

## effect of supplementing arabian and barbe pregnant mares with *Saccharomyces Cerevisiae* on Colostrum IgG1 Concentration in Algerian Breed

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### ABSTRACT

There is limited information on the effect of maternal dietary yeast supplementation on immunoglobulin levels in foals.

The main objective of this study was to evaluate Effect of Feeding Yeast Culture *saccharomyces cerevisiae* on mare colostrum IgG1 concentration, serum IgG1 and weight of their foals in Algerian breed.

In this study forty, Arabian and barbe Horse mares ( $8,54 \pm 3,72$  yr) were randomly assigned to one of two groups: yeast or control. All mares received a basal diet of barley wet due to 3kg morning and 3 Kg evening with water and mixed grass hay *ad libitum*.

In addition to the daily diet, from 10 to 15h: 30 mares go to pasture (paddock) for exercise and grazing grass. Mares in the yeast treatment group also received 10g/d of a live culture of *Saccharomyces cerevisiae* Sc 47 BIOSAF® HEAT RESISTANT CONCENTRATE OF LIVE YEAST (LESAPRE, FEED ADDITIVES, LESAPRE-France) ( $1.10^{10}$  UFC/g) from 300 d of gestation to 180 d post-foaling. Colostrums samples 20 ml were collected from mare udder in 50ml glass tube immediately pre suckling, the Blood samples were collected from the foals via jugular venipuncture immediately after parturition (d 0), at 12 and 24 hr. samples were analyzed for total IgG1 concentrations using commercial IDR kits. Data were analyzed using SYSTAT 12 © Copyright 2007 and a p-value of  $\leq 0.05$  was considered statistically significant.

Supplementing the maternal diet with live yeast did influence mare colostrums and foal serum IgG1 concentrations significantly with  $P=0,02$ .

IgG1 concentrations were significantly increased post-foaling weight in foals born from mares fed the yeast supplement compared to controls  $P=0.005$  at five month post foaling  $195.75 \pm 14.21$  Kg &  $176.40 \pm 26.30$  Kg respectively and  $P=0.009$  at weaning with  $207.14 \pm 13.17$  Kg in yeast group compared with control  $188.20 \pm 24.28$  Kg.

In this study, dietary yeast supplementation influence significantly mares colostrums and foals serum IgG1 concentration, with an increase foal weight when fed at a target dose of 10g once daily.

**KEY WORDS:** Immunoglobulins, Horse, IDR, Yeast

### INTRODUCTION

Foals are born immunodeficient [1]. Like most farm animals, there is no transfer of immunoglobulins (Ig) through the placenta during gestation. Foals receive immunoglobulins from the dam's colostrum which can be absorbed through the foal's gastrointestinal epithelium for the first 18 – 24 hr of life. If a foal does not absorb sufficient quantities of immunoglobulins, it could develop a condition referred to as failure of passive transfer (FPT) and be at increased risk of morbidity and mortality due to septicemia [2].

IgG is the most prevalent immunoglobulin in equine blood and colostrum [3]. Originally it was thought that there were five IgG subclasses but it has been discovered that there are actually seven subclasses (IgGa = IgG1; IgGb = IgG4 and IgG7; IgG(T) = IgG3 and IgG5; and IgGc = IgG6). In serum, the IgG isotypes rank from greatest to lowest concentration as follows: IgGb > IgG(T) > IgGa > IgGc [3].

IgM is the first antibody to respond to a pathogen to the system [4]. If the pathogen is novel, IgM will be the most prevalent immunoglobulin at the beginning of the immune response while the body is initiating production of IgG. IgA is the primary immunoglobulin found in mucosal membranes and secretions and can also be found in the blood. It is the second most prevalent immunoglobulin next to IgG in colostrum. As the colostrum changes into milk, IgA becomes the most prevalent while IgG becomes second [1]. The main function of IgA is to prevent attachment of microbial pathogens to mucosal surfaces which help to protect suckling animals from gastrointestinal infections [4]. IgE is reported to have the lowest concentration in serum compared

to the previously described immunoglobulins and is associated with allergic reactions [3, 4]. The function of IgE in foals is still being determined.

