INFLUENCE OF *YEAST* AND *LACTOBACILLUS* PRODUCTS AS FEED SUPPLEMENTS ON BLOOD PARAMETERS AND REPRODUCTIVE PERFORMANCE OF LACTATING EGYPTIAN BUFFALOES

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SUMMARY

The present study is a trial to improve blood parameters and the reproductive performance of lactating Egyptian Buffaloes following the administration of probiotics during late gestation and early postpartum period. Fifteen lactating buffaloes at one-month pre-partum were divided randomly into three groups (5 each) until five months postpartum. The control group fed basal diet without supplements (G1), the 2^{nd} group (G2) was given the basal diet plus 20 g/h/d of Saccharomyces cerevisiae yeast culture (YC), the 3^{rd} group (G3) was given the basal diet plus 20 g/h/d of Lactobacillus acidophilus (LB). Blood samples were collected monthly during the postpartum period (PP) from calving to the 5th month for estimation of enzymes activities, hematological and blood parametersas well as reproductive measurements, while colostrum samples were taken from dams on days 1, 2 and 3 postpartum to measure the concentration of immunoglobulin. Results showed that the values of all hematological parameters were significantly (P < 0.05) improved in G3 followed by G2 then control. Also, Data indicated that the values of all biochemical blood parameters for buffaloes supplemented with LB in G3 were significantly (p < 0.01) higher than G2, while G1 was lower significantly (p < 0.05) than other treated groups. Enzymes activities and reproductive measurements percentage increased insignificantly in G3 and G2 compared with G1. There were highly significant immunoglobulin concentrations in colostrum of dams in G3 followed by G2 than control. It could be concluded that Lactobacillus acidophilus supplementation to rations of lactating buffaloes had beneficial effects on health status, immunity response, blood parameters and the reproductive performance under the local farm feeding conditions in Egypt.

Keywords: Yeast, lactobacillus, lactating buffaloes, blood parameters, reproduction, immunity response

INTRODUCTION

There are challenges facing pregnant animals during the interval from three weeks pre-partum to three weeks post-partum, this period is indicated by the presence of many changes in the physiological conditions, metabolism and endocrine enough to accommodate parturition and lactogenesis (Goff and Horst, 1997; Drackely, 1999). If nutrition management cannot manage these challenges, these animals will be vulnerable to a wide range of health problems soon after parturition. These problems include retained placenta, metritis, postpartum anoestrus, milk fever, ketosis, and severely suppressed immune function (Grummer, 1995; Goff and Horst, 1997). It is of importance to notice that the proper management during the transition period (Gordon, 2004) helps in responding the immune system (Lowry et al., 2005; Chae et al., 2006), improving the reproductive performance, decreasing the incidence of metabolic and infectious diseases and overcoming stress of milk production (Axford, 2001; Hsueh et al., 2010).

In Egypt, one of the most critical problems in animal production is the lack of sufficient feeds to meet animal's nutritional requirements (Yousef and Fayed, 2001). Application of biotechnology in the field of ruminant nutrition has made possible the use of probiotics, prebiotics and enzymes as feed additives in the dairy industry for efficient utilization of nutrients. These probiotics are live microbial feed supplements that used as growth promoters to reduce the widely used of antibiotics and synthetic chemical of feed supplements (Sumeghy, 1995; Strzetelski, 1996; Dawson, 2002; Fooks and Gibson, 2002), which beneficially affects animal by improving its microbial balance and properties of the indigenous microflora.

In recent years, there are modern trends to using probiotics such as yeast culture and lactobacillus to improve livestock production. It's known that, yeast cells are a rich source of vitamins, enzymes also it stimulates cellulolytic bacteria in the rumen, increases fiber digestion and flow of microbial protein from the rumen. Inclusion of YC in ruminant's diets and non-ruminants improves health status and animals production (Calsamiglia et al., 2006). Hence, yeast culture supplementation has been shown to improve the growth rate and feed conversion efficiency. However, the effect of dietary yeast supplementation on milk yield and composition is varied. In some studies, yeast culture supplementation was shown to increase milk production and its content of fat (Ayad et al., 2013 and Wafa et al., 2020).

