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EFFECT OF *SACCHAROMYCES CEREVISIAE* FEED SUPPLEMENTATION ON HAEMATOLOGY AND REPRODUCTIVE PARAMETERS FOR ALGERIAN RABBITS

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ABSTRACT. This study aims at investigating the effect of *Saccharomyces cerevisiae* (SC) supplementation on reproductive performance, haematological parameters and fertility of rabbits under Algerian conditions. The animals were divided into three groups and received the same feed ration during the experimental period. The control group received a basal diet without feed additives (Group#0) and the two yeast SC groups received 0.3 and 0.6 g/day per head (Group#1 and Group#2, respectively). Semen and blood samples were collected for determination of semen parameters and haematology. The weights of rabbits treated with SC 0.3 g/day were statistically significantly different ($P<$ 0.05) from the control groups and group treated with SC 0.6 g/day. There were significant differences between the treatment groups for (RBCs), haemoglobin (HGB), haematocrit (HCT) and mean corpuscular haemoglobin (MCH) values, with higher values in rabbits supplemented with SC 0.3 g/day and 0.6 g/day, compared to those in the control group. The scrotal diameter did not differ between the dietary treatments. When compared with the control group, feeding rabbits graded levels of SC resulted in an increase in the average semen volume, mass motility and individual motility at day 51 of the experiment. On the other hand, the sperm concentration was significantly lower ($P<$ 0.05) in rabbits supplemented with SC 0.3 g/day and 0.6 g/day during the two months compared to that in the control group. The spermatozoa mortality rate was lower for the rabbits supplemented with SC 0.3 g/day and 0.6 g/day (15.7% and 11.4%, respectively), compared to that in the control group (24%). In conclusion, this study has shown that inclusion of SC 0.3 g/day and 0.6 g/day in the diets of rabbit has positive effects on body weight

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and sperm analysis. Moreover, it increases the level (RBCs), haemoglobin (HGB), haematocrit (HCT) and mean corpuscular haemoglobin (MCH).

Keywords: *Saccharomyces cerevisiae*; feed; haematological parameters; sperm; rabbits.

INTRODUCTION

It is known that high levels of antibiotics have been used in food-producing animals as growth promoters and for disease prophylaxis. However, there is a major problem among human consumers due to the occurrence of antibiotic residues in meat because of the wide use of antibiotics and antibiotic-resistant bacteria in animals. Therefore, the European Union Commission banned the use of antibiotics as a growth enhancer in the diets of animals (Castanon, 2007). It is therefore imperative to replace the overuse of antibiotics and to search for a new safe alternative for health improvement and infection control in animals.

The probiotics (bacterial and yeast cultures) are non-pathogenic microbial adjuncts, which have been used as feed supplements and also as growth promoters, improving the immune system of animals by promoting the composition and microbial balance in its gut. Several studies have garnered attention over the years on the use of probiotics as alternative feed additives in order to replace antibiotics and synthetic chemical feed supplements (Higginbotham and Bath, 1993; Brydt *et al.,* 1995; Sumeghy, 1995; Strzetelski, 1996). Yeasts are being widely used in food, medicine and the cosmetic industry due to their bioactive and nutritional components, such as peptides, amino acids, beta-glucan, glutathione, cerebroside and zinc (Cha *et al.,* 2004, 2008; Kinosita *et al.,* 2007; Lee *et al.,* 2005).

Moreover, it increases the level of some haematological parameters, such as RBCs, HGB, HCT, and GCTs. There has been scientific interest over the last two decades in the supplement *Saccharomyces cerevisiae* (SC), which increases the cellulolytic rumen bacteria (Ogunade *et al.,* 2019). Pinheiro *et al.* (2020) and Arif *et al.* (2020) reported that the strains of SC have potential as probiotics and are adsorbent of aflatoxin B1. The Algerian rabbit represents a significant portion of the agricultural economy. The statistical data revealed that more and more breeders are interested in rabbit farms where the production of rabbit meat is the first activity (Saidj *et al.,* 2013). Rabbit meat is an important source of protein, rich in precious nutrients (essentially amino acids and lipids and low in fat content and cholesterol (< 59 mg/100 g) (Combes and Dalle Zotte, 2005). In the last years, the Algerian Department of Agriculture has adopted a policy of diversifying animal production by encouraging rabbit farmers to invest more. Therefore, this research study was conducted to explore the impact of SC supplementation on some reproductive parameters and haematological balance in male rabbits under Algerian conditions. It is known

that high levels of antibiotics have been used in food-producing animals as growth promoters and for disease prophylaxis.

