

EFFECT OF YEAST-BASED PROBIOTICS SUPPLEMENTATION ON THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF LACTATING BUFFALOES

N.E. El-Bordeny¹, A. Abdou¹, H. Abo-Eid², M.M. Abdo³ and R.M.A. Abdel-Gawad³

¹*Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, 11241, Egypt.*

²*Sustainable Development Department, Environmental Studies and Research Institute, University of Sadat City, Sadat City, Menofiya, 32897, Egypt.*

³*Dairy Science Department, National Research Centre, Dokki, Cairo, 12311, Egypt.*

Crossponding author: ramadangawad2@gmail.com

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SUMMARY

The present study was carried out to investigate the effect of yeast-based probiotics (YBP) supplementation on the productive and reproductive performance of Egyptian buffaloes. Twenty multiparous lactating Egyptian buffaloes, ranged from 2nd to 5th season of lactation, were randomly assigned into 2 groups (10 each). The buffaloes were fed a total mixed ration without or with YBP supplementation. The experimental period started two weeks before expected calving date and extended for three months after parturition. Milk yield, milk composition, blood parameters, and some reproductive parameters were measured. The results indicated that colostrum composition was not significantly ($P>0.05$) affected by YBP supplementation. Actual milk yield, 4% FCM and ECM were significantly ($P<0.05$) increased by YBP supplementation group comparing to control. Total solid, solid not fat, fat and lactose yield were significantly ($P<0.05$) increased for YBP group compared to control. However, milk protein percentage obtained from control group was significantly ($P<0.05$) higher than YBP supplemented group. No significant differences ($P>0.05$) between the two groups in blood metabolites were shown. For reproductive performance, period up to 1st estrus was decreased from 88.5 to 55.7 days for treated group comparing to control. Also, the service period was decreased by YBP supplementation from 224.6 days for control group to 150.4 days for treated group. Further, an improvement on fertility rate was recorded for YBP supplemented group (90%) comparing to control (60%). In conclusion, ration supplementation with YBP had beneficial effects on milk yield and milk composition yield as well as the general reproductive performance of buffaloes with no adverse effects on general animals health.

Keywords: *Lactating buffaloes, probiotics, yeast, milk yield, blood parameters, and reproductive parameters.*

INTRODUCTION

Antibiotics have been used for many years in animal feed as additives to overcome some of the health problems and improve feed utilization and productivity of animals. However, due to increasing safety concerns regarding the risks of antibiotics resistance and chemical residues in animal derivative products (Martínez *et al.*, 2014; Yamamoto, *et al.*, 2014 and Diaz *et al.*, 2018), probiotic additives (bacterial and yeast) have been developed as growth promoters to replace antibiotics and synthetic chemical feed supplements, to improve animal health and productivity (Allen and Ying, 2012; Uyeno *et al.*, 2015 and Dabiri *et al.*, 2016).

Probiotics are defined as cultures of live microorganisms, or nonviable probiotics including cultural extracts, enzyme preparations, or combinations of these, that have health benefits to the host (Sanders, 2008, Poppy *et al.*, 2012, Ezema, 2013 and Suarez, and Guevara, 2018). Several of microorganisms have been reported as probiotics that are used in diet of ruminants to upgrade feed utilization and animal performance (Grochowska, Nowak, Mikula, and Potocka, 2012). Different studies have been reported that

bacterial probiotics give better results in young calves and chickens, whereas yeast and fungal probiotics have an effective action with adult ruminants (Musa *et al.*, 2009 and Shakira *et al.*, 2017).

Although studies on bacterial probiotics were increasing, the most commonly used probiotics in ruminants feeding are based on yeast preparation of *Aspergillus oryzae* and/or *Saccharomyces cerevisiae* (Chiquette, 2009). The yeast-based probiotics (YBP) received the Generally Recognized as Safe (GRAS) status from Food and Drug Administration (FDA), so it can be used in animal feeds. (Shakira *et al.*, 2017). Probiotic yeast has a good advantage is that yeast doesn't has antibiotic resistance gene. It also has ability to colonize in the gastro-intestinal tract (GIT), to neutralize enterotoxin, and to tolerate bile salt and gastric acid, leading to improving the health status and dairy animals productivity (Chiquette, 2009 & Shakira, *et al.*, 2017).

