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SERUM BIOCHEMICAL VARIATION IN MARES HAVING A DIETARY SUPPLEMENTATION OF *Saccharomyces cerevisiae* IN PERI-PARTUM PERIOD

Mohamed Said Saim[1], M.A. Ayad[1], S. Meliani[2*], S. Derrar[1], B. Benallou[1], M. Benmedjahed[3]

[1]Institute of Veterinary Sciences, Ibn Khaldoun University of Tiaret, Algeria
[2]Faculty of Nature Sciences and Life, Ibn Khaldoun University of Tiaret, Algeria
[3]National Haras of Chaouchaoua of Tiaret, Algeria

Abstract. The aim of this study was to assess the effects of *Saccharomyces cerevisiae* supplementation on the metabolic profile of brood mares, raised in Tiaret Algeria. Ninety pregnant mares were used between 2013 and 2015. Animals were assigned to treatment's group and to a control group, the mares were fed with corn, soybean meal, barley, wheat bran, and the second (yeast group) was supplemented with 10g alive yeast (*Saccharomyces cerevisiae* 47) daily from 300 Day of gestation to 30 days post-foaling. For biochemical analyses, jugular blood samples were collected. Each mare was collected five times, 30 days (F-30), 15 days (F-15) before foaling, at the foaling day (F), at 15 (F+15) and 30 days after foaling (F+30). to determine the following parameters, glucose, urea, creatinine, total proteins, cholesterol, triglyceride, albumin, Aspartate Amino-Transferase(AST), Alanine Amino-Transferase (ALT), Gamma-Glutamyl-Transferase (γ-GT), Calcium and inorganic phosphorus. In this study, the serum glucose mean values were significantly higher *($p<0,05$)* at F+30 with 0,85±0,16 g/l in the treatment group against 0,73±0,09 g/l in the control one. In this study, urea serum levels at F-15 and at foaling were significantly lower *($p<0,05$)* in the treatment group with 0,24±0,05 g/l and 0,27±0,05 g/l respectively against 0,33±0,10 g/l and 0,38±0,12 g/l respectively in the control one. This experiment showed that adding *Saccharomyces cerevisiae* to mare's meal in the peripartum period enhanced the metabolism of glucose and proteins and decreased urea serum levels.

Keywords: *Saccharomyces cerevisiae, probiotics, mares, biochemical, serum, calcium.*

Corresponding Author: *Meliani Samia, Faculty of Nature Sciences and Life, Ibn Khaldoun University of Tiaret, Algeria, Phone:+213542891290, e-mail: melianisamia@hotmail.com*

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1. Introduction

Nutritional additives effects on animal species have been studied by many nutrition companies, as well as research institutions (Rezende, 2012). Probiotics have been used in the equine industry as a nutritional strategy to improve diet assimilation, performance and to encourage growth, among other things (Biel *et al.*, 1990; Art *et al.*, 1994). Administration of probiotic strains separately and in combination significantly improved feed intake, feed conversion rate, daily weight gains and total body weight in chicken, pig, sheep, goat, cattle and equine (Chiofalo *et al.*, 2004; Bontempo *et al.*, 2006; Casey *et al.*, 2007; Stella *et al.*, 2007,Torres-Rodriguez *et al.*, 2007, Quarantelli *et al.*, 2008, Agazzi *et al.*, 2009). Research indicates that adding yeast culture to the diet of horses can improve nutrient digestibility (Switzer *et al.*, 2003), increase microbial populations (Medina *et al.*, 2002, Lattimer *et al.*, 2005), and maintain caecal pH (Medina *et al.*, 2002, Hall & Miller-Auwerda, 2005). However, other reports observed

no improvement in nutrient digestibility when yeast culture was supplemented to horses in vivo (Glade *et al.,* 1990) and in vitro (Lattimer *et al.,* 2005). The use of probiotics aiming to improve animal health has become routine, while research has shown their benefits in the better absorption of equine diets (Moura *et al.,* 2009; Moura *et al.,* 2011; Rezende *et al.,* 2012).

Most pregnancy diseases are likely to occur during the last trimester of pregnancy (Le Blanc, 1991), when foetal growth accelerates dramatically, resulting in a significant increase in the nutritional requirements of the mare (Lewis, 1995). Therefore, study was conducted to assess the effects of *Saccharomyces cerevisiae* supplementation on the metabolic profile of brood mares, raised in Tiaret Algeria, by focusing on the last month of gestation and early postpartum.