Probiotics have been used to stimulate the immune system in many animal species [5]. *Saccharomyces cerevisiae* has been shown to be a general immunostimulant in that it increases immunoglobulin concentration in horses and cattle [5- 8]. Due to yeast probiotic's immunostimulatory effect, it is theoretically possible that an increased immunoglobulin level in the mare could potentially cascade down to the foal via the colostrum by passive transfer to increase foal serum immunoglobulin concentrations. The objective of this study was to determine if maternal dietary yeast supplementation during late gestation and early lactation affect serum immunoglobulin levels in mares colostrums, serum of their foals and weight during six Months post foaling .

## MATERIALS AND METHODS

**Horses and Supplementation** – forty pregnant Arabian and Barbe Horses mares (8,54 ± 3,72 yr) were used in a completely randomized design to evaluate the effect of dietary live yeast supplementation on colostrum immunoglobulin concentrations before suckling, foal serum immunoglobulin concentrations pre and 24-48h post suckling of age. Each mare received a basal diet of barley wet due to 3kg morning and 3 Kg evening with water and mixed grass hay *ad libitum*.

In addition to the daily diet, from 10 to 15h: 30 mares go to pasture (paddock) for exercise and grazing grass. Mares were randomly assigned to one of two treatments from d 300 of gestation to 180 d (+/- 15d) post-foaling: Mares in the yeast treatment group also received 10g/d (1.10<sup>10</sup> UFC/g) of a live culture of *Saccharomyces cerevisiae* Sc 47 BIOSAF® HEAT RESISTANT CONCENTRATE OF LIVE YEAST (LESAPRE, FEED ADDITIVES, LESAPRE-France). Prior to parturition, and within 180 d after parturition, mares were housed in 3.5 x 3.3 m box stalls. with access to paddocks to shelter, mares were acclimated to grass pasture. Throughout the study, foals have access to the mares' concentrate. Starting at approximately 14 d of age.

### SERUM AND COLOSTRUM SAMPLES:

#### SERUM COLLECTION

Serum samples from 40 foals were collected before the first nursing and once again between 24 and 48 hr after birth. In the case of 40 foals in which a total of 2 blood samples were taken, blood samplings were performed by venipuncture using a 21 G x 11/2" needle (Improvacuter®, evacuated blood collection tube for in vitro diagnostic use). The blood sample was transferred to reproduction laboratory immediately after blood collection and then centrifuged at 1,500 × g for 10 min to obtain serum. Serum was divided on 2 eppendorf tube 2ml and conserved at -20 °C until further analysis.

#### COLOSTRUMS COLLECTION

Colostrum sampling was performed manually from both mammary glands separately. The udder area and end of the teat were cleaned using gauze dipped in 70% alcohol solution to prevent infection before sampling. The first 2 flows of colostrums were not collected, and subsequent flows were collected until the total volume reached approximate 20 ml. All colostrum samples were frozen within 1 hr and stored at -20°C until analyzed.

#### FOALS WEIGHT MEASURE

Weight was performed manually to all foals at first month post partum to 6 month to observe effect of yeast culture on foal growth.

#### RID KITS :

Serum and colostrum samples were evaluated by the use of commercially available SRID kits for equine serum and colostrum: IDRing® HORSE IgG TEST by IDBiotech - Immuno Diffusion Biotechnologies Avenue Marie Curie 63 500 Issoire – FRANCE

#### DATA ANALYSIS

The different results are described by the mean and standard error (SE, calculated from the standard deviation, using the formula  $SD=SE \cdot \sqrt{n}$ , 5, n being the size of the workforce per lot). The homogeneity of variance between treatments was verified by the Bartlett test. The results were subjected to analysis of variance (ANOVA 1), to determine the effect of yeast supplementation on the parameters considered. Significance was declared at  $P < 0.05$ . All these tests are conducted using the statistical software SYSTAT 12 © (SYSTAT 12 © Copyright 2007).

