



## Effect Of Supplementation Of B-Galacto-Oligosaccharide And Probiotic Mixture On Growth Performance Of Physiologically Stressed Rabbits

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### Abstract

This study explored the effects of prebiotic  $\beta$ -Galacto-oligosaccharide ( $\beta$ -GOS) and probiotic supplementation as a nutritional strategy to mitigate stress-induced impacts on growth performance in rabbits. Twenty male rabbits were randomly assigned to four groups: A (negative control, no stress), B (positive control, Dexamethasone-stressed 15 mg/kg [D-S 15]), C (D-S 15 + probiotic mixture), and D (D-S 15 + 0.2%  $\beta$ -GOS). Initial body weight did not differ significantly ( $P > 0.05$ ) among the groups. However, a significant difference ( $P < 0.05$ ) in weekly body weight was observed from weeks 1 to 12. Body weight increased linearly across all groups, with the final body weight at week 12 being highest in group A ( $2.32 \pm 0.52$  g), followed by group C ( $2.09 \pm 0.32$  g) and group D ( $2.01 \pm 0.51$  g), while group B had the lowest weight ( $1.87 \pm 0.61$  g). A significant difference ( $P < 0.05$ ) in average daily gain was also recorded, with group A showing the highest gain ( $5.7 \pm 0.43$  g), while groups C and D showed lower gains ( $5.3 \pm 0.24$  g and  $5.0 \pm 0.61$  g, respectively). Group B exhibited the lowest daily gain ( $4.2 \pm 0.17$  g). Feed intake followed a similar pattern, with group A having the highest intake at week 12 ( $126 \pm 0.13$  g), followed by groups C ( $124 \pm 0.13$  g), D ( $123 \pm 0.03$  g), and B ( $116 \pm 0.09$  g). Feed efficiency was significantly different ( $P < 0.05$ ), with group A showing the best feed efficiency ( $4.9 \pm 0.14$ ), while group B recorded the poorest efficiency ( $5.0 \pm 0.17$ ). Overall, stress significantly reduced growth performance in rabbits by decreasing body weight gain, feed

<b>CC License</b> CC-BY-NC-SA 4.0	intake, and feed efficiency, while probiotic and prebiotic $\beta$ -GOS supplementation improved these parameters, indicating its potential to mitigate the adverse effects of stress.  <b>Key words:</b> <i><math>\beta</math>-galacto oligosaccharide, Probiotic, Stressed Rabbits</i>
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## INTRODUCTION

Rabbits are small creatures that are common across the world and belong to the family Leporidae of the order Lagomorpha. Meadows, woods, forests, and grasslands all serve as their habitats. In comparison to the cost of larger animals, rabbits are extremely docile, non-aggressive, easy to manage, and affordable. According to Mapara *et al.* (2012), the reproductive, lactation, and puberty cycles in rabbits are brief. Due to their rapid development and great proficiency, rabbits are regarded as superior to other animals (Sharma & Choudhary, 2017). Different animals' meat when compared, have high quality for output and good feed conversion ratio. Normal slaughter age for rabbits is 18 to 20 weeks, or 3 kg, of body weight. Rabbit meat is highly delicious, lesser in fat and also in cholesterol level which is (4%) and it is easily to digest, higher in protein pleased (25%), and lower in caloric value (160 Kcal/100g meat). As a result, heart patients and toddlers can ingest it. All rabbits are monogastric hindgut fermentator and its digestive mechanism is allowing to the absorb vitamins and proteins via caecotrophy. These key characteristics, rabbits' utilization is declining globally, owing generally to consumer acceptability and necessary cooking time (Petracci *et al.*, 2018). The rabbit is one of most captivating production animals since its meat-producing animals in ideal theory. Rabbit has a small life cycle, are highly productive, and have a brief gestation period, and it has high feed conversion ratio (2-2.3) on high essence diet, (3-3.8) on higher forages, and grain-free diet (Lebas *et al.*, 1997; Cullere & Dalle, 2018). Prebiotic and probiotic are auspicious treatment which have the probable to minimise gastrointestinal illnesses in cattle while also increasing production. Those chemicals which have been recommended to aid in avoidance of carcass impurity and to rise animal invulnerable responses (Huang *et al.*, 2004).