2. Materials and method

This experiment was conducted in the Chaouchaoua's national Haras located in Tiaret at the western region of Algeria. A total of ninety pregnant mares were used between 2013 and 2015. The age of animals was about 8,54±3,72 years and the range weight was about 479,8± 33,69Kg. All animals were healthy and maintained under the same management conditions. Animals were assigned to treatment's group and to a control group, the mares were fed with corn, soybean meal, barley, wheat bran, and the second (yeast group) was supplemented with 10g alive yeast (*Saccharomyces cerevisiae* 47) daily from 300 Day of gestation to 30 days post-foaling.

For biochemical analyses, jugular blood samples were collected in the morning using heparinized Venoject glass tubes (improvacuter®, evacuated blood collection tube for in vitro diagnostic use). Each mare was collected five times, 30 days (F-30), 15 days (F-15) before foaling, at the foaling day (F), at 15 (F+15) and 30 days after foaling (F+30). Immediate centrifugation was made and serum was stored at -20°C until analysis. For clinical chemistry an automatic analyzer *Cobas Roche*® was used to determine the following parameters, glucose, urea, creatinine, total proteins, cholesterol, triglyceride, albumin, Aspartate Amino-Transferase (AST), Alanine Amino-Transferase (ALT), Gamma-Glutamyl-Transferase (γ-GT), Calcium and inorganic phosphorus. The means and standard deviations for all samples were made and analyzed with the one-way ANOVA test using SPSS© V.25 IBM Software.

3. Results and discussion

All results of our study are reported in the Table 1. The average values for the measured parameters for all mares were respectively for glucose 0,75±0,17 g/l, urea 0,30±0,08 g/l, creatinine 11,18±3,08 mg/l, total proteins 46,89±12,17 g/l, cholesterol 0,74±0,26 g/l, triglycerides 0,38±0,33 g/l, albumin 24,00±5,73 g/l, AST 55,49±27,58 UI/l, ALT 1,97±0,89 UI/l, γGT14,69±10,02 UI/l, calcium 76,74±21,35 mg/l and inorganic phosphorus 21,25±4,73 mg/l. All the values recorded in this work are in the average values reported in bibliography (Appendix VIII, 2008; Desjardins et Caddoré, 2006; University of Edinburgh, 2015, UC Davis school of veterinary medicine, 2015; Rodríguez *et al.*,2007).

Table 1. Mean±SD levels for the serum parameters variation between the control group and the treated group

Groups	*Control (12)*	*Treatement (12)*	*Total (24)*
	Parameters		
Samples time	***Glucose g/l***		
F-30	0,76±0,09	0,85±0,16	0,80±0,13
F-15	0,77±0,16	0,84±0,24	0,81±0,20
Foaling	0,68±0,13	0,73±0,18	0,70±0,16
F+15	0,61±0,10	0,66±0,16	0,63±0,14
F+30	0,73±0,09	0,85±0,16*	0,79±0,14
	Urea g/l		
F-30	0,30±0,09	0,26±0,05	0,28±0,07
F-15	0,33±0,10	0,24±0,05*	0,29±0,09
Foaling	0,38±0,12	0,27±0,05*	0,32±0,11
F+15	0,27±0,04	0,27±0,04	0,27±0,04
F+30	0,36±0,09	0,30±0,06	0,33±0,08
	Creatinine g/l		
F-30	12,11±2,21	13,40±2,74	12,75±2,52
F-15	13,18±3,00	12,61±4,29	12,89±3,63
Foaling	10,14±1,90	9,45±4,27	9,80±3,25
F+15	11,50±2,67	9,62±1,64	10,56±2,37
F+30	10,27±1,77	9,52±1,94	9,90±1,86
	Total Proteins g/l		
F-30	45,92±10,02	45,92±10,02	45,92±9,80
F-15	47,36±14,32	47,36±14,32	47,36±14,01
Foaling	41,25±11,69	41,25±11,69	41,25±11,43
F+15	43,67±7,18	43,67±7,18	43,67±7,02
F+30	51,07±12,44	61,41±10,85*	56,24±12,58
	Cholesterol g/l		
F-30	0,71±0,16	0,71±0,16	0,71±0,16
F-15	0,83±0,31	0,83±0,31	0,83±0,30
Foaling	0,71±0,30	0,71±0,30	0,71±0,29
F+15	0,58±0,19	0,58±0,19	0,58±0,18
F+30	-	0,98±0,17	0,98±0,17
	Triglycerides g/l		
F-30	0,38±0,17	0,38±0,17	0,38±0,17
F-15	0,35±0,24	0,36±0,22	0,35±0,22
Foaling	0,44±0,64	0,39±0,55	0,41±0,58
F+15	0,16±0,01	0,17±0,02	0,16±0,02
F+30	-	0,64±0,18	0,64±0,18
	Albumine g/l		
F-30	24,67±4,98	24,67±4,98	24,67±4,87
F-15	24,20±7,02	24,00±6,38	24,09±6,52
Foaling	22,20±6,60	21,33±6,43	21,73±6,36
F+15	22,20±4,32	22,83±4,13	22,65±4,06
F+30	-	28,58±3,48	28,58±3,48
	ALT UI/l		
F-30	66,08±30,59	66,08±30,59	66,08±29,92
F-15	67,10±33,52	65,33±31,94	66,14±31,89
Foaling	46,20±17,78	46,92±16,48	46,59±16,67
F+15	32,80±16,89	40,58±15,93	38,29±16,10
	AST UI/l		
F-30	1,92±0,51	1,92±0,51	1,92±0,50
F-15	1,90±0,88	2,08±0,90	2,00±0,87
Foaling	1,60±0,97	2,00±1,28	1,82±1,14
F+15	1,20±0,45	1,58±0,67	1,47±0,62
F+30	-	2,75±0,87	2,75±0,87
	δ GT UI/l		
F-30	9,67±6,41	9,67±6,41	9,67±6,27
F-15	20,50±11,13	20,83±10,21	20,68±10,38

