

## EFFECT OF USING SOME FEED ADDITIVES (TW- PROBIOTICS) IN DAIRY COW RATIONS ON PRODUCTION AND REPRODUCTIVE PERFORMANCE

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### SUMMARY

This study aimed to evaluate the effects of supplementing commercial yeast culture (*S. cerevisiae*) namely BGY 35 or a product of lactic acid bacteria and enzymes namely AVI-BAC® to the diet of lactating crossbred cows, for the last 2 months pre-partum and the first 4 months post-partum (from July to October), on body weight, feed and water intakes, milk production, some blood parameters, reproductive performance and physiological response. A total of 12 cows with average live body weight (LBW) of 440 kg and 2-6 parities, were used in this study. Cows were divided into three groups, 4 animals in each group. During pre- and post-partum period, cows in the 1<sup>st</sup> group (G1) were fed the control diet (untreated), while those in the 2<sup>nd</sup> (G2) and 3<sup>rd</sup> (G3) groups were fed the control diet daily supplemented with 3 g AVI-BAC/cow and 20 g BGY 35/cow, respectively. During pre- and post-partum, LBW, feed and water intakes, rectal temperature (RT), respiration rate (RR) and pulse rate (PR), yield and composition of milk were recorded. Blood samples were collected for determination of total proteins (TP), albumin (AL), creatinine, urea and glucose concentrations in serum. Activity of transaminases (AST and ALT) and alkaline phosphatase (ALP) as well as concentrations of thyroid hormones (T3 and T4), estradiol (E2) and progesterone (P4) were also determined in blood serum. Post-partum 1<sup>st</sup> estrous interval (PPFEI), number of services/conception (NSC), days open (DO) and conception rate (CR) were calculated. Results showed insignificant effect of probiotics supplementation on LBW of cows during pre-partum, calving and post-partum as well as on birth weight of produced calves. During pre-partum, both probiotics supplementations increased ( $P<0.05$ ) feed intake of rice straw (RS) and total DM relative to LBW. Intakes from concentrate feed mixture (CFM), corn silage (CS), total DM/h or total DM relative to metabolic body weight were not affected. During post-partum period, probiotics supplementation increased ( $P<0.05$ ) RS and total DM intakes. Total DM intake relative to LBW or metabolic body weight was not affected. There was insignificant effect of probiotics supplementation on milk yield and milk composition, although average daily milk yield tended to increase by about 17 and 15 % and all milk components increased for G2 and G3 as compared to G1. During pre-partum period, concentrations of all blood biochemicals were not affected by probiotics supplementation. During post-partum, only serum AL concentration was increased ( $P<0.05$ ) in both supplemented groups as compared to the control one. Activities of AST, ALT and ALP were not affected by probiotics supplementation during pre- and post-partum periods. There was a reduction ( $P<0.05$ ) in T4 concentration in G2 during pre-partum, and reduction ( $P<0.05$ ) in T3 and T4 concentrations in both supplemented groups as compared to the control group during post-partum period. Within 120 days post-partum, PPFISI was earlier by about 21.5 and 25 d, NSC was less by about 0.5 and 0.75 and DO was shorter by about 21.0 and 36.5 d in G2 and G3 than in G1. The CR was higher in G3 (100%) than in G2 and G1 (50% in each). Probiotics supplementation resulted in slight reduction in RR, RT and PR of cows during pre- and post-partum periods as compared to un-supplemented diet, but the differences were not significant. Both probiotics supplementation decreased water consumption as compared to control diet, but the differences were significant ( $P<0.05$ ) only between G2 and G1.

In conclusion, dietary supplementation of probiotics AVI-BAC (3 g/h/d) to diet of dairy cows, during 2 months pre-partum and 4 months post-partum, seemed to have a beneficial effect on milk yield and fat yield, while BGY 35 (20 g/h/d) seemed to have pronounced improvement on reproductive performance of dairy cows in terms of increasing conception rate and shortening days open.

**Keywords:** dairy cow, additives, Tw- probiotics, enzymes, production, reproductive.

### INTRODUCTION

The growth promoters are substances when fed to animals improve their production performance parameters. The growth promoter substances include antibiotic growth promoters as flavomycin, probiotics, acidifiers, enzymes, herbal products, beta agonists, microflora enhancer and immunomodulators (Devegowda, 1996). Probiotics are described as live microbial feed supplements (Fuller, 1989) in a mono or mixed culture of living microorganisms (Havenaar *et al.*, 1992) in group of

microorganism strains (Fooks and Gibson, 2002) based on *Lactobacillus*, *Bacillus* and *Saccharomyces* (Burns, 1995), which beneficially affects the host animal by improving its microbial balance and properties of the indigenous microflora.

Interest in the use of direct-fed microbials as feed supplements for dairy cows had a great attention in recent years. Yeast cultures (YC) are frequently used as additives in diets of dairy cows. Inclusion of YC in diets of ruminants and non-ruminants leads to improve healthy status and productivity of animals (Calsamiglia *et al.*, 2006). Results on performance

results of ruminants fed YC products have been variable. Improvements in dry matter intake, DMI (Wohlt *et al.*, 1991 and 1998), milk production and components (Piva *et al.*, 1993), and reproductive performance (Abdel-Khalek, 2003) have been noted when cows were fed YC. In contrast, no differences were found in DMI (Piva *et al.*, 1993), milk production and composition (Robinson and Garrett, 1999) in other studies when cows were fed YC.

