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EFFECT OF DIET SUPPLEMENTATION WITH LIVE YEAST (*SACCHAROMYCES CEREVISIAE*) ON PERFORMANCE OF RABBIT DOES AND THEIR PROGENIES

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Abstract: A study was conducted to determine the effect of live yeast supplementation in the diet of rabbit does on their mortality and reproductive performance and the performance of their progeny. A total of 52 cross-bred rabbit does (New Zealand×Californian) were divided into 2 groups differing in diet offered during 2 reproductive cycles and containing (group S; n=26) or not (group C; n=26) 1 g of yeast (Actisaf Sc 47, S.I. LESAFFRE, France)/kg of feed. Natural mating was performed 11 d after kindling and kits were weaned at 28 d of age. Body weight of litters was measured at birth, 21 d and at 28 d of age (weaning). Mortality of kits and rabbit does was monitored daily, and fertility of rabbit does and viability rate of kits at birth were also determined. Weight and litter size at birth and at weaning, litter weight gain during lactation and length of gestation were similar between the 2 groups during the 2 cycles. The mortality of does during the experiment was higher in group C than in group S (27 *vs.* 4%; $P<0.05$). Fertility rate of rabbits does and viability rate of kits at birth were higher ($P<0.05$) in rabbits fed with the supplemented diet than those with the control diet during the second lactation. In the first cycle, kit mortality was lower in S group (15.5%) than the C group (24.7%) during the first 21 d ($P<0.05$). However, no difference was observed during the second lactation. In conclusion, our results suggest that the inclusion of yeast in the diet of rabbit does could trigger positive effects on the fertility and mortality of rabbit does, as well as on the viability rate of kits at birth.

Key Words: rabbit does, live yeast, reproductive performance.

INTRODUCTION

Intensive breeding of rabbits can alter the environment, causing physiological stress, increasing the frequency of enteric diseases which subsequently lead to high mortality and decreased reproductive and productive performance of rabbit does. The control of enteric diseases was shown to have been achieved by supplementing diets with antibiotics (Kritas *et al.,* 2008). Due to the ban on the use of antibiotics as growth promoters (EU, 2005), intensive research has focused on the development of alternative strategies with the aim of maintaining a high productivity and reducing the morbidity and mortality under intensive production. The application of probiotics as dietary supplements could serve as a possible solution. Some probiotics exert a barrier effect against pathogenic microorganisms by preventing their development and colonisation within the digestive tract and by stimulating the immune system in human (Vanderpool *et al.,* 2008). As a probiotic, *Saccharomyces cerevisiae* yeast has been known to improve growth performance in weanling pigs and rabbits (Van Heugten *et al.,* 2003; LeMieux *et al.,* 2010; Combes *et al.,* 2013) and immunological status in pigs (Monroy-Salazar *et al.,* 2012). It also improved nitrogen metabolism, fibre digestion and milk production in ruminants (Cole *et al.,* 1992) and enhanced growth performance and limited morbidity or/and mortality in growing rabbits (Maertens and De Groote, 1992; El-Hindawy *et al.,* 1993; Kimsé *et al.,* 2012). However, studies on the use of yeast as a probiotic in rabbit does are scarce. Indeed, the results of Ayyat *et al.* (1996) suggest that the inclusion of a probiotic (Lacto-Sacc) in diets of lactating rabbit does increased milk production, litter size and weight at weaning. The objective of our study was to evaluate the effects of diet supplemented with live yeast *Saccharomyces cerevisiae*

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Table 1: Ingredients and chemical composition of control diet.

	%
Ingredients	
Soybean meal	15.5
Alfalfa	19.2
Wheat bran	38.5
Barley	22.0
Vegetable oil	1.8
Vitamin and minerals mixture*	3.0
Chemical composition	
Crude protein	17.9
Crude fat	3.1
Crude fibre	16.0
Phosphorus	0.6
Calcium	1.3
Methionine	0.3
Lysine	0.9

*Premix provided per kg of diet: calcium carbonate: 300 mg, Fe 40%: 75 mg, Cu: 15 mg, manganese: 39 mg, Zn: 75 mg, sodium selenite: 0.15 mg, Co: 1 mg, calcium iodate: 1.5 mg, Vit A: 10000 IU, Vit D3: 2500 IU, Vit E: 50 mg, K3: 1.35 mg, B1: 2 mg, B2: 5 mg; B5: 15 mg, B6: 2 mg, B12: 0.03 mg, B3: 40 mg, B9: 4 mg, Biotine: 0.03 mg. Chemical composition of the diet was estimated following ISO: Crude protein (ISO 5983-1:2005), Crude fat (ISO 6492: 1999), Crude fibre (ISO 6865: 2000),Phosphorus (ISO6491: 1998), Calcium (ISO 6490-1: 1985).

