Phytases). *International Journal of Food Science & Technology*, **37**, 791-812. https://doi.org/10.1046/j.1365-2621.2002.00617.x

[13] Vohra, A. and Satyanarayana, T. (2003) Phytases: Microbial Sources, Production, Purification, and Potential Biotechnological Applications. *Critical Reviews in Biotechnology*, **23**, 29-60. https://doi.org/10.1080/713609297

[14] Pandey, A., Szakacs, G., Soccol, C., Rodriguez-Leon, J. and Soccol, A. (2001) Production, Purification and Properties of Microbial Phytases. *Bioresource Technology*, **7**, 203-214. https://doi.org/10.1016/S0960-8524(00)00139-5

[15] Wodzinski, R.J. and Ullah, A.H.J. (1996) Phytase. *Advances in Applied Microbiology*, **42**, 263-302. https://doi.org/10.1016/S0065-2164(08)70375-7

[16] Amoikon, T.L.S., Aké, M.D.F., Djéni, N.T, Grondin, C., Casaregola, S. and Djè,

K.M. (2019) Diversity and Enzymatic Profiles of Indigenous Yeasts Isolated from Three Types of Palm Wines Produced in Côte d'Ivoire. *Journal of Applied Microbiology*, **126**, 567-579. https://doi.org/10.1111/jam.14154

[17] Latta, M. and Eskin, M. (1980) A Simple and Rapid Colorimetric Method for Phytate Determination. *Journal of Agricultural and Food Chemistry*, **28**, 1313-1315. https://doi.org/10.1021/jf60232a049

[18] Desch, G. (2001) Aspects biochimiques et analytiques du diagnostic et de la surveillance du diabète. *Médecine Nucléaire—Imagerie Fonctionnelle et Métabolique*, **25**, 61-72.

[19] Kaplan, A. and Glucose, K. (1984) ClinChem. The CV Mosby Co., St Louis, Toronto, Princeton, 436 p.

[20] Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, without Use of the Preparative Ultracentrifuge. *Clinical Chemistry*, **18**, 499-502. https://doi.org/10.1093/clinchem/18.6.499

[21] Koller, A. (1984) Total Serum Protein. In: Kaplan, L.A. and Pesce, A.J., Eds., *Clinical Chemistry*: *Theory*, *Analysis*, *and Correlation*, Mosby Company, St. Louis, 1316-1319.

[22] Valdiguie, P. (2000) Biochimie Clinique. In Editions Médicales Internationales, France. p. 340.

[23] Jaffe, M. (1886) Ueber den Niederschlag welchen Pikrinsäure in normalen Harn erzeugt und über eine neue reaction des Kreatinins. *Biological Chemistry*, **10**, 391-400. https://doi.org/10.1515/bchm1.1886.10.5.391

[24] Oso, A., Idowu, O., Haastrup, A., Ajibade, A., Olowonefa, K., Aluko, A., Ogunade, I. and Osho, S. (2013) Growth Performance, Apparent Nutrient Digestibility, Caecal Fermentation, Ileal Morphology and Caecal Microflora of Growing Rabbits Fed Diets Containing Probiotics and Prebiotics. *Livestock Science*, **157**, 184-190. https://doi.org/10.1016/j.livsci.2013.06.017

[25] Al-Khalaifah, H. (2018) Benefits of Probiotics and/or Prebiotics for Antibiotic-Reduced Poultry. *Poultry Science*, **97**, 3807-3815. https://doi.org/10.3382/ps/pey160

[26] Morales, J. (2004) Application of a Probiotic Supplement in the Recovery of a Layer Replacement. Fourth Congress of Aviculture, Santiago de Cuba.

[27] Haygarth, P.M., Jarvis S.C., Chapman P.J. and Smith R.V. (1998) Phosphorus Budgets for Two Contrasting Grassland Farming Systems in the UK. *Soil Use and Management*, **14**, 160-167. https://doi.org/10.1111/j.1475-2743.1998.tb00635.x

[28] Miles, D.M. and Sistani, K.R. (2002) Broiler Phosphorus Intake versus Broiler Phosphorus Output in the United State: Nutrition or Soil Science? *World's Poultry Science Journal*, **58**, 493-500. https://doi.org/10.1079/WPS20020035

[29] Bouwman, L., Goldewijk, K., Van Der Hoek, K., Beusen, A., Van Vuuren, D. and Willems, J. (2011) Exploring Global Changes in Nitrogen and Phosphorus Cycles in Agriculture Induced by Livestock Production over the 1900-2050 Period. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 20882-20887. https://doi.org/10.1073/pnas.1012878108

[30] Brejnholt, S., Giuseppe, D., Vibe, G., Lars, K. and Brinch-Pedersenb, H. (2011) The Degradation of Phytate by Microbial and Wheat Phytases Is Dependent on the Phytate Matrix and the Phytase Origin. *Journal of the Science of Food and Agriculture*, **91**, 1398-1405. https://doi.org/10.1002/jsfa.4324