The main action of yeast culture supplementation to ruminants diets include improvement of gut health and ecology through rumen maturity by favoring microbial establishment (Dabiri *et al.* 2016). Also, YBP have a prebiotic action by providing vitamins and organic acids to support and stimulate the growth of rumen fungi (Mao *et al.*, 2013), rumen protozoa (Kumar *et al.*, 2013), and cellulolytic bacteria (Hristov *et al.*, 2013). Moreover, stabilization of rumen pH (Musa *et al.*, 2009, Grochowska *et al.*, 2012 and Diaz *et al.*, 2018) and interaction with ammonia and lactate utilizing bacteria were reported (Dawson, 1992, Yang *et al.*, 2004 and Chaucheyras *et al.*, 2012). These effects of using yeast-based probiotics are leading to increase fiber digestion, protein synthesis in the rumen, and thereby, animal productivity (Hillal *et al.*, 2011 and Shakira *et al.*, 2017). Limited studies were concerned to investigate the association between probiotic yeast and ruminants reproductive performance (Zouagui *et al.*, 2017). Some of studies reported that certain strains of bacteria and yeasts have parietal structures capable of binding to mycotoxins, Zelaronen in particular, which has a positive effect on animals reproductive performance (Jouany and Morgavi, 2007 and Zaleska *et al.*, 2015).

However, buffaloes are considered the main producing animal for milk in Egypt and other countries, the effect of probiotics on its productive and reproductive performance was not extensively studied. The main objective of this study is evaluating effect of YBP supplementation on the productive and reproductive performance of lactating buffaloes.

MATERIALS AND METHODS

The present study was carried out at Al-Eman farm for animal production, Al-Nobaria - Al-Behira Governorate and the laboratories of the Dairy Science Department, National Research Centre, Dokki, Giza, Egypt.

Animals, diets, feeding and experimental design:

Twenty multiparous lactating Egyptian buffaloes (2-5 lactation seasons) with an average live body weight of 519.0 ± 18.0 kg, were randomly assigned into two groups (10 buffaloes each). The experimental animals started to get their experimental feed approximately 15 days before expected calving date and continued until the day 90 after parturition. The buffaloes were fed a total mixed ration (TMR, Table 1) without or with 10g/h/day YBP. The commercial YBP product, based on a *Sacharomyses cerevisiae*¹⁰²⁶ strain has a 1×10^9 cfu/g as a minimum concentration, as well as vitamin complex and minerals (AlltechInc, Lexington, KY, USA). The rations were formulated to cover the energy and protein allowances according to Paul *et al.* (2002). The ration was offered twice daily at 08:00 and 17:00 and the animals had continuous access to clean fresh water.

Sampling:

Samples of the TMR were collected biweekly, pooled and dried at 55°C for 48 hours, then ground in a Wiley mill to pass a 1 mm screen, then, stored for subsequent analysis. Body weights, daily milk yields (MY) and milk composition were recorded at 15, 30, 45, 60, 75 and 90 days in milk (DIM). During the first four-days after parturition, samples of colostrum were hand milked at time of morning suckling. After the first week, the buffaloes were milked two times daily at 03:00 and 16:00 using the DeLaval milking units. Milk samples were composed for each animal, which milk from the morning and evening milking was mixed according to the relative production and stored in a refrigerator (+4°C) until chemical analysis.

Blood samples were taken from seven experimental animals from each group monthly up to three months' post parturition. A 10 ml blood sample was withdrawn from jugular vein directly into a clean dry, glass tube 3 h post morning feeding. Blood serum samples were obtained by centrifuging blood samples 2 h after sampling at 4000 rpm for 15 min, then, stored at -20°C in a clean, dry, glass vials up to subsequently analysis.

Table (1): Ingredient and chemical composition of experimental total mixed ration.

Ingredient (g/kg)	Control
Berseem	757
Rice straw	60
Yellow corn	100
Soybean meal	40
Wheat bran	23
Sunflower meal	10
Calcium carbonate	7
Minerals and Vitamins ^a	3
Chemical composition (g/kg DM)	
Dry matter	901.1
Organic matter	896.6
Crude protein	168.5
Ether extract	37.2
Crude fiber	235.9
Nitrogen free extract	455
NE _L (Mcal/kg DM) ^b	1.5

^a Contained 141 g/kg of Ca, 27 g/kg of P, 65 g/kg of Mg, 14 g/kg of S, 120 g/kg of Na, 6 g/kg of K, 944 mg/kg of Fe, 1613 mg/kg of Zn, 484 mg/kg of Cu, 17.48 mg of Mn, 58 mg/kg of I, 51 mg/kg of Co, 13 mg/kg of Se, 248,000 U/kg of vitamin A, 74,000 U/kg of vitamin D3 and 1656 IU/kg of vitamin E.

