

IMPACT OF USING YEAST AS FEED ADDITIVE ON PRODUCTION PERFORMANCE AND IMMUNE RESPONSES OF SHE-CAMELS AND THEIR NEWBORN CALVES DURING PERI-PARTURATION PERIOD

By

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Abstract

She-camels face down-regulation of immune responses during pregnancy that may lead to viral and bacterial infections so increases the dangers affecting their newborn calves. This study aimed to improve the body weight and immune responses of she-camels and their newborn calves. A total of 10 dams were divided into two equal groups; G₁ (fed the basal diet only) and G₂ (fed the basal diet & *Saccharomyces cerevisiae* (SC); 0.5 g/head/day). Dams' and calves' plasma, milk samples were collected. Quantitative analysis of IgG, IgA & IFN- γ levels in samples was done by ELISA and Body weights were recorded monthly. Results revealed significant increases (P<0.05) in body weight, body weight gain of dams and calves' birth weight in G₂ compared to G₁. IgG₂ levels dams were higher than IgG₁ at all durations, IgA levels didn't show any significant change in groups while IFN- γ showed an increased level in G₂ more than G₁ from the time of calving to one month post calving. IgG, IgA & IFN- γ levels in milk didn't show significant differences between the experimental groups. Calves' IgG in G₂ showed higher significant levels compared to G₁ from birth to one month after birth, IgA & IFN- γ levels in G₂ showed significant increase at one month after birth. Conclusion: SC is a good feed additive for she-camels during peri-partum. It improved total body weight, body weight gain and birth weight of calves. Immune responses of dams and calves were enhanced that may help in facing the stressful pregnancy event.

Keywords: She-camel, Yeast, Immunity, Calf, Peri-parturation; Body weight, Parasitosis.

Introduction

Camelus dromedarius (One-humped camel) is one of the important domestic animals in arid and semi-arid regions, due to their superior capability to produce low cost and high quality meat and milk (Muzzachi *et al.*, 2015). FAO (2013) Statistics had recorded that camel population in Egypt is 152,946 head that well worth to focus on its development in the area.

The pregnancy is one of the physiological stressors that might affect she-camel's performance especially during peri-parturient period thus it may adversely affect animal's immunity (Al-Zamely, 2011), damage its biological macromolecules and disruption of normal metabolism and physiology (Abdel-Aal and Eid, 2014). In camels, herd productive efficiency is also greatly influenced by the high mortality rate of camel calves in

their first 3 months due to the harsh desert conditions and infectious diseases. Improving she-camel's health during peri-parturation were important for the health status of the newborn calves that come to life almost deprived of serum immunoglobulins (Igs) and depend on colostrum for virtually all humoral passive immunity (Silva *et al.*, 2013).

Probiotics are one of those feed strategies that have been used for various livestock species to improve animal health and production (Vivek *et al.*, 2014). They are known to be live micro-organisms which when administered in adequate amounts confer a health benefit to the host (FAO, 2009). Probiotics have been reported to improve growth performance, nutrient digestibility, balance of the intestinal microflora and importantly promote the immune function (Zhang and

Kim, 2013). Live yeast, yeast cell wall or their extracts were used in ruminant feeding as a natural protein source to improve animal performance, health and immune responses (Burdick Sanchez *et al*, 2014).

The major components of yeast or its cell wall are polysaccharides such as α or β glucans (Kogan and Kocher, 2007) that can be used as feed additives. They may interact directly with the immune cells or bind to pathogenic bacteria preventing attachment or colonization in Gastro-intestinal tract (Posada *et al*, 2014). Also, yeast cell wall has antioxidant compounds (Kogan *et al*, 2005).

Generally, this unique animal that inhabits arid areas in the world faces some health problems that need to be solved in order to maintain this great animal species from extinction. Hence, there were no applied researches that focus on improving camel production performance by using probiotics; this makes our work novel applied results. The aims of our work were to develop the production performance of camels by improving health of pregnant one by enhancing immunity during stressful event, increasing body weight, improving its milk's immunological value expected to have positive effects on health, birth weights and calves' immunity that form the unit of herd and milk immunological value.

