

**Clinicopathological studies of dietary supplementation of *Saccharomyces cerevisiae* in calves**Osama A. Abdalla<sup>1</sup>, Mohamed E.EL-Boshy<sup>2,3</sup>, Fatma M. Abdel Hamid<sup>3</sup> and Nosa M. Ali<sup>3</sup><sup>1</sup>.Clinical Pathology Department, Faculty of Vet. Medicine, Suez Canal University, Ismailia, Egypt<sup>2</sup>.Laboratory Medicine Department, Faculty of Applied Medical Science, Umm Al-Qura University. Makkah, PB 7296, Makkah 21955, Saudi Arabia<sup>3</sup>. Clinical Pathology Department, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt  
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**Abstract: Background and Objectives:** The present study aimed to evaluate some selective immunological, antioxidant and biochemical parameters as well as growth promoters effects of *Saccharomyces cerevisiae* supplementation as a feed additives in calves. **Material and Methods:** Thirty buffalo calves were divided into 3 groups. Group I: control one, Group II: treated orally with *Saccharomyces cerevisiae* (Sc) at a dose rate 30g once daily/calf for 12 weeks and groupIII: treated with 60g Sc once daily/calf for 12 weeks. **Results:** The treatment with *Saccharomyces cerevisiae* resulted in significant increase in the body weight, blood glucose level, total protein, albumin and A/G ratio, while AST, LDH activities, globulin concentration, uric acid and creatinine levels are insignificantly changed. The level of MDA is significantly decrease. Meanwhile catalase, GSH level, serum lysozyme, bactericidal activity and serum NO level are significantly increased **Conclusions:** we are concluded that *Saccharomyces cerevisiae* treatment of the calves has desirable effects on the body weight, serum antioxidant enzymes and some immunological parameters. Also has no toxic effect on kidney or liver functions. [Osama A. Abdalla, Mohamed E.EL-Boshy, Fatma M. Abdel Hamid and Nosa M. Ali. **Clinicopathological studies of dietary supplementation of *Saccharomyces cerevisiae* in calves.** *J Am Sci* 2013;9(9):298-306]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 39

**Keywords:** *Saccharomyces cerevisiae*; body weight; biochemical parameters; antioxidant; immunological parameters.

**1. Introduction**

Feed additives are widely used in order to increase body weight gain of farm animals, particularly those species known with their slow growth rate. Probiotics have been used as a supplement in animal feeds for improving animal performance (Dawson, 1995). Moreover, many of the beneficial productive responses associated with the use of probiotic supplements can be directly related to their effects on the microbial population in the digestive tract (Nahashon *et al.*, 1992). The mode of action of probiotics may be due to producing antibiotic substances and inhibiting harmful bacteria metabolism and decreased intestinal pH (De-Blas, *et al.*, 1991). Yeast has been known as a probiotic in feed animal (Saegusa *et al.*, 2004).

Dietary live yeast as *Saccharomyces cerevisiae* has been used as a fermenting agent in baking, distilling and brewing industries since ancient times. But today, there are many strains of the organism being used for different purposes (Onifade, 1998). Yeast are known as rich sources of vitamins, enzymes and other important nutrients, also co-factors which make them attractive as digestive enhancers and as a basic source nutrients. Yeast culture has been shown to have several effects in ruminants, since it alters feed performance, nutrient digestibility (El-Talty *et al.*,

2001) and protein synthesis in the digestive tract (Williams, 1988).

Recently, the use of supplemental yeast in animal feeding has various beneficial impacts. The action of live yeast for improving performance is not fully understood, but there are two probabilistic explanations. The first, action of yeast is most probably supporting the growth of lactic acid bacteria. The second one is a competitive exclusion of pathogenic bacteria by yeast and its products especially the cell wall component (Onifade, 1998). Yeast and yeast cultures have been widely used in ruminant to manipulate rumen fermentation to improve animal performance. However, resulted performance of ruminants fed yeast culture had been varied. These differences may depend on many factors such as diet composition, forage to concentrate ratio, type of forage fed, yeast dose, feeding strategy and stage of lactation. Several studies have showed that live yeast and yeast culture supplementation may increase feed intake and milk production of dairy cows (Dann *et al.*, 2000).

Live yeast culture is very active in forming amino acids and vitamins. It promotes fermentation by secreting various digesting enzymes within the ruminant stomach and stabilizes the flora in the stomach and intestines by supplying the nutrients which are necessary for their growth. The addition of

yeast culture increased dry matter intake, daily gain and feed efficiency by beef cattle (**Mir and Mir, 1994**).

