



## The Effect of Symbiotic Drenching Mixture on the Ingestive Behavior, Productive Performance, Blood Biochemical Parameters and Carcass Traits of Growing Rabbits

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### ABSTRACT

Stressors that originated from intensive housing of young rabbits during the growing period have bad effects on rabbit performance and health status. A total of forty-five apparently healthy New Zealand White rabbits were used to investigate the effect of drenching of symbiotic mixture on the behavior, productive performance, blood biochemistry and carcass criteria of growing rabbits. They were allocated into two groups } 1st: control (n=21) and 2ed treated) (n=24) and both groups were housed under conventional cage system. The treated group was weekly drenched by 40mg/rabbit/day (200g/1000liter) symbiotic mixture which was dissolved in 5ml distilled water, while the control group received only 5ml distilled water through the drench and all solutions were at room temperature. Video recording of behaviors were done weekly, in addition to determination of rabbit's body weight and feed intake. At the end of the experiment 10 rabbits from each group were slaughtered for blood biochemical analysis and carcass traits evaluation. The obtained results revealed that there was a significant increase in body weight and body weight gain of the drenched group than control one, however, the feed intake, feed conversion ratio and performance index were decreased in the treated rabbits. Drenched rabbits have a significant ( $P \leq 0.05$ ) higher total proteins, albumin, and globulin and AL/GL ratio than control groups. The behavior of treated animals showed a significant ( $P \leq 0.05$ ) increase in feeding duration (min.) and drinking frequency and duration (min.). This study concluded that drenching of growing rabbits a symbiotic mixture results in improvement of their ingestive behavior, performance and blood biochemical parameters.

### 1. INTRODUCTION

The high reproductive capacity of rabbits can be used as a highly profitable venture for investment in animal production as well as to face the big shortage in animal protein through its high growth rate (Püschel et al., 2010), high carcass yields and their ability to convert 20% of the protein they feed into consumable meat which is higher than beef that only convert 8-12% (Basavaraj et al., 2011).

Intensive housing of growing rabbits during the weaning stage originated a lot of stressors which lead to spreading of enteric pathogens that had a negative

impact on their growth, performance, feed efficiency and health (Licois, 2000; Bovera et al., 2010). It is notable that feed additives can be used safely for rabbits in order to improve performance. Nowadays, it is studied that antibiotics as feed additives can be replaced by probiotics, prebiotics, bacteriocins and organic acids (Marounek et al., 2003).

Probiotics are a live microbial feed additive, which has a beneficial effect on the host animal by means of improving its intestinal microbial balance (Kalma et al., 2016) and encouraging the growth of beneficial bacteria that is needed in a rabbit's natural digestive process (Szendrő et al., 2011). Moreover, improve

productive parameters, enhance sanitary conditions, maintain a balance and allow multiplication of the beneficial microbial population in the gastro-intestinal tract (Corcionivorschi et al., 2010). Metzler et al. (2005) stated that probiotics are used as a nutritional technique to support host organisms during difficult physiological periods, attenuate technological stress and prevent or combat diarrheal syndromes. The mixtures of probiotic and prebiotic are termed symbiotics which work by additive or synergistic effects (Gibson and Roberfroid, 1995).

The effect of probiotic supplement on animal growth performance could be expected to be less effective after weaning for 3 weeks or at 13 weeks of age in cross breed rabbit due to the intestinal microflora becomes stable and normal gut functions have been established (Huang et al., 2004). Moreover, the supplementation of white New Zealand rabbit with probiotic or prebiotic or combination of both with organic acid had no effect on their feed intake (Eiben et al., 2008; Chrastinova et al., 2010; Ewuola et al., 2011). On the other hand, addition of probiotics phytase mixture increased feed intake for Californian breed of rabbit (Iwu et al., 2015). The feed conversion ratio was reduced in NZW rabbit supplemented with probiotics at 6 weeks of age. (Chrastinova et al., 2010; Thanh and Jamikorn, 2012). However, feed conversion efficiency of rabbits supplemented with probiotic (RE 3, 1.0 ml per kg feed) tended to be better than those provided with 1.5 ml per kg feed (Kritas et al., 2008; Wallace et al., 2012). In addition, there were no effect on the blood profile of rabbits (Ahamefule et al., 2008). Moreover, the addition of growth promoting additives (dietary prebiotics and probiotics assymbiotic) had no significant effect on the carcass characteristics measured except the right arms of rabbits (Ewuola et al., 2011).