Materials and Methods

Ethical Approval: All experiments were done in accordance with the ethical guidelines and regulations set forth by the Institutional Animal Care and Use Committee (IACUC) in Egypt.

Probiotic strain: Levucell (SC20), a commercial probiotic product of dried live yeast (Lallemand SAS Co.) was used. Composed ingredient of the live yeast was *Saccharomyces cerevisiae* (SC), (20×10^9 CFU/g).

Animal grouping and experimental durations: Ten healthy pregnant dromedary she-camels at their late gestation period (at 10th month of pregnancy; two months pre-calving) with body weights ranged from 570 to 600 kg and housed at Camels Studies and Production Development Center (CSPDC), Matrouh Governorate, western northern of Egypt. All dams were fed basal diet starting at the transition period of pregnancy (at 10th month of pregnancy) and ended at one month post-calving. Dams were divided into two groups of five dams each: GI (Control): dams fed the basal diet only. GII: dams were fed the basal diet & 0.5gm of SC/ head daily (included in a palm date to avoid loss after manufacturer's recommendations).

Laboratory examination: Urine to diagnose a urinary tract or kidney infections (Wilson and Anderson, 1993) and to exclude balantidiasis (Tajik *et al*, 2013). The stool analysis involved collection and analysis of fecal matter to diagnose the presence or absence of gastrointestinal parasites (El-Naggar *et al*, 2006), as eggs of *Marshallagia* spp. *Nematodirus* spp. *Haemonchus* spp. and *Trichuris* spp. *Trichostrongyle* spp. as well as oocysts of *Eimeria cameli* (Radfar and Gowhari, 2013). Blood was examined for parasite interferes with Igs levels (Morsy *et al*, 2001), as *Babesia* spp. *Theileria* spp. (el Kady, 1998) and *Trypanosoma evansi* (Haridy *et al*, 2011) and *Toxoplasma gondii* (Gebremedhin *et al*, 2014). Also, they were examined carefully for the ecto-parasites (el-Azazy, 1996), especially tick infestation as *Hyalomma* spp. (El Kammah *et al*, 2001) and the nasal nasal-botfly myiasis as *Cephalopina titillator* (Morsy *et al*, 1998). Body weights (Bwt) were recorded monthly. Plasma and milk samples were collected at certain durations as follows (Table 1).

Table 1: Experimental durations at which body weights recorded and plasma samples taken

Time from calving	Dams' plasma sampling	Dams' body weights	Milk Sampling	Calves' plasma sampling	Calves' body weights
Two months pre-calving	√	√	—	—	—
One month pre-calving	√	√	—	—	—
At calving	√	√	√	√	√
One week post calving	√	—	√	√	—
One month post calving	√	√	√	√	—

Blood Sampling and Plasma preparation: Blood samples were collected from the Jugular vein of dams and their calves into vacutainer tubes containing EDTA (an anti-coagulant), centrifuged at 3000 rpm/10min to obtain plasma which were kept at -20° C until analysis.

Milk sampling and Whey preparation: Milk was collected in sterilized tubes and transformed into whey. Ten ml of milk or colostrum were centrifuged at 4000 rpm at 4°C for 30 minutes to remove the floating fat drop then warmed in a water bath at 40-45°C for 30 minutes, after that some drops of freshly prepared Rennet solution were added and incubated in the water bath again. Casein clot aggregated with the remaining fat was filtered to obtain clear whey and stored at -20° C until needed (Hyun *et al*, 2014).

Immuno-Assay: IgG, IgA and the cytokine IFN- γ were quantitatively detected by Sandwich ELISA (Aydin, 2015). Procedures were done according to the manufacturers' instructions (Camel IgG ELISA, Life Diagnostics Inc., Catalog No: IGG-16; Camel IgA ELISA, Mybiosource, Catalog Number: MBS073806 and Camel IFN- γ ELISA, Wuhan Fine Biological Technology Co., Ltd., Catalog Number: ECM0009).