^b Calculated using published values of feed ingredients (NRC, 2001).

Reproductive performance:

Estrus detection was applied by using teaser bull every day, followed by natural insemination for the animal in heat and the data were recorded for each animal. Service per conception, first estrus period, service period, first conception rate and fertility rate were recorded.

Chemical analysis and calculations:

The samples of TMR were analyzed in triplicate to its content of DM, ash, crude protein (CP), crude fiber (CF) and ether extract (EE) according to AOAC (2000.). Blood biochemical analysis was determined using commercial kits (Spectrum, Spain).

Milk samples were analyzed for total solids, fat, total protein and lactose by infrared spectrophotometer (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark). Fat corrected milk (4% fat) and energy corrected milk (ECM) was calculated as follow.

$$4\% \text{ FCM} = 0.4 \text{ milk yield (gm)} + 15 \text{ fat yield (gm)} \text{ (Gaines, 1928).}$$

$$\text{ECM} = 0.327 \times \text{milk yield (kg)} + 12.95 \times \text{fat yield (kg)} + 7.20 \times \text{protein (kg)} \text{ (Tyrrell, and Reid, 1965)}$$

Statistical analysis:

The obtained data were statistic analyzed according to statistical analysis system (SAS, 2003). Data of milk yield, milk composition, milk content yield, feed efficiency, and reproductive efficiency were analyzed using student's t-test (Snedecor, and Cochran, 1994). Whereas, data of colostrum and blood parameters were statistically analyzed using the general linear model (GLM) procedure (model1 and model 2) of SAS (2003) according to the following models:

$$Y_{ijk} = \mu + t_i + d_j + (t*d)_{ij} + e_{ijk} \text{ (model 1),}$$

$$Y_{ijk} = \mu + t_i + a_{j(i)} + d_k + (t*d)_{ik} + e_{ijkl} \text{ (model 2)}$$

Where, Y_{ijk} and Y_{ijkl} : observations, μ : the overall mean, t_i : effect of treatment, d_j , d_k : effect of days, $a_{j(i)}$: effect of animal within treatment, $(t*d)_{ij}$ and $(t*d)_{ik}$: the interaction between treatment and days, e_{ijk} and e_{ijkl} : the experimental error. Duncan's Multiple Range Test (Duncan, 1955) distinguished the differences among means.

RESULTS AND DISCUSSION

Colostrum composition:

The chemical composition analysis of colostrum results are presented in Table (2). Given these results, the colostrum composition was not significantly ($P>0.05$) affected by YBP supplementation, however, it

Table 2

was significantly ($P < 0.001$) affected by progressing days with either control and treatment. Colostrum TS, TP and SNF were significantly ($P < 0.05$) and gradually decreased with days' progress from the 1st day to the 4th day post parturition (Table 2). The absence of probiotic yeast effect on the chemical composition of colostrum may be attributed to the short time of treatment before parturition. Different studies stated that probiotics have beneficial health effect and this is, partly, attributed to the ability of probiotics, bacteria and yeast, to modulate the immune system, increasing either innate and adaptive immune response (Matsuzaki, and Chin, 2000; Dawson, 1992; Pagnini *et al.*, 2010) which it contrasted with increasing immunoglobulins in colostrum and thereby, TP and TS, but this effect was not clear in the recent study. Various in-vivo and in-vitro studies have demonstrated that different probiotic bacteria including different strains of *Lactobacillus casei*, *Streptococcus thermophilus*, *Lactobacillus fermentum* and yeast have been tested to promote gut health via stimulation of the innate immune response (Matsuzaki, and Chin, 2000, Galdeano and Perdigon, 2006).

It is known that colostrum has a high portions of immune globulins content on birth day and gradually decreased with time progress up to getting milk with its known nature. In this connection, Georgiev (2005) found that concentration of colostrum total solid and proteins were decreased in the 3rd day compared to the 1st day after parturition. The present results are similar to the findings of Macedo *et al.* (2012) who reported that colostrum composition was not affected with yeast culture addition but was basically affected by the time from parturition.

