Growth performance of male NZW rabbits fed diets supplemented with beneficial bacteria or live yeast

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Abstract: Sixty male growing NZW rabbits aged eight weeks old, weighed in average 837.0±50.0 g were randomly ranked in four equal groups to feed on four experimental diets for 10 weeks. All groups were fed a uniform rabbits pelleted diet, where R1 diet was without supplement (control), R2 supplemented with 0.1% *Bacillus subtilis*, R3 with 0.1% live *Saccharomyces cerevisiae* and R4 with 0.05% *Bacillus s.* + 0.05% *Sacchromyces c*. mixture. The results indicated that the voluntary feed intake was not influenced by bacteria or yeast supplementations. Meanwhile, body weight gain and feed efficiency were obviously improved (P<0.05) with diets supplemented with yeast, bacteria or their mixture than the control. Nutrients digestion coefficients and dietary nitrogen utilization were (P<0.05) higher in yeast or bacteria supplemented diets group than those of R1 and R4. The lower values concerning weight gain, nutrients digestibility and dietary nitrogen utilization with feeding Yeast + bacterial mixture (R4) than other supplemented diets, which revealed that there was an antagonistic effect between the two microbial types on feed utilization of rabbits. Carcass characteristics, dressing % calculated relative to pre-slaughter or empty body weight, meat, bone ratio and body chemical composition were not statistically different among groups.

Keywords: rabbits, growth performance, probiotics, carcass characteristics, body composition

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1 Introduction

Rabbits are sensitive to enteric diseases especially when they are exposed to productive or environmental stresses.

This problem can be avoided by using antibiotics which its use had been banned as growth promoter in animal feeding by the European legislations. For that reason, direct feeding of some beneficial microbes (probiotics) instead of antibiotics, such as *Bacillus subtilis and Saccharomyces cerevisiae* have been given great attention by nutritionists. Probiotics are bioregulators that can prevent reduction of ruminal pH by increasing the use of lactic acid, also improving the gut microbial balance, animal immune system and health (Falcao E Cunha *et al*. 2007), as well as feed conversion, weight gain and growth performance (Li *et al*. 2009; Sinol *et al*. 2012; Zhang and Kim 2013). The positive effect of probiotics on certain pathogens in animals has been shown in several studies, where they appear to control enteric diseases associated with *Escherichia coli* or other enteric pathogens (Abe *et al*., 1995; Alvarez *et al*., 2001; Kritas and Morrison, 2005; Timmerman *et al*., 2005).

Bacillus spp. has been identified as a suitable probiotic because of the resistance of its spores to harsh conditions and storage in long term at ambient temperature (Sinol *et al*. 2012; Chen *et al*. 2013). The use of *Bacillus subtilis* not only improves health but also promotes intestinal digestion processes by matching nutrients and productivity of rabbits. Several strains of

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Saccharomyces cerevisiae have reduced mortality in various species of animals (Collier *et al*. 2010; Peret *et al*. 1998), and the beneficial effects are more known in conditions of stress or in animal herds with high mortality (Ewing, 2008). However, there are few studies of probiotic supplementation in rabbits and the information about the effects of probiotics on nutrients digestibility, feed efficiency and growth performance of rabbits are still limited.

Therefore, this study was conducted to investigate the influence of supplementation of Bacillus subtilis and live Saccharomyces cerevisiae or their mixture on nutrients digestibility and growth performance of Male NZW rabbits.

2 Materials and Methods

Two different types of probiotics, dry live yeast of 10^8cfu/g (RUMI YEAST –*Saccharomyces cerevisiae Sc47*– Neovia - France) and bacterial dry media of 3×10^7 cfu/g (Enviva PRO- *Bacillus subtillis* –Dupont– USA), were used as growth promoters for rabbits. Yeast or bacterial supplements were added to rabbits' diet at the level recommended by the manufacturers. Four Batches of a uniform rabbits diet 150 kg each were formulated to contain; 30% alfalfa hay, 29% ground barley grains, 8.0% wheat bran, 8.0% ground yellow corn, 20% soybean meal (44%), 3.0% cane-molasses, 1.0% ca. diphosphate, 0.5% sodium chloride, 0.4% minerals and vitamins mixture and 0.1% DL-methionine. The first diet (R1) was free of supplements (served as control), the second (R2) was supplemented with 0.1% *Bacillus subtillis*, the third (R3) was supplemented with 0.1% *Saccharomyces cerevisiae* and the fourth (R4) was supplemented with 0.05% *B. subtilis* + 0.05% *S. cerevisiae.* All diets were thoroughly mixed and pelleted at 0.3 cm diameter cubes.