Most researches indicated insignificant effect for YC on activity of AST and ALT in blood serum of dairy cattle fed diets supplemented with YC (*Saccharomyces cerevisiae*) Faten Abou Ammou *et al.* (2013); Abu-El-Ella *et al.* (2014). Also, most researches indicated significantly effect for probiotic on the improvement of hematological and

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biochemical blood parameters (Zeedan et al., 2008, 2009a, b and 2014; Abu-El-Ella et al., 2014; Ghoniem et al., 2018; Mahrouset et al., 2019 and Wafa et al., 2020). Ahmad Para et al., (2019) found that there was a significant (p < 0.05) difference in plasma metabolic profile, protein and globulin across different periods of experimental study in both treatment groups. Yeast culture usage in ruminant diets, found to improve their performance (Williams, 1989) and it was found to increase protein content in blood (El-Shaer, 2003) and glucose concentration (Sharma et al., 1998 and Mukhtar et al., 2010). While, it decreased the cholesterol levels (Fayed et al., 2005). Also, the reproductive performance was obtained by Abdel-Khalek (2003) and Wafa et al., (2020) in Friesian cows and Ebrahim (2004) in Egyptian buffaloes.

Ahmad Para *et al.*, (2019) in buffaloes and Mostafa *et al.*, (2014) in cows indicated that the birth weight significantly increased in calves born from dams supplemented with Biogen–Zinc compared with the control group. In ewes, Kassabra and Mohammed (2013) and goats, Abu-El-Ella *et al.*, (2014) indicated that the birth weight recorded significant increase in probiotics group while, the lowest value was in control group.

Therefore, the objective of this study was to evaluate the impact on lactating Egyptian Buffalo treated with probiotic (*Saccharomyces cerevisiae* and *Lactobacillus acidophilus*) during pre- and postpartum period on blood parameters health status, immunity response and their reproductive performance.

MATERIALS AND METHODS

The present study was conducted at Animal Production Research Institute, Egypt, in cooperation

with Department of Animal Production, Faculty of Agriculture, Tanta University.

Animals:

Fifteen lactating Egyptian Buffalo-cows (2-4 lactations) with an average live body weight of 550±8.02 kg approximately in three groups, 5 in each were used in this study. Animals were chosen in late gestation period (LP) at approximately day 60 prepartum and divided randomly into three comparable experimental groups (5 animals per group) and postpartum period (five months after parturition). Animals were housed outdoor at day and night.

Feeding system:

Animals were fed their ration individually according to Kearl (1982). The control ration contains concentrate feed mixture (CFM), berseem 3rd cuts (BC) and rice straw (RS), it was offered to buffaloes in the first group without supplementation (G1). The other two experimental groups (G2) and (G3) received the control ration supplemented with 20 g/h/d of YC (Saccharomyces cerevisiae yeast culture; Qlyae, 2016) and 20 g/h/d of LB (Lactobacillus acidophilus; Abo Teba, 2019), respectively. The YC Diamond V-XPC[™] is a yeast culture that contains 1.0×10⁶ CFU of Saccharomyces cerevisiae, it was provided from Diamond V Cedar Rapids, IA52404, USA. LB (Probax®) was offered from Dugok-Ri, Sinam-Myeon Co Ltd., Yesan-Gun, Chungcheongnam-Do 340-861, Korea that contains 1.0×10^{10} CFU (Lactobacillus acidophilus) and Dextrose up to 1kg. The chemical analysis on dry matter basis of different feedstuffs, YC and LB is presented in Table (1) and the formulation of the experimental rations in Table (2).