Prebiotics are non-digestible product of feed elements of profit to the host by selectively boosting development and activity one or few bacteria in colon. It has been Clarify that consuming functional oligosaccharides advances growth concert to improve the host health (Gibson & Roberfroid, 1995). The prebiotics can use as a potential alternative source to the growth-promoting antibiotics by changing intestinal microbes and Immune system response to reduce pathogenic colonisation, improving nutrient utilisation (amino acids and proteins), gut health, and performance (Rafiq *et al.*, 2021).

Stress affects the overall productivity of rabbits and broilers on account of neuroendocrine alterations, immunosuppression and gastrointestinal infections (Quinterio-Filho *et al.*, 2012). Depending upon the severity of the environmental stress, responses of the rabbits and broiler may vary from a mild discomfort to death (Nwachukwu *et al.*, 2021). Rabbits and broilers exposed to stress typically exhibits reduced feed intake, muscle mass, relative weights of viscera and immune organs and poor performance (Malekizadeh *et al.*, 2012; Kulkarni *et al.*, 2013; Nwachukwu *et al.*, 2021). It triggers production of reactive oxygenic species and results in oxidative stress (Lin *et al.*, 2006). It is also reported to increase the gastrointestinal attachment of pathogens like *Salmonella spp.*, *Escherichia coli* and *Campylobacter* which are the pathogens of zoonotic or public health concern (Burkholder *et al.*, 2008). Initially, neuroendocrine alterations mediated via cortisol, corticosterone and thyroid hormones was thought to perform main role in the development of infections during stress. However, the modern concept of microbial endocrinology proposes that the pathogens directly interact with stress hormones hence altering the host-pathogen interaction (Lara & Rostagno, 2013). This altered host-pathogen interaction, alone or in combination with the above mentioned factors is assumed to be triggering factor behind the economic losses during the heat stress.

The majority of a class of carbohydrates known as GOS is oligo-galactose, with minor amounts of the disaccharide and simple monosaccharide. Commercial processing of lactose using  $\beta$ -galactosidase 4 is now taking place. Galacto-oligosaccharides, which naturally occur in human milk, may be an important component in protecting human infants towards gastrointestinal pathogenic bacteria (Niittynen *et al.*, 2007). Galacto-oligosaccharide is a lower in cost of prebiotic that is widely utilised. It is one of three prebiotics that fulfil European Union requirements. The lacto-bifidogenic ability of  $\beta$ -galacto-oligosaccharides ( $\beta$ -GOS) is extensively recognised in people and animals, and it is thought to be the basis for improvements in gastrointestinal health, structure, and immunological function (BrunoBarcena & Azcarate-Peril, 2015). Nonetheless,  $\beta$ -GOS, derived from Bifidobacterium galactosidase, failed to enhance grill performance under

thermoneutral conditions. However, it has been claimed that the benefits of prebiotics on grill performance are varied (Biggs *et al.*, 2007). There is little known about the role and efficacy of -GOS in broilers during heat stress (Varasteh *et al.*, 2015), especially in aspect of growth performance and intestinal barrier components. Current study will evaluate the dietary effects of B-galacto-oligosachtride on development performance, bone morphology and quality of quality is measures in physiologically challenged rabbits.

The word "probiotic" is derivative from Greek and the meaning is "for life." The word "probiotic" was most likely coined by Ferdinand Vergin in 1954, in his article "Anti-und Probiotika," which compared the negative effects ("probiotika") of some helpful bacteria with the positive effects ("harmonious effects") of antibiotics and other antibacterial drugs on the gut microbiota (Paulina & Katarzyna, 2017). The biotechnological behaviour is to increase nutritional content of rabbits feed which enhanced digestibility of stringy by-products of agriculture, also directly or indirectly through the employment of microorganisms or microbial enzymes. Furthermore, when utilised in farm animals, addition of animate yeast in animal feeds which has been proven to increase digestibility, animal performance, feed efficacy ratio, and growing rate, in the form of egg or meat production. The animals' health, reducing pathogenic bacteria, which have negative environmental impact (Ezema & Eze, 2012). The major probiotic microorganisms are straining of useful gram-positive bacteria from the genera of *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, and *Streptococcus Pedococcus*.