AVI-BAC as probiotic is derived from fermentation processes using micro-organisms such as bacteria (*Lactobacillus* and *Bifidobacterium*), and fungi. It is manufactured from *Aspergillus SP.* Published Literature regarding the use of AVI-BAC has been demonstrated for poultry and growing animals (Sretenović *et al.*, 2008).

Generally, supplemented probiotics (YC) may be most beneficial to dairy cows if it is fed before parturition, a period that is characterized by decreased DMI as parturition approaches, and through peak of lactation (Wohlt *et al.*, 1991). Therefore, the objectives of the current study were to determine the effects of supplementing commercial yeast culture (*S. cerevisiae*) namely BGY 35 or a product of lactic acid bacteria and enzymes namely AVI-BAC® to the diet of lactating crossbred cows for the last 2 months pre-partum and the first 4 months postpartum on feed and water intakes, milk yield, milk composition, some blood parameters, reproductive performance and physiological response.

**Table 1. Chemical analysis (% on DM basis) of different feed stuffs of the control diet**

Nutrient	CFM	CS	BH	RS
DM (%)	90.0	35.50	89.0	89.0
OM	93.65	90.90	87.70	86.97
CF	15.03	30.50	30.50	38.50
CP	15.45	9.51	11.30	3.23
EE	3.40	3.15	3.20	1.55
NFE	59.77	47.74	37.70	43.69
Ash	6.35	9.10	12.3	13.03

CFM: Concentrate feed mixture. BH: Berseem hay. CS: Corn silage. RS: Rice straw.

Cows in the 1<sup>st</sup> group were fed the control diet (untreated), while those in the 2<sup>nd</sup> and 3<sup>rd</sup> groups were fed the control diet daily supplemented with 3 g AVI-BAC/cow (AVI-BAC) and 20 g BGY 35/cow (BGY 35), respectively. Supplements of each treatment group were well mixed with the ingredients of daily amount of CFM immediately before feeding. Feeds were offered to animals in all groups twice daily for 2 months pre- and 4 months post-partum period. Cows in all groups were individually fed on different experimental diets and water was individually offered three times/day with daily recording of water consumption.

Cows in all groups were fed based on milk yield according to (NRC, 1988). Amount of feeds were adjusted biweekly based on milk yield and reproductive status.

Yeast culture (BGY 35) is a brewer's dried yeast (*Saccharomyces cerevisiae*) composed of 35% crude protein, 1% crude fat and 8% crude fiber, and contains vitamins, amino acids and minerals.

## MATERIALS AND METHODS

The study was conducted at the Animal Production Research Station, El-Serow, belonging to Animal Production Research Institute, Agricultural Research Center.

### Animals:

A total of 12 crossbred cows (Baladi x Friesian) within the last two months of pregnancy, with an average live body weight of 440±26.434 kg and 2<sup>nd</sup> to 6<sup>th</sup> parities, were used in this study. The experimental cows were divided according to LBW, parity and milk production to three experimental groups, four animals in each group. Animal were housed under semi-open shed. Also, LBW of calves produced from each group was recorded and then weight gain was calculated during post-natal period

### Feeding system:

During pre- and post-partum period, cows in all groups were fed a diet composed of concentrate feed mixture (CFM), corn silage (CS) and berseem hay (BH). Rice straw (RS) was offered *ad lib*. The CFM consisted of 33% wheat bran, 28% yellow corn, 34% uncorticated cotton seed meal, 3% molasses, 1.5% premix and 0.5% common salt. Chemical analysis of CFM, CS, BH and RS are shown in Table (1).

However, probiotic (AVI-BAC®) as growth promoter was produced by ProByn International Inc (USA). Each kg of AVI-BAC contains *Lactobacillus* (100 g *L. acidophilus*, 1.0 x 10<sup>8</sup> CFU/g and *L. plantarum*, 98 g, 9.8 x 10<sup>7</sup>/g), *Bifidobacterium bifidum* (2 g, 2.0 x 10<sup>6</sup>/g), *Bacillus subtilis* fermentation extract (50 g), *Aspergillus oryzae* fermentation extract (50 g), dextrose as diluents (700 g) and enzymes including amylase (25 U/g), cellulose (4.5 U/g), beta-glucanase (2.25 U/g) and hemicellulase (2.75 U/g).

The ambient temperature during the entire length of the experimental period ranged between 23 to 40C°.

### Experimental procedures:

#### Body temperature, respiration rate and pulse rate:

During pre- and post-partum, body temperatures including rectal (RT) using digital precision thermometer (TRD, Ellab Cropcopen Hagen, Denmark) were recorded at 12:00 h. At the same

time, respiration rate (RR) was measured by counting the flank movements for one minute using a stop watch. Pulse rate of the tail vein was also recorded.

#### **Body weight, feed intake and water consumption:**

At the start of the experimental period (2 months pre-partum), experimental cows in all groups were weighed to get the initial LBW, and then animals were weighed during post-partum. After calving, calves of each group were weighed.

Average daily feed intake and water consumption was individually recorded during pre- and post-partum periods.

#### **Milking and milk samples:**

Milk yield was measured after the calves were allowed to suckle colostrums from their dams for the first seven days. Cows were milked by milking machine twice daily at 5 a.m. and 4 p.m. After each milking, milk was weighed on limited day for each week for all lactation period. Milk samples of each animal (mixture from morning and evening milking) were taken during mid-lactation period for the determination of milk composition.

#### **Chemical analysis:**

Chemical analysis of feeds was determined after the official methods of AOAC (1980), while chemical analysis of milk was determined using milko-Scan (Model 133 B).