Itom	DM0/	Chemical analysis on DM basis (%)					
Item	DIVI 70	OM	ASH				
CFM	89.0	88.50	14.32	15.10	4.22	54.86	11.50
Rice straw (RS)	90.63	88.20	3.00	39.33	1.73	44.14	11.80
berseem 3 rd cuts (BC)	17.19	87.29	13.49	23.37	1.68	48.75	12.71
Yeast culture (YC)	92.23	94.77	33.67	6.24	10.42	44.44	5.23
Lactobacillus acidophilus(LB)	93.00	91.60	25.80	12.50	10.60	42.70	8.40

 Table 1. Chemical composition of the ingredients used in the formulation of the experimental rations

CFM= Concentrate feed mixture, DM= Dry matter, OM= Organic matter, CP= Crude protein, CF= Crude fiber, EE= Ether extract, NFE= Nitrogen free extract.

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Ingradiants	The	*	
Ingreutents	G1	G2	G3
CFM %	51.42	54.80	56.81
Rice straw (RS) %	22.42	20.86	19.93
berseem 3 rd cuts (BC) %	26.16	24.34	23.26
	(1) C2 (C1) I D 20 (1 (1)		

G1=Control ration, G2=(G1+YC 20 g/h/d), G3=(G1+LB 20 g/h/d).

Blood samples:

Blood samples were taken before 30 days prepartum and monthly up to the 5^{th} month postpartum from the jugular vein of all animals in different experimental groups into dry and clean test tubes. Blood samples of each buffalo were divided into two parts, the first part was collected in test tubes containing heparin as an anticoagulant and left as a whole blood for determination of hematological determinations. The 2nd parts of blood samples was left to clot for 4 hours at 4-5°C, thereafter centrifuged for 15 min at 3000 rpm for plasma separation, and stored at -20°C until further analysis. The RBCs and WBCs count was determined by haemocytometer and the packed cell volume (PCV%) was determined by micro-hematocrit tube using micro hematocrit centrifuge at 10000 rpm for 5 min, while hemoglobin concentration was carried out using (Super+Ior®, Sahli's method) according to Sahli, (1905). Biochemical in plasma samples were analyzed photmetrically using spectrophotometer and commercial kits to determine concentration of total proteins, TP (Henry, 1964), albumin, Al (Doumas et al., 1971), total lipids, TL (Zollner and Kirsch, 1962), total cholesterol, TC (Richmond, 1973), glucose, Gu (Trinder, 1969) and creatinine, Cr (Bartles et al., 1972). Activity of asprtat (AST) and alanin (ALT) transaminases was determined in blood plasma according to Reitman and Frankel (1957). Concentration of globulin (Gl) was computed by subtraction of albumin from total protein concentration. Concentration of triiodothyronine hormone (T_3) in blood serum was estimated using radioimmunoassay (RIA) commercial kits (Coat-A-Count®-TKT31) and Automatic Mini-Gamma Counter (LKB-1275) according to Saunders (1995). Different types of immunoglobulins (IgG, IgM and IgA) concentrations in samples of colostrum for three consecutive-days post-partum were determined (Killingsworth and Savory, 1972).

Reproductive measurements:

During postpartum period (150 days), the experimental buffalo cows were observed twice daily for estrus and that came in heat were inseminated 12 h after estrus detection. Using rectal palpation,

animals were examined for pregnancy post 50 days of insemination. After parturition, the interval elapsed for fetal membranes drop (hour) complete, uterine cervical closure (day) were recorded and the interval from calving to first estrus (day), number of services per conception and calving interval (day) were recorded.

Statistical analysis:

Data were statistically analyzed by one-way design, the methods of analysis of variance were according to model procedures of SPSS (2013) program. The detected significant differences were performed at (P<0.05) by Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Hematological parameters:

As shown in Tables (3), the present study revealed significant (P < 0.05) increase in RBCs count and percentage of the PCV. While, WBCs count and Hb concentration showed non-significant decrease for lactating buffaloes fed rations supplemented with probiotics in G3 followed by G2 than those in G1 (control).