(Actisaf Sc 47, France) on mortality and reproductive performance of rabbit does and the performance of their progenies over 2 reproductive cycles.

MATERIALS AND METHODS

Diet

Two diets were used in this study: a control diet formulated to meet the requirements of the does (Table 1) and the control diet supplemented with yeast (S group). The Actisaf Sc 47 (CNCMI-4407. S.I. LESAFFRE, France), coated with a fat matrix, was added to vitamin and mineral premix at the level of 1 g of yeast per 1 kg of diet (i.e. 6.5×10^9 CFU/kg feed) and then included in the diet before pelleting. The temperature after pre-pelleting was 53°C and after pelleting was 70°C. After pelleting, 3 samples (500 g each) were collected in a plastic box and sent to LESAFFRE (Marcq en Baroeul, France) for yeast enumeration. For each sample, we took the temperature. We put the feed in a thermos and the temperature was recorded with a thermometer. Rabbit does were fed *ad libitum*, receiving 200 g feed/d from weaning until kindling and 400 g feed/d from kindling until weaning for 2 reproductive cycles. Water was provided *ad libitum*.

Experimental design and animal management

The study was carried out at the experimental rabbitry of the National Institute of Agronomy, Tunisia from the month of March to June year 2013. A total of 52 cross-bred New Zealand×Californian rabbits does at the age of 8 mo and 2nd parity were allocated into 2 groups (26 does/group) with homogenous body weight, 3908 g for the supplemented group and 3932 g for the control group. Rabbit does from the supplemented group received the yeast in the diet from the 2nd natural mating until the weaning of the 2nd reproductive cycle (103 d). Does were mated 11 d post-partum and each doe was palpated 12 d thereafter for pregnancy diagnosis. Those which did not get pregnant were maintained in the experiment and remated after 43 d. Dead rabbit does were not replaced during the experimental period. One rabbit doe from the supplemented group was removed from the study at the beginning of the 2nd cycle due to sickness. After kindling, no adoption of kits was done and all kits were weaned at the age of 28 d.

Housing

Animals were housed in individual mother wire cages equipped with a nipple drinker, feeder and nest box placed outside the cage. The photoperiod was set to 15 h of light and 9 h of dark. During the entire experimental period, the daily temperature in the rabbitry ranged between 19 and 26°C, with an average daily temperature of 23°C. Moreover, the relative humidity ranged between 55 and 66%, with an average daily humidity of 60%.

Methodology for yeast enumeration

Yeast enumeration was performed according to method EN15789 (AFNOR, 2009). The enumeration of yeast in feed samples was consisted of the following steps: Preparation of sterile and dry poured agar plates, and sterile molten agar at 48±1°C for poured plates. Drawing a representative test sample under sterile conditions. Preparation of the initial suspension to obtain a homogeneous distribution of yeast-like cells from the test portion. Preparation of

further decimal dilutions of the initial suspension to reduce the number of microorganisms per unit volume, to allow the counting of colonies after incubation. Inoculation of the prepared plates with an aliquot of the optimum dilutions and dispersion of the inoculum by using a sterile spreader, or poured plate. Aerobic incubation of inverted spread plates for 3 d at 30±1°C, or 35±1°C for 2 d for poured plates. Counting of typical colonies, considering the specific properties of yeast. Morphological verification of isolates of yeast through the use of microscope analysis. Calculation of the colony count per g or kg of feed sample.

Data collection

Litter weight and size were recorded at birth, at 21 d of age and at weaning. During the 1st and 2nd reproductive cycles, does' fertility was recorded. The viability rate of kits at birth and length of gestation period (day) were also recorded. Dead does and kits were recorded daily and the mortality was determined for each reproductive cycle. The mortality of kits due to death of their mothers during the lactation period was excluded from the calculation of kit mortality. All dead rabbits were necropsied.