Feeding Experiment: Sixty male growing New Zealand White rabbits (NZW) aged eight weeks old with an average body weight of 837.0±50.0 g were blocked by weight into four equal groups (15 animals each). Experimental rabbits were housed individually in galvanized metal wire cages equipped with feeding and water troughs, where the first group of rabbits was fed R1, while 2nd, 3rdand 4thgroups were fed R2, R3 and R4, respectively. Experimental diets were offered daily at 8.30 a.m. Feed refusals were daily collected, weekly weighed and recorded. Clean drinking water was freely available at all times. During the whole experimental feeding period, rabbits were kept under proper hygienic conditions.

Digestibility Trials: At the end of the feeding experiment, four digestibility trials were carried out over a period of seven days where three days were for adaptation and the other four days for quantitative collection of feces and urine. Three random rabbits from each group were individually confined in stainless-steel metabolic cages, where feces and urine could separately be collected. Daily amounts of feed intake, feces and urine out-put were determined and daily recorded during the collection period. Individual composite samples of dry feces and acidified urine were kept in glass bottles and stored at 4°C until chemical analysis.

Slaughter Technique: After termination of the feeding experiment, three representative rabbits randomly chosen from each group were fasted for 12 hrs, weighed and hand slaughtered. After complete bleeding, the drained blood was collected and weighed. Slaughtered animals were de-skinned, dressed out and the hot carcass without head was weighed and recorded. Edible offals (liver, heart, spleen and kidneys), non-edible offals (head, lungs& trachea, clean empty G.I.T. and testicles) and trimmings (fur, four legs, blood and G.I.T. contents) were separately weighed and recorded. The whole carcass of each rabbit was de-boned and the resultant amounts of meat and bone were separately weighed and recorded. De-bonded meat of each rabbit was minced, oven dried for 72 hrs, weighed to determine body water content and the dry meat was finally ground to determine protein, fat and ash.

Chemical Analysis: Chemical composition of feeds and feces were determined for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash according to the standard methods of A.O.A.C. (2005). Nitrogen free extract (NFE) was calculated by difference. Urinary nitrogen (UN) was determined by themicro-kjeldahl method. Body chemical composition was determined according to A.O.A.C. (2005). Chemical composition of the basal diet is given in Table 1.

Table 1 Chemical composition of the basal experimental diet

Moisture %	10
Dry matter composition (DM), %	
Organic matter (OM)	93.15
Crude protein (CP)	17
Crude fiber (CF)	13.44
Ether extract (EE)	4.56
Nitrogen free extract (NFE)	58.15
Ash	6.85

Statistical Analysis: Collected data concerning body weight gain, feed efficiency, nutrients digestibility, dietary nitrogen utilization, carcass dressing percentage and body chemical composition were subjected to one way analysis of variance according to Steel and Torrie (1980) applying the General Linear Model procedure of SAS (2000). Significant differences between means were tested using Duncan's Multiple Range Test (1955).

3 Results and discussion

The results of feed intake, body weight gain and feed conversion efficiency of rabbits in experimental groups are given in Table 2. Voluntary feed intake wasn't significantly influenced by bacteria (B. subtilis) or live yeast (S. cerevisiae) supplementation. Pattern of the weekly feed consumption was presented in Figure 1, which illustrated that all experimental groups had nearly similar trend during the whole feeding period and no mortality cases were recorded. Body weight gain or average daily gain (ADG) of rabbits fed alone bacteria or yeast or their mixture supplemented diets were significantly (P<0.05) higher than those fed un-supplemented diet (R1 group). The highest body weight gain was recorded for yeast diet (R3) followed by that supplemented with bacteria (R2) with no significant difference between the two supplements. Regardless of the higher weight (P<0.05) weight gain of rabbits fed (bacteria + yeast mixture) diet (R4) than control (R1), it had much lower (P<0.05) weight gain value than those of R2 and R3 (1207.5 g vs. 1441.0 g and 1486.5 g, respectively). In comparison with the control group, rabbits fed yeast diet were heavier by 37%, bacteria diet 33% and it was only 11% with bacteria + yeast mixture diet. Such result is pointed out to the beneficial action of live yeast (S. cerevisiae) and bacteria (B. subtilis) as growth enhancers of rabbits. Meanwhile, the lower weight gain associates feeding the supplement mixture than other alone supplements, might reveal that there was an antagonistic effect between live yeast and beneficial bacteria on nutrients digestion and absorption sites in the gastrointestinal tract of rabbits.