Statistical Analysis: Data are presented as means \pm Standard error (SE) of each group was calculated from mean values individually. Comparison between groups was done using T test. Analysis was carried out using SAS User's Guide: Statistics procedure of SAS version 9.1 for Windows (SAS, 2004).

Results

Effect of SC feed addition on she-camels' Bwt and its impact on their newborn calves' birth weight: Bwt of dams in the two experimental groups before SC addition were nearly similar. After one month, the Bwt of G₂ dams were significantly higher when compared to G₁ (605 kg vs. 591.33kg, respectively). Bwt gains pre-calving were higher in G₂ (23.67 \pm 0.88 kg) compared to G₁; (11.67 \pm 0.88 kg). But, there was non-significant difference in Bwt of dams in the two experimental groups directly after delivery; however G₂ was numerically higher than G₁. Dams' Bwt in G₂ was significantly higher (578.9 kg) than G₁ (563.67kg) one month post-calving. Bwt gained post-calving was high in G₂ (17 \pm 1kg,) than G₁ (10.33 \pm 0.33 kg).

Details are given in tables (2, 3, 4 & 5) and figures (1, 2 & 3).

Table 2: Body weight of dams in experimental groups at different times from calving.

Dams' Body Weight (kg)	G ₁ (Control)	G ₂ (Yeast)
At start of experiment	579.67 \pm 3.18	581.33 \pm 1.86
One month after starting	591.33 \pm 4.06 ^B	605 \pm 1.16 ^A
Post-partum	553.33 \pm 3.28	561.90 \pm 1.07
One month post-partum	563.67 \pm 3.53 ^B	578.9 \pm 0.86 ^A

^{A, B} (mean \pm SE) within same row with different superscripts differ significantly (P<0.05) from each other. G₁: control group, G₂: group of animals fed *Saccharomyces cerevisiae*.

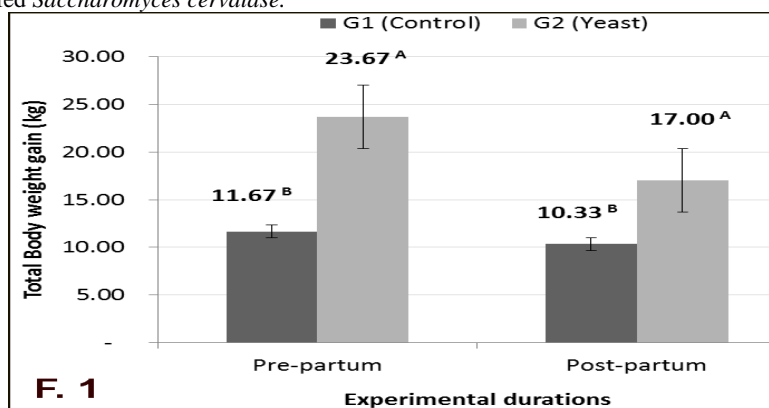


Fig 1: Dams' total body weight (Bwt) gain in two experimental groups pre- and post-calving. Data represented by (mean \pm SE) with different superscripts (^{A, B}) differ significantly (P<0.05) from each other. G₁: control group, G₂: dams fed *Saccharomyces cerevisiae*

