



RESEARCH ARTICLE

Evaluation Study on the Effect of *Saccharomyces cerevisiae*¹⁰²⁶ and *Lactobacillus casei* on Some Ruminal and Biochemical Parameters in Fattening Calves

Ossama I. Eladawi^{1*}, Shaimaa M. Gouda², Elabasy M. Elnaggar² and Sobhy El. Maghawry²

¹MSD Animal Health, 5th Settlement, New Cairo, 11835, Egypt

²Animal Medicine Department, Internal Medicine, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

Article History: Received: 15/12/2019 Received in revised form: 07/01/2020 Accepted: 26/01/2020

Abstract

This study was designed to evaluate the effect of *Saccharomyces cerevisiae*¹⁰²⁶ and *Lactobacillus casei* on some ruminal and biochemical parameters in sixty healthy fattening calves in a private dairy farm at Sharkia Governorate, Egypt. Three groups were included in the experiment, each contains 20 calves. The first Group (G1) served as a control that received a basal diet as total mixed ration. The second (G2) and the third (G3) Groups received the same ration in addition to *Saccharomyces cerevisiae*¹⁰²⁶ and *Lactobacillus casei*, respectively, both were added by 5 g per head per day for three months experiment. Blood samples were collected monthly for three times. The results demonstrated that supplementation of *Saccharomyces cerevisiae*¹⁰²⁶ and *Lactobacillus casei* to calves' feed improved fecal, respiratory and locomotor scores. They increased the ruminal contraction (3.66 ± 0.33/2 minutes), protozoal population and activity and total volatile fatty acids (87.66 ± 1.45 mmol/L). On the other hand, both supplements reduced the ruminal ammonia concentration (25.16 ± 1.12 mmol/L), but ruminal juice pH was elevated in case of *Saccharomyces cerevisiae*¹⁰²⁶ (6.53 ± 0.06) and stabilized in case of *Lactobacillus casei* supplementation. *Saccharomyces cerevisiae*¹⁰²⁶ and *Lactobacillus casei* significantly increased serum levels of β-hydroxy butyric acid (0.29 ± 0.02 mmol/L) while decreased level of non-esterified fatty acids (1.80 ± 0.15 mmol/L and 1.81 ± 0.14 mmol/L, respectively). Aspartate transferase showed significant reduction in *Saccharomyces cerevisiae*¹⁰²⁶ (57.0 ± 21.4 U/L) and *Lactobacillus casei* (68.66 ± 9.49 U/L) supplemented groups, while alanine transferase and gamma-glutamyl transferase showed only reduction (10.33 ± 13.83 U/L and 9.06 ± 0.88 U/L, respectively) in *Lactobacillus casei* supplemented group. There was a significant increase in reduced glutathione GSH and glutathione peroxidase GPX in G2 and G3, while Malonaldehyde MDA of G2 and G3 showed insignificant reduction in comparison with G1. Weight gain was significantly improved in *Saccharomyces cerevisiae*¹⁰²⁶ supplemented group compared with *Lactobacillus casei* and control ones. The results suggested that supplementation of *Saccharomyces cerevisiae*¹⁰²⁶ and *Lactobacillus casei* to fattening calves feed has a positive impact on calves' general health and their weight gain.

Keywords: *Saccharomyces*, *Lactobacillus*, Rumen, Fattening Calves.

Introduction

Nowadays, cattle producers either for beef or milk purposes, do their best to increase the productivity together with decreasing the cost of production, so they always try to follow most recent rearing methods to achieve this purpose [1]. The intensive rearing and specific

managerial methods of fattening calves that feedlot producers follow to increase their profit render calves very susceptible to diseases and retarded growth. To face these challenges, diets have been supplemented with antibiotics, which have been widely used as feed additives

[2]. However, the use of antibiotics may develop antibiotic-resistant strains and interfere with the use of veterinary antibiotics. To avoid these problems, probiotics are used as substitution for replacing antibiotics as growth promoter; improving the general health and lowering the incidence of diseases. Yeast has more than 1000 different species; few of them are used commercially.

The most commonly used yeast species is *Saccharomyces cerevisiae*, also known as "baker's yeast." Although its beneficial effects have been established for centuries, the inclusion of yeast and yeast products in animal diets has only occurred in recent years [3]. Probiotics are live microorganisms conferring a health benefit on the host when administered in adequate amounts [1]. Yeast and *Lactobacilli* are from the commonly and widely used probiotics in animal feed. Yeast supplementations to ruminant diets improve significantly their performance [4], milk production [5] and weight gain [6]. Yeast can alter the patterns of total volatile fatty acids (TVFA) formation [7], stabilize ruminal pH [8], and improve digestibility [9,10]. *Lactobacillus* aids in digestion and compete with potential pathogenic microbes [11], leading to decrease the incidence of diarrhea [12], improve the body weight gain and feed conversion [13] and decrease the mortality rate [14,15]. Therefore, this study was planned to evaluate the effect of *Saccharomyces cerevisiae*¹⁰²⁶ and *Lactobacillus casei* on some ruminal and biochemical parameters in fattening calves.

Materials and Methods

Animals and experimental design

Sixty Holstein calves in a private dairy farm located at Sharkia Governorate were included in the study. The calves were healthy as proved from thorough clinical examination, their life weight ranged from 90 to 110 kg and they were classified into 3 groups in separate yards each of them contains 20 calves. The first group was the control one (G1), which received basal diet without feed supplement and the second group (G2) received Yea-Sacc®¹⁰²⁶ (*Alltech natural*, USA), which is life dry yeast contains 2.8×10^8 CFU of *Saccharomyces cerevisiae* strain

1026 in a rate of 5g per head per day for three months, while the third group (G3) received Probax® (*Microbax*, India), which contains *Lactobacillus casei* not less than 1×10^{10} CFU in rate of 5g per head per day for the three months.