Yeast and fungi, such the strain of *Saccharomyces cerevisiae*, are utilised almost all of the time (Marco *et al.*, 2006), because *bifidobacteriam* which are widely recognised to health-boosting bacteria (Boesten & Vos, 2008), there is a great agreement of benefits in discovering chemicals that can be personally increases their propagation. Many investigations are utilising traditional molecular and cultures approach for bacterial identification which revealed the breast-feeding to neonates has a gut micro biota dominate by *bifidobacteriam* (Benno *et al.*, 1984). Dietary feed additives which have positive impacts on animal health, presentation and immunity since nutritious assessment of animal status may be determined using blood and tissue contents (Ewuola *et al.*, 2011, 2012). Therefore, the experimental design is prompted to investigate dietary feed additives effects on rabbits' health, performance, nutritive digestibility and meat production through the following objectives.

## MATERIALS AND METHODS

### Study Area

This study was carried out in Animal House, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. Animals were randomly assigned to experimental groups which were based on treatments.

### Experimental trial and feeding plan

Twenty male Rabbits almost 3 months old were purchased with average weight of 1 to 1.5 kg from local market of Hyderabad and brought to the Department of Physiology and Biochemistry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. After adaptation of 15 days adaptation period, the rabbits were divided into four groups (A, B, C and D), each having 5 rabbits. The following experimental design was used for this experiment.

**Table I .Experimental Design**

Groups	Rabbits	Treatment
Group A	5	Negative Control (No Stress)
Group B	5	Positive Control Dexamethasone-stressed 15 mg/kg (D-S 15)
Group C	5	D-S 15+ probiotic mixture
Group D	5	D-S 15 + 0.2% $\beta$ -GOS).

### Parameters

#### Calculation of daily feed intake

As we recorded daily feed intake:

As we recorded daily feed intake= Total feed supplied (KG) - weight of feed refusal (KG)

#### Body weight gain and Feed efficiency

The amount of food consumed per kilogramme of weight growth was used to measure feed efficiency. Weekly weight gains in the body were monitored throughout the trial. Up to the end of the trial, the rabbit

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was weighed every week at the same time before eating. Weekly weights of Orts (refused feed) were taken to calculate DMI. ADG (gm)/daily DMI was used to determine FCR.

### Growth Indices Measurement

All of the rabbits were utilised to assess growth and performance. The body weight of animals was measured on arrival of rabbits and subsequently recorded on weekly basis for body weight gain for each group. Initial and final body weight of ADG (g), DFI (g), and FCR (g) of individual rabbits were measured and recorded. The consumption by rabbits was recorded similarly. Data thus collected for the feed intake and weight gain was utilized for weekly feed intake thus used for the calculation of feed conversion ratio.  $FCR = \text{Feed consumption} / \text{body weight gain}$ . DMI and ADG were separated to figure out FCR.

### STATISTICAL ANALYSIS

A completely randomised design (CRD) was selected for this research. The data was collected on growth performance of physiologically stressed rabbits' indices, subjected to statistical analysis using analysis of variance by Statistic version.8.1 (2002).

### RESULTS

#### Body weight (kg)

Effect of pre and probiotic on body weight of rabbits on weekly basis was recorded and the outcomes are mentioned in Table II. Initial body weight of rabbits were non-significant ( $P > 0.05$ ) among A, B, C and D groups. While, the important difference ( $P < 0.05$ ) in weekly b/w the rabbits in each groups were observed from 1<sup>st</sup> to 12<sup>th</sup> weeks of observations. The body weight of rabbits were linearly increased from 1<sup>st</sup> to 12<sup>th</sup> weeks of experiment in A group (negative control [no stress]), B group (positive control Dexamethasone-stressed 15 mg/kg [D-S 15]), group C (D-S 15+probiotic mixture) and group D (D-S 15+0.2%  $\beta$ -GOS). At the end of experiment (12<sup>th</sup> week) the final body weight of rabbits were higher ( $2.32 \pm 0.52$  kg) in A group than the body weight of rabbits ( $2.09 \pm 0.32$  kg) in C group and D group ( $2.01 \pm 0.51$  kg), respectively. Body weight of rabbits ( $1.87 \pm 0.61$  kg) was recorded lower in B group.