Components of the yeast cell wall such as the oligosaccharides glucan and mannan may be beneficial to the local and systemic immune responses (**Murphy et al., 2007**). Furthermore, metabolites produced by *S. cerevisiae* in culture might have antimicrobial activities against pathogens and modulatory effects on the immune system (**Jensen et al., 2007**).

Recently, yeasts have been used as feed ingredients in both humans and animals due to the nutritional value of their nutrients such as proteins, vitamins, pigments, and complex carbohydrates like beta-1,3 glucan and mannan. Mannans and glucans of yeast are potent immunostimulants and also have a good antioxidant activity *in vitro* (**Santacroce et al., 2012**).

This study aimed to evaluate the clinicopathological effect of *Saccharomyces cerevisiae* supplementation as a feed additives in calves, by evaluating its effect on body weight, some selective biochemical parameters and antioxidant parameters as well as some immunological parameters.

## 2. Material and Methods

### 2.1. Experimental animal

Thirty buffalo calves with age 75 days (average body weight 70 kg) of both sexes were used in our study.

### 2.2. Yeast supplements

Yeast (*Saccharomyces cerevisiae*) was purchased from market, as lyophilized powder and stored at 4°C; Sc was dosed at (30 and 60 g once daily /calf for 12 weeks ) dissolved in 0.5 liter of water and given freshly prepared.

### 2.3. Experimental design

The experiment was conducted on thirty buffalo calves with age 75 days (average body weight 70 kg) of both sexes, divided into 3 groups.

as follow:

- Group I (10) animals control group, none treated.
- Group II (10) animals treated with 30 g *Saccharomyces cerevisiae* /calf daily for 12 weeks.
- Group III (10) animals treated with 60 g of *Saccharomyces cerevisiae* /calf daily for 12 weeks.

### 2.4. Collection of blood samples

Blood samples were collected from jugular vein at the end of 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>nd</sup> weeks post treatment with *Saccharomyces cerevisiae*. The samples were put in an inclined position for 20 minutes at room temperature, and then put in refrigerator to avoid glycolysis and for clot retraction. Then the samples were centrifuged at 3000 rpm for 10 minutes then the clear serum samples were separated carefully, collected and stored in Epindorf tubes at -20°C until estimation of serum biochemical parameters.

### 2.5. Body weight detection.

Calves were weighed at the end of 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>nd</sup> weeks post treatment with *Saccharomyces cerevisiae*.

### 2.6. Measurement of liver function markers

Serum AST was determined using readymade kits provided by Randox cat. No (AS 101) according to **Reitman and Frankel (1957)**. LDH using ready made kits provided by (stanbio), according to **Buhl and Jackson (1978)**. Total protein and albumin, were determined using diagnostic kits obtained from (Stanbio laboratory) USA according to **Burtis and Ashwood (1999)** and **Dumas and Biggs (1972)** respectively. Glucose was determined by GOD – PAP method without deproteinization, by using ready made kits provided by Diamond according to **Young (2001)**.

### 2.7. Measurement of kidney function parameters

Uric acid and creatinine were determined using diagnostic kits obtained from Spinrecat, Spain and (Human) Germany, according to **Young, (2001)** and **Szasz et al (1979)**, respectively.

### 2.8. Measurement of antioxidant and oxidative stress markers

#### 2.8.1 Measurement of Malondialdehyde (MDA)

MDA level was determined by enzymatic colorimetric method by using readymade diagnostic kits provided by Bio-diagnostic, Egypt according to **Satoh (1978)**. Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product. MDA was measured spectrophotometrically at 532 nm. The results were expressed as MDA mmol/ml.

#### 2.8.2 Measurement of catalase

Was determined by enzymatic colorimetric method by using ready made kits provided by Alpha according to **Cohen et al., (1970)**. Catalase reacts with a known quantity of H<sub>2</sub>O<sub>2</sub> the reaction is stopped after exactly one minute with catalase inhibitor.

#### 2.8.3. Measurement of Superoxide dismutase (SOD)

SOD was determined by enzymatic colorimetric method by using ready made diagnostic kits provided by Bio-diagnostic, Egypt according to **Nishikimi et al., (1972)**. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate mediated reduction of nitro blue tetrazolium dye. SOD measured spectrophotometrically at 560 nm for 5min. The results were expressed as U/ml.

#### 2.8.4. Measurement of Glutathione (GSH)

GSH was determined by enzymatic colorimetric method by using readymade diagnostic kits provided by Bio-diagnostic, Egypt according to **Beutler et al., (1963)**. The method based on the reduction of 5, 5 dithiobis(2-nitrobenzoic acid) (DTNB) with glutathione (GSH) to produced a yellow compound. The yellow-colored substance formed by the reaction of GSH and DTNB was measured

spectrophotometrically at 412 nm. The results were expressed as GSH mg/dl.