MATERIALS AND METHODS

With regard to the ethical aspects, this experimental procedure was approved by the Scientific Committee at the University of Chlef (Report of Faculty Scientific Council #04 dated 30 September 2015).

Animal groups and feeding

The experiment was conducted in an experimental farm of the Department of Agronomic and Biotechnological Sciences (36°10' N, 1°19' E; University of Chlef, Algeria). This study was conducted from 1 March 2016 to 30 April 2016. A total of nine rabbits aged 10 months were used for this study. The rabbits were in a good condition during the experimental period. The animals were divided into three groups and received the same feed ration during the experimental period. Before the beginning of the feeding experiment, the pre-trial period consisted of an adaptation period of two weeks for animals who were fed SC supplementation. Then, the rabbits were followed over six weeks, during which the collection and analysis of sperm were performed.

The animals were divided into three groups and received the same feed ration during the experimental period. The control group received a basal diet without feed additives (Group#0), and the two yeast SC groups received 0.3 g/day and 0.6 g/day per head (Group#1 and Group#2, respectively). The supplemented SC (CNCM I-1077, Lallemand Animal Nutrition) is marketed by the Vetam Company (Mostaganem, Algeria) and contains 20×10^9 CFU/g of live yeast. The water was distributed *ad libitum* for the study period. The rabbits were weighted at the start of the experiment and then on the following days 1, 15, 29, 44 and 51. Weight gain (WG) was calculated as follows: DWG (g/day) = (Final weight – Initial weight).

Blood and semen sampling

Whole blood samples were withdrawn from the marginal vein into EDTA tubes for haematological analysis. Haematological indices, such as red blood cells (RBCs), platelets (PLTs), haemoglobin (HGB), white blood cells (WBCs), lymphocytes (LCTs), monocytes (MCTs), granulocytes (GCTs), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cell distribution index (RBDI), were measured. All these measurements were performed using an automated haematology analyser (SWELAB alfa, Boule Medical AB, Spanga, Sweden).

The semen was collected on days 21, 35, and 42 to analyse the various parameters of spermatozoa. The collected semen was transported quickly to the laboratory and kept at body temperature. The study protocols of the semen preparation and analysis were done according to Wyrobek and Bruce (1975). The volume of semen was simply measured using the graduations on the collection tube. The pH was measured with a digital pH meter. Sperm motility was estimated after semen collection by examining a small drop of semen placed on a warm slide and examined by light microscope. Sperm concentration was determined using a Thoma cell counting chamber. The determination of live and dead sperm cells was analysed using the eosin-nigrosin staining procedure according to Esteso *et al.* (2006).

Scrotal diameter was measured at the widest point of the scrotum with a graduated tape on days 14 and 45. Sexual activity (libido) was estimated by the time between introduction of the female into the male's cage and ejaculation. At the end of the experiment, the sexual behaviour of rabbit bucks was determined. A sexually receptive female was then dropped from one side of the chamber and allowed 30 min of stimulation (Sanna *et al.,* 2015; Cicero *et al.,* 2001). Mounting latency (ML) was estimated as time from female introduction into the cage to the occurrence of first mount. The intromission latency was defined as the duration interval between the introduction of the female and intromission by the male (Dabhakar and Zade, 2013; Pare *et al.,* 2014; Mutwedu *et al.,* 2019).

Statistical analyses

Statistical analyses of the results were carried out in Statview (Version 4.55). Statistical analysis was performed using t-tests to compare between different groups. The data were expressed as mean ± SE and $P< 0.05$ was considered significant.

RESULTS

The final body weight of the rabbits fed different levels of treatment are presented in *Table 1*. The weights of rabbits treated with SC 0.3 g/day were significantly decreased ($P< 0.05$), compared to the group control and group treated with SC 0.6 g/day.

At the end of the experiment (day 51), the rabbits supplemented with graduated levels of SC had a slightly lower body weight than the control group (3.282 kg). Also, the rabbits supplemented with SC 0.3 g/day showed a continuous increase in body weight, as compared to the other groups.

The mean ± SE values of the blood parameters of treated and non-treated rabbits is shown in *Table 2*. There were significant differences between the treated groups for RBCs, HGB, HCT and MCH values, with higher values in rabbits supplemented with SC 0.3 g/day and 0.6 g/day, compared to the control group. PLT, WBCs and GCTs for the control group were higher than the treated groups. On the other hand, MCTs and LCTs were low in the control group compared to those in the treated groups, which was statistically insignificant ($P> 0.05$). Rabbits supplemented with SC showed slightly higher values in h haematological parameters, such as MCV and RBDI, compared to those reported for the control group.