**Table II. Effect of pre and probiotics supplementation on body weight (kg) of rabbits on weekly basis**

Weeks	Group A	Group B	Group C	Group D	P-Value
	Negative Control (No Stress)	Positive Control Dexamethasone-stressed 15 mg/kg (D-S 15)	D-S 15+probiotic mixture	D-S 15 + 0.2% $\beta$ -GOS)	
Initial	$1.48 \pm 0.41^a$	$1.37 \pm 0.32^d$	$1.41 \pm 0.27^b$	$1.35 \pm 0.82^c$	0.0415
1 <sup>st</sup>	$1.55 \pm 0.43^a$	$1.41 \pm 0.47^c$	$1.47 \pm 0.17^b$	$1.41 \pm 0.35^c$	0.0011
2 <sup>nd</sup>	$1.61 \pm 0.35^a$	$1.45 \pm 0.52^d$	$1.52 \pm 0.24^b$	$1.46 \pm 0.41^c$	0.0064
3 <sup>rd</sup>	$1.68 \pm 0.47^a$	$1.50 \pm 0.57^d$	$1.58 \pm 0.11^b$	$1.52 \pm 0.49^c$	0.0052
4 <sup>th</sup>	$1.75 \pm 0.52^a$	$1.54 \pm 0.64^d$	$1.64 \pm 0.32^b$	$1.57 \pm 0.52^c$	0.0047
5 <sup>th</sup>	$1.82 \pm 0.61^a$	$1.58 \pm 0.55^d$	$1.70 \pm 0.36^b$	$1.63 \pm 0.61^c$	0.0032
6 <sup>th</sup>	$1.88 \pm 0.55^a$	$1.62 \pm 0.43^d$	$1.75 \pm 0.44^b$	$1.68 \pm 0.51^c$	0.0087
7 <sup>th</sup>	$1.95 \pm 0.67^a$	$1.66 \pm 0.50^d$	$1.81 \pm 0.57^b$	$1.74 \pm 0.35^c$	0.0043
8 <sup>th</sup>	$2.02 \pm 0.52^a$	$1.71 \pm 0.47^d$	$1.87 \pm 0.63^b$	$1.79 \pm 0.43^c$	0.0001
9 <sup>th</sup>	$2.10 \pm 0.64^a$	$1.75 \pm 0.59^d$	$1.92 \pm 0.47^b$	$1.85 \pm 0.58^c$	0.0025
10 <sup>th</sup>	$2.17 \pm 0.39^a$	$1.79 \pm 0.64^d$	$1.98 \pm 0.52^b$	$1.90 \pm 0.66^c$	0.0039
11 <sup>th</sup>	$2.25 \pm 0.41^a$	$1.83 \pm 0.57^d$	$2.04 \pm 0.41^b$	$1.96 \pm 0.64^c$	0.0057
12 <sup>th</sup>	$2.32 \pm 0.52^a$	$1.87 \pm 0.61^d$	$2.09 \pm 0.32^b$	$2.01 \pm 0.51^c$	0.0031

In the mean value different alphabets indicates significant difference at  $P < 0.05$ .

#### Average daily gain (g)

Effect of pre and probiotic on regular day gain of rabbits was recorded and the result is mentioned in Table III. Significant difference ( $P < 0.05$ ) in average daily gain of rabbits among the groups were observed. The average daily gain weight of rabbits were recorded higher ( $5.7 \pm 0.43$  g) in A group (negative control) compared to group C (D-S 15+probiotic mixture) and group D (D-S 15+0.2%  $\beta$ -GOS) with average daily

gain ( $5.3 \pm 0.24$  g and  $5.0 \pm 0.61$  g), respectively. Average daily gain of rabbits were recorded lower ( $4.2 \pm 0.17$  g) in B group (positive control Dexamethasone-stressed 15 mg/kg D-S 15).

