## **EFFECT OF PROBIOTIC ON IN VITRO RABBIT CECUM FERMENTATION, CHEMICAL ANALYSIS AND IN VITRO DIGESTIBILITY**

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*(Received 7/5/2024, accepted 31/5/2024)*

### **SUMMARY**

his study aimed to investigate the effect of probiotic s on the in vitro rabbit cecum fermentation by adding some probiotic preparations as yeasts, ZAD® and ZADO® to the rabbit basal diet. The amount of dry matter (DM), ash (ASH), crude fiber (CF), ether extract (EE), and crude protein (CP) in the meal This study aimed to investigate the effect of probiotic s on the in vitro rabbit cecum fermentation by adding some probiotic preparations as yeasts, ZAD® and ZADO® to the rabbit basal diet. The amount of dry matter (DM), a dry matter disappearance (DMD), gases, and interactions with the time obtained for the tested diets. Results revealed that all probiotic treatments (yeasts, ZAD® and ZADO®) improved the in vitro digestibility as increase in DMD (44.09, 46.86, and 46.77%) and decrease in gas production (41.50, 38.41, and 37.83 ml3), respectively compared to that of the control evaluated diets. The highest values of DMD recorded after 24h of incubation time. In addition, ZAD® and ZADO® have the maximum improvement at all times. The findings of the current study concluded that probiotics had an impact on the in vitro fermentation of rabbit cecum and introduced it as a potential low-cost, effective, alternative source of protein-rich diet for rabbits.

*Keywords: Rabbit, Probiotics, ZADO®, ZADO®, Yeast, Cecum fermentation*

# **INTRODUCTION**

The disassociation of protein production from livestock growth areas causes protein deficiencies in some regions of the world, particularly in countries that are developing (Varela- Ortega *et al.*, 2022). As a consequence, and alternative, more sustainable protein feeds are required to meet growing demand and reduce the environmental impact of animal production (Aschemann-Witzel *et al.*, 2021). Extensive researches showed that replacing protein with alternative low-cost, high-quality proteins in animal's diet formulations would strongly alleviate the environmental impact of the food system (Gamboa‐Delgado and Márquez‐Reyes, 2018, Parisi *et al.*, 2020 and Vastolo *et al.*, 2022). In rations with less than 20% grain, rabbits can efficiently use cellulose-rich feed because they occupy a crucial intermediate position between ruminants and non-ruminant animals (Gado *et al.*, 2015). Because rabbit meat is leaner than that of other animals and has a high protein level along with low fat, cholesterol, and sodium content, it is highly valued for human (Cullere and Dalle Zotte, 2018).

Probiotics are live microorganisms (bacteria, yeast or fungi) used as feed additive that promote the host's health, generally considered to be safe and have a beneficial impact on average daily growth ratio of feed to energy (Markowiak and Śliżewska, 2018). Probiotic uses in agriculture have garnered more attention in recent years, and choosing novel probiotic strains and applying them in novel ways has become crucial (Nezamdoost-Sani *et al.*, 2023). In animals, anaerobic bacterial sources of probiotics perform better than fungal sources (Gado, 2020). Recently, probiotics have been added to ruminant rations to break down the fiber content for simpler digestion, to increase the benefits for the microbes and flora, which are the primary sources of microbial protein in the ruminal media, and to lower the ration's cost (Cholewińska *et al.,* 2020). There are presently a lot of natural growth promoters (NGPs) on the market, such as immune-boosters, probiotics, and prebiotics (Abdel-Azeem *et al.*, 2018). From this perspective, antibiotic growth promoters in the feeding concept may be replaced by the anaerobic probiotic technology (ZAD) ® and (ZADO) ®