The effect of SC supplementation on the scrotal diameter, sexual behaviour and sperm analysis of the rabbits is illustrated in *Table 3*. The scrotal diameter did not differ between the dietary treatments. The rabbits fed rations containing 0.3 g/day and 0.6 g/day SC recorded high intromission latency values in comparison to the control group. However, there was a significant difference in sexual behaviour between the controls and rabbits fed different levels of SC supplementation ($P> 0.05$).

Table 1 - Effect of graded levels of *Saccharomyces cerevisiae* (SC) on body weight in rabbits

	Diet treatment (kg)		
	Group#0 (n = 3, ± SE)	Group#1 (n=3, ± SE)	Group#2 (n = 3, ± SE)
Day 1	3.335 ± 0.23[b]	2.305 ± 0.15[a]	3.130 ± 0.25[b]
Day 15	3.303 ± 0.25[b]	2.305 ± 0.17[a]	3.025 ± 0.26[b]
Day29	3.278 ± 0.31[b]	2.382 ± 0.19[a]	3.027 ± 0.25[b]
Day 44	3.302 ± 0.33[b]	2.388 ± 0.19[a]	2.992 ± 0.23[b]
Day 51	3.282 ± 0.27[b]	2.407 ± 0.22[a]	3.027 ± 0.26[b]

[a,b] Values by the same letters between the treated groups in the same row are statistically different ($P< 0.05$).

Table 2 - The impact of *Saccharomyces cerevisiae* (SC) on the haematological parameters of rabbits on different days

	Diet treatment		
	Group#0 (n = 3, ± SE)	Group#1 (n = 3, ± SE)	Group#2 (n = 3, ± SE)
RBCs (10^6/mm^3)	5.21 ± 0.25[a,b]	6.29 ± 0.3[a]	6.09 ± 0.22[b]
HGB (g/dl)	9.5 ± 0.71[a,b]	12.9 ± 0.58[a]	12.6 ± 0.25[b]
PLT (10^3/mm^3)	352 ± 106.7	329.7 ± 85.6	281 ± 83..1
HCT (%)	28.95 ± 1.48[a,b]	36.13 ± 1.43[a]	35.97 ± 1.1[b]
WBCs (10^3/mm^3)	13.7 ± 1.13	9.9 ± 1.61	9.9 ± 3.26
LCTs (10^3/mm^3)	3.75 ± 1.34	5.3 ± 0.87	4.2 ± 1.01
MCTs (10^6/mm^3)	0.65 ± 0.21	1.33 ± 0.47	0.67 ± 0.31
GCTs (10^6/mm^3)	9.3 ± 2.26[a]	3.27 ± 0.47[a]	5.07 ± 2.94
MCV (%)	53.3 ± 3.25	57.53 ± 0.6	59.17 ± 1.62
MCH (pg)	18.35 ± 0.07[a,b]	20.53 ± 0.15[a]	20.7 ± 0.79[b]
MCHC (g/dl)	37.65 ± 2.33	35.73 ± 0.55	35.1 ± 0.6
RBDI (%)	14.85 ± 2.05	14.87 ± 0.40	15.67 ± 0.78

Red blood cells (RBCs), haemoglobin (HGB), platelets (PLT), haematocrit (HCT), white blood cells (WBCs), lymphocytes (LCTs), monocytes (MCTs), granulocyte (GCTs), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution index (RBDI).

[a,b] Values by the same letters between the treated groups on different days are statistically different in the same row ($P< 0.05$).

The pH of semen seems to be stable in rabbits supplemented with SC and in the control group, ranging from 7.33 to 8.00. When compared with control the group, rabbits fed diets with graded levels of SC (Group#1 and Group#2) had increases in mean semen volume, mass motility and individual motility at day 51 of the experiment. On the other hand, the sperm concentration was significantly lower ($P< 0.05$) in rabbits fed SC supplementation at 0.3 g/day and 0.6 g/day over the two months of the experiment compared to the control group. The spermatozoa mortality rate

was lower for the rabbits fed SC supplementation at 0.3 g/day and 0.6 g/day (15.7% and 11.4%, respectively), compared to that in the control group (24%).