Statistical analysis

Data on productive traits were analysed by means of 2-way repeated measures as we have 2 factors (treatment and cycle), in order to derive mean values among control and treatment groups during the 1st and 2nd reproductive cycles. Mean values were considered as significantly different at $P<0.05$. Chi square test was performed for fertility and mortality. Statistical analysis was performed using IBM SPSS software (version 19).

RESULTS AND DISCUSSION

Yeast stability

The comparison between counted (10^6) and expected (6.5×10^6) numbers of colony forming units (CFU) demonstrated an acceptable loss (0.85 log) in viability of yeast cells in the diet. This result demonstrates the stability of the yeast under rabbit feed pelleting process conditions (70°C).

Mortality of rabbit does

Mortality of rabbits does was related to enterotoxaemia. The rate of mortality was lower ($P<0.05$) in rabbit does fed the S diet when compared to the control group (Table 2). In fact, throughout the 2 cycles, 7 rabbit does in the control group died (5 in the 1st cycle and 2 in the 2nd cycle), corresponding to a mortality rate of 27%, and only one rabbit doe died during the 1st cycle in the S group (4%). This result could be explained by a possible positive effect of yeast on the stability of bacteria flora in the caecum.

Fertility of rabbit does

During the 1st cycle, does' fertility did not differ between both groups (Table 2). However, during the 2nd reproductive cycle the S group showed higher fertility rates than control group females (95.8 *vs.* 66.7%, respectively; $P<0.05$). This result might be due to better health condition due to positive actions of live yeast in their gut intestinal tract (Bontempo *et al.* 2006). This observation agrees with the shortened interval from parturition to effective mating of New-Zealand×Californian rabbit does with Toyocerin® observed by Nicodemus *et al.* (2004). On the other hand, it contrasted with the results of Kalmus *et al.* (2009), who reported no effect of yeast supplementation on resumption of ovarian activity in dairy cows. Our results showed that fertility of rabbits does improved in the S group during the 2nd cycle compared to the first one (92.3 to 95.8%. $P<0.05$). Moreover, a reduction in fertility among does in the control group was recorded (79.2 to 66.7%) in the same cycle. This result may demonstrate the beneficial effect of Actisaf Sc47 over the long term. However, because of low observations in this study and the short period of the exposure to the tested probiotic, further studies using larger groups and a longer period of administration are recommended to confirm this effect and to confirm the effects of the yeast supplementation.

Table 2: Effect of dietary supplementation with live *Saccharomyces cerevisiae* on pre-weaning doe performance (litter size and weight) fertility and mortality rate of rabbit does and length of gestation.

	C	S	SEM	*P*-value
Cycle 1	n=26	n=26		
Size of litter				
At birth	8.1	8.3	0.4	NS
At 21 d	6.3	5.7	0.4	NS
At weaning	6.6	5.2	0.4	NS
Weight of litter (g)				
At birth	408	376	26.8	NS
At 21 days	1949	1607	103	NS
At weaning	2864	2361	156	NS
Weight gain of kits (g/d)				
From birth to 21 d	11.3	10.1	0.3	NS
From 21 d to weaning	19.3	20.1	1.4	NS
Fertility of does (%)	79.2	92.3		NS
Mortality of does (%)	19.2	3.8		NS
Length of gestation (d)	31.0	31.0	0.2	NS
Cycle 2	n=21	n=25		
Size of litter				
At birth	7.3	8.2	0.5	NS
At 21 d	5.7	5.8	0.6	NS
At weaning	5.4	5.8	0.6	NS
Weight of litter (g)				
At birth	402	396	26.0	NS
At 21 d	1681	1610	165	NS
At weaning	2616	2662	284	NS
Weight gain of kits (g/d)				
From birth to 21 d	10.6	10.4	0.6	NS
From 21 d to weaning	27.4	25.1	2.0	NS
Fertility of does (%)	66.7	95.8		<0.05
Mortality of does (%)	9.5	0		NS
Length of gestation (d)	31	31	0.2	NS

C: control group, S: Supplemented group with live yeast. NS: Not significant ($P>0.05$).
n: number of females studied per treatment. SEM: standard error of the mean.