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(treated to have a wide range of positive health effects on humans as well as enhance the nutritional content of feedstuffs, facilitate better digestive systems, and preserve animal health (Alagawany *et al.*, 2021). Probiotics contain a range of fibro-lytic enzymes that have been shown to enhance intestinal nutrient absorption and the nutritional content of non-ruminant animal diets (Feizi *et al.*, 2022). Taye and Etefa (2020) developed probiotic products ZAD® and ZADO®, to increase the nutritional content of fibrous materials and to enhance animal digestion in general. In addition to treating agricultural waste items including bagasse, corn stalks, and rice straw, ZAD® was also used in animal feed (Sheikh *et al.*, 2018). The ZAD anaerobic bacteria contributed to the breakdown of the feed's secondary chemical compounds. Additionally, it increases the number of microorganisms, which raises the amount of microbial protein in the reticulo-rumen area or outside the digestive tract (Lu *et al.*, 2019). In developing lambs fed a high concentrate diet, the effects of a probiotic mixture (ZADO) were investigated with respect to in vitro dry matter degradability (DMD), in vitro gas production (GP), metabolizable energy (ME), and short-chain fatty acid (SCFA) synthesis (Abd-Elkerem *et al.*, 2021). In addition, probiotics can produce an improvement in the animal's immunity in addition to the recorded improvement in the total digestible nutrients of the ration (Elghandour *et al.*, 2020).

Probiotics may help improve the health of rabbits, who regularly exhibit post-weaning alimentary problems and typically exhibit a fragile equilibrium in their gut function due to the unique physiology of their digestive system (Colombino *et al.*, 2022). Probiotics, or cellulolytic enzymes, when added to rabbit diets have a noticeable positive impact on weight increase in both backyard and cage-raised rabbits (Gado *et al.*, 2015). Considering that juvenile rabbits' capacity to digest fiber and starch is restricted (Abdel-Aziz *et al.*, 2015), enzyme addition improved the dietary digestion and performance of young rabbits on starter diets (El-Sagheer and Hassanein, 2014). Enzymes act on distinct parts of the rabbit gut by decreasing the pH of the stomach, nevertheless, the digestive parameters evaluated were unaffected by an enzyme complex consisting of amylase, xylanase, β-glucanase, and pectinase (Boontiam *et al.*, 2022). Even in the time after early weaning, exogenous enzymes typically fail to significant alter the enzyme activity in the stomach, intestinal, and caecal contents of rabbits (Gidenne and Fortun-Lamothe, 2022). Probiotics have been shown in earlier studies to enhance rumen and intestinal function, which may increase the nutritional utilization of high concentrate diets provided to growing animals. Therefore, the goal of the current study was to determine the chemical analysis of rabbit feed in order to forecast the feed's in vitro digestibility, dry matter, and gases, as well as to evaluate the effect of probiotics ZAD®, ZADO®, and Yeast® on in vitro rabbit cecum fermentation.

### **MATERIALS AND METHODS**

#### *Study area:*

This study's fieldwork completed in the rabbit production unit in Sahl al-Tina in the city of Qantara, east of the Suez Canal, for the project "Using seaweed in the production of saline fodder, milk, meat and fish under saline conditions" affiliated to the Egyptian Center of Excellence for Bio-saline Agriculture at the Desert Research Center. The laboratory part was completed at the laboratories of the Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

### *Probiotic preparation and addition:*

ZAD is liquid compound while ZADO is biotechnical powder product both are made from natural sources of cellulose enzyme from anaerobic bacteria. They improve nutrients coefficients digestibility and nutritive values of fibers. ZAD® & ZADO® (Ruminococcus flavefaciens), are anaerobic bacterium: exogenous enzymic preparation (EZ) containing cellulases, xylanases, amylase, and proteases, coated with starch and glycol (Patent No. 22155 of Egypt). They were produced at the Animal Production Department's laboratory at the Rumen Ecology Center at Ain-Shams University in Cairo, Egypt's Faculty of Agriculture. Before giving the rabbits their meal, the EZ probiotic complex products, ZAD®, ZADO®, and Yeasts (Saccharomyces), were placed directly over the feed at dosages of 0.25, 0.5, and 1.0 ml/ rabbit/day. The products became active right away (Abdel-Aziz *et al.*, 2015). N.B. Yeast used as active dry form, which once rehydrated will be reactivated and able to ferment the fibers and carbohydrate materials.

#### *Experimental rations:*

Table (1) Llists the ingredients of experimental rations nations.