Table 3 - Effect of graded levels of SC on scrotal diameter, sexual behaviour and sperm analysis in rabbits

	Diet treatment		
	Group#0 (n = 3, ± SE)	Group#1 (n = 3, ± SE)	Group#2 (n = 3, ± SE)
Scrotal diameter (mm)			
Day 15	5.00 ± 0.30	5.67 ± 1.03	4.82 ± 0.72
Day 45	5.45 ± 0.60	5.98 ± 0.71	5.55 ± 1.14
Sexual behaviour			
Mounting latency (sec)	5.0 ± 3.0	5.0 ± 2.8	11 ± 2.83
Intromission latency (sec)	5.33 ± 4.5	13.00 ± 2.8	21.7 ±18.5
Sperm analysis			
pH			
Day 21	7.33 ± 0.06	7.33 ± 0.58	7.50 ± 0.50
Day 51	8.00 ± 0.00	7.47 ± 0.50	8.00 ±0.00
Semen volume (ml)			
Day 21	0.67 ± 0.06	0.53 ± 0.12	0.43 ± 0.15
Day 51	0.40 ± 0.00	0.47 ± 0.29	0.67 ± 0.00
Mass motility			
Day 21	2.67 ± 1.53*	4.33 ± 0.58	6.00 ± 1.00*
Day 51	5.00 ± 0.00	5.33 ± 3.51	6.67 ± 0.58
Individual motility			
Day 21	2.00 ±1.73	1.67 ± 1.15*	4.00 ± 0.00*
Day 51	2.00 ± 0.00	2.67 ± 1.53	4.00 ± 0.00
Sperm concentration (10^6/ml)			
Day 21	269.3 ± 122.5*	52.9 ± 38.9*	206.3 ± 173.6
Day 51	390.0 ± 0.0	148.3 ± 51.3	259.4 ± 81.1
Mortality of spermatozoa (%)			
Day 21	43.3 ± 15.3	39.3 ± 4.6	25.3 ± 13.0
Day 51	24.0 ± 0.0	15.7 ± 9.1	11.0 ± 6.9

*Values by asterisks between the treated groups on different days are statistically different in the same row (P< 0.05).

DISCUSSION

The present study was to investigate the effects of inclusion of the yeast SC in the diet on the haematological and sperm parameters of rabbits. According to the results of this study, the body weight gain of rabbits was not significantly affected by dietary SC supplementation. This confirmed the previous findings of several studies, which noted no

significant differences in the growth performance of rabbits fed a ration containing SC supplementation (Kimsé *et al.,* 2008; Brümmer *et al.,* 2010; Darwish *et al.,* 2011; Seyidoglu *et al.,* 2013; Seyidoglu and Galip, 2014; Belhassen *et al.,* 2016). Kimsé *et al.* (2012) noted that yeast did not affect final body weight, daily weight gain or feed intake in New Zealand rabbits. Another study reported that the administration of SC to mice showed an insignificant improvement in growth performance (Abdel-Aziz *et al.,* 2010). On the one hand, these different results may be due to the dose or kind of yeast used, strain of broilers, basal diet, and environmental conditions or refer to the mechanisms of growth promotion of yeast culture in rabbits, whereas generally, the positive relationship between SC and animal performance characteristics have been reported by several authors (Omat *et al.,* 2010; Saied *et al.,* 2011; Abou El-Naga *et al.,* 2012; Ezma *et al.,* 2012; Onwurah and Okejim, 2014, Mohamed *et al.,* 2015). On the other hand, Onifade and Babatunde (1996) reported that a diet supplemented with SC at 6.0 g/kg improved the feed quality in broiler chicks. Likewise, Waché *et al.* (2006) observed that the addition of SC can enhance diet and protein digestibility, which revealed better growth and food efficiency with yeast supplements. Moreover, Mohammadi *et al.* (2016) confirmed that using a 2% concentration of yeast in the diet improved growth and food utilization in three spots cichlid (*Cichlasoma trimaculatum*). The divergence of yeast results on weight gain could be due to the age of the rabbit, livestock conditions, type of feed, and type and dose of yeast incorporated into the food (Akpa *et al.,* 2012).

Data are presented in *Table 2* and demonstrate that the RBCs, HGB, HCT, GCT and MCV were significantly different in rabbits receiving graded SC diets as compared to the control group. This is in divergence with the previous results revealing that the use of SC in the diets of rabbits had no effect on some haematological parameters (Seyidoglu and Galip, 2014; Belhassen *et al.,* 2016). Seyidoglu *et al.* (2013) revealed no significant variations in haematological parameters in treatment groups, although they noted a slight rise in haematocrit and haemoglobin concentrations in rabbits supplemented with yeast. Other findings are supported by researchers where the values of HGB, HCT, RBCs, MCV, MCH and MCHC were unchanged in broiler chicks fed diets containing probiotics (Gheisari *et al.,* 2008; Shareef *et al.,* 2009; Saied *et al.,* 2011). Moreover, Onifade *et al.* (1999) noted that HCT, HGB, MCV and MCH rose significantly ($P< 0.05$) in rabbits receiving SC supplementation. In another investigation, Paryad *et al.* (2008) observed that the addition of both 1.5% and 2% SC yeast significantly elevated WBCs and decreased the LCT ratios of chicks. Moreover, Mulatu *et al.* (2019) revealed that WBCs, packed cell volume (PCV) and HGB were higher in chickens fed a diet containing SC. In addition, Saied *et al.* (2011) showed that dietary yeast has no effects on the