Milk yield, milk composition, and blood parameters:

The Results of the actual milk yield, 4% FCM and ECM obtained from YBP supplemented group were significantly ($P < 0.05$) higher than control group (Table 3). Milk yield and 4% fat corrected milk were increased by 16.3% and 16.9%, respectively by YBP supplementation than control. In this study, milk composition was not significantly ($P > 0.05$) affected by YBP supplementation except total protein content from control group which was significantly ($P < 0.05$) higher than YBP supplemented group. As a result of increasing milk yield, milk composition yield was also significantly ($P < 0.05$) increased by YBP supplementation.

Table (3): Effect of yeast culture supplementation on milk yield and composition, milk content yield and feed efficiency of lactating buffaloes.

Items	Control	YBP	±SE	P value
Body weight changes				
Initial weight, kg	493	545	24.65	0.15
Final weight, kg	521	570.1	25.18	0.19
Body weight changes, kg/d	0.384	0.335	0.064	0.59
Milk yield, kg/d				
Actual milk yield, kg/d	7.78 ^b	9.05 ^a	0.384	0.03
4% FCM, kg/d	11.10 ^b	12.98 ^a	0.562	0.03
ECM, kg/d	11.93 ^b	13.86 ^a	0.596	0.03
Milk composition%				
Fat, %	6.86	6.90	0.09	0.75
Protein, %	4.44 ^a	4.34 ^b	0.029	0.02
Lactose, %	4.66	4.76	0.086	0.42
TS, %	16.75	16.78	0.043	0.65
SNF, %	9.90	9.88	0.094	0.91
Milk content yield (g/d)				
Fat, g/d	533 ^b	624 ^a	27.5	0.03
Protein, g/d	346	392	16.8	0.07
Lactose, g/d	363 ^b	429 ^a	320.7	0.03
SNF, g/d	771 ^b	893 ^a	39.7	0.04
TS, g/d	1304 ^b	1517 ^a	65.3	0.03
Feed efficiency				
kg MY/kg DM intake	0.607	0.693	0.0370	0.98
kg FCM/kg DM intake	0.864	0.991	0.0500	0.09
kg ECM/kg DM intake	0.929	1.062	0.0542	0.10

Means sharing the same letter, within a row, do not differ significantly from each other at $P \leq 0.05$.

supplement than control group. Also, the results presented in Table (3) showed that the feed efficiency was enhanced by YBP supplementation, however, the differences were not significant ($P>0.05$). In this study, milk and FCM yield were improved by supplementing YBP to buffaloes diets. In a similar study, milk yield was increased by 23% for dairy cows supplemented yeast probiotics two weeks peripartum and for six weeks postpartum (Ayad *et al.*, 2013). The increasing milk yield during this stage, where energy reserves are heavily used to support milk production, can be explained that cows supplemented with yeast can maintain weight and body condition better than controls, leading to lower mobilization of endogenous reserves of cows supplemented with yeast, meaning that a greater availability of energy for milk production. Different studies also reported a good response in milk yield (Rossow *et al.*, 2014 and Bernard, 2015), but it was a relatively lower response (3-9%) in other studies (Bernard, 2015). Responses to YBP supplementation to lactating animals are depending on several factors, such as stage of lactation, age, DMI, feed composition, and probiotic supplementation dose (Desnoyers *et al* 2009; Nocek, Holt, and Oppy, 2001; Ayad *et al.*, 2013 and Rossow *et al.*, 2014). The results of milk composition in this study are in agreement with Nour (2015) who reported that yeast culture supplementation to lactating animal ration led to improve milk yield without any significant effect on milk composition. Several studies reported that the increase in milk production induced by dietary supplementation with *Saccharomyces cerevisiae*, is not always associated with changes in protein and fat content of milk (Vandehaar *et al.*, 1999; Ayad *et al.*, 2013). Nevertheless, an increase of fat in the milk of cows fed the probiotic yeast was reported by Piva *et al.* (1999) and Putnam *et al.* (1997). The increasing in milk fat % in these studies may be due to that yeast is associated with a positive effect of the stimulation of cellulolytic bacteria, and a preferred orientation of fermentation to acetic acid, the main precursor of milk fat synthesis, production. As a result of increasing milk yield, milk composition yield was also significantly ($P<0.05$) increased by probiotic yeast supplement than control group. In agreement with this, Helal and Abdel-Rahman (2010) reported that the buffaloes fed yeast culture supplemented diets produced, significantly ($p<0.05$), more fat, protein, lactose, SNF and TS yields compared to those fed the control ration. Feed efficiency results presented in Table (3), declared that feed efficiency was improved by YBP supplementation to animals diets but the differences were not significant ($P>0.05$). In consistent with that, some studies with lactating animals indicated no significant response in feed efficiency by adding yeast culture (Yalcin *et al.*, 2011; Nour, 2015).