#### *Chemical analyses:*

The feed samples were ground in a laboratory grinder after being dried for 24 hours at 60 0C in a laboratory oven (Retsch, Newtown, PA, USA) with a 0.5 mm screen size. Dry matter (DM), ash (ASH), crude fiber (CF), curd protein (CP), and ether extract (EE) in feed determined by chemical analysis (AOAC, 2023). The NFE= {100-(DM+CP+EE+CF+Ash)}. Fibre proportion was determined in accordance with Tuncil (2020).

#### *In vitro fermentation characteristics:*

The in vitro digestibility of rabbit cecum was evaluated through determination of dry matter disappearance (DMD) and gases obtained for the evaluated control, yeasts ZAD®, and ZADO®. After slaughtering experimental rabbits, two hundred grams of caecal content were diluted with 320 ml of artificial saliva solution (8 g of NaHC03, 4 g of K2HP04, 0.5 g of NH4) 2HP04, 1.5 g of NaCI and 0.5 g of MgS04·1H20 per I of distilled water) under a stream of CO2 gas. 1 g of ground feed sample was added to 50 ml of caecal inoculum. Digestion glasses were closed under a constant stream of CO2, and incubated in an orbital bath at  $40^{\circ}$ C for (2, 4, 6, 24 and 48) hours on the in vitro DMD and produced gasses for the evaluated diets (%) were estimated. For each incubation time, three blank containers were prepared for each incubation time, each sample consisted of three replicates. The difference between the sample's DM content and that of the residual after incubation / sample DM content \* 100 was used to compute the in vitro dry matter disappearance (% DMD) The displacement of a 200 ml lubricated syringe piston, which attached to serum flasks holding buffered cecum fluid and treated feed samples (substrate), estimates the total amount of gas produced. To calculate the amount of gas produced, the total gas produced in the vessels holding the fermented treated samples (with substrate) was subtracted from the gas produced in blank vessels (without substrate) (El-Nile *et al.*, 2021).

#### *Data analysis:*

Statistical analysis was performed on all the experiment's data using the Statistical Package for Social Sciences (SPSS®) Version 24.0, software, employing one- and two-way Analysis of Variance (ANOVA), and expressed as mean  $\pm$  standard deviation (George and Mallery 2019). Means considered statistically significant at a  $p < 0.05$ . The differences among treatment groups estimated using the following model: Yi  $= \mu + Ti + eij$  where

Yi: is the dependent variable.

μ: is the overall mean.

Ti: The effect of treatment. eij: is the experimental error. Yij=μ+Ri+Pj+RPjj+eijk Where:

Yij: dependent variable. μ: overall mean.

Ri: The effect of ration. Pj: The effect of probiotic.

RPij: interaction between ration and probiotic. eijk: is the experimental error.

### **RESULTS AND DISCUSSION**

#### *Determination of chemical analysis of feed:*

Table (2) show the chemical composition of the rabbit feed revealed non-significant difference between detected dry matter (DM), ash (ASH) and ether extract (EE) in control and those that recorded in the evaluated prebiotic samples. However, there is a significant increase (p<0.01) of crude protein content (CP) and degradation(p<0.01) in crude fiber (CF) values in all prebiotic samples compared to control ones. ZAD® and ZADO® treated samples reveled the most significant difference (P<0.05 & p<0.001) among evaluated samples (Table 2). As well as crucial knowledge about how probiotic bacteria can regulate and boost food waste fermentation (Du *et al.*, 2021). The findings of the chemical study match those stated by Jazi *et al.*, (2018) and Drazbo *et al.* (2020). It would assumed that exogenous fibrolytic enzymes would improve the digestion of fiber and consequently, fermented feed meal with probiotics showed an increase in CP and would be expected to increase fiber digestion, resulting in a decrease in CF concentration induced by fermentation of diet and feces. In addition, the presents studs reported that the detected ZAD® and ZADO® treated samples had higher CP values. As opposed to the assertion made by Abdelaty *et al.*, (2021) that ZADO® enzymes are a biotechnical product manufactured from natural sources (Ruminococcus flavefaciens) that improves overall animal digestion. The current findings are consistent with those of premature Hy-Plus rabbit trials conducted by Gado *et al.* (2016), in which ZADO® up to 5 g/kg food added without compromising performance. Furthermore, Gado *et al.* (2017) confirmed that ZAD®'s blend of anaerobic bacteria-derived enzymes had a positive impact on the polysaccharide's conversion into monomers in growing rabbits through the catalytic process of enzymes.