PCV values of broiler chickens. However, Elghandour *et al.* (2019) observed that yeast-fed rabbits had more WBCs and LCTs, compared to the rabbited fed the control diet. It is noted that the LCT content may be a signal of improvement of humoral immune responses in rabbits fed diets supplemented with SC. Also, a relationship between dietary levels of SC and haematological parameters has been reported, and it could be a promoter of supplemental yeast in rabbits (Onifade *et al.,* 1999). Likewise, our results show that SC does not affect animal health. This was demonstrated by Krzysztof *et al.* (2012), who observed that probiotics have immunostimulatory effects.

The results of our present study were in agreement with those of Sharawy *et al.* (2015), who reported a non-significant increase in testicular volume in the probiotics group, compared to that in the non-treated groups, while other researchers noted that scrotal circumference in rams was significantly greater in the probiotics fed group than in the control group (Fernandez *et al.*, 2004; Kerban, 2008). Moreover, Kheradmand *et al.* (2006) indicated that both the testicular size and scrotal circumference of rams were influenced by alimentation, and the scrotal circumference was greater in the treated groups than in the control group. This result can be explained by testicular growth being associated with the nutritional value of food. The present study revealed significant differences in the sperm analysis, namely, mass motility, individual sperm motility and sperm concentration ($P< 0.05$) and were similar to those seen in rams (Sharawy *et al.,* 2015). This result is in accordance with Emmanuel *et al.* (2019) and Helal *et al.* (2018), who observed remarkable enhancement in the sperm concentration and motility of rabbit bucks, which were supplemented with SC. These results are in agreement with results obtained previously, which recorded that the ingestion of probiotics might be recommended to improve sperm motility in human males (Valcarce *et al.,* 2017). The antioxidant activity of SC may be the key to improvement in the sperm parameters (Uskova and Kravchenko, 2009; Spyropoulos *et al.,* 2011; Ewuola, 2013; Mymrin *et al.,* 2017; Shehu *et al.,* 2016). It has been reported that yeasts and their extracts are sources of natural antioxidant cmpounds (Nishino and Ishikawa, 1998; Gazi *et al.,* 2001).

Our results are similar to those obtained by Helal *et al.* (2018), who reported insignificant differences in dead spermatozoa in the experimental groups ($P> 0.05$), while the lowest proportions of dead spermatozoa were recorded in the SC groups. Nevertheless, studies claim that compounds with antioxidant properties, such as SC, have the potential to inhibit oxidative damage in the cell membranes of sperm cells and fragmentation of sperm DNA caused by free radicals (Castellini, 2008; Mourvaki *et al.,* 2010). Our study showed an insignificant difference ($P> 0.05$) in semen pH values in rabbits fed dietary inclusions

of SC. However, Emmanuel *et al.* (2019) showed a significant reduction in the pH of rabbits on a SC diet. Then, the pH in spermatozoa plays a principal role in regulating sperm motility and fertility competence (Holm *et al.,* 1998).

Generally, both mount and intromission latencies were used as indicators of sexual motivation (Dabhakar and Zade, 2013; Nchegang *et al.,* 2016). The effect of the addition of SC on male sexual capability measured by sexual behaviours, such as mounting and intromission latencies, were insignificant ($P > 0.05$), compared to controls. Helal *et al.* (2018) showed identical observations, with SC having a non-significant effect on the libido of rabbits. In another investigation, the mount and intromission latencies of the *Monsonia angustifolia* extract-treated groups showed insignificant differences compared to the control group (Gerda Fouche *et al.,* 2015).

CONCLUSION

The results of the present study showed that inclusion of SC in the diets of rabbits containing 0.3 g/day and 0.6 g/day have positive effects on the body weight and semen analysis of rabbits. Moreover, it increases the levels of some haematological parameters, such as RBCs, HGB, HCT and GCTs. More investigations are desired to document the benefits of dietary inclusion of SC required in rabbits under diverse environmental conditions using an appropriate number of animal experimental units.

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