Clinical examination

Thorough clinical examination was done for all calves before and during periods of the experiment, particularly, the assessment of the vital parameters (body temperature, heart rate, mucous membrane, respiratory rate and ruminal contractions) according to the method described previously [16]. Some clinical scores as fecal score was observed once daily for calves and the results were recorded by fecal fluidity (0=normal, 1=soft, 2=runny, 3=watery) according to a previously published study [17]. Moreover, respiratory and locomotors scores measured from 0 to 3 depending on the severity of the disease were detected as described previously [18]. The calves were weighted using digital balance (BOCSHE, Germany) at the beginning of the experiment then once monthly to evaluate the daily weight gain in different groups.

Blood samples

Blood samples (n=10) were collected monthly (three times) from all calves from the jugular vein in dry, clean and sterile labeled glass tubes with rubber stoppers. The collected serum samples were used for determination of β -hydroxy butyric acid (β HBA) using commercial spectrophotometric Kits (Pointe Scientific, Inc. USA) [19], non-esterified fatty acids (NEFA) using colorimetric kits (IVD DIALAB, Austria) [20], total serum protein [21], serum albumin concentration [22], serum globulin [23], some liver enzymes; aspartate transferase (AST), and alanine transferase (ALT) as described elsewhere [24]. Moreover, gamma-glutamyl transferase (GGT) was measured calorimetrically using kit (colorimetric assay kit ab241029) produced by Abbott Core Laboratories, USA [25]. Peroxidase enzymes; Malonaldehyde (MDA) [26], glutathione peroxidase (GPX) [27] and reduced glutathione (GSH) [28] were measured as well.

Ruminal juice samples

Twenty mL of ruminal fluid were collected by stomach tube three times monthly, to determine the pH, which was measured directly by using a pH meter \ (Mettler Toledo, Germany) [18]. Physical examination of ruminal juice (smell, color, consistency, viability and potentiality of ruminal protozoa) [29], ammonia-nitrogen concentration was performed using a modified phenol-hydrochloride reaction [30]. Total volatile fatty acids (TVFA) concentration was measured by Gas Liquid Chromatography (GLC) using Shimadzu GC 2010 equipped with 15-m EC-1000 column with an internal diameter 0.53 mm and a film thickness of 1.2 μm ; the reagent preparation procedures and temperature gradient for TVFA were previously determined [31,32].

Statistical analysis

All data were analyzed using one-way ANOVA and the differences between means were tested by Duncan's multiple-range test. The results were displayed as mean values with their standard errors (mean \pm SE) using the statistical package SPSS 16.0 [33].

Results**Clinical scoring and vital parameters of different groups of calves under the experiment**

The vital parameters (temperature, heart rates and respiratory rate), showed insignificant changes between the three groups, while ruminal contraction began to be significantly improved in G2 at 2nd month (3.66 ± 0.33 / 2 minutes) and extended with significance till the end of the experiment. The significant improvement in G3 (3.63 ± 0.33 / 2 minutes) was only appeared at the 3rd month of the experiment (Table 1).

On the other hand, a significant improvement in fecal, respiratory and locomotor scores of G2 and G3 was observed in the 3rd month of the experiment when compared with the control group (Table 1).

In comparison with G1 and G3, average monthly gain (AMG) and average daily gain (ADG) of G2 started to be significant from the 2nd month and extend to the 3rd month of the experiment, where AMG of G2 was 39.0 ± 1.08 kg and 44.37 ± 1.25 kg in 2nd and 3rd month, respectively and ADG was 1.3 ± 0.036 kg and 1.47 ± 0.041 kg in 3rd and 2nd month, respectively.

Table 1: Clinical scoring, and average daily gain (ADG) in different groups of fattening calves under investigation

Criteria Groups	First month			Second month			Third month		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
Fecal score	0.65 $\pm 0.18^a$	0.45 $\pm 0.17^a$	0.45 $\pm 0.14^a$	0.6 $\pm 0.13^a$	0.4 $\pm 0.11^a$	0.4 $\pm 0.112^a$	0.6 $\pm 0.168^a$	0.3 $\pm 0.11^b$	0.3 $\pm 0.11^b$
Respiratory score	1.4 $\pm 0.26^a$	1.1 $\pm 0.23^a$	1.1 $\pm 0.22^a$	1.7 $\pm 0.47^a$	1.2 $\pm 0.39^a$	0.95 $\pm 0.33^a$	1.3 $\pm 0.44^a$	0.9 $\pm 0.20^b$	0.65 $\pm 0.19^b$
Locomotors score	0.35 $\pm 0.09^a$	0.25 $\pm 0.099^a$	0.3 $\pm 0.09^a$	0.35 $\pm 0.13^a$	0.2 $\pm 0.091^a$	0.25 $\pm 0.091^a$	0.4 $\pm 0.16^a$	0.2 $\pm 0.091^b$	0.2 $\pm 0.12^b$
Temperature ($^{\circ}\text{C}$)	38.53 $\pm 0.145^a$	38.4 $\pm 0.115^a$	38.66 $\pm 0.09^a$	38.36 $\pm 0.15^a$	38.5 $\pm 0.115^a$	38.36 $\pm 0.31^a$	38.5 $\pm 0.15^a$	38.46 $\pm 0.23^a$	38.46 $\pm 0.09^a$
Heart rate (per min)	73.66 $\pm 2.96^a$	73.33 $\pm 1.76^a$	72.33 $\pm 2.4^a$	73.0 $\pm 3.6^a$	71.66 $\pm 2.18^a$	72.33 $\pm 1.45^a$	73.0 $\pm 1.52^a$	72.33 $\pm 3.8^a$	72.0 $\pm 3.6^a$
Respiratory rate (per min.)	23.95 $\pm 0.85^{ab}$	23.0 $\pm 0.45^{ab}$	23.25 $\pm 0.50^{ab}$	25.8 $\pm 1.35^a$	24.4 $\pm 0.58^{ab}$	23.65 $\pm 0.45^{ab}$	24.15 $\pm 0.73^{ab}$	23.65 $\pm 0.45^{ab}$	22.75 $\pm 1.30^b$
Ruminal contractions (per 2 min.)	2.33 $\pm 0.33^b$	2.66 $\pm 0.33^b$	2.66 $\pm 0.33^b$	2.66 $\pm 0.33^b$	3.66 $\pm 0.33^a$	3.0 $\pm 0.00^b$	3.0 $\pm 0.00^b$	3.66 $\pm 0.33^a$	3.63 $\pm 0.33^a$
ADG (Per Kg)	0.72 $\pm 0.02^d$	0.76 $\pm 0.024^d$	0.73 $\pm 0.03^d$	1.2 $\pm 0.055^c$	1.3 $\pm 0.036^b$	1.2 $\pm 0.038^c$	1.3 $\pm 0.036^{bc}$	1.47 $\pm 0.041^a$	1.33 $\pm 0.04^b$