Table 2 Feed intake, body weight gain and feed efficiency of rabbits in experimental groups

Item	R1	R2	R3	R4
Initial BW, g	821.0±62.6	835.0±57.6	851.5±46.2	839.5±40.4
Final BW, g	1907.0^{c}±82.8	2276.0^{a}±48.4	2338.0^{a}±56.5	2047.0^{b}±50.4
BW gain, g	1086.0^{c}±36.2	1441.0^{a}±29.5	1486.5^{a}±32.9	1207.5^{b}±17.7
ADG, g/d	15.5^{c}±0.5	20.6^{a}±0.4	21.2^{a}±0.4	17.3^{b}±0.3
Total intake, g	8125.6±120.0	8489.6 ±127.8	8521.3±126.2	8255.1±64.8
Daily intake, g	116.1±1.7	121.3±1.8	121.7±1.8	117.9±0.9
Feed conversion efficiency: (g intake/g gain)	7.5^{a} ±0.2	5.9^{c} ±0.2	5.7^{c} ±0.1	6.7^{b}±0.1

Note: a,b,c Means have different superscripts in the same row are significantly different at (P<0.05).

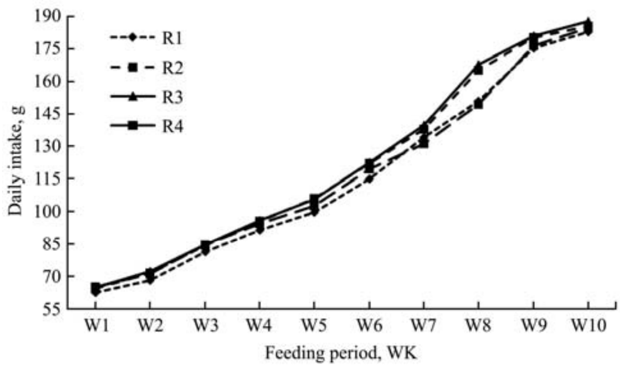

Figure 1 Daily feed intake of rabbits fed experimental diets

By tracing development of the body weight in experimental groups (Figure 2), the superiority of R2 and R3 rabbits is clearly observed. However, the real difference among groups was started after the 3rd week of the feeding period. The results of feed intake and weight gain were currently reflected on values of the feed conversion efficiency (g intake/g gain), where it was better for R3 and R2 being 5.75 and 5.92 respectively, than 6.75 for R4 and 7.55 for R1. Digestion coefficient of most measured nutrients (DM, OM, CP, NFE) were significantly (P<0.05) higher for R2 and R3 than those of R1 and R4. In general, the lowest values of all nutrients were recorded on rabbits fed bacteria + yeast mixture supplemented diet (Table 3). On the other hand, bacteria and yeast supplements didn't improve either ether extract or crude fiber digestibility values. Dietary N utilization calculated as N balance relative to N-Intake or digestible-N shown in Table 4 was significantly (P<0.05) higher for rabbits fed diets with alone supplements of bacteria or yeast (R2 and R3) than other groups (R1 and R4). Although, the difference between values of N-utilization for R2 and R3 groups didn't reach any significance, the corresponding values of R3 were better than those of R2. Carcass characteristics, meat: bone ratio and meat chemical composition of slaughter rabbits in experimental groups were presented in Tables 5, 6 and 7, respectively. There was no significant difference of alone or combined bacteria yeast supplements on carcass traits or rabbit's meat chemical composition. However, aslitebetter dressing percentage values were recorded on slaughtered rabbits of R2 and R3 than R1 and R4.