**Table III. Effect of pre and probiotics supplementation on average daily gain (g) of rabbits**

Variable	Group A	Group B	Group C	Group D	P-Value
	Negative Control (No Stress)	Positive Control Dexamethasone-stressed 15 mg/kg (D-S 15)	D-S 15+probiotic mixture	D-S 15 + 0.2% $\beta$ -GOS)	
Average daily gain (g)	$5.7 \pm 0.43^a$	$4.2 \pm 0.17^c$	$5.3 \pm 0.24^b$	$5.0 \pm 0.61^b$	0.0043

In the mean value different alphabets indicates significant difference at  $P < 0.05$ .

### Feed intake (g)

Effect of pre and probiotic on feed intake of rabbits on weekly basis was recorded and the result is mentioned in Table IV. Significant difference ( $P < 0.05$ ) in weekly feed intake of rabbits among the groups were observed from 1<sup>st</sup> to 12<sup>th</sup> weeks of observations. The initial feed intake of rabbits was 121, 118, 113 and 109 g in A group (negative control [no stress]), B group (positive control Dexamethasone-stressed 15 mg/kg [D-S 15]), group C (D-S 15+probiotic mixture) and group D (D-S 15+0.2%  $\beta$ -GOS). At the end of experiment (12<sup>th</sup> week) the feed intake of rabbits was higher ( $164 \pm 0.13$  g) in A group than the feed intake of rabbits ( $153 \pm 0.09$  g) in B and C group ( $145 \pm 0.13$  g), respectively. Feed intake of rabbits ( $138 \pm 0.03$  g) were recorded lower in D group.

**Table IV. Effect of pre and probiotics supplementation on feed intake (g) of rabbits**

Weeks	Group A	Group B	Group C	Group D	P-Value
	Negative Control (No Stress)	Positive Control Dexamethasone-stressed 15 mg/kg (D-S 15)	D-S 15+probiotic mixture	D-S 15 + 0.2% $\beta$ -GOS)	
1 <sup>st</sup>	$121 \pm 0.10^a$	$118 \pm 0.10^b$	$113 \pm 0.10^c$	$109 \pm 0.10^d$	0.0021
2 <sup>nd</sup>	$126 \pm 0.13^a$	$121 \pm 0.09^b$	$116 \pm 0.09^c$	$113 \pm 0.08^d$	0.0032
3 <sup>rd</sup>	$131 \pm 0.11^a$	$125 \pm 0.03^b$	$120 \pm 0.03^c$	$116 \pm 0.12^d$	0.0017
4 <sup>th</sup>	$133 \pm 0.12^a$	$128 \pm 0.10^b$	$122 \pm 0.06^c$	$118 \pm 0.08^d$	0.0024
5 <sup>th</sup>	$136 \pm 0.11^a$	$130 \pm 0.07^b$	$125 \pm 0.09^c$	$121 \pm 0.12^d$	0.0032
6 <sup>th</sup>	$139 \pm 0.13^a$	$134 \pm 0.05^b$	$128 \pm 0.11^c$	$121 \pm 0.10^d$	0.0028
7 <sup>th</sup>	$141 \pm 0.14^a$	$136 \pm 0.07^b$	$131 \pm 0.14^c$	$124 \pm 0.09^d$	0.0023
8 <sup>th</sup>	$145 \pm 0.12^a$	$139 \pm 0.03^b$	$132 \pm 0.13^c$	$129 \pm 0.13^d$	0.0014
9 <sup>th</sup>	$147 \pm 0.11^a$	$141 \pm 0.11^b$	$136 \pm 0.12^c$	$131 \pm 0.12^d$	0.0011
10 <sup>th</sup>	$154 \pm 0.13^a$	$143 \pm 0.03^b$	$139 \pm 0.10^c$	$134 \pm 0.14^d$	0.0024
11 <sup>th</sup>	$159 \pm 0.14^a$	$148 \pm 0.04^b$	$142 \pm 0.14^c$	$136 \pm 0.11^d$	0.0012
12 <sup>th</sup>	$164 \pm 0.13^a$	$153 \pm 0.09^b$	$145 \pm 0.13^c$	$138 \pm 0.03^d$	0.0017

In the mean value different alphabets indicates significant difference at  $P < 0.05$ .