Values with different superscripts within rows are significantly different ($P < 0.05$). G1: Group 1 (control); G2: Group 2 (yeast supplemented group); G3: Group 3 (*lactobacillus* supplemented group), ADG: average daily gain

Biochemical analysis

Table 2 shows significant increase in β HBA and a significant reduction in the level of non-esterified fatty acids (NEFA) in G2 and G3 compared to G1. While, serum total protein, albumen and globulin levels showed insignificant changes between the three groups all over the experimental period. The AST liver enzyme showed a significant reduction (57.0 ± 21.4 U/L and 68.66 ± 9.49 U/L, respectively) in G2 and G3 respectively in comparison with G1 (80.6 ± 17.2 U/L). While ALT and GGT levels were significantly

reduced (10.33 ± 13.83 U/L and 9.06 ± 0.88 U/L, respectively) only with G3 all over the experiment, while those of G2 showed insignificant changes all over the experiment when compared with G1. GSH and GPX were significantly increased (50.4 ± 5.82 U/ml and 53.53 ± 7.22 U/ml, respectively) in G2 and G3 respectively, while MDA of G2 and G3 showed insignificant reduction (30.8 ± 3.41 nmol/L and 27.33 ± 2.46 nmol/L, respectively) in comparison with G1 (32.75 ± 3.7 nmol/L).

Table (2): Biochemical changes in different groups of calves under investigation

Criteria Groups	First month			Second month			Third month		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
Total serum protein (g/dL)	7.03 $\pm 0.28^a$	7.3 ± 1.15^a	6.78 ± 0.42^a	6.4 ± 0.35^a	6.43 $\pm 0.48^a$	6.08 $\pm 0.4^a$	6.73 $\pm 0.53^a$	6.56 $\pm 0.12^a$	6.33 $\pm 0.64^a$
Serum albumin g/dL	2.24 $\pm 0.09^{ab}$	2.4 $\pm 0.20^{ab}$	1.98 ± 0.28^b	2.41 ± 0.07^{ab}	2.75 $\pm 0.18^{ab}$	2.46 $\pm 0.32^{ab}$	2.63 $\pm 0.21^{ab}$	3.03 $\pm 0.09^a$	2.93 $\pm 0.43^a$
Serum globulin g/dL	4.8 $\pm 0.36^a$	4.9 $\pm 0.95^a$	4.80 ± 0.58^{ab}	4.99 ± 0.33^{ab}	4.68 $\pm 0.308^{ab}$	4.62 $\pm 0.68^{ab}$	4.7 $\pm 0.404^{ab}$	3.53 $\pm 0.176^b$	3.4 $\pm 1.05^{ab}$
Albumin/ globulin ratio %	0.47 $\pm 0.05^a$	0.51 $\pm 0.05^a$	0.56 ± 0.13^a	0.6 ± 0.05^a	0.74 $\pm 0.01^a$	0.77 $\pm 0.26^a$	0.89 $\pm 0.16^a$	1.21 $\pm 0.11^a$	1.34 $\pm 0.78^a$
AST (U/L)	86.66 $\pm 5.45^b$	62.33 $\pm 5.81^c$	81.33 $\pm 10.72^c$	85.33 $\pm 10.39^b$	62.6 $\pm 14.6^c$	80 $\pm 10.11^c$	80.6 $\pm 17.2^b$	57.0 $\pm 21.4^a$	68.66 $\pm 9.49^a$
ALT (U/L)	18.0 $\pm 3.0^a$	17.0 $\pm 4.16^{ab}$	12.33 $\pm 2.02^b$	18.0 ± 2.08^a	15.66 $\pm 4.48^{ab}$	10.33 $\pm 5.45^b$	17.66 $\pm 1.20^a$	15.0 $\pm 6.92^{ab}$	10.33 $\pm 13.83^b$
GGT (U/L)	14.0 $\pm 1.15^a$	13.66 $\pm 4.17^a$	9.33 ± 4.09^b	13.66 $\pm 1.85^b$	12.0 $\pm 1.73^{ab}$	9.33 $\pm 2.4^a$	14.33 $\pm 2.6^a$	13.0 $\pm 2.08^a$	9.06 $\pm 0.88^b$
MDA (nmol/L)	25.38 ± 1.98^a	24.26 ± 0.59^a	24.94 ± 3.28^a	29.84 ± 3.03^a	28 ± 1.85^a	25.9 ± 1.39^a	32.75 ± 3.7^a	30.8 ± 3.41^a	27.33 ± 2.46^a
GSH (U/mL)	35.2 ± 1.9^b	37.76 ± 2.23^a	36.83 ± 2.64^a	41.16 ± 3.29^b	43.34 ± 2.86^a	44 ± 5.58^a	45.46 ± 8.45^b	50.4 ± 5.82^a	53.53 ± 7.22^a
GPX (U/mL)	22.64 ± 0.5^b	30.77 ± 1.61^a	24.96 ± 1.04^{ab}	30.48 ± 3.94^b	33.1 ± 3.23^{ab}	33.5 ± 2.81^{abc}	35.99 ± 6.69^b	39.61 ± 4.6^a	42.38 ± 5.71^a
βHBA (mmol/L)	0.22 ± 0.014^a	0.26 ± 0.016^b	0.27 ± 0.012^b	0.22 ± 0.020^a	0.27 ± 0.013^b	0.29 ± 0.01^b	0.22 ± 0.017^a	0.29 ± 0.02^b	0.29 ± 0.02^b
NEFA mmol/L	2.81 $\pm 0.09^a$	2.39 $\pm 0.29^b$	2.41 ± 0.15^b	2.31 ± 0.08^b	2.09 $\pm 0.20^c$	2.106 $\pm 0.11^c$	2.07 $\pm 0.13^b$	1.80 $\pm 0.15^c$	1.81 $\pm 0.14^c$