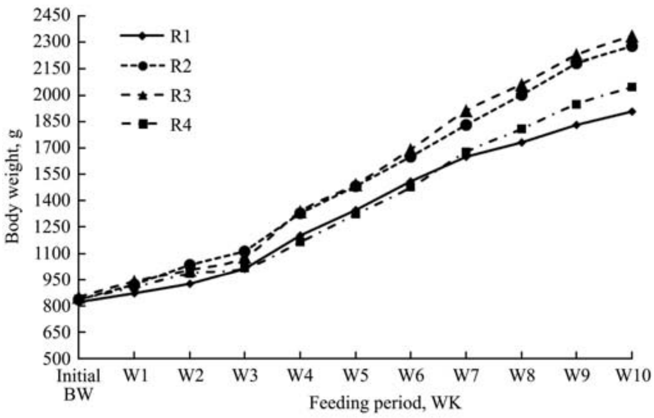

Figure 2 Body weight development of rabbits fed experimental diets

Table 3 Nutrients digestibility of experimental diets

Item	R1	R2	R3	R4
Nutrients digestibility, %				
DM	60.18^{ab}±2.11	64.59^{a}±1.33	65.83^{a}±1.91	52.81^{b}±1.29
OM	64.73^{ab}±2.73	68.22^{a}±2.68	69.94^{a}±2.48	57.95^{b}±2.22
CP	76.34^{ab}±1.86	80.90^{a}±1.19	82.43^{a}±1.87	72.87^{b}±1.89
EE	81.28^{a}±2.64	82.65^{a}±1.20	81.97^{a}±1.71	74.63^{b}±2.26
CF	56.00^{a}±3.62	52.50^{b}±2.61	58.72^{a}±3.06	49.83^{b}±2.34
NFE	62.54^{b}±0.93	67.36^{a}±0.86	67.50^{a}±1.24	54.04^{c}±1.89

Note: ^{a,b} Means have different superscripts in the same row are significantly different at (P<0.05).

Table 4 Apparent dietary nitrogen (N) utilization of rabbits fed experimental diets

Item	R1	R2	R3	R4
N intake	3.18±0.01	3.32±0.03	3.32±0.01	3.17±0.05
Fecal nitrogen	0.75±0.09	0.64±0.04	0.58±0.07	0.90±0.12
Urinary nitrogen	1.09±0.12	1.01±0.38	0.92±0.24	0.94±0.20
Nitrogen balance	1.34±0.21	1.67±0.34	1.82±0.29	1.33±0.19
Dietary N utilization, %:				
of N intake	42.14^{b}±0.21	50.30^{a}±0.21	54.82^{a}±0.29	42.00^{b}±0.21
of digestible N	55.14^{b}±0.23	62.31^{a}±0.34	66.42^{a}±0.25	58.60^{b}±0.19

Note: ^{a,b,c} Means have different superscripts in the same row are significantly different at (*P*<0.05).

Table 5 Carcass characteristics of rabbits fed experimental diets

Item	R1	R2	R3	R4	Sign.
pre-slaughter wt., g	1763.3	1833.3	1910.0	1765.0	---
slaughter wt., g	1720.0	1785.0	1858.3	1717.3	---
carcass wt., g	831.7	883.3	983.3	835.0	---
Edible offals, g:					
giblet wt.	85.3	93.3	87.7	85.7	---
Non-edible offals wt., g:					
Fur + legs	185.0	190.0	203.3	188.3	---
Head	128.3	128.3	143.3	134.7	---
Clean empty G.I.T	118.3	106.7	108.3	108.3	---
Total	431.6	425.0	454.9	431.3	---
Trimmings wt., g					
Blood	43.3	48.3	51.7	47.7	---
G.I.T. cont.	371.3	383.3	332.3	365.3	---
Total	414.6	431.6	384.0	413.0	---
Dressing,%					
Of pre- slaughter wt.	47.16±0.43	48.02±1.6	51.23±1.56	46.49±2.06	NS
Of empty body wt.	59.75±0.4	60.79±1.03	62.15±0.89	58.71±2.33	NS

Note: NS = No significant difference.

Table 6 Physical traits of rabbit's carcass in experimental groups

Item	Meat%	Bone %	Meat : Bone	Sign.
R1	62.90	37.10	1.70	NS
R2	61.96	38.04	1.63	NS
R3	63.55	36.45	1.75	NS
R4	61.25	38.75	1.58	NS

Note: NS = No significant difference.

Table 7 Chemical composition of rabbit's lean meat in experimental groups (on fresh basis)

Item	Moisture %	CP %	EE %	Ash %	Others %
R1	72.13±0.22	20.30±0.56	4.21±0.25	1.28±0.01	2.07±0.06
R2	72.49±0.34	20.13±0.30	4.12±0.16	1.26±0.03	2.00±0.09
R3	72.68±0.24	19.81±0.23	3.92±0.14	1.31±0.02	2.28±0.09
R4	72.51±0.06	19.88±0.04	3.77±0.32	1.30±0.01	2.54±0.31

Note: ^{a,b} Means have different superscripts in the same column are significantly different at (*P*<0.05).