## 2.9. Estimation of some selective humoral immunological parameters

### 2.9.1. Measurement of serum lysozyme activity

Serum lysozyme was determined by the turbidometric assay according to Henry *et al.*, (2000). The lysozyme substrate was 0.75 mg of gram positive bacterium *Micrococcus Lysodeikticus* Lyophilized Cells which was suspended in 1 ml of PBS, pH5.8. In round bottom microtitre plate 25 µl of serum was added to each well with 175 µl of substrate solution at 25°C. The reduction in absorbance at 450 nm was read after 0 and 20 min using microtitre plate ELISA reader. The unite of lysozyme in serum is µg /ml was obtained from lysozyme curve made by Lyophilized Hen egg-White Lysozyme.

### 2.9.2. Measurement of serum bactericidal activity:

Bactericidal activity was determined by using 200 µl of serum or Hank's Balanced Salt Solution for controls were added to 50 µl of suspension live a 24 hr culture of *E. coli 3X10<sup>8</sup>* in round bottom well microtiter plate and incubated at 37°C for 2.5 hours 25 µl diphenyltetrazolium bromide solution (MTT; 2 mg/ml) was added to all well and incubated for 30 min at room temperature to allow the formation of formazan. The supernatant was discarded and the precipitate was dissolved in 200 µl of dimethyl sulfoxide (DMSO). The absorbance of the dissolved formazan was read at 560 nm with spectrophotometer and reported as absorbance units (Fotakis and Timbrell, 2006).

### 2.9.3 Measurement of Nitric oxide (NO)

Nitric oxide is determined by colorimetric determination of nitrite method by readymade diagnostic kits provided by Bio-diagnostic, Egypt according to (Ignarro *et al* 1987). In acid medium and in the presence of nitrite the formed nitrous acid diazotise sulphanilamide and the product is coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has a bright reddish – purple color which can be measured at 540 nm.

## 3. Statistical analysis

Data were analyzed by using statistical software program (SPSS for Windows, version 20, USA). Means and standard error for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. Dissimilar superscript letters in the same column show a significance ( $P < 0.05$ ).

## 4. Results:

### 4.1. The effect of *Saccharomyces cerevisiae* on body weight

As presented in Table -1 the body weight was significantly increased at the end of 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>nd</sup> week post treatment with *Sc* in (Gp- II ) and (Gp- III) comparing by control group. Also at the end of 12<sup>nd</sup> week post treatment with *Sc* there was a Significant increase in body weight in ((Gp- III ) comparing by (Gp- II).

### 4.2. The effect of *Saccharomyces cerevisiae* on serum biochemical parameters

AST&LDH enzymes activities are insignificantly changed in all groups allover the experiment comparing by control one. Meanwhile Glucose concentration is significantly increase in (Gp-III) at 4<sup>th</sup> week comparing by control one and significantly increase in both (Gp- II ) and (Gp- III) at 8<sup>th</sup> and 12<sup>nd</sup> week post treatment (PT). Total protein, albumin and A/G are significantly increase in both (Gp- II) and (Gp-III) comparing by control group but the globulin is insignificantly changed in between all groups at 4<sup>th</sup> week PT. Meanwhile at 8<sup>th</sup> and 12<sup>nd</sup> week PT the <sup>total</sup> protein, albumin, globulin, A/G ratio are insignificantly changed in both (Gp- II ) and (Gp-III) comparing by control group. The uric acid and creatinine levels are insignificantly changed in between all groups till the end of the experiment. Table -2.

### 4.3. The effect of *Saccharomyces cerevisiae* on antioxidant and oxidative stress markers

The level of MDA is significantly decrease in (Gp- III) at the 4<sup>th</sup> and 12<sup>nd</sup> week PT meanwhile it is significantly decrease in (Gp- II) and (Gp- III) at 8<sup>th</sup> week PT. The catalase activity is significantly increase in (Gp- II) and (Gp- III) but SOD activity significantly decrease in (Gp-III) comparing by control group at 4<sup>th</sup> week PT. SOD and catalase activities are insignificantly change at 8<sup>th</sup> and 12<sup>nd</sup> week PT. Also GSH level significantly increase in (Gp- III) comparing by control one at 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>nd</sup> week PT. Table -3.