Values with different superscripts within rows are significantly different ($P < 0.05$). G1: Group 1 (control); G2: Group 2 (yeast supplemented group); G3: Group 3 (*lactobacillus* supplemented group). AST: Aspartate Transaminase; ALT: Alanine Transaminase; GGT Gamma-Glutamyl Transferase; MDA: mMalone Dialdehyde; GSH: Reduced Glutathione; GPX: Glutathione Peroxidase; β HBA: β -hydroxy butyric acid; NEFA: non-esterified fatty acids.

Ruminal juice analysis

The results of physical, microscopical and chemical examinations of ruminal juice are documented in Table 3. In comparison with G1, a visible improvement in odour, protozoal population and activity were observed in G2 from the 1st month of experiment, while an improvement was observed in the 3rd month with G3. The colour showed no changes between different groups. The ruminal pH of G2 showed significant elevation in 2nd and 3rd

month of the experiment, while G3 showed stabilization in pH value all over the experiment. Ammonia concentration of G2 showed significant decrease in all over the period of the experiment, while in G3, it showed insignificant changes along the period of the experiment. However, TVFA concentration was significantly increased in G2, while it insignificantly changed in G3 all over the period of the experiment in comparison with G1.

Table 3: Ruminant juice analysis of calves in different groups under investigation

Criteria	First month			Second month			Third month		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
Colour	Greenish yellow	No change	No change	Greenish yellow	No change	No change	Greenish yellow	No change	No change
Odour	Weakly aromatic	Typically aromatic	Weakly aromatic	Weakly aromatic	Typically aromatic	Weakly aromatic	Weakly aromatic	Typically aromatic	Weakly aromatic
Protozoal motility and population	Mild active with low number	Highly active, massive number	Moderate active with moderate number	Moderate active with moderate number	Highly active, massive number	Moderate active with moderate number	Moderate active with moderate number	Highly active, massive number	Highly active, massive number
PH	6.46 ±0.27 ^{abc}	6.83 ±0.17 ^a	6.93 ±0.14 ^a	6.13 ±0.07 ^c	6.61 ±0.11 ^{ab}	6.53 ±0.07 ^{abc}	6.1 ±0.15 ^c	6.53 ±0.06 ^{ab}	6.31 ±0.06 ^{bc}
Ammonia mmol/L	18.13 ±0.81 ^b	15.8 ±0.93 ^a	18.0 ±2.13 ^b	25.06 ±0.99 ^a	21.23 ±0.66 ^b	24.33 ±0.44 ^{ab}	27.43 ±1.16 ^a	25.16 ±1.12 ^b	27.1 ±1.45 ^a
TVFA mmol/L	66.33 ±4.05 ^b	77.0 ±3.6 ^a	66.66 ±1.76 ^b	76.0 ±3.21 ^b	86.0 ±3.05 ^a	76.0 ±4.35 ^b	81.66 ±3.52 ^b	87.66 ±1.45 ^a	82.33 ±4.48 ^b

Values with different superscripts within rows are significantly different ($P < 0.05$). G2: Group 2 (yeast supplemented group); G3: Group 3 (*lactobacillus* supplemented group), pH: Hydrogen ion concentration; TVFA: Total volatile fatty acids.

Discussion

Farmers and feedlot managers are always looking for nutritional strategies to improve performance of feedlot. The utilization of probiotics as an alternative to feeding antibiotics to improve feedlot performance and decrease cost of feed has gained interest in the feedlot industry [3]. However, because of the results obtained when probiotics are included in calves' diets have not been consistent, further research is needed for validation of this technology for the feedlot industry to test the effects of probiotics (*Lactobacillus* and active dry yeast) in calves' diets.

In the current study, Ruminal contractions were significantly improved in *Saccharomyces cerevisiae*¹⁰²⁶ and lactobacilli casei groups (3.66 ±0.33/ 2 minutes and 3.63 ±0.33/ 2minutes respectively) compared to the control group (3±00 /2 minutes). Those results were agreed with previous studies [4,15] in which, the improvement was related to enhancement of the food digestibility. *Saccharomyces cerevisiae*¹⁰²⁶ stimulates lactic acid utilizing bacteria, which consume excess rumen lactate leading to proper rumen environment for action of other digestive bacteria so improved digestibility and rumen motility.