#### **Blood sampling:**

Animals in each experimental group were bled on two weeks period pre- and one month post-partum. Bleeding was done before morning feeding from each animal by jugular vein-puncture into test-tubes. Blood was allowed to clot and the serum was separated. Serum samples were stored in deep freezer ( $-20^{\circ}$  C) before being analyzed for total proteins (Gornal *et al.*, 1949), albumin (Doumas and Theodore Peters, 2009), creatinine (Bartles *et al.*, 1972), urea (Fawcett and Soctt, 1960) and glucose (Trinder, 1969). Also, activities of aspartate (AST), alanine (ALT) transaminases (Murray, 1984) and alkaline phosphatase (ALP, Belfield and Goldberg, 1971) as well as thyroid hormone ( $T_4$ ) concentration, triiodothyronine ( $T_3$ , Sterling, 1975), tetraiodothyronine (thyroxin,  $T_4$ , Liewendahl, 1990), estradiol (E2, Batzer, 1980) and progesterone (P4, Boganic *et al.*, 1991) were also determined in blood serum. However, concentration of globulin was computed. Blood biochemicals were determined using spectrophotometer and commercial kits.

#### **Reproductive measurements:**

Natural insemination was used as a method of breeding in the station under the current study for all cows in heat 50 days post-parturition. For each cow, the date of service was recorded and thereafter followed up for estrus return 21 days later. The non-return animals were rectally examined 50 to 60 days after the first breeding for pregnancy diagnosis and in any doubtful case, the examination was repeated 2 weeks later.

During post-partum period, interval from calving to 1<sup>st</sup> service (PPFSI) or to conception (days open) and number of services per conception were recorded. Also, conception rate within 120 day-postpartum period was recorded.

#### **Statistical analysis:**

Statistical analysis was done using the General Linear Model (GLM) procedures of the statistical Analysis Systems (SAS, 2002). Data obtained were tested by analysis of variance with one way design to test the group differences according to the following model:

$Y_{ij} = \mu + A_i + e_{ij}$ ; **where:**  $Y_{ij}$ = observed values,  $\mu$ = overall mean,  $A_i$ = experimental group) and  $e_{ij}$ = Random error. Values were given as mean  $\pm$  standard error. All statements of significance were based on  $P < 0.05$  using Duncan Multiple Range Test within the computer program.

## **RESULTS AND DISCUSSION**

#### **Live body weights:**

Results presented in Table (2) show insignificant effect of dietary supplementation of the two probiotics on live body weight (LBW) of cow in different experimental groups during pre-partum, calving and post-partum as well as on birth weight of produced calves. Supplementation with the two probiotics did not affect pre- or post-partum or pre-partum body weight. This is partially in agreement with the results of Dann *et al.* (2000) who reported that initial body condition score (BCS) of cows was not affected when cows were fed a diet supplemented with YC. Also, Abdel-Khalek (2003) found that Yea-Sacc supplementation as YC fed to primi- and multiparous Friesian cows had no significant effect on birth weight of calves. In addition, Ahmed *et al.* (2008) showed insignificant effect of bacterial feed additive (Lecture) on LBW of Zairabi goat does during late pregnancy and lactation period.

#### **Feed intake:**

Results regarding feed intake as dry matter (DM) during pre-partum period (Table 3) show significant ( $P < 0.05$ ) increase in feed intake from rice straw (RS, which was allowed *ad libitum* for cows in all groups) of cows fed diet supplemented with probiotics (AVI-BAC and BGY) as compared to the control diet. This increase did not affect total DM intake, because cows in all groups were fed on similar amounts of concentrate feed mixture (CFM) and corn silage (CS). However, the observed increase DM intake from RS resulted in a significant ( $P < 0.05$ ) increase in total DM intake relative to LBW and insignificantly relative to metabolic body weight.

During post-partum period (lactation period), feed intake from RS was also significantly ( $P < 0.05$ ) increased in treated groups (AVI-BAC and BGY) as compared to the control. This increase led to a significant ( $P < 0.05$ ) increase in total DM intake without significant effect on total DM intake relative to LBW or metabolic body weight (Table 3).

**Table 2. Effect of dietary supplementation of probiotics (X±S.E) on live body weight (LBW) of cows at pre- and post-partum periods and of produced calves at calving**

Item	Control	AVI-BAC	BGY35
<b>Average LBW of cows (kg):</b>			
Two months pre-partum	440.00±28.284	437.00±33.558	434.75±16.007
One month pre-partum	455.00±28.104	452.75±34.376	450.25±15.548
At calving	513.25±28.412	506.50±37.728	490.00±12.227
Post-calving	445.00±32.275	435.00±35.355	435.25±14.979
One month post-partum	456.75±31.579	448.50±32.116	449.50±12.073
<b>Average LBW of calves (kg):</b>			
At calving	28.75±3.146	31.00±1.172	29.50±0.957

All differences among groups are not significant.