### 4.4. Immunological result

The level of serum lysozyme is insignificantly changed in between groups at 4<sup>th</sup> and 12<sup>nd</sup> week PT but significantly increased in (Gp- III) comparing by the control one at 8<sup>th</sup> week PT. While serum bactericidal activity is significantly increase in (Gp- II) at 4<sup>th</sup> week PT and in (Gp-III) at 8<sup>th</sup> week PT comparing by control. The serum NO level significantly increases in both (Gp- II) and (Gp- III) comparing by control at 8<sup>th</sup> and 12<sup>nd</sup> week PT. Table-4.

Table (1): Body weight (Kg) (Mean  $\pm$  S.E) of calves treated with *Saccharomyces cerevisiae* for 12 weeks.

Groups	4 <sup>th</sup> week	8 <sup>th</sup> week	12 <sup>nd</sup> week
(GP- I) Cont.	81.57 <sup>b</sup> $\pm 0.97$	94.57 <sup>b</sup> $\pm 0.78$	109.43 <sup>c</sup> $\pm 0.97$
(GP - II) 30g Sc	85.14 <sup>a</sup> $\pm 1.33$	101.29 <sup>a</sup> $\pm 1.14$	118.14 <sup>b</sup> $\pm 0.91$
(GP- III ) 60g Sc	85 <sup>a</sup> $\pm 1.02$	102.57 <sup>a</sup> $\pm 1.06$	122.2 <sup>a</sup> $\pm 1.2$

Table (2): Some selective serum biochemical parameters (Mean  $\pm$  S.E) at the end of 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>nd</sup> week post treatment with *Saccharomyces cerevisiae* in calves.

	Groups	AST U/L	LDH U/L	Glucose mg/dl	T. Protein g/dl	Albumin g/dl	Globulin g/dl	A/G ratio	Uric acid mg/dl	Creatinine mg/dl
At the end of 4 <sup>th</sup> week	GP I (Cont.)	8.38 <sup>a</sup> $\pm 0.47$	258.6 <sup>a</sup> $\pm 7.9$	83.6 <sup>b</sup> $\pm 0.97$	8.2 <sup>b</sup> $\pm 0.33$	3.09 <sup>b</sup> $\pm 0.02$	5.1 <sup>a</sup> $\pm 0.34$	0.61 <sup>b</sup> $\pm 0.042$	14.2 <sup>a</sup> $\pm 2.3$	1.18 <sup>a</sup> $\pm 0.056$
	(GP II) (30g Sc)	9.78 <sup>a</sup> $\pm 0.70$	268.8 <sup>a</sup> $\pm 27.6$	91.6 <sup>b</sup> $\pm 0.67$	9.27 <sup>a</sup> $\pm 0.19$	4.47 <sup>a</sup> $\pm 0.106$	4.7 <sup>a</sup> $\pm 0.16$	0.93 <sup>b</sup> $\pm 0.04$	11.37 <sup>a</sup> $\pm 1.02$	1.03 <sup>a</sup> $\pm 0.086$
	(GP III ) (60g Sc)	8.50 <sup>a</sup> $\pm 0.67$	251.5 <sup>a</sup> $\pm 14.95$	112 <sup>a</sup> $\pm 1.5$	9.5 <sup>a</sup> $\pm 0.34$	4.48 <sup>a</sup> $\pm 0.22$	5.04 <sup>a</sup> $\pm 0.107$	0.89 <sup>a</sup> $\pm 0.04$	9.75 <sup>a</sup> $\pm 0.19$	1.13 <sup>a</sup> $\pm 1.16$
At the end of 8 <sup>th</sup> week	GP I (Cont.)	10.54 <sup>a</sup> $\pm 1.07$	253 <sup>a</sup> $\pm 67.7$	87.3 <sup>b</sup> $\pm 5.4$	8.06 <sup>a</sup> $\pm 0.58$	3.7 <sup>a</sup> $\pm 0.28$	4.3 <sup>a</sup> $\pm 0.39$	0.88 <sup>a</sup> $\pm 0.076$	4.36 <sup>a</sup> $\pm 0.66$	0.99 <sup>a</sup> $\pm 0.01$
	(GP II) (30g Sc)	11.67 <sup>a</sup> $\pm 1.59$	239.26 <sup>a</sup> $\pm 29.7$	101.7 <sup>a</sup> $\pm 3.2$	9.2 <sup>a</sup> $\pm 0.38$	3.8 <sup>a</sup> $\pm 0.21$	5.40 <sup>a</sup> $\pm 0.29$	0.65 <sup>a</sup> $\pm 0.09$	3.7 <sup>a</sup> $\pm 0.36$	1.23 <sup>a</sup> $\pm 0.2$
	(GP III ) (60g Sc)	11.56 <sup>a</sup> $\pm 1.01$	205.76 <sup>a</sup> $\pm 24.3$	109 <sup>a</sup> $\pm 3.39$	8.8 <sup>a</sup> $\pm 0.93$	4.1 <sup>a</sup> $\pm 0.44$	4.7 <sup>a</sup> $\pm 0.65$	0.90 <sup>a</sup> $\pm 0.15$	3.8 <sup>a</sup> $\pm 0.76$	1.20 <sup>a</sup> $\pm 0.84$
At the end of 12 <sup>nd</sup> week	GP I (Cont.)	25.05 <sup>a</sup> $\pm 3.4$	232.6 <sup>a</sup> $\pm 30$	87.6 <sup>b</sup> $\pm 1.6$	10.6 <sup>a</sup> $\pm 0.76$	4.5 <sup>ab</sup> $\pm 0.32$	6.16 <sup>a</sup> $\pm 0.65$	0.65 <sup>b</sup> $\pm 0.89$	7.14 <sup>a</sup> $\pm 1.39$	1.07 <sup>a</sup> $\pm 0.04$
	(GP II) (30g Sc)	19.4 <sup>a</sup> $\pm 7.5$	185.1 <sup>a</sup> $\pm 22.29$	105.4 <sup>a</sup> $\pm 2.02$	9.5 <sup>a</sup> $\pm 0.52$	3.9 <sup>b</sup> $\pm 0.23$	5.23 <sup>a</sup> $\pm 0.67$	.79 <sup>ab</sup> $\pm 1.29$	6.16 <sup>a</sup> $\pm 1.8$	.99 <sup>a</sup> $\pm 0.09$
	(GP III ) (60g Sc)	31.3 <sup>a</sup> $\pm 10.3$	238.4 <sup>a</sup> $\pm 14$	100.4 <sup>a</sup> $\pm 1.02$	9.9 <sup>a</sup> $\pm 0.65$	4.9 <sup>a</sup> $\pm 0.25$	5 <sup>a</sup> $\pm 0.53$	1.02 <sup>a</sup> $\pm 1.02$	6.54 <sup>a</sup> $\pm 2.07$	1.06 <sup>a</sup> $\pm 0.09$