Foaling	17,30±13,48	17,33±12,20	17,32±12,48
F+15	10,40±3,91	10,75±3,14	10,65±3,26
	Calcium mg/l		
F-30	73,33±12,33	73,33±12,33	73,33±12,06
F-15	81,00±21,40	83,25±20,07	82,23±20,21
Foaling	71,00±31,55	70,25±28,65	70,59±29,27
F+15	68,80±21,06	68,42±16,68	68,53±17,38
F+30	-	96,62±6,18	96,62±6,18
	Phosphorus mg/l		
F-30	19,42±3,00	19,42±3,00	19,42±2,93
F-15	20,30±5,14	20,50±4,72	20,41±4,80
Foaling	22,00±5,44	21,92±5,20	21,96±5,19
F+15	22,25±4,99	21,75±3,28	21,88±3,59
F+30	-	24,27±6,47	24,27±6,47

**Refers to a significant difference in the same line (p<0,05)*

Haematology and biochemistry were studied in heavy draft horses (Aoki & Ishii 2012) and Standard bred mares (Mariella *et al.,* 2014) showing a significant change in some electrolytes and energy parameters during the last month of gestation and at the beginning of the postpartum. In this study, the serum glucose mean values were significantly higher *(p<0,05)* at F+30 with 0,85±0,16 g/l in the treatment group against 0,73±0,09 g/l in the control one. Pregnancy is always associated with an altered metabolic status when compared with the non-pregnant mares one, thus it is necessary to get sufficient reserves and energy substrates for pregnancy requirements, a good foetal development, exercise and breastfeeding (Hadden & McLaughlin, 2009). Aoki and Ishii (2012) noted a postpartum elevation of blood glucose and hypothesized that it was related to the physical stress associated with the foal, the physical stress increases the level of cortisol promoting gluconeogenesis (Wong *et al.*, 1992), and it is the result of the development of insulin resistance, which improves the placental transfer of glucose to meet the increasing demands of the foetus (Fowden *et al.,* 1984; Hoffman *et al.,* 2003).

In this study, urea serum levels at F-15 and at foaling were significantly lower *(p<0,05)* in the treatment group with 0,24±0,05 g/l and 0,27±0,05 g/l respectively against 0,33±0,10 g/l and 0,38±0,12 g/l respectively in the control one. Urea is an end product of protein catabolism, synthesized in the liver and excreted by the kidneys. The serum urea concentration is determined by the balance between protein catabolism and renal excretory function. Changes in serum urea may reflect an increase in energy demand and a higher requirement for amino acids for anabolic processes according to Mariella *et al.,* (2014). Urea serum values still high due to the energy demand at the beginning of lactation and it was suggested that it may be related to changes in energy metabolism rather than renal function (Aoki & Ishii, 2012). In our study the lot supplemented with yeast showed stable values of urea compared to the control group, this may be due to the effect of yeasts which have an important role in carbohydrate and protein metabolism by reducing the rate of urea by reducing gluconeogenesis with sufficient glucose intake for the mare and the foal.

In the present work, protein mean values were lower than the range of 57-77 g/l values considered normal in the literature (Appendix VIII, 2008; Desjardins et Caddoré, 2006; University of Edinburgh, 2015, UC Davis school of veterinary medicine, 2015). In this study, proteins mean levels at F+30 were significantly lower (p<0,05) with 51,07±12,44 g/l than 61,41±10,85 g/l in the treatment group. Many studies conducted in

mare's protein and energy metabolism illustrate the potential for adjusting the use of these nutrients to maintain a foetal diet (Larsson *et al.,* 2008), however, the most significant changes in maternal metabolism occur when foetal nutrient demand increases significantly at the last trimester of pregnancy (King, 2000). Undernutrition during pregnancy can permanently affect the cell's number of a developing organs which critical growth periods coincide with the lack of essential nutrients (Panzani *et al.,* 2009).