Prebiotics are non-digestible food that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Moreover, Tomasic and Tomasic (2003) stated that they are natural factors belonging to the group of dietary fiber (inulin with low degree of polymerization, fructose derived oligosaccharide and resistant starch). They use have many advantages; because they are not affected by the thermal processing of the feed as well as acid production by stomach, they don't introduce foreign microbes into the gut, they prevent the attachment of pathogens to the gut mucosa and they can stimulate the gut immune system (Forchielli and Walker, 2005).

It is hypothesized that probiotic prebiotic mixture supplement to weaning rabbits could possibly improve gut microbial population and digestion resulting in enhanced feed efficiency, body weight gain (BWG), and performance index. So that, the aim of this work is to investigate the effect of the drenched mixture of probiotic and prebiotic on the behavior, productive performance, blood biochemistry and carcass criteria of growing rabbits.

## 2. MATERIALS AND METHODS

### 2.1. Experimental animals

This study was carried out at the Rabbit farm, faculty of veterinary medicine, Suez Canal University, Ismailia, Egypt, from January to April 2017. The protocol used were approved by Animal Care and Use Committee of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. A total number of forty-five apparently healthy New Zealand White growing rabbits at 30 day of age their body weight ( $324 \text{ g} \pm 17$ ). They were allocated into two groups. The first group was the control group (C; n=21 rabbit) and the second group was the treated group (T; n=24 rabbit). The first group was drenched 5 ml distilled water 2 times / week, while the second group was drenched bactocell drink, which manufactured by LALLEMAND SAS animal nutrition company, France. This mixture contains *Pediococcus Acidilactici* MA18/5M lactic acid bacteria ( $2.5 \times 10^9 \text{ CFU/g}$ ), inactive yeast extracts (*Saccharomyces Cerevisia*) and Fructo-oligosaccharides. Each rabbit was drenched by the aid of clean and sterile syringe with a dose of 40mg/rabbit/day dissolved in 5ml distilled water in room temperature for five weeks.

### 2.2. Housing conditions

All rabbits were kept in groups of three animals per cage with 15 replicates (7 replicates for control and 8 for drenched one). Each group was allocated in a wire mesh cage ( $0.40 \text{ m} \times 0.36 \text{ m} \times 0.25 \text{ m}$ ). All animals were kept under similar adequate managerial and hygienic conditions until the end of the experiment (72 days old). All rabbits were housed under natural light/dark cycle (app. 16/8 hrs) and ambient temperature ( $20 \pm 6^\circ\text{C}$ ) and relative humidity ( $60 \pm 5\%$ ).

### 2.3. Feeding System and ration composition

The cages were located in separate rows. Rabbits were maintained in their respective treatment group. Feed was added in each feeder every day. Feeders were completely separated between cages. Each feeder was divided into four compartments, resulting in

simultaneous access by four animals. All groups received a balanced commercial diet (Table:1). It was formulated to contain adequate levels of nutrients for growing rabbits as recommended by the National Research Council, (NRC, 1977).

There were one nipple drinkers and one feeder (36 cm width, 10 cm length and 10 cm height, content: about 3 kg for each cage).

**Table 1:** Chemical composition of commercial diet

Diet ingredients	Percent (%)
Protein	18%
Fat	2.69%
Fiber	11.99%
Digestible energy	2575 kcal/kg ration.

#### 2.4. Behavioural observations and productive performance.

Twelve rabbits from each group were randomly selected for focal behavioral observation (5 min/hr) for 24 hr/wk for five weeks using video camera (Panasonic WV Ns202ae) for each group connected to computer through DVR card (Altmann, 1974). The mean frequency and duration (min.) spent in the following behavioral patterns were scored according to Ribikauskas *et al.* (2010).

- 1- Eating: Consumption of feed from the feeder, gnawing the pellet.
- 2- Drinking: Drinking water from nipple drinkers.

The animals were weighed once per week after weaning for five weeks till the slaughtering day at the end of the fattening period in all groups. Live body weight and the amount of feed were recorded for all groups at the same age from 5<sup>th</sup> to 10<sup>th</sup> weeks. Weekly weight gain (WWG), feed intake (FI) and feed conversion ratio (FCR) were calculated, such ratios were expressed on the basis of the remaining living rabbits. In addition, Performance index (PI) was calculated as live body weight (kg)  $\times$  100/feed conversion ratio (FCR) (Amber *et al.*, 2004).

#### 2.5. Blood sampling and biochemical parameters

To study the influence of treatments on *biochemical parameters*, blood samples from ten rabbits from each group were collected on day of slaughtering. After that, the collected blood samples immediately centrifuged at 2000 $\times$ g for 10 min. Serum samples were stored at -20 °C until analyzed.