The data of Table (4) showed that total blood serum proteins, total albumens, globulin concentration, AG ratio, urea, and creatinine concentrations were not significantly ($P>0.05$) affected by YBP supplementation and days of sampling. The results indicated that the experimental animals were not in a catabolism situation and kidney function was not adversely affected by YBP supplementation. The present values of AST and ALT activity reflected normal activity of the animal hepatic tissues. Consequently, YBP supplementation in the present investigation had no an adverse effect on the liver function, expressed by levels of AST and ALT enzymes, which they are considered the most important indicator for liver activity (Maxine, 1984).

The present results are in agreement with Maxine (1984), Ayad *et al.* (2013) and Azzaz *et al.* (2015) whom reported that glucose, urea, cholesterol, total protein and albumin in blood serum were not affected by yeast culture supplementation. While, Abou-Elenin *et al.*, (2011) found that yeast culture supplementation had a significant effect on some blood metabolites in lactating cows. The differences between the results of different studies may be due to the effect of lactation stage, environmental conditions, diet composition, forage type and dose and type of supplemented yeast.

Reproductive performance:

Data of Table (5) showed nonsignificant ($P>0.05$) reduction in number of service per conception (8.15%), 1st estrus period (37.05%) and service period (33.04%) for the group supplemented by YBP compared to control group. Moreover, the data showed no difference in 1st estrus conception rate which was 20% for both groups, while, a great improvement in fertility rate was recorded for YBP supplemented group (90%) compared to the control group (60%). The results indicated that the reproduction performance was improved, however, the differences were not significant ($P>0.05$). The non-significantly results may be due to the low number of the experimental animals parallel to the high variation within each group. It was noticed that the service per conception in this study was highest (2.23 ± 1.17) compared to other system ranged from 1.78 ± 0.32 to 1.76 ± 0.42 service/animal (Meena *et al.*, 2016). This may be due to un-identification of heat, post-partum complication in the buffalo and may also indicative of poor post-partum management.

Moreover, the data showed that the service period of buffalo supplemented by YBP in this study (150.43 ± 19.48) were lower than that obtained by Meena *et al.* (2016) which were ranged from 189 ± 15 to 199 ± 18 days / animal, respectively but higher than that recorded by Jamuna *et al.* (2013) which the average service period was 139.91 ± 2.96 days for Murrah buffalo. The improvement of reproductive

Table 4

performance in the recent study may be explained that certain strains of bacteria and yeasts have parietal structures capable of binding to mycotoxins, Zearalenone in particular (Jouany and Morgavi, 2007 & Zouagui *et al.*, 2017). Zearalenone is a major toxin produced by the *Fusarium* molds and its chemical structure is similar to that of the estrogen hormones and this chemical structure is well known by its estrogenic activities (Jamuna *et al.*, 2013) so, it causes some reproductive disorders and various modifications at the genital organs, mainly when its concentration in feed is near to 400 ppb (Whitlow and Hagler, 2001; Sporsen and Towers, 1995 & Towers, Sprosen, and Webber, 1995).

Some of studies reported an improvement in reproductive performance for Friesian cows (Abdel-Khalek, 2003 & Zaleska *et al.*, 2015) and Egyptian buffaloes (Ibrahim, 2004), supplemented yeast culture on their diets. While, Kalmus *et al.* (2009) found that yeast culture supplementation had no effect on post-partum metabolic status, bacterial elimination from the uterus nor the resumption of ovarian activity were found in the treated cows.

Table (5): Effect of yeast culture supplementation on some reproductive parameters of lactating Buffaloes.