*DM, Dry matter* ، *ASH, Ash* ، *CF, Crude fiber* ، *EE, Ether extract* ، *CP, Crude protein*

*a-b Means with different superscripts within each row are significantly different (Ns= non-significant, \*\*=p<0.01*

### *Determination of in vitro digestibility, dry matter and gasses of feed:*

The effect of treatment method and time on the in vitro digestible values dry matter disappearance (DMD) and gases for the evaluated diets revealed that all probiotic treatments, (yeasts, ZAD® and ZADO®), improved the in vitro digestibility, as evidenced by an increase in DMD (44.09, 46.86 and 46.77 %) and decrease in gas (41.50, 38.41 and 37.83%), respectively. Compared to that of the control evaluated diets in DMD (39.14) and in gas (50.00 %). The highest values of DMD were recorded after 24 h of incubation time. In addition, ZAD® and ZADO® have the maximum improvement at all times (Table 3 and Fig 1 a & b). The higher percentage of DMD and when employing tested probiotic-treated meals, increased gas production was obtained, which strongly suggests a higher fermentation activity by rabbit caecal bacteria (Yacout *et al.*, 2021). Our findings are in line with earlier research that shown the advantageous effects of enzymes on ruminal fermentation were increased when diets were treated with enzymes before to feeding or when ruminal fluid was incubated (Giraldo *et al.*, 2008, Hassan *et al.*, 2020). As pointed out by El-Sanhoury and Ahmed (2017) poultry (FBW) benefited from the application of ZADO® at different dosages in broiler feeding up to 0.5 kg/ton. They explained it by saying that the ZADO® groups' larger stomachs and intestines led to better- feed utilization.

<b>Treat</b>	<b>DMD</b>	Gas
Control	39.14 $\pm$ 4.45 $\degree$	$50.00 \pm 9.55$ <sup>a</sup>
Yeast	$44.09 \pm 4.76$ <sup>b</sup>	$41.50 \pm 7.72$ <sup>b</sup>
Zad	$46.86 \pm 5.05^{\text{a}}$	$38.41 \pm 7.20$ °
Zado	$46.77 \pm 4.90^{\mathrm{a}}$	$37.83 \pm 7.33$ <sup>d</sup>
	Time	
$0$ hrs.	$\Omega$	$\Omega$
$2$ hrs.	$34.57 \pm 1.24$ <sup>d</sup>	$11.25 \pm 0.25$ <sup>e</sup>
4 hrs.	$43.78 \pm 1.50^{\circ}$	$21.75 \pm 0.91$ <sup>d</sup>
6 hrs.	$56.29 \pm 1.01^b$	$32.62 \pm 1.21$ °
24 hrs.	$65.16 \pm 2.07$ <sup>a</sup>	$80.75 \pm 1.55^b$
48 hrs.	$65.49 \pm 1.04$ <sup>a</sup>	$105.25 \pm 3.79^{\circ}$

**Table (3): Effect of treatment and time on the in vitro DMD and gas production for the evaluated diets (%).**

*a-b Means with different superscripts within each column are significantly different (\*\*\*=p<0.001).*



**Fig (1): Effect of treatment method (a) and time (b) on the in vitro digestible values DM and gases obtained for the evaluated diets (%).**

The interactions of in vitro digestibility with the time obtained for the evaluated diets showed a significant difference ( $P < 0.05$ ) at 2, 4, 6 and 24 hours of incubation but not significantly at 48 hours of incubation. The higher values of DMD recorded after 24h of incubation time for all treatment methods (yeasts, ZAD® and ZADO®), (65.26 %, 69.27% and 67.63%) respectively compared with control (58.51%) at same time (Table 4 and Fig 2 a & b). A good indicator of microbial fermentability, digestibility, and rumen protein synthesis is DMD and gas generation (Salem *et al.*, 2014). The significant differences in DM values at the different times were due to punctual variations on the caecal and fecal digestibility values, however, the more disappointing results over time and the lower precision with the caecal fermentation indicate that it is necessary to improve its standardization (Pascual *et al.*, 2000). Consequently, the use of suitable, probiotic concentrations and reaction times suggested achieving a suitable modeling of the complex in vitro digestibility present in the rabbit cecum (Moyano *et al.*, 2015, Brodkorb *et al.*, 2019, Pogány *et al.*, 2020).