Pre-weaning productive parameters

There was no difference in the viability rate of kits at birth between the supplemented and control groups during the 1st lactation (Table 3). However, in the 2nd lactation, a higher viability rate of kits was observed in the yeast supplemented group (95.3 *vs.* 87.7%. $P<0.05$). Kritas *et al.* (2015) did not observe any effect of 3×10^5 CFU of *Bacillus subtilis* C-3102/g of diet on neonatal mortality of piglets.

In the 1st lactation, the mortality of kits from 1 to 21 d of age was lower ($P<0.05$) in the supplemented does compared to the control group (15.5 *vs.* 24.7%). However, in the 2nd lactation, there was no difference in kit mortality between the 2 groups. In a similar study using Toyocerin®, Pinheiro *et al.* (2007) observed a decrease in mortality of kits from birth to 18 d of age (18.5 *vs.* 11.1%) in control and treated groups, respectively. Moreover, Stamati *et al.* (2006), observed higher pre-weaning mortality among piglets in the control group (14%) than in the treated batch (7.4%) when applying 0.5 kg of Toyocerin/ton of feed.

A summary of the effect of live yeast on pre-weaning performance of kits is shown in Table 2. Litter size at birth (8.2±0.7 and 7.7±0.7), respectively, during the 1st and 2nd cycles was not different between groups. This suggests that live yeast had no effect on the survival rate of embryos and foetuses during gestation. Likewise, during the 1st and

Table 3: Effect of dietary supplementation with live «Saccharomyces cerevisiae » on viability rate of kits at birth and mortality of kits (%) during the lactation periods.

	C	S	*P*-value
Viability rate of kits at birth (%)			
1st cycle	93.6	90.6	NS
2nd cycle	87.8	95.6	<0.05
Mortality of kits (%)			
1st cycle			
1-21 d	24.7	15.5	<0.05
21-28 d	2.9	2.9	NS
2nd cycle			
1-21 d	18.4	15.2	NS
21-28 d	1.4	1.4	NS

C: control group, S: Supplemented group with live yeast. NS: Not significant ($P>0.05$).

2nd cycles, litter weight and size at 21 d (1778.2±202.6 g and 6.0±0.8, respectively; 1645.7±186.0 g and 5.7±0.8, respectively) and at weaning were not affected by dietary live yeast supplementation. These results are in conformity with those of Nicodemus *et al.* (2004), who found that a probiotic Toyocerin® included at the rate of 0.2 g/kg of diet had no effect on these parameters at birth and at weaning. Likewise, Pinheiro *et al.* (2007), in rabbit, observed the same litter size and weight at birth and weaning among the control and treatment groups during the 1st reproductive cycle when testing 0.2 g and 1 g of Toyocerin®/kg of diet. Additionally, Jang *et al.* (2013) noted no effect of live yeast on these parameters at birth and weaning for suckling piglets. Moreover, Jurgens *et al.* (1997) noted that active dry yeast included in sow diets did not improve sow reproductive performance as measured by number of pigs born, litter birth weight or litter weaning weight. On the contrary, in other studies Maertens *et al.* (1994) and Ayyat *et al.* (1996) observed a significant ($P<0.05$) improvement in litter size and weight at 21 d and at weaning due to Paciflor® and to Lacto-Sacc supplementation. Furthermore, Zaleska *et al.* (2015) observed an increase in prolificacy in sheep fed yeast. Supplementation of rabbit does' diet with Actisaf Sc 47 did not affect the kits' weight gain in the first 21 d of age and no marked changes were observed during the last week before weaning throughout the 1st and 2nd cycles. These results showed firstly a possible lack of effect of Actisaf Sc 47 on milk production from birth to 21 d, when kits do not consume solid feed and their weight gain is related directly to the milk consumption, and secondly on solid consumption from 21 d until the weaning. The growth performance results were similar to those reported by Jang *et al.* (2013), who did not observe any effect of yeast on the growth performance of nursing piglets. However, Pinheiro *et al.* (2007) observed a higher weight gain in treatment group with Toyocerin® from 18 d of age to weaning in kits.

CONCLUSION

The addition of 1 g of yeast (Actisaf Sc47, LESAFFRE)/1 kg of diet enhanced fertility and reduced mortality of rabbit does, while improving the viability rate of kits at birth. However, diet supplemented with the tested probiotic had no effect on the other reproductive performance traits in rabbit does. Further research is necessary to confirm these results and understand the mode of action of yeast as a probiotic.

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