**Table 3. Effect of dietary supplementation of probiotics (X±S.E) on feed intake of cows during pre- and post-partum periods**

Feed intake	Control	AVI-BAC	BGY35
<b>During pre-partum period:</b>			
CFM (kg DM/h/d)	4.50±0.000	4.50±0.000	4.50±0.000
RS (kg DM/h/d)	1.69±0.005 <sup>b</sup>	1.75±0.010 <sup>a</sup>	1.77±0.010 <sup>a</sup>
CS (kg DM/h/d)	2.75±0.000	2.75±0.000	2.75±0.000
Total DM intake (kg DM/h/d)	8.94±0.005	9.00±0.010	9.02±0.010
Total DM intake (% of LBW)	1.87±0.123 <sup>b</sup>	1.97±0.154 <sup>a</sup>	1.93±0.0682 <sup>a</sup>
Total DM intake (g/kg W <sup>0.75</sup> )	87.60±4.125	89.97±5.356	89.75±2.375
<b>During post-partum period (kg/h):</b>			
CFM (kg DM/h/d)	4.95±0.000	4.95±0.000	4.95±0.000
RS (kg DM/h/d)	1.68±0.027 <sup>b</sup>	1.77±0.008 <sup>a</sup>	1.78±0.000 <sup>a</sup>
CS (kg DM/h/d)	3.33±0.000	3.33±0.000	3.33±0.000
Total DM intake (kg DM/h/d)	9.96±0.027 <sup>b</sup>	10.05±0.008 <sup>a</sup>	10.06±0.000 <sup>a</sup>
Total DM intake (% of LBW)	2.24±0.169	2.31±0.187	2.29±0.050
Total DM intake (g/kg W <sup>0.75</sup> )	102.908±5.694	105.613±6.298	104.76±1.734

a and b: Means having different superscripts within the same row are significantly different at P<0.05.

In agreement with the present results, some authors observed an improvement in DM intake when lactating cows were fed YC (Williams *et al.*, 1991; Wohlt *et al.*, 1991 and 1998; Robinson and Garrett, 1999; Dann *et al.*, 2000). In addition, similar results were reported on lactating buffaloes fed Biovet as micro-organisms added to their diets (Gujjar *et al.*, 2006) or dairy goats fed Lecture as a bacterial feed additive (Ahmed *et al.*, 2008). However, Aikman *et al.* (2008) observed no difference in DM intake between the control and treated cows fed two TMR's differing in level of concentrate and supplemented with direct-fed microbial (DFM) during the first 14 weeks of lactation. The significant increase in DM intake from RS may reflect higher ruminal fermentation in treated groups than in control. Feeding yeast products may be most beneficial to dairy cows during late gestation and early lactation because of their effects on rumen fermentation and nutrients digestion (Dann *et al.*, 2000) in term of increasing the digestibility of CP and ADF (Wohlt *et al.*, 1998). The most cited benefit of yeast cultures on ruminal digestion is support of the growth and activity of anaerobic, namely cellulolytic bacteria. Yeasts would utilize residual oxygen introduced into the rumen with feeds, thus contributing to maintain anaerobic environment (Calsamiglia *et al.*, 2006). On the other hand, applying of the complex lactic acid bacteria improved

fermentation quality and *in vitro* DM digestibility (Yongkai *et al.*, 2012).

#### **Milk production:**

Results shown in Table (4) revealed insignificant effect of dietary supplementation of probiotics on milk yield and milk composition of cows, although cows in both treatment groups increased their average daily milk yield by about 17 and 15 % for AVI-BAC and BGY groups as compared to the control cows, respectively. Also, dietary supplementation of both probiotics insignificantly increased milk components including fat, protein and lactose as compared to the control diet. These results indicated higher fat, protein and lactose yields in milk of cows in the treatment groups than in controls. It is of interest to record that the observed increase in milk yield and milk component percents of treated groups compared with the control one was associated with increase in DM intake of RS (Table 3). Similarly, Soder and Holden (1999) found no effects of YC on DM intake or milk yield and composition of primi-parous and multi-parous cows. Consequently, the effects of YC supplementation during the pre-partum period and through peak lactation remain controversial and have not been adequately researched.

**Table 4. Effect of dietary supplementation of probiotics (X±S.E) on average daily milk yield and chemical composition of milk produced by cows during lactation period**

Item	Control	AVI-BAC	BGY35
Average daily milk yield (kg)	8.055±0.850	9.430±0.548	9.260±0.752
<b>Milk composition (%):</b>			
Fat	3.603±0.199	4.003±0.150	3.830±0.158
Protein	2.595±0.063	2.748±0.034	2.773±0.114
Lactose	4.595±0.105	4.838±0.080	4.885±0.048
Solids not fat	7.883±0.158	8.293±0.106	8.355±0.136
Total solids	11.480±0.115	12.288±0.196	12.178±0.115
Ash	0.710±0.003	0.704±0.005	0.706±0.005

All differences among groups are not significant.

**Table 5. Effect of dietary supplementation of probiotics (X±S.E) on some biochemicals in blood serum of cows during pre- and post-partum periods**

Item	Control	AVI-BAC	BGY35
<b>During pre-partum period:</b>			
Total protein (g/dl)	7.391±0.310	6.504±0.111	7.697±1.017
Albumin (g/dl)	2.891±0.091	3.641±0.279	3.486±0.254
Globulin (g/dl)	4.500±0.254	2.863±0.325	4.211±0.876
Creatinine (mg/dl)	1.030±0.091	0.837±0.039	0.899±0.104
Urea-N (mg/dl)	20.969±1.615	26.099±7.935	21.893±4.883
Glucose (mg/dl)	83.29±1.028	81.91±0.348	82.56±0.188
<b>During post-partum period:</b>			
Total protein (g/dl)	7.419±0.403	8.013±0.781	6.532±0.229
Albumin (g/dl)	2.677±0.136 <sup>b</sup>	3.368±0.075 <sup>a</sup>	3.384±0.077 <sup>a</sup>
Globulin (g/dl)	4.742±0.391	4.645±0.854	3.148±0.216
Creatinine (mg/dl)	0.987±0.072	0.879±0.081	0.790±0.038
Urea-N (mg/dl)	16.794±0.530	14.978±1.357	17.113±1.053
Glucose (mg/dl)	73.71±0.311	74.15±0.195	74.47±0.169

a and b: Means having different superscripts within the same row are significantly different at P<0.05.