Similar trend of the increase in PCV and RBCs count was observed in ruminants fed dietary probiotic supplementation (Abd El-Ghani *et al.*, 2004; Wafa, 2008 and Wafa *et al.*, 2020). On the other hand, Ghorbani *et al.* (2002) and Beauchemin *et al.*, (2003) found no significant effect for dietary supplementation. Generally, the average of all hematological parameters in different experimental groups was in the normal range as reported by several authors (Rowlands *et al.*, 1996; Bhosale *et al.*, 1997 and Bhat, 1999).

 Table 3. Effect of dietary probiotics on hematological parameters of lactating buffaloes

Itom]	Overall mean		
Item	G1	G2	G3	Overall lifeall
RBCs count $(x10^{6}/mm^{3})$	7.55±0.17 ^b	7.83 ± 0.12^{b}	8.34±0.13 ^a	0.001**
WBCs count $(x10^3/mm^3)$	7.98±0.13	8.33±0.14	8.35±0.21	0.212^{NS}
Hb (g/dl)	10.38 ± 0.47	10.88±0.33	11.38±0.48	0.275^{NS}
PCV (%)	34.53 ± 0.78^{b}	36.87±0.91 ^a	37.67 ± 0.58^{a}	0.017^*

a-b: Means within the same row with different superscripts are significantly different at P<0.05. ** Significant at P<0.01. *Significant at P<0.05. NS: Non-significant.

Blood metabolites, enzyme activity and hormone:

Data presented in Table (4) showed that blood metabolites (TP, Al, Gl, Gu, TC and TL) for buffaloes fed G3 ration was significantly (p<0.05) higher than those fed on G2 and G1 rations. Concentration of creatinine being lower in G3 (0.85 mg/dl) than in G1 and G2 (1.15 and 1.10 mg/dl), respectively.

In good agreement with these results, several investigators found that feeding different ruminants on diet supplemented with YC significantly increased concentrations of blood TP (Abd El-Ghani *et al.*, 2004; Ebrahim, 2004; El-Ashry *et al.*, 2001) compared with non-supplemented group. On the

other hand, some authors found no significant effect on blood total protein when dairy cows fed diet with YC supplementation (Ayala-Oseguera *et al.*, 2001 and Bonadaki *et al.*, 2004). Wafa *et al.*, (2020) showed that the concentrations of TP and AL were significantly (P<0.01) higher in dairy cows fed 40g of YC than those fed 20g or controls. Zeedan *et al.* (2008, 2009a, b and 2014) and Abu-El-Ella *et al.*, (2014) reported that plasma TP, Al and Gl recoded significant increase (P<0.05) in buffaloes with Biogen–zinc supplementation. In lactating buffaloes, Ghoniem *et al.* (2018) found that the daily supplementation of dry yeast at level of 10 g per head had no significant effects on plasma total protein, albumin, and globulin. Mahrous *et al.* (2019) concluded that, the statistical evaluation showed significant increase (P<0.05) in group fed diet with RY supplementation for total protein, albumin and globulin. Ahmad Para *et al.* (2019) found that there was a significant (p<0.05) difference in plasma metabolic profile (protein and globulin) across different experimental periods.

Abu-El- Ella *et al.* (2014) reported that activity of AST and ALT increased (P<0.05) in response to YC supplementation during different physiological stages. Zeedan *et al.*, (2008, 2009a, b and 2014); Mahrous *et al.* (2019) and Mostafa *et al.* (2014)

reported that values of serum AST and ALT were not significantly affected by using probiotics treatments. Also, they found that the concentrations of AST and ALT in all treated animals were within the normal range of healthy animals that may indicate good nutritional status of experimental animals. Faten Abou Ammou *et al.*, (2013) indicated that, the addition of yeast culture to Damascus goats ration at levels of 2.5 and 5 g/h/d increased the concentrations of AST and ALT. On the other hand, Wafa *et al.*, (2020) indicated a significant decrease in both ALT and AST concentration in dairy primi-parous cow's blood.