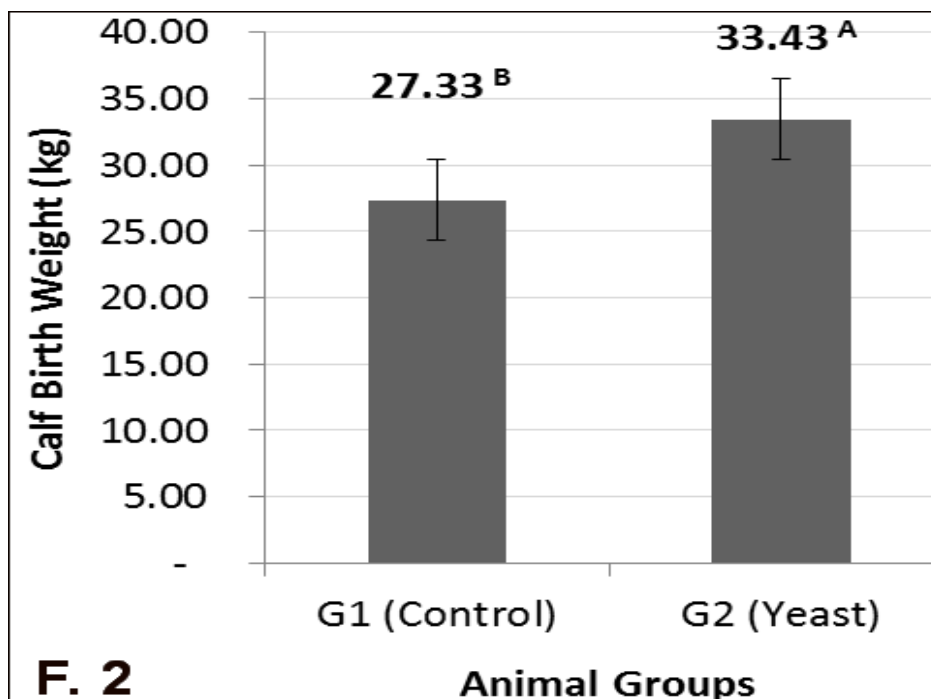


Fig 2: Calves' birth weight in the experimental groups. Data represented by (mean \pm SE) with different superscripts (^{A, B}) differ significantly ($P < 0.05$) from each other. G₁: calves of control dams, G₂: calves of dams fed *Saccharomyces cerevisiae*.

Calves' birth weights showed significant differences between experimental groups, calves birth weights in G₂ dams were higher (33.43 ± 0.69 kg) than in G₁ (27.33 ± 0.88 kg)

Table 3: Concentrations of IgG, IgA and IFN- γ (ng/ml) in she-camels' plasma of experiments at different times from calving

Time from calving	Parameter	Animal groups	
		G ₁ (Control)	G ₂ (Control)
		Mean \pm SE	Mean \pm SE
Two months pre-calving	IgG	1.61 \pm 0.18	2.92 \pm 0.74
	IgA	3.34 \pm 0.34 ^A	2.14 \pm 0.37 ^B
	IFN- γ	0.01 \pm 0.001	0.01 \pm 0.006
One month pre-calving	IgG	2.45 \pm 0.22 ^B	4.73 \pm 0.04 ^A
	IgA	2.94 \pm 0.27	2.29 \pm 0.39
	IFN- γ	0.02 \pm 0.003	0.02 \pm 0.002
At calving	IgG	3.38 \pm 0.42 ^B	4.31 \pm 0.09 ^A
	IgA	2.81 \pm 0.11	3.20 \pm 0.46
	IFN- γ	0.03 \pm 0.001 ^B	0.04 \pm 0.002 ^A
One week post-calving	IgG	3.75 \pm 0.41 ^B	5.44 \pm 0.63 ^A
	IgA	2.33 \pm 0.08	2.44 \pm 0.28
	IFN- γ	0.04 \pm 0.001 ^B	0.07 \pm 0.004 ^A
One month post-calving	IgG	2.25 \pm 0.36 ^B	4.07 \pm 0.54 ^A
	IgA	2.14 \pm 0.09	2.08 \pm 0.11
	IFN- γ	0.03 \pm 0.001 ^B	0.05 \pm 0.002 ^A

^{A, B} (mean \pm SE) within same row with different superscripts differ significantly ($P < 0.05$) from each other. G₁: control group, G₂: dams fed *Saccharomyces cerevisiae*.

Effect of SC feed addition on IgG, IgA and IFN- γ levels in she-camels' plasma during peri-parturition: At one month before the expected calving date (after one month of SC addition), IgG; the most important Ig in camels, showed a significant increase ($P < 0.05$) in G₂ (4.73ng/ml) compared to G₁ (2.45ng/ml). Neither the concentration of IgA nor the concentration of IFN- γ showed

any significant changes between the experimental groups. At calving, the concentration of IgG and IFN- γ showed significant increases ($P < 0.05$) in G₂ compared to G₁ while IgA concentration did not show any change in the two groups (Table 3). At one week post-calving, IgG concentrations in G₂ began to increase again when compared to G₁, while IgA concentrations didn't show any

significant differences between the two groups. The levels of IFN- γ continued to show high and peak levels in G₂ (0.07 ng/ml) compared to G₁ (0.04 ng/ml). At one

month post-calving, IgG and IFN- γ levels decreased but continued to maintain higher levels in G₂ more than G₁ but IgA was still showing no change at all.