In accordance with the present results of YC, Robinson and Garrett (1999) did not observe any beneficial effects of yeast cultures on the milk production and its composition in dairy cows. However, improvements in milk components have been noted when cows were fed YC (Piva *et al.*, 1993). In this respect, Wohlt *et al.* (1991) observed that primi-parous Holstein cows fed YC starting 30 d pre-partum and continuing through wk 18 of lactation had greater milk yield through 18 wk lactation period. In a similar study, Wohlt *et al.* (1998) found that YC supplementation during early lactation improved milk yield. In a subsequent study, Robinson and Garrett (1999) observed trends for increased DM intake and milk production during early lactation for cows fed YC pre- and postpartum. Similar results were reported by Dann *et al.* (2000). However, Swartz *et al.* (1994) reported that daily supplementation of two yeast culture preparations (*Saccharomyces cerevisiae*, at about 5 x [10.sup.10] cfu/d per cow) did not significantly improve the production parameters of lactating dairy cows under the nutritional management programs of the farms.

The obtained results of cows fed AVI-BACK, Aikman *et al.* (2008) did not observe a positive response in milk production, and fat and protein percentages in milk of cows fed diet supplemented with DFM as compared to the control cows. Similar full lactation results were reported by others when Holstein cows were fed lucerne maize based diets (Krause *et al.*, 2002). Also, Hagg and Henning

(2007) reported no difference in milk fat percentage when cows were fed DFM as compared to control cows. However, Biovet as a DFM has favorable effect on milk yield and feed efficiency due to beneficial micro-organisms (BM) and combined function for increased digestibility of concentrate mixture and fodder in lactating buffaloes (Gujjar *et al.*, 2006).

#### **Blood parameters:**

##### **Biochemicals in blood serum:**

During pre-partum period, results presented in Table (5) show that concentrations of blood biochemicals including total protein (TP), albumin (AL), globulin (GL), creatinine, urea-N and glucose in serum of cows were not significantly affected by dietary probiotics supplementation. However, during lactation period (post-partum), concentration of AL in serum was significantly (P<0.05) increased, while GL concentration insignificantly decreased in both treatment groups as compared to the control group.

In ruminants, concentration of plasma TP can be an index to evaluate nutrients when fed both adequate and low levels of crude proteins (Kumar *et al.*, 1980). Positive correlation between dietary proteins and plasma TP concentration was reported by Bush (1989). In agreement with the present results, Ibrahim (2004) found that YC supplementation showed insignificant effect on TP concentration in plasma of lactating buffalo cows. Fayed (2001) found that blood serum TP was insignificantly increased

with Yea-Sacc supplementation in sheep and goats. Also, no effect of premix containing *Sac. cerevisiae* ( $6 \times 10^8$  cfu/g of premix, Doreau and Jouany, 1998) or YC (Yea-Sacc<sup>1026</sup>, Iwanska *et al.*, 1999) on TP concentration in plasma lactating cows.

Regarding the level of TP fraction, El-Ashry *et al.* (2001a) indicated that YS (Yea-Sacc<sup>1026</sup>, Lacto-Sacc and bakery yeast) significantly increased AL concentration in lactating buffaloes. Farag (2004) found YC supplementation decreased concentration of GL in blood serum of buffalo calves, while average concentration of blood AL slightly increased. On the other hand, YC supplementation increased TP concentration of lactating buffaloes (Ibrahim, 2004; Salem *et al.*, 2002) and lambs (El-Shaer, 2003), while AL and GL levels in blood were not affected by YC supplementation in lactating buffaloes (Ibrahim, 2004) and sheep (El-Shaer, 2003). However, YC supplementation significantly decreased plasma AL concentration in growing buffalo-calves (El-Ashry *et al.*, 2001b) and in Friesian calves fed dietary *Lacto-Sacc* (Ragheb *et al.*, 2003).

Concerning the effect of YC on blood glucose, some authors reported that glucose concentration in blood was slightly improved in dairy cows and buffalo cows fed rations containing YC (Ahmed, 2001; Ragheb *et al.*, 2003) or lactating cows fed DFM in lactating cows, Iwanska *et al.* (1999) also reported insignificant differences in blood glucose level as affected by YC (*Sac. cerevisiae*<sup>1026</sup>) with or without a vitamin premix and mineral bioplex. In sheep fed YC diet, El-Shaer (2003) indicated no significant effect of YC on glucose concentration. On the other hand, concentration of serum glucose was increased ( $P < 0.05$ ) by increasing level of YC supplementation in the diets of lactating Friesian cows (Ahmed, 2001), in Friesian calves fed diet supplemented with *Lacto-Sacc* (Ragheb *et al.*, 2003)

and in lactating buffaloes fed ration supplemented with Yea-Sacc, Lacto-Sacc and baker's yeast (El-Ashry *et al.*, 2001a). Finally, Strusinska *et al.* (2003) found a positive influence of added yeast cultures, mineral and vitamin supplements on selected biochemical indicators in the blood of dairy cows.