[31] Vielma, J., Ruohonen, K., Gabaudan, J. and Vogel, K. (2004) Top-Spraying Soybean Meal-Based Diets with Phytase Improves Protein and Mineral Digestibilities But

Not Lysine Utilization in Rainbow Trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*, **35**, 955-964. https://doi.org/10.1111/j.1365-2109.2004.01106.x

[32] Selle, P.H. and Ravindran, V. (2007) Microbial Phytase in Poultry Nutrition. *Animal Feed Science and Technology*, **135**, 1-41. https://doi.org/10.1016/j.anifeedsci.2006.06.010

[33] El-Aziz, A., Mahrose, K., El-Kasrawy, N. and Alsenosy, A. (2021) Yeast as Growth Promoter in Two Breeds of Growing Rabbits with Special Reference to Its Economic Implications. *Anais da Academia Brasileira de Ciências*, **93**, 13 p. https://doi.org/10.1590/0001-3765202120190274

[34] Bhatt, R.S., Agrawal, A.R. and Sahoo, A. (2016) Effect of Probiotic Supplementation on Growth Performance, Nutrient Utilization and Carcass Characteristics of Growing Chinchilla Rabbits. *Journal of Applied Animal Research*, **45**, 304-309. https://doi.org/10.1080/09712119.2016.1174126

[35] Kozasa, (1986) Les probiotiques pour demain. *Revue de l'Alimentation Animale*, No. 397, 38-39.

[36] Marionnet, D. and Lebas, F. (1990) Un probiotique: qu'est-ce que c'est? *Cuniculture*, **17**, 255-258.

[37] Mottet, C. and Michetti, P. (2005) Probiotics: Wanted Dead or Alive. *Digestive and Liver Disease*, **37**, 3-6. https://doi.org/10.1016/j.dld.2004.09.010

[38] Özkan, Ö. and Pekkaya, S. (2019) Normal Values of Biochemical Parameters in Serum of New Zealand White Rabbits. *Türk Hijyen ve Deneysel Biyoloji Dergisi*, **76**, 157-162. https://doi.org/10.5505/TurkHijyen.2018.53254

[39] Mohri, M., Sharifi, K. and Eidi, S. (2007) Hematology and Serum Biochemistry of Holstein Dairy Calves: Age Related Changes and Comparison with Blood Composition in Adults. *Research in Veterinary Science*, **83**, 30-39. https://doi.org/10.1016/j.rvsc.2006.10.017

[40] Özkan, C., Kaya, A. and Akgül, Y. (2012) Normal Values of Haematological and Some Biochemical Parameters in Serum and Urine of New Zealand White Rabbits. *World Rabbit Science*, **20**, 253-259. https://doi.org/10.4995/wrs.2012.1229

[41] Onifade, A.A., Obiyan, R.I., Onipede, E., Adejumo, D.O., Abu, O.A. and Babatunde, G.M. (1999) Assessment of the Effects of Supplementing Rabbit Diets with a Culture of *Saccharomyces cerevisiae* Using Growth Performance, Blood Composition and Clinical Enzyme Activities. *Animal Feed Science and Technology*, **77**, 25-32. https://doi.org/10.1016/S0377-8401(98)00244-2

[42] Chebab, S. (2005) Etude de l'effet Lb. plantarum «BJ0021» et St. Thermophilus «R4» sur les paramètres plasmatiques et sanguins du lapin local. Mémoire de fin d'étude, soutenu le 02/07/2005 à l'université de JIJEL, Algérie, 59 p.

[43] Harcourt-Brown, F. (2004) Biological Characteristic of Domestic Rabbit/Digestive Physiology. In: *Textbook of Rabbit Medicine*, Elsevier Science, Oxford, 3.

[44] Bagóné-Vántus, V., Kovács, M. and Zsolnai, A. (2014) The Rabbit Caecal Microbiota: Development, Composition and Its Role in the Prevention of Digestive Diseases—A Review on Recent Literature in the Light of Molecular Genetic Methods. *Acta Agraria Kaposváriensis*, **18**, 55-65.

[45] Gidenne, T. and Perez, J.M. (1994) Effect of Dietary Starch Origin on Digestion in the Rabbit. 2. Starch Hydrolysis in the Small Intestine, Cell Wall Degradation and Rate of Passage Measurements. *Animal Feed Science and Technology*, **42**, 249-257. https://doi.org/10.1016/0377-8401(93)90102-P

[46] Gidenne, T., Combes, S., Licois, D., Carabaño, R., Badiola, I. and Garcia, J. (2008)

Ecosystème caecal et nutrition du lapin: Interactions avec la santé digestive. *INRAE Productions Animales*, **21**, 239-250.
https://doi.org/10.20870/productions-animales.2008.21.3.3398