Fecal score showed an improvement in G2 and G3 at 3rd month of experiment, which were consistent with previous researches [34, 35]. It is possible that feeding of *Saccharomyces cerevisiae*¹⁰²⁶ may decrease the risk of diarrhea by reducing the attachment and invasion of intestinal cells by these pathogens, because they may bind to the oligosaccharides present in the yeast cell wall, minimizing the growth of enteric pathogens by the metabolites produced by yeast or reduce the inflammatory response in the gut because of the metabolites of yeast. Moreover, another study [13] found an improvement in fecal score of calves supplemented with *Lactobacillus* in their feed. Respiratory score was significantly improved with addition of *Saccharomyces cerevisiae*¹⁰²⁶ and *Lactobacilli casei*, which was similar to that reported in a previous work [12] in which, feeding of

Lactobacilli decreases the respiratory affections due to the improvement of general health and immune status of the treated calves and competing properties of probiotics with pathogenic bacteria. Locomotors score was significantly improved in G2, in a previous study [36], it was reported that feeding of yeast culture decreased hoof affection as the yeast is O₂ scavenger and stabilize the pH for the rumen, so optimize the living environment for ruminal bacteria then minimize the dead bacteria which become endotoxins causing inflammation of the hoof and udder. Moreover, *Saccharomyces cerevisiae*¹⁰²⁶ minimizes the chance for acidosis that causes hoof inflammation. The body temperature of calves under investigation was insignificantly changed between the three groups

We recorded a significant increase in average daily gain (ADG) of G2 (1.47±0.041 kg). Previously, [6] an increase in weight gain after using yeast in animal feed was documented. This increase in weight gain with supplementing yeast to animal diet is due to increase of dry matter intake (DMI) and improvement of digestion, as the animals can ingest more food, at the same time the outflow rate of digesta increased from the rumen to the duodenum by improvement of digestion process. In contrast, we recorded that addition of *Lactobacilli casei* had no effect on ADG of calves.

βHBA serum concentration significantly increased in G2 and G3 (0.29±0.02 mmol/L and 0.29±0.02mmol/L, respectively) calves in comparable to the control group (0.29±0.01 mmol/L). In a previous study [37], βHBA concentrations were high in probiotic supplemented treatments, and this considered as an indicator for greater development of rumen. Most of βHBA is formed from conversion of butyrate in the rumen wall before releasing into portal circulation, but the rumen in the newborn calves is metabolically nonfunctional. So, βHBA concentrations were low in the early age of calves. After initiation of solid feed intake by calves and the subsequent establishment of microbial population and ruminal fermentation, the

rumen is then physically and metabolically developed, and the ruminal epithelium becomes the primary source of β HBA production. β HBA concentrations were high in probiotic supplemented treatments, and this considered as an indicator for greater development of rumen. Serum level NEFA showed significant reduction in G2 and G3 (1.80 ± 0.15 mmol/L and 1.81 ± 0.14 mmol/L respectively) calves, which was similar to the results reported in another study [37] in which, a reduction in the value of NEFA when using probiotic in animal feed and a decrease in NEFA levels as indication of a more efficient use of dietary energy and greater dry matter intake in the probiotic received groups were reported. A significant reduction was recorded in AST value in both G2 and G3 (57 ± 21.4 U/L and 68.66 ± 9.49 U/L, respectively) in comparison with G1 (80.6 ± 17.2 U/L). Moreover, a significant reduction in GGT and ALT (9.06 ± 0.88 U/L and 10.33 ± 13.83 U/L respectively) values in G3 all over the experiment was documented [32]. This reduction in liver enzymes in treated calves while using probiotics is due to the suggestion that probiotic promote integrity of the gut mucosa to prevent gut permeability, endotoxemia, and pro-inflammatory cytokine production and liver injury [38]. In comparison with G1, GSH and GPX were significantly increased in G2 (245.46 ± 8.45 U/ml and 35.99 ± 6.69 U/ml, respectively) and G3 (53.53 ± 7.22 U/ml and 42.38 ± 5.71 U/ml, respectively) all over the period of experiment, while the mean values of MDA of G2 and G3 showed insignificant reduction. These results were consistent with a previous study [39] in which, the antioxidants activities were accelerated, while MDA levels were decreased by probiotics supplementation. As explained previously [40], probiotics have antioxidant activity, as they have enzymatic and non-enzymatic effect and they can produce GPX as non-enzymatic antioxidants which, reduce reactive oxygen intermediates. Also, they deal with oxygen radicals by producing superoxide dismutase that dismutates the superoxide radicals to oxygen and hydrogen peroxide. while, [41] stated that *Lactobacilli* can

produce a haem-dependent catalase that can quickly degrade the hydrogen peroxide. Further researches are needed to measure antioxidants activities in calves after using probiotics.

An improvement in odour and protozoal population and activity in G2 and G3 was observed all over the period of experiment. A previous study [42] emphasized that *yeast* and probiotics have a positive effect on number and activity of rumen protozoa. The accelerated activity and increased proliferation of protozoa with supplementing yeast attributed to the proper environment provided by slight increases and/or stabilization of rumen pH and TVFA with reduction in ammonia concentration as reported in the current study. Ruminal pH in our work showed significant elevation and stabilization in G2 and G3 (6.31 ± 0.06 and 6.53 ± 0.06 , respectively) in 2nd and 3rd month of experiment, which was similar to previous studies [42- 44]. Conversely, other researchers [45] observed a stabilization in pH value with addition of *yeast* and direct feed microbial DFM. The increases and stabilization of ruminal pH when adding yeast and probiotics to animal feed may be attributed to the stimulation of lactic acid utilizing bacteria in the rumen [45]. Ruminal juice ammonia concentration in the current study recorded a significant decrease in G2 all over the period of experiment. This result matched with those reported in a previous work [46] as a reduction in ruminal ammonia concentration with adding DFM and yeast to calf diet was observed. Decreasing rumen ammonia concentrations is attributed to the high ruminal microbial proliferation by yeast [47].