#### **Enzymatic activity and hormonal profile:**

Results presented in Table (6) show that activities of transaminases (AST and ALT) and alkaline phosphatase (ALP) were not significantly affected by dietary supplementation of probiotics during pre- and post-partum periods. However, significant ( $P < 0.0$ ) reduction in concentration of thyroid hormone ( $T_4$ ) was observed only in AVI-BAC group as compared to the control group during pre-partum. Also, significant ( $P < 0.05$ ) reduction was observed in  $T_3$  and  $T_4$  in both treatment groups as compared to the control group during post-partum period.

The determined values of AST and ALT activities are within the physiological limits of transaminases. According to Pechová *et al.* (2002), the activity of AST increases in dairy cows suffering from liver steatosis or in cows with disturbed energy metabolism. Its value is therefore very individually variable. Similarly, the activity of ALP enzyme in the blood serum did not exceed the reference volume and was apparently not affected by the addition of probiotics. In agreement with the present results of AVI-Back group, Sretenović *et al.* (2008) reported unaffected activity of AST and ALT in blood of dairy Holstein-Friesian cows. In spite of significant differences in the individual blood indicators, their concentrations are apparently not connected with YC supplementation but rather with the diet and with the individuality of cows.

**Table 6. Effect of dietary supplementation of probiotics (X±S.E) on activity of some enzymes and hormones in blood serum of cows during pre- and post-partum periods**

Item	Control	AVI-BAC	BGY35
<b>During pre-partum period:</b>			
AST (U/ml)	15.66±0.667	23.33±4.410	20.33±3.180
ALT (U/ml)	14.33±0.296	14.60±0.351	14.26±0.260
ALP (IU/l)	20.99±6.491	38.95±21.008	35.46±10.601
$T_3$ (nmol/l)	1.83±0.149	1.53±0.336	1.99±0.640
$T_4$ (nmol/l)	42.54±4.911 <sup>a</sup>	25.70±2.270 <sup>b</sup>	40.50±2.522 <sup>a</sup>
<b>During post-partum period:</b>			
AST (U/ml)	14.60±0.360	14.93±0.145	15.23±0.176
ALT (U/ml)	14.23±0.176	14.40±0.265	14.46±0.384
ALP (IU/l)	16.79±0.530	8.59±2.507	8.22±1.989
$T_3$ (nmol/l)	2.16±0.135 <sup>a</sup>	1.46±0.163 <sup>b</sup>	1.31±0.098 <sup>b</sup>
$T_4$ (nmol/l)	84.73±2.894 <sup>a</sup>	40.36±3.543 <sup>b</sup>	46.16±6.832 <sup>b</sup>

a and b: Means having different superscripts within the same row are significantly different at  $P < 0.05$ .

**Table 7. Effect of dietary supplementation of probiotics (X±S.E) on reproductive performance of cows**

Item	Control	AVI-BAC	BGY35
<b>Reproductive measurements:</b>			
PPFSI, day	91.00±3.81	68.5±21.79	66.00±9.21
NSC	2.5±0.42	2.00±0.00	1.75±0.31
DO	118.00±19.41	97.00±22.51	81.5±11.623
Conception rate (%)	50	50	100

**Table 8. Effect of dietary supplementation of probiotics (X±S.E) on progesterone and estradiol in blood serum of cows during pre- and post-partum periods**

Item	Control	AVI-BAC	BGY35 (G3)
<b>During pre-partum period:</b>			
Progesterone (ng/ml)	18.997±3.454	10.133±4.499	21.109±5.180
Estradiol (pg/ml)	4.664±0.751	4.881±0.591	6.239±1.214
<b>During post-partum period:</b>			
Progesterone (ng/ml)	1.480±0.194	1.702±0.173	1.732±0.194
Estradiol (pg/ml)	0.989±0.215	0.928±0.079	1.563±0.368

All differences among groups are not significant.

**Table 9. Effect of dietary supplementation of probiotics (X±S.E) on physiological response of cows during pre- and post-partum periods**

Item	Control (G1)	AVI-BAC	BGY35
<b>During pre-partum period:</b>			
Respiration rate (times/min)	27.25±2.926	26.00±2.273	24.75±1.315
Rectal temperature (°C)	39.27±0.229	38.90±0.367	38.92±0.368
Pulse rate (times/min)	70.50±6.344	64.25±3.276	70.40±4.113
<b>During post-partum period:</b>			
Respiration rate (times/min)	26.50±1.258	26.25±2.926	26.00±0.816
Rectal temperature (°C)	38.82±0.249	38.77±0.165	38.75±0.126
Pulse rate (times/min)	84.00±1.826	76.25±4.768	78.50±0.957

All differences among groups are not significant.

#### **Reproductive performance:**

Results regarding the reproductive performance of cows in different experimental groups show that postpartum first service interval (PPFSI) was earlier by about 22.5 and 25 d, number of services per (NSC) was less by about 0.5 and 0.75 and days open (DO) was shorter by about 21.0 and 36.5 d of cows in AVI-BAC and BGY groups than those of the control cows, respectively (Table 7). On the other hand, conception rate was higher in BGY (100%) than in AV-BAC groups (50% in each).