## RESULTS

### COCLOSTRUM IGG1 CONCENTRATIONS

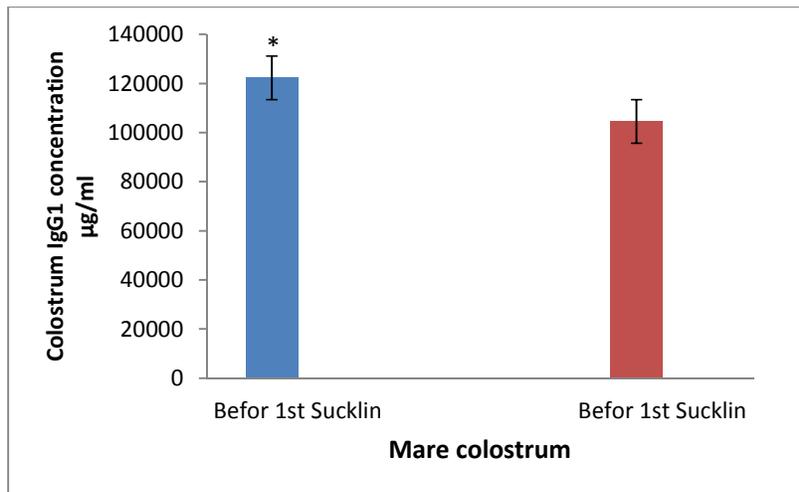
Colostrums IgG1 concentration between Yeast and Control mares before 1<sup>st</sup> suckling are shown in **Figure 1**. There is a significant differences due to dietary supplementation with regard to IgG1 concentration ( $P = 0,02$ ) with a concentration  $122,25 \pm 145,59$  g/l &  $104,51 \pm 157,15$  g/l respectively for supplemented and control mares .

### SERUM IGG1 CONCENTRATIONS

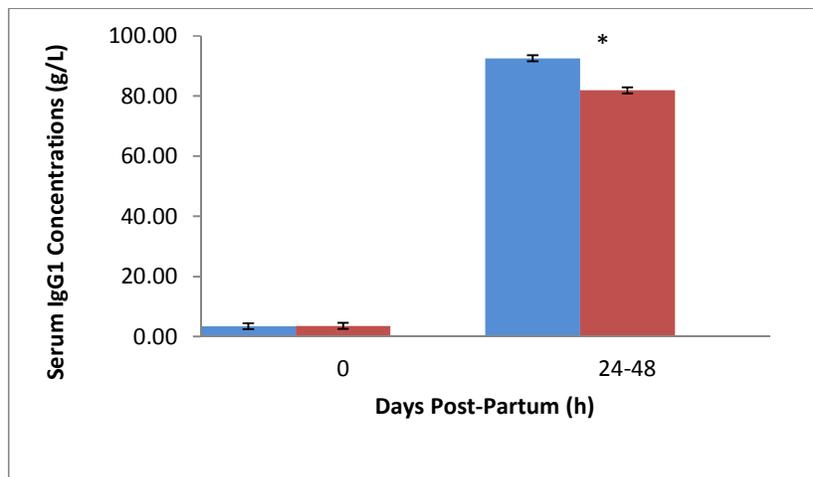
serum IgG1 concentration between Yeast and Control foals before 1<sup>st</sup> suckling (0 h) and (24-48 h) post foaling are shown in **Figure 2**. There are a similarity between the control groupe and the supplemented groupe in IgG1 concentration at 0 h before 1<sup>st</sup> suckling bat at 24-48 h after birth yeast groupe show an increase concentration IgG1 compared to the control group and appear to be statistically significant differences due to dietary supplementation with regard to IgG1 concentration ( $P = 0,008$ ).