Table 4: Comparison between the concentrations of IgG, IgA and IFN- γ (ng/ml) detected in milk of she-camels in experimental groups.

Time from calving	Parameter	Groups	
		G1	G2
		Mean \pm SE	Mean \pm SE
At calving	IgG	1.46 \pm 0.03	1.24 \pm 0.09
	IgA	2.04 \pm 0.51	2.75 \pm 0.15
	IFN- γ	0.01 \pm 0.002	0.02 \pm 0.004
One week post-calving	IgG	1.32 \pm 0.14	2.28 \pm 0.35
	IgA	1.74 \pm 0.15	2.37 \pm 0.15
	IFN- γ	0.02 \pm 0.003	0.03 \pm 0.010
One month post-calving	IgG	1.69 \pm 0.08	2.24 \pm 0.54
	IgA	2.11 \pm 0.40	1.84 \pm 0.06
	IFN- γ	0.03 \pm 0.009	0.04 \pm 0.010

Note: no statistical differences were detected between different groups concerning ($P > 0.05$) in the concentrations of IgG, IgA or IFN- γ passively transferred into milk of dams of the two groups. G₁: control group, G₂: dams fed *Saccharomyces cerevisiae*.

Effect of SC feed addition on IgG, IgA and IFN- γ levels in she-camels' milk: IgG levels showed non-significant difference between G₁ and G₂ at all but was found to be numerically higher in G₂ compared to G₁ at one week post-calving and one month post-calving ng/ml respectively. IgA levels also revealed non-significant differences between

groups while the numerical values showed that G₂ had higher values compared to G₁ at calving until at one week post-calving respectively. For the concentration of IFN- γ , there was no significant difference between groups at all the studied periods but there were numerically higher in G₂ more than G₁ at all durations.

Table 5: Concentrations of IgG, IgA & IFN- γ (ng/ml) in plasma of calves born to dams of experimental groups during lactation.

Time from birth	Parameter	Animal groups	
		G ₁ (Control)	G ₂ (Control)
		Mean \pm SE	Mean \pm SE
At birth	IgG	2.21 \pm 0.91 ^B	4.26 \pm 0.19 ^A
	IgA	1.99 \pm 0.43	1.61 \pm 0.50
	IFN- γ	0.01 \pm 0.002	0.01 \pm 0.002
One week after birth	IgG	2.59 \pm 0.71 ^B	4.95 \pm 0.30 ^A
	IgA	1.69 \pm 0.34	2.03 \pm 0.41
	IFN- γ	0.02 \pm 0.002	0.03 \pm 0.010
One month after birth	IgG	2.03 \pm 0.48 ^B	4.99 \pm 0.19 ^A
	IgA	3.01 \pm 0.38 ^B	5.41 \pm 1.10 ^A
	IFN- γ	0.02 \pm 0.003 ^B	0.04 ^A 0.004 ^A

^{A,B} (mean \pm SE) within the same row with different superscripts differ significantly ($P < 0.05$) from each other. G₁: calves of control dams, G₂: calves of dams fed *Saccharomyces cerevisiae*.

Impact of SC feed addition to she-camels on the immune responses (IgG, IgA & IFN- γ) of the newborn calves: Plasma IgG, IgA & IFN- γ levels in calves of experimental groups from birth to one month after birth. Significant differences ($P < 0.05$) between the two experimental groups in concentrations of IgG were shown, where calves born to G₂ dams had higher IgG levels than calves born

to G₁ dams at all durations. Besides, at birth the IgA & IFN- γ levels in calves' plasma didn't show any significant differences between the experimental groups. At one week after birth, IgA & IFN- γ levels didn't differ significantly between two experimental groups. At one month after birth, both IgA & IFN- γ levels in G₂ were significantly higher as compared to G₁.