Rabbit's sera were examined for some biochemical parameters including: total protein (TP), albumin

(ALB), globulin (GLOB), creatinine (CREAT) and glucose (GLUC). They were measured calorimetrically using endpoint kits (Diamond Diagnostic) and UV1100 spectrophotometer (Young, 2001).

#### 2.6. Carcass traits

Ten rabbits from each group were arbitrarily taken and weighed before slaughter (live body weight). After slaughter weight of carcass (dressed weight), head, liver and viscera were recorded then dressing percentage was calculated.

#### 2.7. Statistical analysis

The data were analyzed by T-independent test using SPSS 16.0 software package according to (Argyrous, 2005).

### 3. RESULTS AND DISCUSSION

#### 3.1. Weekly body weight and weight gain

The results shown in Table 2, indicated that the treated group had a significant increase in body weight and weight gain compared with control one which could be attributed to the beneficial effect of probiotics administration through balancing the gut microflora barrier that improves the soundness of intestinal mucosal barrier and digestion by intestine thus improves the function of intestinal transportation system. Ashayerizadeh *et al.* (2009) reported that prebiotics and probiotics as growth promoters can be used as alternatives for antibiotics because they improve growth performance of broiler chickens without any effect on consumers. Similar results for the improvement of growth performances have been reported by Sieo *et al.* (2005); Apata (2008) and Yu *et al.* (2008). Similarly, Amber *et al.* (2014) observed that rabbits received MIX1 (mixture of prebiotic) at treatment recorded the highest value of daily weight gain, followed by rabbits received MOS1 (mannan oligosaccharide) treatment, but no significant differences between them could be observed, while the lower value was observed for rabbits received control treatment (28.9 and 28.6 vs. 24.1 gm,  $P < 0.001$ ). On the other hand, rabbits fed probiotic supplemented diets at early period (3 weeks of age) had higher ( $P < 0.05$ ) daily weight gain as compared with those at later period (5 weeks of age). Feed intake was increased ( $P < 0.05$ ) with supplementing Bio-Mos, Bio-Plus or their mix in diets (Amber *et al.*, 2014).

Similar results on the positive effect of probiotics on growth performances have been reported by Oso *et al.* (2013), who concluded that the highest ( $P < 0.05$ )

final live weight and weight gain of growing rabbits were recorded when they fed diet containing Prebiotic (MOS at 1.0 g/kg feed) when compared to those rabbits fed on arabixonylans oligosaccharides (Axe 1.0 g/kg feed) or Probiotic (*Pediococcus acidilactis* as  $1 \times 10^{10}$  cfu/g; 0.5g/kg feed or *Bacillus Cereus* as  $1 \times 10^9$  cfu/g; 0.5 g/kg feed) or even other dietary combinations, moreover Kritas *et al.* (2008) showed that probiotic-treated rabbits were heavier than control group (54 g and 123 g) at the end of the growing and finishing phases, respectively, with higher average daily gain and better feed conversion ratio ( $p < 0.05$ ), in addition Sarat Chandra *et al.* (2015) reported that

supplying diet with probiotics had a positive effect on body weight gain of weaning rabbits (28 days) in New Zealand white, Grey Giant, and Flemish Giant.

On contrary, Dorra *et al.* (2013) stated that use of dietary organic acids did not significantly influence growth performance, carcass traits or blood parameters of New Zealand White  $\times$  California experimental rabbits, moreover Seyidoglu and Galip (2014) observed that final body weight and total weight gain was not affected significantly ( $P > 0.05$ ) for a period of 90 days when male New Zealand White rabbits (aged 5-6 weeks) raised on supplemented diet with live yeast culture.

**Table 2:** Effect of symbiotic mixture on productive performance of growing rabbits (mean + S.E)

Items	Group A (Control)	Group B (Treated)	P-value
Initial Body weight (g)	288.92	289.12	
Final Body weight (g)	849.31 <sup>b</sup> $\pm 33.68$	898.02 <sup>a</sup> $\pm 36.28$	0.029*
Total weight gain (g)	212.06 <sup>b</sup> $\pm 10.55$	242.93 <sup>a</sup> $\pm 11.76$	0.05*
Feed intake (g)	567.07 $\pm 41.85$	555.60 $\pm 39.52$	0.91
Feed conversion ratio	2.75 $\pm 0.14$	2.68 $\pm 0.14$	0.73
Performance index (%)	31.91 $\pm 2.23$	30.67 $\pm 2.05$	0.49

Means in the same row with different superscripts are significantly different ( $P \leq 0.05$ ). \*  $P \leq 0.05$