These results indicated that feeding lactating cows on diets supplemented with YC (BGY 35) had beneficial effects on reproductive performance of lactating cows as compared to AVI-BAC did. It is of interest to note that dietary supplementation of YC (BGY 35) markedly increased concentration of progesterone and estradiol in blood serum of cows in BGY group during pre- and post-partum periods as compared to AVI-BAC and control groups, but the differences were not significant (Table 8). Previous studies have identified a strong relationship between the extent of negative energy balance (NEB) in early lactation and decreased conception rate (Butler and Smith, 1989), which may indicate improving energy balance of cows fed both supplements, reflecting increase in reproductive performance of treated cows as compared to the controls

In accordance with the present results, Abdel-Khalek, 2003) found that Yea-Sacc (*Saccharomyces cerevisiae*) supplementation as YC fed to multiparous Friesian cows improved PPFSI, DO, service period and NSC, but the differences were not significant. Also, Dann *et al.* (2000) observed that days to first breeding (PPFSI) averaged 74.9 and was not affected by YC (*Saccharomyces cerevisiae*) treatment. Treatment also did not affect services per pregnancy, which averaged 2.1 services. The tendency of improvement in reproductive performance of supplemented cows, in particular

with BGY 35, may be related to mineral content of YC. The potential for minerals to play a significant role in cow fertility is indisputable. Reproductive problems are frequently reported in association with trace mineral deficiencies (Boland, 2002). Zinc deficiency in ruminant may be impairing conception rate and ovarian function. The YC acts as a highly concentrated form of Zn in which the element is correlated to components in the yeast cells. This may lead to an improvement in reproductive performance when dairy cows were fed YC (Williams, 1988).

#### **Physiological response:**

Physiological response of cows to treatments was expressed as changes in respiration rate (RR), rectal temperature (RT) and pulse rate (PR) as shown in Table (9) as well as amount of consumed water as presented in Table 10 in comparison with the control group. Results in Table (9) show that dietary supplementation of AVI-BAC and BGY resulted in a slight reduction in respiration rate (RR), rectal temperature (RT) and pulse rate of cows during pre- and post-partum periods as compared to unsupplemented diet (Control group), but the differences were not significant.

As a result of decreasing RR, RT and PR, water consumption of cows in AVI-BAC and BGY groups showed marked decrease as compared to the control group, but the differences were significant ( $P < 0.05$ ) only between cows in AVI-BAC and control group (Table 10). The observed increase in water consumption of the control cows was mainly due to increasing RR and RT and consequently water loss to regulate body temperature. These findings may indicate beneficial effect of feeding lactating cows on diets supplemented with probiotics to eliminate heat stress during summer season in Egypt.

**Table 10. Effect of dietary supplementation of probiotics (X±S.E) on water consumption of cows during pre- and post-partum periods**

Water intake	Control	AVI-BAC	BGY35
<b>During pre-partum period:</b>			
Total water intake (l/h/d)	46.75±2.323 <sup>a</sup>	32.25±3.425 <sup>b</sup>	40.00±2.915 <sup>ab</sup>
As ml/kg LBW	97.77±5.840 <sup>a</sup>	67.89±2.373 <sup>b</sup>	86.20±8.716 <sup>ab</sup>
As ml/kg W <sup>0.75</sup>	456.64±23.468 <sup>a</sup>	316.38±16.665 <sup>b</sup>	399.97±37.600 <sup>ab</sup>
As ml/ g DM intake	5.225±0.258 <sup>a</sup>	3.58±0.380 <sup>b</sup>	4.44±0.320 <sup>ab</sup>
<b>During post-partum period:</b>			
Total water intake (l/h/d)	53.25±3.258 <sup>a</sup>	41.50±2.174 <sup>b</sup>	48.75±2.831 <sup>ab</sup>
As ml/kg LBW	118.51±5.563 <sup>a</sup>	94.33±2.922 <sup>b</sup>	111.24±7.997 <sup>a</sup>
As ml/kg W <sup>0.75</sup>	544.83±24.843 <sup>a</sup>	435.66±14.883 <sup>b</sup>	512.73±33.892 <sup>a</sup>
As ml/ g DM intake	5.34±0.386 <sup>a</sup>	4.13±0.274 <sup>b</sup>	4.85±0.250 <sup>a</sup>

a and b: Means having different superscripts within the same row are significantly different at P<0.05.

Generally, it was suggested that several factors affect the response of dairy cows to supplemental YC, such as stage of lactation, type of forage fed, feeding strategy, and the forage-to-concentrate ratio (Piva *et al.*, 1993). The efficiency of YC depends among other factors also on the conditions of cultivation, on the concentration of live yeast cells (CFU) as well as on the dose of the culture used (Doležal *et al.* 2011). Uses and benefits of probiotics were mentioned by Sainsbury (1992) as follows: (1) there is a proven ability to promote growth and productivity in livestock in a perfectly natural way. (2) Probiotics protect against *Salmonella* infections, including the worst types such as *enteritidis* and *typhimurium*. (3) They can protect against toxins produced by harmful forms of *E. Coli*. (4) Probiotics stimulate immunity to infections by boosting interferon production, immunoglobulin concentration and macrophage activity. (5) They have an activity suppressing *Clostridial* infection, often associated with intensive livestock production. (6) Probiotics have also been shown to be antagonistic to many other harmful bacteria, such as *Klebsiella*, *Proteus* and *Campylobacter*. (7) There is research evidence that Probiotics are active against the development of cancers in animals.

In addition, the beneficial effect of probiotics could be produced in two ways. They could operate by: (1) Suppressing harmful bacteria; this could manifest itself in reduced numbers of bacteria or in a decreased concentration of harmful metabolites such as enterotoxin. (2) Stimulation of bacteria which are engaged in beneficial activities such as production of essential nutrients like vitamins or in digestion of food components (Mulder, 1991). Increasing levels of probiotics may induce a "barrier" influence against common pathogens. Mechanism of the effect are likely to include the excretion of acids (lactate, acetate), competition for nutrients and gut receptor sites, immunomodulation and the formation of specific antimicrobial agents. Probiotics suppress enzymes responsible for genotoxin formation (Fooks and Gibson, 2002). Soluble products present in yeast culture have been shown to inhibit microbial growth and activity and modulate the immune system (Jensen *et al.*, 2007). Yeast cells also improve

digestibility and absorption of minerals such as phosphorus, magnesium, calcium, copper, potassium, zinc and manganese (Kinal *et al.*, 2007).