Discussion

The present results showed that oral administration of *SC* gave positive effects on dams' Bwt after one month of administration pre-calving. Dams' Bwts were higher in treated animals than control at calving and one month post-calving. *SC* administration had improved dam's daily Bwt gain pre/post-calving. The results agreed with Casey *et al.* (2007) who tested some probiotics separately or in combination on growth performance of sheep, goat and cattle. Improvement of growth performance may be related to feed higher consumption, microbial ecology improvement of the animal and nutrients absorption resulting in better weight gain (Musa *et al.*, 2009). Yeast and their products strongly enhanced animal performance and increased body weight in beef cattle (Finck *et al.*, 2014).

The present results showed that birth weights of calves related to dams administered *SC* were higher than control group. *SC* administration enhanced nutrient transfer from dams to the newborn during pregnancy, thus was expected to have a positive impact on birth of newborns and general health status. This result agreed with Shen *et al.* (2011) who found that yeast administration to sows during gestation increased piglets weaning weight and average daily gain.

The effect of adding *SC* to feed affected some immune responses of late pregnant she-camels and during early lactation period. Levels of IgG, IgA or cytokine IFN- γ in plasma of dams were positively influenced by yeast against exposure to various stress events (pregnancy, delivery or lactation). For IgG levels in plasma of dams, *SC* addition had positive effects on G₂ compared to G₁. This effect appeared after one month after oral administration and continued at calving, peak levels at one week post-calving and decreased slightly at one month post-calving. Plasma IgA levels in G₂ dams increased slightly at calving. This result agreed with Cakiroglu *et al.* (2010) who reported that *SC* feed supplementation acted as

an immune-stimulant when given daily to dairy cows. Yin *et al.* (2008) found that galactomannan-oligo-saccharides (a yeast product) feed supplementation, enhanced serum levels of Igs.

The present results of addition *SC* to diet of late pregnant she-camels had positive effects on the pro-inflammatory IFN- γ levels by the time calving, continued greatly to one week after calving and one month after calving. This result agreed with Collier *et al.* (2011) who found that yeast elicited inflammatory immune responses and reduced pigs' mortality. *SC* cell wall as an immunomodulators interacted directly or indirectly with pathogens and the organism's immune system, and stimulated synthesis and release of pro-inflammatory cytokines from macrophages, neutrophils or T lymphocytes in the swine (Xiao *et al.*, 2004).

Mammalian milk is known to be enriched with Igs to give passive immune protection against antigens and micro-organisms in the GIT of mother or environmental antigens coming in contact. The Igs are present at the highest concentrations in the first few days post-partum (colostrum) and then falls progressively (Kleinman and Walker, 1997).

The present results showed that the effect of oral administration of *SC* dams on IgG levels in the milk possess similar trends such as the plasma IgG levels. The treated group showed variable higher milk IgG levels than the untreated ones during supplying colostrum. IgG levels in milk of dams administered *SC* showed a peak one week and one month post-calving. In dams' milk fed *SC*, IgA levels didn't show significant changes but, IFN- γ levels released in milk were higher in dams given *SC* more than untreated dams starting from calving, continued to one week post-calving up to one month post-calving.

The presence of immunological factors in milk illustrated where B & T cells induced by an antigen such as *SC*, were able to migrate via different lymphatics and via the mesenteric nodes reached the systemic cir-

ulation through the thoracic duct and repopulated in mammary glands hence the assumption of an increased IgG production in milk of dams. This fact agreed with the phenomenon "Common Mucosal System" (Cebra *et al.*, 1991). She-camel, unlike other ruminants' females, has an impermeable epitheliochorial placenta, which prevented intra-uterine passage of antibodies and other immunological factors from dam to fetus during pregnancy (Moffett and Loke, 2006). This impermeability is related to the unique structure of six layers of cells between maternal and fetal circulations (maternal capillary endothelium, uterine connective tissue, uterine epithelium, chorionic epithelium, fetal connective tissue and fetal capillary endothelium); so the calves were born with the hypogammaglobulinemia and immature immunity (Enders and Carter 2004). So, the maternal protection to fetus during pregnancy postponed calf specific immunity (Silva *et al.*, 2013). Also, the innate immune system of the fetus or newborn calves was immunosuppressed due to maternal hormones released during late pregnancy (Benesi *et al.*, 2012).