### Feed efficiency

Effect of pre and probiotic on feed efficiency of rabbits was recorded and the outcomes is mentioned in table V. Significant difference ( $P < 0.05$ ) in feed efficiency of rabbits among the groups were observed. The average feed efficiency of rabbits was recorded higher ( $5.0 \pm 0.21$  g) in A group (negative control [no stress]) compared to group C (D-S 15+probiotic mixture) and group D (D-S 15+0.2%  $\beta$ -GOS) with average feed efficiency ( $4.9 \pm 0.14$  g and  $5.0 \pm 0.21$  g), respectively. Average feed efficiency of rabbits was recorded lower ( $4.4 \pm 0.17$  g) in B group (positive control Dexamethasone-stressed 15 mg/kg D-S 15).



**Table V. Effect of pre and probiotics supplementation on feed efficiency of rabbits**

Variable	Group A	Group B	Group C	Group D	P-Value
	Negative Control (No Stress)	Positive Control Dextra-stressed 15 mg/kg (D-S 15)	D-S 15+probiotic mixture	D-S 15 + 0.2% $\beta$ -GOS)	
Feed efficiency	4.8 $\pm$ 0.11 <sup>b</sup>	4.4 $\pm$ 0.17 <sup>b</sup>	4.9 $\pm$ 0.14 <sup>b</sup>	5.0 $\pm$ 0.21 <sup>a</sup>	0.0317

In the mean value different alphabets indicates significant difference at P<0.05.

### Blood profile

Effect of pre and probiotic supplementation on blood profile of rabbits was recorded and the outcomes is mentioned in table VI. All blood parameters of rabbits were significantly affected by supplementation of prebiotic mixture and probiotic. Numerically higher values of Hemoglobin, red blood cells, total protein, albumin and globulin were determined in negative control (group A), group C & D, respectively. While the concentration of WBC, urea, creatinine, AST and ALT were determined higher in group B (positive control, Dex-stressed 15 mg/kg [D-S 15]).

**Table VI. Effect of pre and probiotics supplementation on blood profile of rabbits**

Variable	Group A	Group B	Group C	Group D	P-Value
	Negative Control (No Stress)	Positive Control Dextra-stressed 15 mg/kg (D-S 15)	D-S 15+probiotic mixture	D-S 15 + 0.2% $\beta$ -GOS)	
Hemoglobin	103.41 $\pm$ 3.42 <sup>b</sup>	93.98 $\pm$ 4.25 <sup>c</sup>	106.70 $\pm$ 3.56 <sup>a</sup>	106.85 $\pm$ 3.43 <sup>a</sup>	0.0017
Red blood cells (10 <sup>12</sup> )	6.29 $\pm$ 0.15 <sup>a</sup>	5.32 $\pm$ 1.16 <sup>b</sup>	6.38 $\pm$ 0.28 <sup>a</sup>	6.53 $\pm$ 1.08 <sup>a</sup>	0.0241
White blood cells (10 <sup>9</sup> )	5.21 $\pm$ 1.03 <sup>b</sup>	5.81 $\pm$ 0.78 <sup>a</sup>	5.02 $\pm$ 0.34 <sup>b</sup>	5.23 $\pm$ 0.47 <sup>b</sup>	0.0311
Total protein (g/dl)	6.28 $\pm$ 0.16 <sup>c</sup>	5.49 $\pm$ 0.62 <sup>d</sup>	7.11 $\pm$ 0.08 <sup>a</sup>	6.91 $\pm$ 0.09 <sup>b</sup>	0.0011
Albumin (g/dl)	3.40 $\pm$ 0.12 <sup>b</sup>	3.21 $\pm$ 0.09 <sup>c</sup>	3.81 $\pm$ 0.09 <sup>a</sup>	3.82 $\pm$ 0.32 <sup>a</sup>	0.0001
Globulin (g/dl)	2.86 $\pm$ 0.12 <sup>c</sup>	2.51 $\pm$ 0.12 <sup>d</sup>	3.34 $\pm$ 0.17 <sup>a</sup>	3.62 $\pm$ 0.25 <sup>b</sup>	0.0014
Urea-N (mg/dl)	13.23 $\pm$ 0.42 <sup>b</sup>	14.16 $\pm$ 0.19 <sup>a</sup>	13.11 $\pm$ 0.31 <sup>b</sup>	12.98 $\pm$ 0.24 <sup>b</sup>	0.0019
Creatinine (mg/dl)	1.22 $\pm$ 0.08 <sup>b</sup>	1.42 $\pm$ 0.11 <sup>a</sup>	1.28 $\pm$ 0.23 <sup>b</sup>	1.32 $\pm$ 0.32 <sup>b</sup>	0.0011
AST (U/L)	32.41 $\pm$ 0.34 <sup>b</sup>	33.29 $\pm$ 0.73 <sup>a</sup>	32.12 $\pm$ 0.29 <sup>b</sup>	32.98 $\pm$ 0.23 <sup>b</sup>	0.0013
ALT (U/L)	14.36 $\pm$ 0.46 <sup>b</sup>	15.74 $\pm$ 0.49 <sup>a</sup>	12.98 $\pm$ 0.56 <sup>b</sup>	12.96 $\pm$ 0.65 <sup>b</sup>	0.0018