### FOAL GROWTH

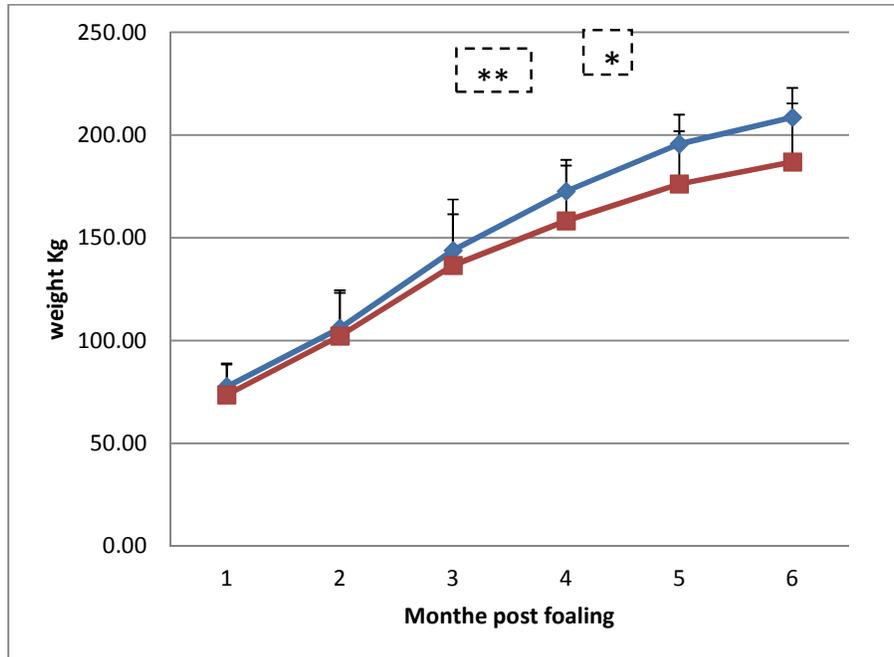
Effect of supplementation SC on weight of foal in Kg from the 1<sup>st</sup> month to six month post-partum is shown in **Figure 3**. Body weight of foals in the control group averaged  $73,57 \pm 14,81$  kg at first month compared to  $77,55 \pm 11,25$  kg for foals in the saccharomyces cerevisiae group. Yeast supplementation of the mares was significantly increase weight from month five to month six post foaling  $195,75 \pm 14,21$  Kg at five month in yeast group compared to control group  $176,11 \pm 25,73$  Kg with  $P=0,003$  and  $208,59 \pm 14,35$  Kg at month six (weaning) compared to control group  $186,93 \pm 28,50$  Kg with  $P=0,04$ .



**Figure 1:** effect of supplementation SC on colostrum IgG(1) concentrations in mg/dl before 1<sup>st</sup> suckling ( $n = 20$  per time point per group). Bars represent mean  $\pm$  SE. \* $p < 0,05$



**Figure 2:** effect of supplementation SC on serum IgG(1) concentrations in g/L from pre suckling (0) through 24-48 post-partum ( $n = 20$  per time point per group). Bars represent mean  $\pm$  SE. \* $p < 0,05$



**Figure 3:** Effect of supplementation SC on weight of foal in Kg from the 1<sup>st</sup> month to six month post-partum (n = 20 per time point per group). curve represent mean  $\pm$  SE. \* $p < 0,05$  \*\* $p < 0,01$

## DISCUSSION

All of the serum immunoglobulins concentrations analyzed in the present study were consistent with normal ranges previously reported for foals [9 - 11]. Although it is widely recognized that there is no placental transfer of immunoglobulins in the horse [9, 12, 13], low concentrations of immunoglobulins were detected prior to the ingestion of colostrum in the present study. These findings are supported by [1, 14] that found detectable amounts of serum IgM, IgG, and occasionally IgG(T) suggesting that there are some fetal immunoglobulins produced in utero that can be measured before colostrum uptake immediately. The initial increase in immunoglobulins from immediately after birth to 24 hr post-partum supports the absorption of maternal immunoglobulins via colostrum.