Table (3): Some selective oxidative stress parameters (Mean  $\pm$  S.E) at the end of 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>nd</sup> week post treatment with *Saccharomyces cerevisiae* in calves.

	Groups	MDA nmol/ml	SOD U/ml	CAT U/L	GSH mg/dl
At the end of 4 <sup>th</sup> week	GP I (Cont.)	18.7 <sup>a</sup> $\pm 0.84$	2428 <sup>a</sup> $\pm 45.2$	236.5 <sup>b</sup> $\pm 11.09$	1.06 <sup>b</sup> $\pm 0.075$
	(GP II) (30g Sc)	16.47 <sup>a</sup> $\pm 0.94$	2380 <sup>a</sup> $\pm 56.8$	392.2 <sup>a</sup> $\pm 20.5$	1.14 <sup>b</sup> $\pm 0.033$
	(GP III ) (60g Sc)	8.28 <sup>b</sup> $\pm 0.10$	1860 <sup>b</sup> $\pm 33$	350.8 <sup>a</sup> $\pm 34.1$	1.63 <sup>a</sup> $\pm 0.148$
At the end of 8 <sup>th</sup> week	GP I (Cont.)	20.03 <sup>a</sup> $\pm 0.91$	2097.9 <sup>a</sup> $\pm 174.8$	251 <sup>a</sup> $\pm 36.8$	0.45 <sup>b</sup> $\pm 0.043$
	(GP II) (30g Sc)	17.2 <sup>b</sup> $\pm 1.6$	2247 <sup>a</sup> $\pm 178.9$	244.6 <sup>a</sup> $\pm 33.6$	0.76 <sup>b</sup> $\pm 0.20$
	(GP III ) (60g Sc)	10.2 <sup>c</sup> $\pm 0.89$	2450 <sup>a</sup> $\pm 198$	234.2 <sup>a</sup> $\pm 24$	1.19 <sup>a</sup> $\pm 0.08$
At the end of 12 <sup>nd</sup> week	GP I (Cont.)	19.8 <sup>a</sup> $\pm 1.7$	1997 <sup>a</sup> $\pm 91$	372.9 <sup>a</sup> $\pm 44.3$	0.115 <sup>b</sup> $\pm 0.01$
	(GP II) (30g Sc)	16.7 <sup>a</sup> $\pm 1.08$	2217 <sup>a</sup> $\pm 133$	353.2 <sup>a</sup> $\pm 35.6$	0.116 <sup>b</sup> $\pm 0.01$
	(GP III ) (60g Sc)	10.7 <sup>b</sup> $\pm 0.67$	2040 <sup>a</sup> $\pm 124$	275.6 <sup>a</sup> $\pm 35.4$	0.75 <sup>a</sup> $\pm 0.02$

Table (4): Some selective immunological parameters (Mean  $\pm$  S.E) at the end of 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>nd</sup> week post treatment with *Saccharomyces cerevisiae* in calves.