**Table 4:** Effect of symbiotic mixture on carcass traits of growing rabbits (mean + S.E).

Items	Group A (Control)	Group B (Treated)	P-value
Live body weight (g)	1637.14 $\pm 49.70$	1644.25 $\pm 49.45$	0.79
Carcass weight (g)	953.14 $\pm 31.56$	966.50 $\pm 28.46$	0.55
Dressing percentage (%)	58.22 $\pm 2.04$	58.78 $\pm 1.88$	0.29
Visceral weight (g)	645.00 $\pm 22.84$	629.25 $\pm 23.84$	0.81
Head weight (g)	131.71 $\pm 11.94$	105.00 $\pm 3.98$	0.19
Liver weight (g)	37.86 $\pm 3.85$	47.50 $\pm 4.63$	0.60

There was no significant difference between means ( $P > 0.05$ ).

**Table 3:** Effect of symbiotic mixture on biochemical parameters of growing rabbits (mean + S.E).

Items	Group A (Control)	Group B (Treated)	P-value
Total proteins, g/dl	6.89 <sup>b</sup> $\pm 0.63$	7.93 <sup>a</sup> $\pm 0.76$	0.043*
Albumin (AL), g/dl	4.97 <sup>b</sup> $\pm 0.54$	5.83 <sup>a</sup> $\pm 0.54$	0.036*
Globulin (GL), g/dl	1.92 <sup>b</sup> $\pm 0.20$	2.11 <sup>a</sup> $\pm 0.26$	0.044*
AL/GL	2.86 <sup>b</sup> $\pm 0.53$	3.14 <sup>a</sup> $\pm 0.47$	0.019*
Glucose (mg/dl)	86.42 $\pm 4.17$	90.15 $\pm 4.41$	0.74
Creatinine (mg/dl)	0.70 $\pm 0.06$	0.63 $\pm 0.08$	0.45

Means in the same row with different superscripts are significantly different ( $P \leq 0.05$ ). \*  $P \leq 0.05$

**Table 5:** Effect of symbiotic mixture on ingestive behavior of growing rabbit (mean + S.E).

Items	Group A (Control)	Group B (Treated)	P-value
Feeding behavior frequency	46.58 $\pm 8.38$	47.50 $\pm 6.84$	0.61
Feeding behavior duration (min.)	24.33 <sup>b</sup> $\pm 3.89$	29.06 <sup>a</sup> $\pm 4.31$	0.05*
Drinking behavior frequency	7.83 <sup>b</sup> $\pm 1.64$	12.67 <sup>a</sup> $\pm 1.48$	0.02*
Drinking behavior duration (min.)	2.19 <sup>b</sup> $\pm 0.25$	4.14 <sup>a</sup> $\pm 0.44$	0.03*

Means in the same row with different superscripts are significantly different ( $P \leq 0.05$ ). \*  $P \leq 0.05$

The structure of digestive tract of rabbits make them exposed to suffer from digestive disturbances especially after weaning, thus feed additives can be used to improve their performance. There is a challenge to scientists to replace the use of antibiotics as feed additive by less harmful compounds such as probiotics which have many advantage like decreasing of toxin production, incitement of enzyme production, vitamins production, increase resistance to colonization and reduce stress for rabbits (Falcao-e-Cunha *et al.*, 2007; Shehata and Tawfeek, 2010).

### 3.2. Feed intake

The data presented in Table 2 revealed that feed intake decreased non-significantly in treated group than control ones. Eiben *et al.* (2008) showed that feed intake of New Zealand White rabbit breed was not affected by addition of feed additives which were, bacteria of *Bacillus subtilis* and *Bacillus licheniformis*, prebiotic inulin, organic acids, and tannin. In accordance with the present results, El-Katcha *et al.* (2011) showed that dietary supplementation of probiotic at 0.1 or 0.15 g/kg diet from *E. faecium* containing products (Protexin) and *Lacto bacillus acidophilus* and other *L. strain* with enzyme (AM Phi-Bact) had decreased daily feed intake by 9.1 percent, 7.6 percent, 16.2 percent and 11.3 percent, respectively when compared with control. Similarly, Ewuola *et al.* (2011) postulated that daily feed intake was not differed significantly among treated group fed probiotics, prebiotics and combination of both.