Table 4. Effect of dietary probiotics on blood metabolites, enzyme activity and hormone of lactating buffaloes

Itom]	Overall mean		
Item	Gl	G2	G3	Overall mean
Blood metabolites				
total protein (g/dl):	$7.63 \pm 0.05^{\circ}$	8.02 ± 0.04^{b}	$8.44{\pm}0.08^{a}$	0.000^{***}
albumin (g/dl):	$4.33 \pm 0.05^{\circ}$	4.53 ± 0.04^{b}	$4.83{\pm}0.07^{a}$	0.000****
globulin (g/dl):	$3.30\pm0.03^{\circ}$	3.49 ± 0.04^{b}	3.61 ± 0.02^{a}	0.000^{***}
glucose (mg/dl):	51.49 ± 1.43^{b}	53.39±1.57 ^b	59.17±1.46 ^a	0.002^{**}
total cholesterol (mg/dl):	176.10±6.38 ^c	212.25±14.83 ^b	252.58±9.94 ^a	0.000^{***}
creatinine (mg/dl):	1.15 ± 0.05^{a}	1.10 ± 0.06^{a}	0.85 ± 0.04^{b}	0.000^{***}
total lipids (g/dl):	590.33±22.87 ^c	677.80±29.10 ^b	813.67±17.57 ^a	0.000^{***}
Enzyme activity and Hormone				
AST (IU/ml)	39.80±2.41 ^a	27.60 ± 2.06^{b}	$21.33 \pm 1.62^{\circ}$	0.000^{***}
ALT (IU/ml)	9.33±0.61	9.43±0.24	8.80±0.24	0.495 ^{NS}
(T3) (ng/dl):	62.94±2.15	65.93±5.24	73.93±4.31	0.160^{NS}

a-c: Means within the same row with different superscripts are significantly different at P<0.05.

Immunogloblins concentrations in colostrum:

In comparison with control (G1), probiotic supplementation (G3 and G2) increased significantly (P<0.05) the concentration of colostrum IgA, IgG and IgM. The statistical analysis revealed significant effect for probiotic treatment on all colostrum immunoglobulins concentrations. In general, colostrum immunoglobulins concentration was higher in treated groups as compared to control, being the highest in G3 (Table 5).

The obtained results in this study indicated heavier calves at birth in all groups of dams treated with LB or YC than in control group that fed non-supplemented diet which may be attributed to the improvement in immune, health status and weight of their dams (Wafa, 2017). Fröhdeová *et al.*, (2014) indicated that, the addition of low amount of *Saccharomyces cerevisiae*

increased the level of IgG in serum at higher lactating cows. Also, similar effects were reported by some previous investigations after feed supplementation with prebiotics in different species (Franklin *et al.*, 2002; Spearman, 2004). In bovine, Baines *et al.*, (2011) confirmed that probiotic (Celmanax) increased level of both IgG and IgM after parturition which may be due to transport of immunoglobulins from blood into mammary gland secretion in the period around calving (Heriazon *et al.* 2011). According to the findings of Murphy *et al.* (2005) and El-Hawary and Abd El-Hady (2018) the present results indicated that immunoglobulin (Ig) amount in buffalo colostrum depend on immune potentiators addition to dam ration at pre-partum period.

Table 5. Effect of dietary probiotics on immunogloblins (IgG, IgA and IgM) concentrations in colostrum of lactating buffaloes

Immunoglobuling (g/dl)	Experimental groups				
minulogiobulins (g/ul)	G1	G2	G3		
IgA	0.209 ± 0.041^{b}	0.359 ± 0.63^{a}	0.379±0.81 ^a		
IgG	2.308 ± 0.30^{b}	3.113 ± 0.28^{a}	3.273 ± 0.29^{a}		
IgM	0.321 ± 0.10^{b}	$0.514{\pm}0.12^{a}$	0.548 ± 0.12^{a}		
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a and b: Means within the same row with different superscripts are significantly different at P<0.05.