Several studies have shown that *Saccharomyces cerevisiae* will impact the immune system [5, 6, 15]. When sows were supplemented with *S. cerevisiae*, varying effects were observed on the immunoglobulin concentrations of their piglets. [16] was found that the level of IgG in colostrum of immunostimulated sows was higher in comparison with a control group. A statistically significant difference occurred only in the colostrum of sows, treated with TFX and HMB, but in the sows treated with isoprinosine the differences in the level of IgG were statistically insignificant. In the study [17] a total of 47 F<sub>1</sub> sows (Yorkshire  $\times$  Landrace) were allotted to five dietary treatments and two different levels ( $10^6$  or  $10^7$  CFU/g of diet) of live yeast (*Saccharomyces cerevisiae* Sc47) were supplemented during gestation, lactation or both. Treatments were (1) no yeast supplementation during gestation and lactation, (2) gestation—basal diet, lactation— $10^6$  CFU of yeast/g of diet, (3) gestation—basal diet, lactation— $10^7$  CFU of yeast/g of diet, (4) gestation— $10^6$  CFU of yeast/g of diet, lactation— $10^6$  CFU of yeast/g of diet, (5) gestation— $10^7$  CFU of yeast/g of diet, lactation— $10^7$  CFU of yeast/g of diet. There was no significant difference in milk composition during overall lactation except that IgG concentration in colostrum tended to be higher in the groups receiving live yeast supplementation ( $P=0.10$ ) compared with the control group, resulting in higher IgG concentration in plasma of piglets at 24 h postpartum ( $P < 0.05$ ). Tortora found that mares injected with 1,3/1,6 glucan, a component of yeast cell walls, had increased colostrum IgG and IgG(T) concentrations and their foals had increased serum IgG(T) levels. Although immunoglobulin concentrations significantly differed over time in the present study, there were differences in colostrum and foal immunoglobulin concentrations due to the addition of *S. cerevisiae* to the maternal diet during late gestation and early lactation.

Maternal nutrition has been shown to affect insulin-glucose regulation, predispose offspring to metabolic disorders and affect fetal growth, as well as lead to a more rapid establishment of certain microflora [18 - 23]. Studies in swine and sheep suggest that the establishment of the intestinal microbial environment in 28 young animals could have an effect on the bacterial ecosystem of the adult animal [24, 25]. The main microbial environments that the foal is exposed to in early life are from the mare, because the neonatal gastrointestinal tract is thought to be sterile until it is exposed to bacteria from the mare vaginal secretions, feces, saliva and/or milk

as well as the environment and neonatal diarrhoea in foals does not coincide with postpartum oestrus in their dams but with changes in intestinal bacteria [26 - 29]. When a foal is born, it is exposed to bacteria from the mare's vagina, feces, saliva and/or milk [27, 29]. Milk can contain up to  $10^9$  microbes/L with the most abundant microbes including *Streptococci* and *Lactobacilli* [26, 30]. Research has shown that supplementing the maternal diet with yeast culture increased milk production and nutrient content [31]. A study by Glade fed eight, pregnant mares 20 g per head per day of a commercial yeast supplement four weeks pre-parturition until 4 weeks post-parturition. Dry matter and crude protein digestibility significantly increased when the lactating mares were supplemented with yeast. Increases in energy content, sugars, total lipids, total nitrogen and total amino acids were also observed in the milk of the mares that were supplemented with yeast. This study also demonstrated an increased growth rate of the foals whose dams were treated with yeast culture. It has been suggested that the foal's GI microflora could be optimized by modifying the mare's microbial ecosystem [22]. In mice, supplementation of the maternal diet during gestation and lactation with prebiotics may have influenced the maternal intestinal microflora as well as the microflora of the offspring in a positive manner [32, 33]. It was observed in mice, that adding 50 g per kg of fructo-oligosaccharides (FOS) to the maternal diet affected the offsprings' intestinal microflora for up to two weeks 29 post-parturition, as shown through distinct clustering in a dendrogram analysis [33]. Recent research by Faubladier et al showed that probiotic supplementation of pregnant mares can affect their offspring's gastrointestinal microflora during the first few days of life. Foals of the mares supplemented with probiotics had a greater establishment of total anaerobes and lactate utilizers at an earlier time than the foals whose dams were not supplemented, which may be caused by the shift in the bacterial ecosystem of the mare and/or the change in lactation. The researchers proposed that the effect on early establishment of total anaerobes and lactate utilizers could be caused by the modification of the bacterial ecosystem of the mare and/or the alteration in milk production. Research has also shown an increased body weight observed in foals of mares supplemented with probiotics from late gestation to early lactation [23]. When mares were supplemented from d 300 of gestation to 60 d postparturition, their offspring were heavier than those foals of non-supplemented mares from 19 d to 60 d post-parturition.

## CONCLUSION

Overall, dietary yeast supplementation did influence mares colostrums, foals serum IgG1 concentration and increase foal weight when fed at a target dose of 10 g once daily.

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