Based on the foregoing results, dietary supplementation of probiotics as yeast culture, *S. cerevisiae* (20 g BGY 35/h/d) or as a product of lactic acid bacteria and enzymes (3 g AVI-BAC<sup>®</sup>/h/d), during 2 months pre-partum and 4 months post-partum, improved productive and reproductive performances of lactating cows. Bacterial additive (AVI-BAC) seemed to have a beneficial effect on milk yield and fat yield, while yeast culture (BGY 35) seemed to have pronounced improvement on reproductive performance of dairy cows in terms of increasing conception rate and shortening days open.

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## تأثير استخدام بعض الإضافات الغذائية على الأداء الإنتاجي والتناسلي لأبقار اللبن

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تهدف هذه الدراسة إلى تقييم آثار إضافة الخميرة التجارية (BGY 35) وبكتيريا حمض اللاكتيك والانزيمات (AVI-BAC) إلى علائق الأبقار الخليطة، خلال الشهرين الأخيرين قبل الولادة و خلال 4 أشهر الأولى بعد الولادة (من يوليو الى أكتوبر)، على وزن الجسم والعلف المأكول والماء وإنتاج اللبن وبعض مكونات الدم والأداء التناسلي والاستجابة الفسيولوجية. تم استخدام 12 بقرة بمتوسط وزن الجسم الحي عند الولادة 440 كجم وعدد المواسم من 2-6. تم تقسيم الأبقار إلى ثلاث مجموعات، 4 أبقار فى كل معاملة، خلال فترة ما قبل وبعد الولادة، تم تغذية الأبقار في المجموعة الأولى على عليقة المقارنة ( غير معالجة)، في حين أن أولئك في المجموعة الثانية تم تغذيتها على علف مضاف إليه AVI-BAC بمعدل 3 جم/حيوان/يوم، وتم إضافة الخميرة (BGY 35) بمعدل 20 جم خميره/حيوان/يوم لحيوانات المجموعة الثالثة خلال مرحلة ما قبل وبعد الولادة تم تسجيل الأعلاف المأكولة والماء المشروب ودرجة حرارة المستقيم ومعدل التنفس ومعدل النبض ومحصول اللبن والتحليل الكيماوى للبن. تم جمع عينات الدم لتقدير البروتين الكلى والألبومين والكرياتينين واليوريا والجلوكوز في مصل الدم وكذلك تركيز انزيمات AST و ALT والفوسفاتيز القاعدى وكذلك تركيز هرمونات الغدة الدرقية ( T3 و T4 ) والاستراديول والبروجسترون في مصل الدم. أظهرت النتائج تأثير غير معنوى لإضافة البروبيوتيك على الوزن الحى للأبقار خلال مرحلة ما قبل الولادة والولادة وبعد الولادة، وكذلك على وزن العجول عند الولادة. خلال مرحلة ما قبل الولادة إضافة كلا من الخميرة وبكتيريا حمض اللاكتيك يزيد المؤكول من قش الأرز والمادة الجافة الكلية نسبيا لوزن الحيوان. ولم يتأثر المؤكول من العلف المركز أو سيلاج الذرة والمادة الجافة نسبيا إلى وزن الجسم التمثلى. لم يكن هناك تأثير معنوى للإضافات الغذائية على محصول اللبن أو تركيب اللبن، في حين متوسط إنتاج الحليب اليومي ارتفع بنحو 17 و 15%، على التوالي لإضافة الخميرة أو بكتريا حمض اللاكتيك. خلال فترة ما قبل الولادة ، لم يتأثر تركيز جميع مكونات الدم بالإضافات الغذائية. خلال فترة ما بعد الولادة ، ارتفع تركيز البيومين الدم معنويا (5%) بالإضافات الغذائية، في حين تركيز البروتين الكلى والجلوبيولين ونشاط AST و ALT وانزيم الفوسفاتيز القاعدى لم تتأثر معنويا. تركيز كلا من T<sub>3</sub> و T<sub>4</sub> انخفض معنويا في مجموعة الحيوانات التى تغذت على عليقة مضاف إليها بكتريا حمض اللاكتيك في مرحلة قبل الولادة. إضافة كلا من الخميرة والبكتريا أدى إلى انخفاض طفيف في معدل التنفس ودرجة حرارة المستقيم ومعدل النبض فى الأبقار خلال فترات ما قبل وبعد الولادة بالمقارنة مع مجموعة المقارنة، ولكن كانت الفروق غير معنوية.

وتوصى هذه الدراسة ان أضافة بكتريا حمض اللاكتيك (3 جم/حيوان/يوم) خلال الشهرين الأخيرين قبل الولادة و 4 أشهر الأولى بعد الولادة إلى علائق الأبقار الحلابه له تأثير مفيد على إنتاج اللبن ونسبه الدهن فيه، بينما أضافة الخميره (20 جم/حيوان/يوم) خلال نفس الفتره حسن من الكفاءه التناسليه للأبقار الحلابه من حيث زيادة معدل الأخصاب وتقليل عدد الأيام المفتوحه.