Item	Control	YBP	P value
Number of animal	6	7	
Service per conception	2.33 ± 0.61 ^a	2.14 ± 0.34 ^a	0.78
Period up to 1 st estrus, day	88.5 ± 35.24 ^a	55.71 ± 4.37 ^a	0.34
Service period, (day)	224.67 ± 64.29 ^a	150.43 ± 19.48 ^a	0.26
1 st service conception rate, %	20	20	
Fertility, %	60	90	

Means sharing the same letter, within a row, do not differ significantly from each other at P≤0.05.

Moreover, Bruno *et al.* (2009) did not find any effect of using live *Saccharomyces cerevisiae* cultures, did not find any effect on the ovulation cycle, efficacy of insemination or the number of abortions. Allbrahim *et al.* (2010) observed a higher pre-ovulatory surge of estradiol in cows administered live yeast, although this supplement did not affect the size of the ovulatory ovarian follicles.

CONCLUSION

Based on the findings of this study, it can be concluded that yeast-based probiotic (YBP) supplementation has a potential to being a good alternative for antibiotics, feed supplement and used for different purposes, as it has a probiotic and prebiotic properties, to improve lactating buffaloes production and reproduction performance.

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تأثير إضافة البروبيوتك المكون من الخمائر علي الاداء الإنتاجي و التناسلي للجاموس الحلاب

نصر السيد البرديني¹ ، عاصم عبده¹ ، حسني أبو عيد² ، محمد محمود عبده³ و رمضان محمد أحمد عبد الجواد³

¹قسم الانتاج الحيواني- كلية الزراعة- جامعة عين شمس- شبرا الخيمة- محافظة القليوبية- مصر.

²قسم التنمية المستدامة- معهد الدراسات و البحوث البيئية- جامعة مدينة السادات- مدينة السادات- محافظة المنوفية- مصر.

³قسم الألبان- شعبة الصناعات الغذائية و التغذية- المركز القومي للبحوث- الدقي- القاهرة- مصر.

أجريت هذه الدراسة علي الجاموس المصري لدراسة تأثير إضافة المحفزات الحيوية المكونة من الخمائر علي الأداء الإنتاجي و التناسلي. تم استخدام عشرون حيوان من الجاموس المصري في موسم الحليب من الثاني الي الخامس و تم تقسيمها عشوائيا الي مجموعتين (10 حيوانات بكل مجموعة). المجموعة الأولى و هي المجموعة الضابطة و تم تغذيتها علي عليقة المزرعة بدون اي اضافات. المجموعة الثانية و تم تغذيتها علي عليقة المزرعة مع إضافة المحفزات الحيوية المكونة من الخمائر. بدأت التجربة قبل الولادة بأسبوعين و استمرت ثلاث شهور بعد الولادة. تم تسجيل انتاج اللبن اليومي و قياس المكونات الكيميائية للسرسوب و اللبن و بعض مقاييس الدم و بعض المقاييس التناسلية. أظهرت النتائج المتحصل عليها ان التركيب الكيميائي للسرسوب لم يتأثر معنويا باستخدام المحفزات الحيوية. زاد انتاج اللبن و انتاج اللبن المعدل نسبة الدهن (4%) و اللبن المعدل للطاقة زيادة معنوية باستخدام المحفزات الحيوية المكونة من الخمائر. زادت كمية الجوامد الكلية و الدهن و اللاكتوز زيادة معنوية في المجموعة المعاملة عن المجموعة الضابطة و حدث العكس مع كمية بروتين اللبن. مكونات الدم لم تتأثر معنويا بإضافة المحفزات الحيوية الي علائق الجاموس المصري. أدي استخدام المحفزات الحيوية في علائق الجاموس الحلاب الي انخفاض متوسط الفترة حتي اول شياح من 88.5 الي 55.7 يوم للمجموعة المعاملة مقارنة بالمجموعة الضابطة. أيضا انخفضت الفترة حتي التلقيح المخصبة من 224.6 للمجموعة الضابطة الي 150.4 للمجموعة المعاملة. ارتفعت نسبة الاخصاب الي 90% للمجموعة المعاملة مقارنة بالمجموعة الضابطة (60%). خلصت هذه الدراسة الي إمكانية استخدام الخمائر كمحفزات حيوية لتحسين الاستفادة من التغذية و تحسين الاداء الإنتاجي و التناسلي للجاموس المصري الحلاب بدون أي تأثيرات سلبية علي الصحة العامة للحيوانات. يجب تكرار التجربة علي عدد أكبر من الحيوانات و في فترات انتاجية مختلفة لتأكيد النتائج.