Colostrum is the only source for immune protection which ensures the survival of calves in their first months of life via the natural passive transfer of the majority of immune factors such as Igs and cytokines from dams to them (Silva *et al.*, 2013). During early lactation, Igs in colostrum are absorbed via unselective permeability of epithelial cells in calf's small intestine then are transported through intestinal lymphatic tissue to reach its circulatory system (Riddle, 2003). The pro-inflammatory cytokines such as IFN- γ are also present in maternal colostrum and possibly absorbed by the intestinal mucosa of calves, reaching the highest concentrations in newborn bloodstream at the first 72 hours of their life (Madureira, 2011). But, a calf might have IFN- γ levels prior to colostrum intake, probably due to antigen contact during pregnancy where some microorganisms could cross the placenta and

innate immune system of calves was then the main defense during pregnancy (Gomes *et al.*, 2014).

Knowledge of passive transfer of immunity to young calves led to focus on improving the immune factors in pregnant dams by the probiotics usage as immuno-stimulators.

The presented study also showed that plasma IgG levels in calves (related to G₂ dams) were greatly enhanced compared to the untreated counterparts from birth (during colostrum suckling), to one week after birth and up one month after birth (during milk suckling). Also, plasma IgA levels in calves (related to dams fed SC) were higher than calves related to untreated dams at one week after birth and reached the peak levels one month after birth. The plasma IFN- γ levels in calves (dams fed SC) were higher in levels starting from one week after birth and continued up to one month after birth but also calves were born with plasma containing IFN- γ . This agreed with Gomes *et al.*, (2014) who reported the probability of microorganism passage via the placenta and potentiating the innate immune system of fetus during pregnancy.

The addition of yeast to diets of dairy cattle enhanced performance of the newborn calves and decreased morbidity and mortality (Magalhaes *et al.*, 2008). Also, when gestating sows were supplemented with SC, an improvement of newborns health was achieved (Shen *et al.*, 2011).

The mode of action by which yeast or their products affect immunity and health of an animal may be one of the following postulations. It must be known that yeast composing polysaccharides are indigestible by the digestive system's enzymes in mammals and are supposed to reach the large intestine where they may be fermented by commensal microbiota that in turn caused changes into the bacterial composition and the released metabolic compound (de verse *et al.*, 2008). The yeast also alter with the microbiome in the GIT of a ruminant and promote beneficial bacteria; thus aid in development of the

healthy intestinal flora enhancing nutrient uptake and improving immune function (Mullins *et al*, 2013). Live yeast cell wall polysaccharides directly bound to pathogenic bacteria and inhibited their binding and colonization in the GIT (Posada *et al*, 2014).

A third postulation suggested that some yeast cell wall components as mannans and β -glucans might have direct effects on leukocytes and secretions of pro-inflammatory cytokines through the intestinal mucosal epithelium to play a major role in organizing gut immune system. Intraepithelial lymphocytes and dendritic cells (DC) protruded their dendrites through the epithelial lining and became in contact with gut lumen, thus, direct interaction with β -glucans occurs (Qi *et al*, 2011) and transporting them to gastrointestinal lymph nodes to the circulating blood to the bone marrow, lymph nodes and spleen (Sandvik *et al*, 2007). Yeast cell wall compounds was taken up by specialized epithelial cells; Mast cells in lymphoid follicles associated within Peyer's patches (Volman *et al*, 2008).

Conclusion

The outcome results showed that the inclusion of *Saccharomyces cerevisiae* as feed additive to regular diets of pregnant dromedary she-camels is effective for improvement of their body weights at the periparturition period, for enhancement of immune responses of them during stressful events and positively reflects on birth weight and health of their newborn calves.

Therefore, it is recommended to add Yeast in the regular diet of pregnant she-camels in order to recover their health and in turn the newborns' health. This systemic chain improved economic value of this great animal.

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