### 3.3. Feed conversion ratio (FCR)

Results shown in Table (2) indicated a non-significant improvement of FCR in treated group than control one. This result was in agreement with findings of El-Sagheer and Hassanein (2014) who concluded that addition of 1 or 2 g Veta-zyme/kg diet significantly ( $P < 0.05$ ) improved FCR than those of un-supplemented diet. Also, Shrivastava *et al.* (2012) reported that addition of 60 gm probiotic to food may improve feed conversion ratio of weaned rabbits. While, Onu and Oboke (2010) showed that rabbits fed 50 % maize processing waste based diet (MPW) supplemented with 200 mg of enzyme (grindazym) or 200 mg probiotic (yeast) per kg feed had significantly ( $P < 0.05$ ) increased feed conversion ratio than MPW or non-MPW diet without supplementation, Ezema and

Eze (2015) concluded no significant differences between groups of cross-bred rabbit fed different doses of probiotic at 13 weeks of age.

### 3.4. Biochemical parameters

Results in Table (3) showed that concentrations of total protein (TP), albumin (AL), and globulin (GL) were significantly higher in treated group than control one while a non-significant increase of blood glucose level in treated than control group. The results were in compliance with the finding of Abdel-Khalek *et al.* (2012) who studied the effect of two levels (1 and 1.5% EM) of microorganisms supplementation (EM1) for growing New Zealand White rabbit (7 weeks of age) and found an increase in concentration of total protein, albumin, globulin and glucose in the group fed 1.5% EM1 treatment. Seyidoglu and Galip (2014) reported a non-significant increase for blood glucose for those received *S. cerevisiae* (3 g/kg diet) from control. Moreover, Sarat Chandra *et al.* (2015) reported that there was non-significant increase in blood glucose level for rabbits when fed with probiotics (*Saccharomyces boulardi* 50% and *Pediococcus acidilacticii* 50%, 109 CFU/g of feed) and enzymes (Kemzyme HF at 500 g/Ton of feed). On contrary, Simonova *et al.* (2013) showed a significant decrease ( $P < 0.05$ ) for glucose among groups which received *Enterococcus faecium* CCM7420 (EF) and *E. senticosus* extract (ES) or their combination. Abd-El-Hady and El-Abasy (2015) postulated a significant reduction ( $P < 0.001$ ) of glucose level for rabbits supplemented with prebiotic (Bio-Mos®, mann oligosaccharide), probiotic (Bio-Plus® 2B, *Bacillus subtilis* and *Bacillus licheniformis*) and their mixture compared with control group.

### 3.5. Carcass traits

There was a non-significant ( $P > 0.05$ ) difference in carcass traits between groups as described in Table (4). These results agreed with Onbasilar and Yalcin (2008) who studied the effect of dietary supplementation with probiotic and anticoccidial on performance of 48 NZW rabbits for 6 weeks. They noted that carcass yield and weight percentage of lung, heart, kidney and small intestine are not different among groups. As well as, El-Sagheer and Hassanein (2014) reported a non-significant differences in carcass traits such as its dressing weight, pancreas, heart, liver,

spleen and head weight percentages for treatment groups (supplemented with enzymes and probiotic mixture supplementation Veta-zyme/kg 1 g Veta-zyme/kg commercial diet). However, Brzozowski and Strzemecki (2013) reported that the addition of *Bacillus Cereus* Var. Toyoi, as a probiotic for young rabbit's diet at a level of 400 mg/kg of a probiotic preparation showed positive higher results on dressing percentage. Matusevicius and Jeroch (2009) studied the effect of Probiotic Toyocerin® 1 x 10<sup>10</sup>CFU/g in 60 New Zealand white rabbits for 56 days and noted that carcass weight as well as weight of valuable carcass parts increased.

### 3.6. Ingestive behavior

Ingestive behavior which includes both solid food and liquids varied significantly ( $P < 0.05$ ) from treated to control group as the drenched rabbits showed a higher feeding duration (min) than control ( $29.06 \pm 4.31$  and  $24.33 \pm 3.89$  min) respectively. This finding could be explained by Shehu *et al.* (2014) and Alcock *et al.* (2014) who reported that microbes in the gastrointestinal tract are easily manipulatable by prebiotics, probiotics and they are under selective pressure to manipulate host eating behavior by generating cravings for foods that they specialize on or foods that suppress their competitors. Moreover, drenched rabbit group has a significantly ( $P < 0.05$ ) higher drinking behavior frequency ( $12.67 \pm 1.48$ ) and duration ( $4.14 \pm 0.44$  min) than control group during the growing period of rabbits. This result was due to the good taste and the stimulation of palatability of drenched mixture which allowed rabbits to drink more and spent more time drinking (Wallace and Newbold, 1992).

## 4. CONCLUSION

It was concluded that the administration of symbiotic mixture which is a combination of probiotic and prebiotic improved performance, biochemical parameters and ingestive behavior of growing rabbits.

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