[47] Elmasry, A., Mendoza, G., Miranda, L., Vázquez, G., Salem, A. and Hernández, P. (2016) Effects of Types and Doses of Yeast on Gas Production and *in Vitro* Digestibility of Diets Containing Maize (*Zea mays*) and Lucerne (*Medicago sativa*) or Oat Hay. *South African Journal of Animal Science*, **46**, 391-397.
https://doi.org/10.4314/sajas.v46i4.7

[48] Bellier, R., Gidenne, T., Vernay, M. and Colin, M. (1995) *In Vivo* Study of Circadian Variations of the Ceacal Fermentation Pattern in Post Weaned and Adult Rabbits. *Journal of Animal Science*, **73**, 128-135. https://doi.org/10.2527/1995.731128x

[49] Gidenne, T., Jehl, N., Lapanouse, A. and Segura, M. (2004) Inter-Relationship of Microbial Activity, Digestion and Gut Health in the Rabbit: Effect of Substituting Fibre by Starch in Diets Having a High Proportion of Rapidly Fermentable Polysaccharides. *British Journal of Nutrition*, **92**, 95-104.
https://doi.org/10.1079/BJN20041173

[50] Gong, Y., Liao, X., Liang, J., Jahromi, M., Wang, H., Cao, Z. and Wu, Y. (2013) *Saccharomyces cerevisiae* Live Cells Decreased *in Vitro* Methane Production in Intestinal Content of Pigs. *Asian-Australasian Journal of Animal Sciences*, **26**, 856-863.
https://doi.org/10.5713/ajas.2012.12663

[51] Gidenne, T. (1997) Caeco-Colic Digestion in the Growing Rabbit: Impact of Nutritional Factors and Related Disturbances. *Livestock Production Science*, **51**, 73-88.
https://doi.org/10.1016/S0301-6226(97)00111-5

[52] Lila, Z.A., Mohammed, N., Yasui, T., Kurokawa, Y., Kanda, S. and Itabashi, H. (2004) Effects of a Twin Strain of *Saccharomyces cerevisiae* Live Cells on Mixed Ruminal Microorganism Fermentation *in Vitro*. *Journal of Animal Science*, **82**, 1847-1854.
https://doi.org/10.2527/2004.8261847x

[53] Lila, Z.A., Mohammed, N., Takahashi, T., Tabata, M., Yasui, T., Kurihara, M., Kanda, S. and Itabashi, H. (2006) Increase of Ruminal Fiber Digestion by Cellobiose and a Twin Strain of *Saccharomyces cerevisiae* Live Cells *in Vitro*. *Journal of Animal Science*, **77**, 407-413. https://doi.org/10.1111/j.1740-0929.2006.00366.x

[54] Coenen, T.M. (2000) Safety Evaluation of Enzyme Preparation Derived from Lactate *Kluyveromyces lactis*. *Food and Chemical Toxicology*, **38**, 671-677.
https://doi.org/10.1016/S0278-6915(00)00053-3

[55] Bovill, R., Bew, J. and Robinson, S. (2001) Comparison of Selective Media for the Recovery and Enumeration of Probiotic Yeasts for Animal Feed. *International Journal of Food Microbiology*, **67**, 55-61.
https://doi.org/10.1016/S0168-1605(01)00448-2

[56] Campeanu, G. (2002) Biotechnological Studies Obtaining Concerning the Role of Biomass with Probiotic Yeasts and Bacteria Form. *Romanian Biotechnological Letters*, **7**, 795-802.

[57] Kumura, H., Tanoue, Y., Tsukahara, M., Tanaka, T. and Shimazaki, K. (2004) Screening of Yeast Strains for Probiotic Applications. *Journal of Dairy Science*, **87**, 4050-4056. https://doi.org/10.3168/jds.S0022-0302(04)73546-8

[58] Hamm, R. and Grau, R (1953) Eine einfache methode zur bestimmung der wasserbindung im muskeol. *Die Naturwissenschaften*, **40**, 29-30.

[59] Fernández-Pacheco, P., Cueva, C., Arévalo-Villena, M., Moreno-Arribas, M. and Briones, P. (2019) *Saccharomyces cerevisiae* and *Hanseniaspora osmophila* Strains as Yeast Active Cultures for Potential Probiotic Applications. *Food & Function*, **10**,

4924-4931. https://doi.org/10.1039/C9FO00732F

[60] Ouoba, L., Nielsen, D., Anyogu, A., Kando, C., Diawara, B., Jespersen, L. and Sutherland, J. (2015) *Hanseniaspora jakobsenii* sp. nov., a Yeast Isolated from Bandji, a Traditional Palm Wine of *Borassus akeassii*. *International Journal of Systematic and Evolutionary Microbiology*, **65**, 3576-3579. https://doi.org/10.1099/ijsem.0.000461

[61] Medirabbit.com (2021) Complete Blood Count and Biochemistry Reference Values in Rabbits. http://www.medirabbit.com/EN/Hematology/blood_chemistry.htm