	Groups	Lysosomal $\mu$ g/mL	Bactericidal activity	NO $\mu$ mol/ l
At the end of 4 <sup>th</sup> week	GP I (Cont.)	0.72 <sup>a</sup> $\pm$ 0.14	4.5 <sup>b</sup> $\pm$ 0.17	4.65 <sup>a</sup> $\pm$ 0.81
	(GP II) (30g Sc)	0.68 <sup>a</sup> $\pm$ 0.15	5.8 <sup>a</sup> $\pm$ 0.37	3.6 <sup>a</sup> $\pm$ 0.62
	(GP III ) (60g Sc)	0.81 <sup>a</sup> $\pm$ 0.13	4.7 <sup>b</sup> $\pm$ 0.37	4.62 <sup>a</sup> $\pm$ 0.50
At the end of 8 <sup>th</sup> week	GP I (Cont.)	0.70 b $\pm$ 0.194	3.17 b $\pm$ 0.144	6.6 b $\pm$ 0.90
	(GP II) (30g Sc)	0.66 b $\pm$ 0.165	3.47 b $\pm$ 0.629	11.7 a $\pm$ 1.22
	(GP III ) (60g Sc)	1.2 a $\pm$ 0.73	6.15 a $\pm$ 0.495	10.4 a $\pm$ 1.2
At the end of 12 <sup>nd</sup> week	GP I (Cont.)	0.53 a $\pm$ 0.11	5.6 a $\pm$ 0.46	5.7 c $\pm$ 2.6
	(GP II) (30g Sc)	0.63 a $\pm$ 0.06	5.7 a $\pm$ 0.33	10.1 b $\pm$ 1.5
	(GP III ) (60g Sc)	0.61 a $\pm$ 0.03	6.4 a $\pm$ 0.17	13.5 a $\pm$ 2.2

## 5. Discussion

Feed additives are useful for dairy producers for improving the nutrition of dairy animals and increased profits when used correctly. Yeast was used only at a lower level as a feed supplement, which was later named as probiotic. It was presumed that the yeast supplemented in diet, as a probiotic multiplied in the gastrointestinal tract and released useful products, which may affect nutrient utilization from the feed. Alternately if the yeast culture (YC) was not able to multiply in the prevailing conditions of the intestine, at least the components of yeast cells may affect the fermentation process favorably (Magalhaes *et al.*, 2008). Yeast culture stimulates desirable microbial growth in the rumen and stabilizes the rumen pH. The ruminal fermentation and end products production can be altered. The increase in nutrient flow post ruminally, nutrient digestibility, and the alleviation of stress through enhanced immune response are other benefits of direct microbial feeding. Also, yeast cultures have biologically valuable proteins combined with high potency vitamin B-complex and important trace minerals (Kamra and Pathak, 2005). In the present study, our result show that *Saccharomyces cerevisiae* has desirable effect on body weight in calves after 4 weeks of treatment and trend continue to increase significantly at 12<sup>nd</sup> weeks in both (Gp- II ) and (Gp-III) which be indicator for improve feed intake, digestibility and feed conversion rate. Increase body weight gain may be due to YC provides soluble

growth factors (i.e., organic acids, vitamin B and amino acids), which would stimulate growth of rumen bacteria that utilize lactate and digest cellulose (Callaway and Martine, 1997). This explanation confirmed by Ismaiel *et al.* (2010) who reported that yeast culture increased average daily gain of lambs might be due to yeast or yeast culture are rich source in vitamins, enzymes and other important nutrients and co-factors which make them attractive as digestive enhancers. Also Hossain *et al.* (2012) mentioned that the increase body weight gain may be due to increase palatability of feed as glutamic acid produced by yeast is responsible for improvement in the taste of feed stuffs and a pleasant odour. Meanwhile Ognik & Krauze (2012) suggested that improved body weight effects are related to the beneficial influence of the components of the cellular wall of *Saccharomyces cerevisiae* yeast on the development, morphology and metabolism indices in turkeys and chickens. As Lecewicz *et al.* (2008) suggested that the changes in the morphology of intestines and metabolic indices resulted in better digestion, better absorption of nutrients in the small intestine, improved specific immunity and increased weight gains. Our result agree with Lesmeister *et al.* (2004) who reported increase in starter, total dry matter intake, average daily gain, daily hip height and hip width changes indicate a positive influence of yeast culture on dairy calf growth, especially at the concentration (2 %YC) daily inclusion level. Also agree with Mousa *et al.* (2012) who reported that