The results obtained from this study could be summarized that the alone supplements at 0.1% of B. subtilis or *S. cerevisiaein* diets of growing NZW rabbits had clear positive effects on weight gain, feed conversion efficiency, digestibility of DM, OM, CP and NFE and dietary N utilization. Meanwhile, all supplements had no effect on voluntary feed intake, carcass characteristics, meat, bone ratio and the chemical composition of rabbit's meat. It's worth saying that the supplementation effect has started to appear after three weeks of feeding. There was a noticeable competition between bacteria and yeast mode of action resulted in lower measured parameters of bacteria – yeast combination treatment than alone supplements.

In agreement with our results concerning, feed intake, weight gain and feed conversion efficiency, feeding diets supplemented with *Lactobacillus acidophilus* and *Lacto coccus lactis* showed that bacterial probiotics improved weight gain and feed conversion ratio without significant effect on feed consumption (Bhatt et al., 2017). Same conclusion was also noted by Thanh and Uttra (2017) on weaning rabbits fed by supplements of *Bacillus subtilis* and *Lactobacillus acidophilus*. Onifade et al. (1999) observed that Saccharomyces cerevisiae improved growth performance and feed intake in domestic rabbits. Nicodemus et al. (2004) explained that the positive effect of Bacillus cereus var. Toyoi supplementation could be regarded to that supplemented probiotics had positive effect on the development of an optimum bacteria flora in the gastrointestinal tract, which allowed an improvement of feed utilization. Kritas et al. (2008) explained that feeding probiotics may have a growth promoting activity by competing with harmful flora and stimulating the immune system. Copeland et al. (2009) explained that probiotic fortified diets were effective in decreasing pathogenic bacteria colonization. Similar conclusion was also stated by Kritas et al. (2008) in their study on rabbits fed supplemented diets with *Bacillus licheniformis* and *B. subtilis*. They reported that the supplements had significant influence in improving growth rate, which suggesting better health status, and more specifically gastro-intestinal tract health.

In the contrast, Kimsé et al. (2008) found that the *S. cerevisiae* supplementation did not affect body weight, daily weight gain and feed efficiency of rabbits.

The present findings of nutrients digestibility and dietary nitrogen utilization are in conformity with the findings of Dihigo et al. (2014) with *Bacillus Subtilis* and Endospores, Bhatt et al. (2017) with *Lactobacillus acidophilus* and *Lactococcus lactis* and Thanh and Uttra (2017) with *Bacillus* subtilisand *Lactobacillus acidophilus* in rabbit diets. In the contrary, Kimsé et al. (2008) did not find any significant effect of tow supplementations levels of *S. cerevisiae* on nutrient digestibility of growing rabbits. In addition to that, Campos-Morales et al. (2014) in his study on Volcano rabbits indicated that feeding diets supplemented with S. cerevisiae was negatively affected digestion and mortality of rabbits in captivity.

The present results of carcass parameters were in agreement with the findings of El-Sagheer and Hassanein (2014) and Bhatt et al. (2017), who found that dietary supplementation with probiotic in rabbit diets, had no significant effect on carcass traits. It seems to be interesting that the lag time needed by rabbits to respond for treatments was three weeks in the present study. In some earlier studies, Jensen (1998) noted positive effects of probiotic supplementation on the growth performance and feed efficiency of weaning rabbits in the first two weeks after feeding, while no significant difference was observed in the last four weeks. In other research on pigs, Pluske et al. (1997) stated that the intestinal microflora became stable for normal gut functions during two to three weeks of post-weaning period. Similar conclusion was noted by Huang et al. (2004). The antagonistic effect between bacteria and yeast investigated from results of R4 treatment might suggest that there was a competition between *S. cerevisiae* and *B. subtilis* in sites of digitation and absorption along the gastro intestinal tract where live yeast has better effect than bacteria particularly in the cecum of rabbits, which might explain the superior results of yeast supplemented diet than other treatments.

In conclusion, it's fair to state that the alone supplement at 0.1% of live yeast (*S. cerevisiae*) or (*B. subtilis*) is recommended to enhance growth performance and improve feed conversion efficiency of NZW rabbits.

CONFLICT OF INTEREST: We certify that there is no conflict of interest with authors or any financial organization regarding the material discussed in this manuscript.

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