Conclusion

The supplementation of *Saccharomyces cerevisiae*¹⁰²⁶ and *Lactobacillus casei* to the feed of fattening calves has a positive impact on calves' general health and their weight gain. Additionally, an improvement of the fecal, respiratory and locomotors scores, ruminal contraction, rumen protozoal population, ruminal pH and ruminal TVFA concentrations

with a reduction in liver enzymes, NEFA and rumen ammonia were also reported.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgement

It gives me a great pleasure to express my appreciation and sincere gratitude to the staff members (specially Prof. Dr. Ahmed Abdelaal) of Animal Medicine Department, Internal Medicine, Faculty of Veterinary Medicine, Zagazig University, for their assistant and continuous support. Special thanks to Prof. Dr. Mohamed Badr, professor of Biochemistry, Animal Health Institute, Zagazig for his support and cooperation in analysis of the research samples. All thanks to staff member of Samy Asaad farm where we have done the experiment for their cooperation and assist in samples collection and recording the results all over the experiment.

References

- [1] FAO/WHO, (2002): Guidelines for the Evaluation of Probiotics in Food. Food and Agriculture Organization of the United Nations/World Health Organization, London, Ontario. www.who.int/foodsafety/publications/fs_management/en/probiotic_guidelines Pdf, 1-11.
- [2] Quigley, J.D.; Drewry, J.J.; Murray, L.M.; and Ivey, S.J. (1997): Body weight gain feed efficiency and fecal scores of dairy calves in response to galactosyl-lactose orantibiotics in milk replacers. J Dairy Sci, 80 (8): 1751–1754.
- [3] Berge, A.C.; Lindeque, P.; Moore, D.A.; and Sisco, W.M. (2005): A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of preweaned calves. J Dairy Sci, 88 (6): 2166–2177.
- [4] Denev, S. A.; Tz. Peeva, P.; Rrdulova, P.; Stancheva, G.; Staykova, G.; Beev, P.; Todorovaand S.; and Tchobanova, (2007): Yeast cultures in ruminant nutrition. Bulg J Agric Sci, 13: 357-374.
- [5] Dawson, K.A.; Newman, K.E.; and Boling, J. A. (1990): Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. J Anim Sci, 68: 3392-3398.
- [6] Ali K.; Ihsan U.; Hayaz U.; Shakira G.; and Mustansar A.G. (2017): Study on the effects of dietary supplementation of inactive dry yeast (*Saccharomyces cerevisiae*) on feed intake, body weight gain and fecal microbiota of crossbreds steers. International J of Biosciences (IJB), 10 (4): 288-294.
- [7] Biricik, H.; and Yavuz, H.M. (2001): Effects of *Saccharomyces cerevisiae* yeast culture on milk production, milk composition *Saccharomyces cerevisiae* live cells on mixed ruminal microorganism fermentation in vitro. J of Animal Science, 82:1847-1854.
- [8] Harrison, G.A.; Hamken, R.W.; Dawson, K.A.; Harmon, A.J. and Barber, K.B. (1988): Influence of addition of yeast supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci., 71(11): 2967-2975.
- [9] Joseph, B.S. (2002): The effect of yeast *saccharomyces cerevisiae* culture in a free choice mineral mix on intake digestibility and milk production for beef cattle on fescue basal pasture. A Thesis presented in partial fulfillment of the requirements for the degree Master of Science in the graduate school of the Ohio state University.
- [10] Ghazanfar S.; Anjum, M. I.; Azim1, A. and Ahmed, I. (2015): the effects of dietary supplementation of yeast (*saccharomyces cerevisiae*) culture on growth performance, blood parameters, nutrient digestibility and fecal flora of dairy heifers. J Anim Plant Sci, 25(1): 53-59.
- [11] Savage, D.C. (1987). Microorganisms associated with epithelial surfaces and the stability of the indigenous gastrointestinal microflora. J Die Nahrung, 31(5-6):383-95.