Reproductive measurements:

As shown in Table (6), the present study revealed a significant (P<0.05) increase in calves BW being the highest in G3 and the lowest in G1 while, G2

showed the moderate value. The reproductive measurements including DFM, CC, PFEI and CCI were significantly (P<0.05) decreased for lactating buffaloes fed rations supplemented with probiotics in

G3 followed by G2 than those in G1 (control). Data in Table (6) showed a slightly decrease in NS/C for buffaloes fed diet supplemented with probiotics (G2 and G3) compared with G1 but the differences were non-significant.

Itom		Overall mean		
	G1	G2	G3	
BWC (kg)	33.50±1.00 ^c	37.40 ± 1.18^{b}	40.40 ± 0.90^{a}	0.000****
DFM (h)	8.05 ± 0.71^{a}	$4.80{\pm}0.44^{b}$	$2.80\pm0.44^{\circ}$	0.000^{***}
CC (d)	37.40 ± 0.87^{a}	27.20 ± 1.20^{b}	22.30±0.47 ^c	0.000^{***}
PFEI	112.50±14.65 ^a	64.90±5.32 ^b	$35.00\pm5.25^{\circ}$	0.000^{***}
CCI	148.70 ± 11.16^{a}	81.40±5.44 ^b	47.20±8.03°	0.000^{***}
NS/C	1.90 ± 0.87	1.40±0.51	1.30±0.48	0.105^{NS}

 Table 6. Effect of dietary probiotics on reproductive measurements of lactating buffaloes

BWC: Body weight of calves, DFM: Drop fetal membranes, CC: Cervical closure, PFEI: Postpartum first estrus interval, CCI: Calving to conception interval, NS/C: Number of services per conception.

a-c: Means with different superscripts within the same row are significantly different at P<0.05. *** P<0.001. NS: Non-significant.

In agreement with the present results, several investigators found that feeding different farm animals on diet with probiotics significantly improved all reproductive measurements. Zeedan et al. (2009a and 2014) indicated higher values (P≤0.05) of (BWC, PFEI, NS/C) in buffalo cows supplemented with Biogen-Zinc (BZ) compared with control group. Mousa et al. (2012) reported that supplementation of yeast to ewes diets increased litter weight at birth of their offspring. Kassabra et al. (2013) found the highest birth weight with 8 g/h DY supplemented group followed by 4 g/h DY group then the lowest values was in control one (P < 0.05). Faten Abou Ammou et al. (2013) indicated that addition of yeast culture (YC) at levels 2.5 g/h/d or 5g/h/d to Damascus goats ration increased birth weight of kids born. Mostafa et al. (2014) showed that supplementing commercial yeast culture (S. cerevisiae) namely BGY at rate 35 g/d or a product of lactic acid bacteria and enzymes namely AVI-BAC® (two probiotics) to the diet of lactating cows led to a significant increase in birth weight of produced calves. Abu-El-Ella et al., (2014) indicate that supplementation of biogen-zinc significantly (P≤0.05) improved weight of Damascus kids born or weaned.

Results of Qlyae (2016) showed that the average of buffalo's birth weight ranged between 33.07 to 37.62 kg and the additives treatment did not affect significantly the newborn birth weight. Ahmad Para *et al.* (2019) indicated that, the calves birth weight in supplemented group (24 g/day FYC; Diamond V XPC Yeast Culture) was higher significantly (P \leq 0.05) being 36.4 kg than the control group (34.98kg). Also, yeast treatment improved all reproductive measurements, Ebrahim (2004) and Wafa *et al.* (2020) confirmed that average number of services per conception within 120 days postpartum, was a higher value in control than in yeast culture treated cows (1.5 vs. 1.0).

CONCLUSION

It could be concluded that *Lactobacillus* acidophilus supplementation in Egyptian buffalo

ration had beneficial effects on blood parameters, health status, immunity response and their reproductive performance under the local farm feeding conditions in Egypt.

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تأثير منتجات الخميرة واللاكتوباسيللوس كإضافات غذائية على قياسات الدم والأداء التناسلي للجاموس المصري الحلاب

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