In our work, no significant differences were observed between the experiment groups. However, it was indicated that blood cholesterol varies shortly after supplementation with probiotic yeast (Piva *et al.,* 1993, Galip, 2006). Triglycerides mean values decreased before foaling and at F+15, but no significant difference was observed which is consistent with previous report supposing that is due to the start of milking and the increase in energy consumption in the body (Maijó *et al.,* 2012).

The use of probiotic yeast had no effect on the blood triglycerides values in our study, in contrast of the results obtained in rats and chicken where the addition of yeast to the food decreased triglycerides levels, phospholipids and the proportion of abdominal fat (Onifade, 1997; Galip, 2006), however, the mechanisms of the interactions involved in these variations still unknown.

Albumin is a major protein of total protein, it is synthesized by the liver and degraded by most tissues. It is responsible for eighty percent of the oncotic pressure, allows the transport of proteins, fatty acids, bile acids, bilirubin, calcium, hormones and drug molecules (Lassen *et al.,* 2004). In this work, mean albumin levels for all mares was 24,00±5,73 g/l and no significant differences were recorded between the experiment group.

Aspartate Amino-Transferase (AST) is an induced enzyme, found in high concentration in the liver and in the muscles (heart and skeletal) of all species, it is a non-specific enzyme that needs to be analysed in combination with other variables (Lassen *et al.,* 2004). A significant increase in activity may be due to liver injury or muscle, or haemolysis. AST is used in combination with Creatinine Kinase to assess muscle pain.

The serum AST mean levels recorded in our work were lower than those reported as usual normal values (Desjardins & Caddore, 2006, UC davis school of veterinary medicine, 2015; appendix VIII, 2008; University of Edinburgh, 2015). In addition to species, race, and age, AST activity is influenced by muscle activity (Weigert *et al.,* 1980). Working horses have about 60% higher activity (112 IU/l) than horses that rest for several days (70 IU / L) (Weigert *et al.,* 1980).

Alanine Amino-Transferase (ALT) is a liver cytosolic specific enzyme increasing in blood within specific changes in hepatocytes, in primates, dogs, cats, rabbits and rats, in contrast, but in the horses (Kramer & Hoffman, 1997). ALT levels in blood is influenced by age and muscles activity (Weigert *et al.,* 1980). Serum ALT levels in our study were in the range of reported values (Appendix VIII, 2008; Desjardins & Caddore, 2006; UC davis school of veterinary medicine, 2015; University of Edinburgh, 2015) but no significant differences were recorded in this work due to yeast addition. We noted that in both groups, the mean level of ALT was higher with 66,08±29,92 UI/l and decreased fifteen days after foaling to 38,29±16,10 UI/l without a significant difference *(p>0,05).*

Gamma-Glutamyl-Transferase (γGT) an induced enzyme, synthesized by the majority of tissues but mainly by the liver. The kidneys also synthesize GGT but it is

then excreted only in the urine; the pancreas synthesizes it but it is then excreted rather in the pancreatic secretions only in the circulatory torrent (Lassen *et al.,* 2004). The plasma concentration can increase with the stress of training, particularly in thoroughbred's mares (Carlson, 2009).

The serum γGT concentrations recorded in our mares were in the range of usual values reported (Desjardins & Caddore, 2006, UC Davis School of Veterinary Medicine, 2015, University of Edinburgh, 2015), and significantly lower than the values cited by (Appendix VIII, 2008). In this study, a gradual increase in the calcium mean levels for all mares was recorded from F-30 with 73,33±12,06 mg/l, F-15 with 82,23±20,21 mg/l, at foaling with 70,59±29,27 mg/l decreased at F+15 surely due to lactation to 68,53±17,38 mg/l and increased to 96,62±6,18 mg/l thirty days after foaling. These finding are consistent with Berlin and Aroch (2009), who observed a decrease in serum calcium in pregnant mares compared to non-pregnant mares. Phosphorus mean levels, for all mares, also increased from 19,42±2,93 mg/l at F-30 to 24,27±6,47 mg/l at F+30 always without any significant difference between groups.

4. Conclusion

This experiment showed that adding *Saccharomyces cerevisiae* to mare's meal in the peripartum period enhanced the metabolism of glucose and proteins and decreased urea serum levels. Those findings suggest that the use of *Saccharomyces cerevisiae* in the last month of pregnancy can improve the mare metabolism and the foetal development.

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