- [12] Timmerman H.M, Mulder L., Evert H., Van Espen D.C., Van Der Wal E., Klassen G., Rouwers S. M., Hartemink R., roubouts F.M., and Beynen A. C (2005): health and growth of veal calves fed milk replacer with or without probiotics. *J Dairy Sci.*, 88 (6), 2154-2165.
- [13] Abe, F., Ishibashi, N., Shimamura, S., (1995). Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. *J. Dairy Sci.* 78 (12), 2838–2846.
- [14] Gorgulu, M., Siuta, A., Ongel, E., Yurtseven, S., Kutlu, H.B., (2003). Effect of probiotic on growing performance and health of calves. *Pak. J. Biol. Sci.* 6, 651–654.
- [15] Vanbelle, M.; Teller, E.; and Focant, M. (1990): Probiotics in animal nutrition: a review. *Arch Tierernahr* 40(7):543-67.
- [16] Edward, A. (2012): Vital Signs in Animals, what Cattle Producers Should Know About Them, cattle producer's handbook, 3 rd Edition, 610,1-3.
- [17] Larson, L.L.; Owen, F.G.; Albright, J.L.; Appleman, R.D.; Lamb, R.C. and Muller, L.D. (1977): Guidelines toward more uniformity in measuring and reporting calf experimental data. *J Dairy Sci*, 60 (6): 989-991.
- [18] Radostitis, O.M; Gay, C.C.; Hinchcliff, K.W. and Constable, PD. (2007): *Veterinary Medicine-A textbook of the disease of cattle, horse, sheep, pigs and goats*, 10th Edition. Saunders Elsevier, Philadelphia Pp. 1448-1773.
- [19] Koch D. D. and Fledbruegge D.H. (1987); Optimized kinetic method for automated determination of beta-hydroxybutyrate, *J Clin Chem*, 33(10): 1761.
- [20] Smith, S.R. and Wilson, P.W. (2006); free fatty acids and atherosclerosis. *J Clin Endocrinol Metab*, 91(7):2506-8.
- [21] Cannon, D.C.; Henry, R. J. and Winkelman, J. W. (1974): *clinical chemistry-principals and Techniques*, 2nd Edition, Harper & Row, Hagerstown, M. D. pp 411-421.
- [22] Bartholomew, R. J. and Delaney, A. M. (1966): Sulphonphthaleins as specific reagents for albumin: determination of albumin in serum. *Proceedings of the Australian Association of clinical Biochemists*.1:214.
- [23] Dumas, B.T.; Watson, W.A. and Biggs, H.G. (1997): Albumin standards and the measurement of serum albumin with bromcresol green. *Clin Chem Acta*, 258(1):21-30
- [24] Reitman, S. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Path.*28 (1): 56-63.
- [25] Theodorsen L, Stromme J.H. (1976); Gamma-Glutamyl-3carboxy-4-nitroanilide: the substrate of choice for routine determinations of gamma-glutamyl-transferase activity in serum? *Clin Chim Acta*,72 (2):205-210
- [26] Esterbauer, H.; Cheeseman, K.H.; Danzani, M.U.; Poli, G. and Slater, T.F. (1985): Separation and characterization of the aldehydic products of lipid peroxidation stimulated by carbon tetrachloride or ADP-iron in isolated rat hepatocytes and rat liver microsomal suspensions. *Biochem J*, 227(2): 629–638.
- [27] Paglia, D.E. and Valentine, W.N. (1967): Studies on qualitative and quantitative characterization of erythrocytes glutathione peroxidase. *J.lab clin. Med.* 70 (1),158- 169
- [28] Beutler, E.; Duron, O. and Kelly, B.M. (1963): Improved method for the determination of blood glutathione. *J Lab Clin Med*, 61,882-888.
- [29] Kleen, J.L.; Hooijer, G.A.; Rehage, J. and Noordhuizen, j.P. (2009): Subacute ruminal acidosis in Dutch dairy herds. *Vet Rec*, 164 (22): 681-684.
- [30] Broderick, G.A. and Kang, J.H. (1980): Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63 (1):64-75.
- [31] Grigsby, K.N., M.S. Kerley, J.A. Paterson, and J.C. Weigel. (1992): Site and extent of nutrient digestion by steers fed a low-quality brome grass hay diet with incremental levels of soybean hull

- substitution. J. Anim. Sci. 70 (6):1941-1949.
- [32] Bateman, H.G.; Williams, C.C. and Chung, Y.H. (2002): Effects of supplemental zinc in high quality diets on ruminal fermentation and degradation of urea *in vitro* and *in vivo*. Prof Anim Sci 18 (4):363-367.
- [33] Snedecor, G.W. and Cochran, W.G. (1982): Statistical Methods. 7th Edition, Iowa State University Press, Towa, 511.
- [34] Krol, B. (2011): Effect of Mannooligosaccharides, Inulin and yeast nucleotides added to calf milk replacers on rumen microflora, level of serum immunoglobulin and health condition of calves. EJPAU, 14(2):18.
- [35] Renaud, D.L.; Kelton, D.F.; Weese J.S.; Noble C. and Duffield T.F. (2019): Evaluation of a multispecies probiotic as a supportive treatment for diarrhea in dairy calves: A randomized clinical trial. J of dairy sci, 102(5):4498-4505.
- [36] Alshaikh, M.A.; Alsiadi M.Y.; Zahran, S.M.; Mogawer, H.H. and Aalshowime T.A. (2002): Effect of feeding yeast culture from different sources on the performance of lactating Holstein cow in Saudi Arabia. Asian-Australas. J Anim Sci, 15: 352–356.
- [37] Bayatkouhsar, A. M.; Tahmosebi, A.A. Nasreriyan, A.A. and Mokarram, R. R. (2013): Effect of supplementation of lactic acid bacteria on growth performance, blood metabolites fecal coliform and lactobacilli of young dairy calves, animal feed science and technology, 186 (s 1-2), 1-11
- [38] Demeyer, D.I., and van Nevel, C.J. (1979): Effect of defaunation on the metabolism of rumen microorganisms. Br. J. Nutr. 42 (3), 515-524
- [39] Yang, Y. W.; Baiku, W.W.; Xuefang, C.; Aikun, F.; Yali, L. and Weifen, L. (2017): Effects of Probiotic Bacillus as substitute for antibiotics on antioxidant capacity and intestinal autophagy of piglets. AMP Express 7(1):52.
- [40] Stecchini, M. L., del Torre, M. and Munari, M. (2001): Determination of peroxy radical-scavenging of lactic acid bacteria. Int J food Microbial, 64 (1-2), 183-188.
- [41] Knauf, H.J., Vogel, R. F. and Hammes, W. P. (1992): Cloning, sequencing and phenotypic expression of *katA*, which encodes the catalase of lactobacillus sake LTH677. Appl Environ Microbial 58 (3), 832-839.
- [42] Kamra, D.N.; Chaudhary, L.C.; Neeta-Agarwal; Singh, R.; Pathak, N.N. and Agarwal, N. (2002): Growth performance, nutrient utilization, rumen fermentation and enzyme activities in calves fed on *Saccharomyces cerevisiae* supplemented Diet. Indian J. Anim. Sci., 72 (6), 472–475.
- [43] Mir, Z. and Mir, P.S. (1994). Effect of the addition of live yeast (*Saccharomyces cerevisiae*) on growth and carcass quality of steers fed high-forage or high grain-diets and on feed digestibility and *in situ* degradability. J. Anim. Sci., 72 (3), 537-45.
- [44] Abdel-Ghani AA. (2004): Influence of diet supplementation with yeast (*Saccharomyces cerevisiae*) on performance of Zaraibi goats. Small Ruminant Research, 52 (3):223-229.
- [45] Marden, J.P.; Bayourthe, C.; Enjalbert, F.; Monocoulon, R. (2005): A new device for measuring kinetics of ruminal pH and redox potential in dairy cattle. J Dairy Sci, 88 (1): 277-281.
- [46] Doležali, P.; Doležali, J. and Třináctý J. (2005): The effect of *Saccharomyces cerevisiae* on ruminal fermentation in dairy cows. Czechs. Journal of Animal Science, 50 (11):503-510.
- [47] Crocker, L.M.; DePeters, E.J.; Fadel, J.G.; Perez-Monti, H.; Taylor, S.J.; Wyckoff, J.A. and Zinn, R.A. (1998): Influence of processed corn grain in diets of dairy cows on digestion of nutrients and milk composition. J. Dairy Sci. 81(9), 2394–2407.