Table (2): Effect of yeast culture supplementation and sampling day on milk colostrum composition of lactating buffaloes.

Item	Day 1		Day2		Day3		Day4		SE	P value		
	Control	YBP	control	YBP	Control	YBP	Control	YBP		Treatment	Day	Interaction
Total solid, %	28.51 ^a	28.72 ^a	24.48 ^b	24.76 ^b	19.58 ^c	20.08 ^d	18.63 ^e	18.70 ^e	0.10	0.13	0.00	0.19
Total protein, %	14.55 ^a	14.20 ^b	10.50 ^c	10.30 ^c	6.60 ^d	6.71 ^d	5.13 ^e	5.15 ^e	0.11	0.34	0.00	0.14
Lactose, %	5.14 ^b	5.46 ^{ab}	5.80 ^{ab}	6.03 ^a	5.32 ^{ab}	5.58 ^{ab}	5.89 ^a	5.98 ^a	0.18	0.34	0.00	0.93
Fat, %	8.01 ^{ab}	8.27 ^a	7.35 ^{cd}	7.68 ^{bc}	6.85 ^{de}	7.00 ^{de}	6.81 ^{de}	6.75 ^e	0.14	0.43	0.00	0.53
Solid not fat, %	20.49 ^a	20.45 ^a	17.12 ^b	17.09 ^b	12.73 ^c	13.08 ^c	11.81 ^d	11.94 ^d	0.15	0.68	0.00	0.51
Ash, %	0.80 ^a	0.87 ^a	0.82 ^a	0.76 ^a	0.81 ^a	0.78 ^a	0.80 ^a	0.81 ^a	0.03	0.17	0.92	0.52
Gross energy, kcal	1772.74 ^a	1789.99 ^a	1510.83 ^b	1538.05 ^b	1223.78 ^c	1254.84 ^c	1161.02 ^d	1160.43 ^d	8.98	0.19	0.00	0.30

Means sharing the same letter, within a row, do not differ significantly from each other at P≤0.0

Table (4): Effect of yeast culture supplementation and sampling day on some blood metabolites of lactating Buffaloes.

<i>Item</i>	Day 30		Day60		Day90		P value		
	Control	YBP	Control	YBP	Control	YBP	Treatment	Day	Interaction
Total protein, g/dL	7.10 ^a ±0.14	7.37 ^a ±0.14	7.20 ^a ±0.15	7.36 ^a ±0.14	7.46 ^a ±0.15	7.20 ^a ±0.14	0.67	0.68	0.05
Albumin, g/dL	3.64 ^a ±0.35	3.48 ^a ±0.35	3.94 ^a ±0.37	3.86 ^a ±0.35	3.92 ^a ±0.37	3.41 ^a ±0.35	0.62	0.60	0.78
Globulin, g/dL	3.46 ^a ±0.32	3.88 ^a ±0.32	3.25 ^a ±0.35	3.50 ^a ±0.32	3.54 ^a ±0.35	3.79 ^a ±0.32	0.46	0.53	0.88
A/G Ratio	1.12 ^a ±0.20	1.19 ^a ±0.20	1.24 ^a ±0.22	1.22 ^a ±0.20	1.17 ^a ±0.22	1.00 ^a ±0.20	0.96	0.73	0.86
Creatinin, mg/dL	1.00 ^a ±0.17	1.12 ^a ±0.19	0.83 ^a ±0.18	1.13 ^a ±0.17	0.82 ^a ±0.17	0.62 ^a ±0.18	0.78	0.08	0.15
Urea, mg/dL	58.09 ^a ±9.79	43.53 ^a ±9.79	47.20 ^a ±10.46	48.11 ^a ±7.79	42.96 ^a ±9.79	46.27 ^a ±9.79	0.80	0.70	0.41
AST, unit/L	36.54 ^a ±6.96	49.97 ^a ±7.44	46.33 ^a ±7.44	55.15 ^a ±6.96	43.85 ^a ±7.44	41.80 ^a ±6.96	0.55	0.38	0.34
ALT, unit/L	130.54 ^a ±4.61	122.02 ^a ±4.61	130.43 ^a ±4.92	134.18 ^a ±4.61	131.49 ^a ±4.92	129.61 ^a ±4.61	0.56	0.40	0.39

Means sharing the same letter, within a row, do not differ significantly from each other at P≤0.0