<b>Item</b>	<b>DMD</b>	Gas
Control 0 hr.	0j	0 <sub>n</sub>
Control 2 hrs.	$29.78 \pm 1.30^i$	$12.50 \pm 0.28$ <sup>m</sup>
Control 4 hrs.	$35.10 \pm 0.43$ <sup>h</sup>	$26.50 \pm 0.28$
Control 6 hrs.	$51.86 \pm 2.72$ <sup>d</sup>	$40.50 \pm 0.28$ <sup>h</sup>
Control 24 hrs.	$58.51 \pm 6.24$ c	$90.50 \pm 0.28$ <sup>d</sup>
Control 48 hrs.	59.57 $\pm$ 0.61 $\rm{c}$	$130.00 \pm 1.15^{\text{a}}$
Yeast 0 hrs.	0i	0 <sub>n</sub>
Yeast 2 hrs.	$34.57 \pm 3.43^h$	$11.50 \pm 0.28$ <sup>m</sup>
Yeast 4 hrs.	43.94 $\pm$ 0.89 <sup>f</sup>	$23.50 \pm 0.28$ <sup>k</sup>
Yeast 6 hrs.	$56.05 \pm 1.16$ <sup>c</sup>	$31.50 \pm 0.28$ <sup>i</sup>
Yeast 24 hrs.	$65.26 \pm 3.67^b$	$80.50 \pm 0.28$ <sup>e</sup>
Yeast 48 hrs.	$64.73 \pm 0.30^b$	$102.00 \pm 0.57$ <sup>b</sup>
Zad 0 hrs.	0i	0 <sub>n</sub>
Zad 2 hrs.	$35.90 \pm 1.25^h$	$11.00 \pm 0.01^m$
Zad 4 hrs.	$47.91 \pm 1.85$ <sup>e</sup>	$19.50 \pm 0.28$ <sup>1</sup>
Zad 6 hrs.	$58.33 \pm 1.12$ <sup>c</sup>	$29.50 \pm 0.28$ <sup>i</sup>
Zad 24 hrs.	$69.27 \pm 3.13$ <sup>a</sup>	$77.50 \pm 0.28$ <sup>f</sup>
Zad 48 hrs.	$69.79 \pm 1.20^a$	$93.00 \pm 0.57$ <sup>d</sup>
Zado-0 hrs.	0i	0 <sub>n</sub>
Zado 2 hrs.	$38.03 \pm 1.91$ <sup>g</sup>	$10.00 \pm 0.01^{\rm m}$
Zado 4 hrs.	$48.15 \pm 1.89$ <sup>e</sup>	$17.50 \pm 0.28$ <sup>1</sup>
Zado 6 hrs.	$58.94 \pm 0.60$ <sup>c</sup>	$29.00 \pm 0.57$ <sup>i</sup>
Zado 24 hrs.	$67.63 \pm 1.08^a$	$74.50 \pm 0.28$ <sup>g</sup>
Zado 48 hrs.	$67.89 \pm 0.30$ <sup>a</sup>	$96.00 \pm 0.57$ <sup>c</sup>

**Table (4): The interaction between treatment and time on the in vitro digestible values DMD and gases.**

*a,bMeans with different superscripts within each column are significantly different.*



**Fig (2): Interaction between treatment method and time on the in vitro digestible values DM (a) and gases (b) obtained for the evaluated diets (%).**

#### *The Interaction effect between nutrient contents and in vitro incubation time on gas production:*

Total gas production relative to dieters CF and CP contents showed significant difference (P>0.05) for all terminated experimental diets in the most applied in vitro incubation times. The values of DMD, EE, ASH, OM, and NFE did not change significantly amongst the various treatments. However, the value of