supplementation of live dried yeast to diets of sheep at levels (5 or 7.5 g/head/day) had positive and beneficial effects on enhance digestion and nutritive values, rumen fermentation as well as daily weight gain for lambs These results are in accordance with **komonna (2007)** who found that addition yeast culture for diets of ewes during nursing period resulted in improving its feed utilization and resulted in satisfactory ewe live weight and lamb growth rate.

The AST&LDH enzymes activities are insignificantly changed in all groups allover the experiment which consider an indicator for healthy situation of liver. This result agree with (**Doležal et al., 2010**) who said that AST &LDH concentrations are apparently not connected with the yeast culture supplementation but rather with the diet and with the individuality of cows. Also **Abdel-Khalek et al. (2000)** reported that value of serum AST was not significantly affected by using yeast treatments. While disagrees with **Abu EL-Ella and Kommonna (2013)** who found with pregnant ewes fed diets supplemented with YC increase the activity of serum AST that may be due to several factors such as feeding practices, genetics control, response to stress, age, liver function disorder and body weight.

The serum glucose level is an indicator of physiological condition of the animals. Glucose represents the synthesis of carbohydrates and is in the form in which carbohydrate is supplied to cell from body fluids. In the present study, at end of 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>nd</sup> weeks post treatment with *Saccharomyces cerevisiae* there is a significant increase in serum glucose concentration in all group comparing by control. This result may be related to rapid rate of hydrolysis and absorption of the dietary carbohydrates in alimentary tract that may be resulted from the effect of YC on activity of amylase which lead to increase carbohydrates hydrolysis in the small intestine (**Abdel-Khalek et al., 2000**). Otherwise, this may be attributed to increase the activity of cellulolytic bacteria that act on cellulose fibers degradation and thus produced more glucose and increased the glucogenic precursor propionate in rumen or decreased plasma insulin and insulin-glucose ratio leading to an increase in gluconeogenesis (**Dawson, 1993**).

The serum proteins are considered reliable index reflecting health and performance characteristics of the animals (**Talha et al., 2009**). Our result show significant increase in total protein at end of 4<sup>th</sup> weeks post treatment with *Sc* in all groups. This may be resulted from the increase in digestibility of crud protein (**Yousef and Zaki, 2001**). This confirmed by **Kumar et al. (1980)** who said that, there was a positive correlation between dietary protein and serum protein. Another explanation reported by

**Putnam and Schwab (1994)** who reported that YC stimulates rumen microbes that altered microbial protein synthesis and increased protein passage as well as protein yield. Also **Komonna, (2007) and Mousa et al. (2012)** who found that there was a better utilization of dietary protein through digestive tract after treatment with *Sc*. Serum total protein consists mainly of albumin and globulin. They are the most groups of protein and carry out a wide variety of biological functions. The main function of albumin in blood is to act as a buffer and assist in ion transport and in particular, those of water insoluble vitamins (**Erwin et al., 1961**). At the end of 4<sup>th</sup>, 12<sup>nd</sup> weeks post treatment with *Saccharomyces cerevisiae* there are significant increase in albumin and A/G ratio. This result may attributed to the role of YC as growth promoter on decreasing the deamination of amino acids As albumin acts as a significant mobile protein store for amino acids (**El-Ashry et al., 2003**). This result agree with **Abu El-Ella and Kommonna, 2013** who reported that, the significant increase in blood albumin suggested normal status of liver function, since liver is the main organ of albumin synthesis. Also The obtained results are in accordance with those reported by **El-Shaer (2003)** who mentioned that The increase of albumin in response to YC supplementation may be associated with improved nitrogen absorption. Our result show insignificant change in globulin level at end of 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>nd</sup> week post treatment in treated groups comparing by control one. This result agree with (**Mousa et al., 2012**).

The serum creatinine level is a useful indicator of glomerular filtration in the kidney, creatinine is the main metabolite of skeletal muscles, resulting from metabolic changes that occur in muscle (**Ognik and Krauze, 2012**). In our result uric acid and creatinine level are insignificantly change in all groups comparing by control along the treatment with *Saccharomyces cerevisiae*. Our result agree with **Mousa et al. (2012)** and **Darwish et al. (2011)** as treatment with *Sc* showed insignificant change in creatinine and uric acid levels.