الملخص العربي

تقييم تأثير السكراروميسيس سيرفيسي^{١٠٢٦} واللاكتوباسيلس كازي على مكونات الكرش وكيمياء الدم

في عجول التسمين

أسامة اسماعيل العدوى^{١*}، شيماء محمد جوده^٢، العباسي محمد عباس النجار^٢ وصبحي المغاوري محمد^٢

^١ شركة أم اس دى لصحة الحيوان ، القاهرة الجديدة ، التجمع الخامس ، ١١٨٣٥ ، مصر

^٢ قسم طب الحيوان ، الأمراض الباطنة ، كلية الطب البيطري ، جامعة الزقازيق ٤٤٥١١ ، مصر

تم في هذه الدراسة تجربة الخميره الحية (السكراروميسيس سيرفيسي^{١٠٢٦} - اى ساك) واللاكتوباسيلس كازي (بروباكس) على ٦٠ عجل هولشتاين ألماني في أحد المزارع بمحافظة الشرقية. كانت هذه العجول تتمتع بصحة جيدة طبقاً للفحص الظاهري وتتغذى على عليقة متوازنة من حيث المكونات والاضافات ويتراوح أوزانهم ما بين ٩٠ و ١١٠ كيلو جرام وقد قسمت هذه العجول الى ثلاث مجموعات متساوية العدد كلا منها عشرون عجل وهم المجموعة الأولى وهي المجموعة الضابطة و التي تتغذى على عليقة دون اضافة و المجموعة الثانية والتي تحتوى عليقتها على الخميرة ثم المجموعة الثالثة والتي تحتوى عليقتها على اللاكتوباسيلس. استمرت التجربة لمدة ثلاث شهور لتقييم تأثير الخمائر واللاكتوباسيلس في علائق عجول التسمين على معدلات الأوزان والصورة الدموية والبيوكيميائية وكذلك مكونات الكرش المختلفة. وكانت نتائج الفحص الاكلينيكي للعجول تحت التجربة مرضية حيث وجد تحسن معنوي سجل البراز والحركة وايضا معدل الاصابة بالأمراض التنفسية كما لوحظ أيضا تحسن في حركة الكرش ($3.66 \pm 0.33/2$ minutes) للمجموعة الثانية والثالثة. ولقد وجد بفحص سيرم الدم لعجول المجموعة الثانية والثالثة مقارنة بالمجموعة الاولى زيادة في نسبة حامض البيتا هيدروكسي بيوتيرك β HBA (0.29 ± 0.02 mmol/L) و انخفاض ملحوظ في نسبة الاحماض الدهنية الغير مكلورة NEFA (1.80 ± 0.15 mmol/L). أما انزيمات الكبد (AST, ALT & GGT) فقد سجلت انخفاضا معنويا في المجموعة الثالثة (9.06 ± 0.88 U/L) و (10.33 ± 13.83 U/L) و (68.66 ± 9.49 U/L) بالترتيب أما في المجموعة الثانية فقد انخفضت نسبة AST فقط (57.0 ± 21.4 U/L). أيضا سجل ارتفاع في معدل انزيمات GSH و GPX وانخفاض في معدل انزيم MDA في المجموعتين الثانية والثالثة وهذا يدل على تحسن في مناعة العجول. ولقد لوحظ ارتفاع في الكثافة العددية وكفاءة ميكروبات الكرش خلال مدة التجربة في المجموعة الثانية مقارنة بالمجموعة الأولى والثالثة التي أظهرت تحسن في الشهر الثالث من التجربة فقط. بتحليل سائل الكرش وجد ارتفاع ملحوظ في قيمة الاس الهيدروجيني في المجموعة الثانية (6.53 ± 0.06) وتوازن وثبات خلال فترة تجربه في المجموعة الثالثة. وعن نسبة الأمونيا في الكرش فقد لوحظ انخفاض معنوي في حالة المجموعة الثانية (25.16 ± 1.12 mmol/L) مقارنة بالمجموعة الأولى والثالثة أما بالنسبة للأحماض الدهنية الطيارة فقد سجلت زيادة معنوية (87.66 ± 1.45 mmol/L) في المجموعة الثانية فقط وهذا يشير الى تحفيز ميكروبات الكرش المسؤولة عن تخليقها. أما بالنسبة لمعدلات الأوزان فقد سجلت زيادة معنوية في المجموعة الثانية مقارنة بالمجموعة الاولى والثالثة. ولهذا فاننا ننصح مربى العجول باستخدام الخمائر واللاكتوباسيلس في علائق العجول للاستفادة من فاعليتها في زيادة معدلات النمو وتقليل الاصابه بالأمراض وتحسين الصحة العامة للعجول.