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gas concentration in relation to the nutrient content of diet was more significantly different (P<0.05) with ZADO and ZAD treated groups than yeast and control groups of evaluated diets (Table 5). The level of total gas production depends on the composition of nutrient content and the in vitro gas production technique could be useful for studying the nutritive characteristic of rabbit diets (Fahmy *et al.*, 2019). The results which were attained agreed with the conclusions of Saeed *et al.* (2018), who claimed that probiotics plays a role in the digestion of CP and CF and have the ability to digest fiber more thoroughly and quickly. These seemingly incompatible results are most likely caused by the fact that not all of the organic matter lost during in vitro degradation fermented into gas and volatile fatty acids (VFA). Additionally, the type of VFA that created could have influenced how much gas was produced (Russouw *et al.*, 2020). In accordance with current results, the absence of high CP in fermented meal may be attributable to the degradation of polypeptide chains by the proteolytic enzymes from probiotics and decrease in the fiber content in probiotic- treated diets which functioned synergistically since several enzymes were secreted (Hassaan *et al.*, 2017 and Jha *et al.*, 2020).





*DM, Dry matter – OM, Organic matter – EE, ,Ether extract – CP, Crude protein – CF, Crude fiber –– NFE, Nitrogen free extract*

*a,b Means with different superscripts within each row are significantly different (\*\*\*=p<0.001).*

### **CONCLUSION**

The results of the present investigation concluded that the three used probiotics were effective on in vitro rabbit cecum fermentation by improving portion digestibility. Moreover, among the used probiotics, it was found that ZAD and ZADO® showed a significantly increase in crude protein contents, improved dry matter and reduction in gaseous content. Therefore, this study provided basic data to ssuccessfully support the idea of developing low-cost, efficient, alternative protein-rich feed for rabbits.

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**تأثير البريبيوتيك على التخمرات في أعور األرانب، التحليل الكيميائي وقابلية الهضم في المختبر** 

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تهدف هذه الدراسة إلى تأثير البروبيوتيك على عملية التخمرات في أعور األرانب في المختبر عن طريق إضافة بعض مستحضرات البروبيوتيك مثل الزاد (ZAD (®، الزادو (ZADO (®، والخميرة )إلى النظام الغذائي األساسي لألرانب. وتم قياس كمية المادة الجافة (DM(، والرماد(ASH (، واأللياف الخام(CF (، ومستخلص األثير (EE(، والبروتين الخام (CP (في وجبة األرانب التجريبية كيميائيا. وتم دراسة تأثير المعاملة والزمن على اختفاء المادة الجافة والغازات في المختبر وتفاعلاتها مع الزمن الناتج للعلائق المختبرة. أظهرت النتائج أن جميع المعالجات بالبروبيوتيك تحدث تحسن في قابلية الهضم في المختبر، حيث يوجد زيادة في هضم المادة الجافة (DMD( بنسب 44,99)، 44,84 و 44,77٪ (مع وجود انخفاض في انتاج الغازات بنسب 44,09)، 88,44 و (7,88، في كل العينات المعالجة بالبروبيوتكYeasts ، ZADو ZADO على التوالي مقارنة مع تلك العينة الضابطة التي تم تقييمها في علف األرانب حيث كانت نسبة هضم العينة الضابطة. ) 39.14( وفيما يتعلق بعمل بفترة الحضانة للعينات، تم تسجيل أعلى قيم هضم المادة الجافة (DMD (بعد 84 ساعة من فترة الحضانة. باإلضافة إلى ذلك، يتمتع الزاد (ZAD (®والزادو(ZADO (®، بأقصى قدر من التحسين في جميع األوقات. وخلصت نتائج الدراسة الحالية إلى أن البروبيوتيك كان له تأثير على التخمرات في المختبر لأعور الأرانب وقدمته كمصدر بديل محتمل منخفض التكلفة وفعال لنظام غذائي غني بالبروتين لألرانب.

ا**لكلمات المفتاحية:** الأرانب، البروبيوتيك، الزاد@، الزادو @، الخميرة، تخمير الأعور