The body has an effective defense mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzyme such as SOD, CAT and GPx, which constitute a supportive team of defense against ROS (**Gowri et al., 2008**). In the present study at end of 4<sup>th</sup> weeks post treatment with *Sc* SOD level significantly decrease in (Gp- II) comparing by control. This result may be due to increase ROS (reactive oxygen species) production as Glucans from yeast may enhance the phagocytic activity and increase the production of reactive oxygen metabolites by macrophages and this could be responsible for the observed decrease in SOD activity

(Santacroce *et al.*, 2011). Catalase is an enzyme responsible for reducing hydrogen superoxide to water and oxygen. In the present study catalase activity at the end of 4<sup>th</sup> weeks post treatment with *Sc* show significant increase in (Gp- II) and (Gp- III) comparing by control group and insignificantly change till the end of the experiment. the increase in the activity of catalase in the blood may caused by environmental burdens to which animals are exposed during their growth. As Czech *et al.* (2006) recorded an increase in catalase during pregnancy of sheep fed a feed mixture enriched with mannanoligosaccharides (Bio-Mos) which isolated from the cellular wall of baker's yeast as *Saccharomyces cerevisiae* limits the intensity of pathological conditions owing to the elimination of pathogenic agents and the fighting of intestinal infections, and thus reducing the stimulation of antioxidation processes.

MDA is an end product of lipid peroxidation, and it is considered a late biomarker of oxidative stress and cellular damage. The Oxidative stress arises when the generation of ROS by-products of the oxidative metabolism primarily produced in the mitochondria, exceeds the cellular ability to eliminate them and to repair cellular damage, thus leading to oxidation of biomolecules including DNA, lipids and proteins (Ognik and Krauze, 2012). Our study appear significant reduction in MDA in all groups comparing by control at end of 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>nd</sup> weeks post treatment with *SC*, this result agree with Darwish *et al.* (2011) who found that *Sc* treatment alone in mice resulted in significant reduction in the level of MDA in the kidney tissues and disagree with Ognik and Krauze (2012) who reported that Bio-Mos (mannanoligosaccharides which isolated from the cellular wall of baker's yeast) did not increase the concentration of Malonedialdehy. In our study GSH level has significant increase in (Gp-III) comparing by control at the end of 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>nd</sup> weeks post treated with *SC*. This result agree with Darwish *et al.* (2011) who reported that dietary yeast stimulates both immune and antioxidant responses in juveniles of gilthead sea bream and in leopard grouper *Mycteroperca rosacea* after exposure to pathogens. Lysozyme is mainly secreted by phagocytes and is a nonspecific immune effector. In our work there is a significant increase in serum lysozyme activity observed at the end of 8<sup>th</sup> weeks post treatment with *Saccharomyces cerevisiae*. As (Gao *et al.*, 2009) found an increase in serum lysozyme levels in (*Sc*) treated birds as a result of more phagocytes were activated with the inclusion of (*Sc*). Therefore, (*Sc*) may enhance the nonspecific immunity of birds at some levels. Also there is a positive effect of *Sc* on the serum lysozyme activity exhibited a linear response in the birds irrespective of the infection.

Although at end of 4<sup>th</sup>, 12<sup>nd</sup> weeks post treatment *Sc* serum lysozyme level insignificantly changed and this agree with Peng and Delbert (2003) who observed that fish (hybrid striped bass) fed the diet with 2% brewers yeast showed no significant differences in serum lysozyme level in between treated groups. At the end of 4<sup>th</sup> and 8<sup>th</sup> weeks post treatment with *SC* there is significant increase in bactericidal activity in (Gp- II) and (Gp- III) respectively comparing by control one, while at the end of 12<sup>nd</sup> weeks no significant changes was observed. Nitric oxide in the present study doesn't show any insignificant changes at the end of 4<sup>th</sup> weeks, Meanwhile there is significant increase at the end of 8<sup>th</sup> and 12<sup>nd</sup> weeks post treatment with *SC* and this result agree with Jung *et al.*, 2004 and disagree with El-Boshy *et al.*, 2010).

#### 6. Conclusion:

Our results indicate that feed supplementation with *Saccharomyces cerevisiae* in the calves has desirable effects on the body weight, serum antioxidant enzymes and some immunological parameters especially the high dose of *Saccharomyces cerevisiae*. Moreover *Saccharomyces cerevisiae* treatment has no toxic effect on kidney or liver functions.

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