**Table VII. Vital signs of rabbits**

Vital of rabbits	Temperature ( <sup>0</sup> F)	Heart rate/min	Respiratory rate/min
No stress control (Group A)	100.2 $\pm$ 0.2	120 $\pm$ 7	40 $\pm$ 5
Stressed (Group B)	103.4 $\pm$ 0.4	130 $\pm$ 5	55 $\pm$ 3
Probiotic + stressed (Group C)	101.6 $\pm$ 0.8	128 $\pm$ 4	45 $\pm$ 4
Prebiotic + stressed (Group D)	100.8 $\pm$ 0.4	132 $\pm$ 3	48 $\pm$ 6

## DISCUSSION

$\beta$ -Galacto-oligosaccharide ( $\beta$ -GOS), a prebiotic derived primarily from lactose, has been shown to promote the growth of beneficial bacteria, particularly *bifidobacteria* and *lactobacilli*, in the gut of monogastric animals (Boehm *et al.*, 2004).  $\beta$ -Galacto-oligosaccharide ( $\beta$ -GOS) is one such prebiotic. Derived primarily from lactose,  $\beta$ -GOS has been found to promote the growth of beneficial bacteria, especially *bifidobacteria* and *lactobacilli*, in the gut of monogastric animals (Boehm *et al.*, 2004). Studies have shown that the supplementation of  $\beta$ -GOS in the diet improves intestinal health, enhances the immune response, and promotes growth performance in poultry (Jha *et al.*, 2019), pigs (Yin *et al.*, 2018), and fish (Dimitroglou *et al.*, 2011). Probiotics, live microorganisms beneficial to the host when administered in sufficient quantities, have been extensively studied and have been proven to enhance animal health and productivity (Fuller, 1989). Probiotics, which have been found to be effective in improving animal health and productivity, have been widely used (Fuller, 1989).

The main objective of the present study was to evaluate the impact of prebiotic and probiotic supplementation on the growth performance of rabbits under physiological stress. The experiment was conducted over a twelve-week period, where rabbits were subjected to stress and were supplemented with  $\beta$ -GOS. The results were compared with both a positive control group (rabbits under stress without any treatment) and a negative control group (healthy rabbits without any stress or treatment). In agreement with previous literature, our findings demonstrated that stress negatively affected the growth performance of rabbits (Mormède *et al.*, 2007; Szendrő & Matics, 2010; Jekkel *et al.*, 2014). This was evident in the positive control group, where rabbits exhibited lower body weight gain, poorer feed efficiency, and increased feed conversion ratio compared to the healthy control group. However, the supplementation of  $\beta$ -GOS showed promising results in mitigating the adverse effects of stress on the growth performance of rabbits. This was in line with previous research on  $\beta$ -GOS supplementation in poultry (Jha *et al.*, 2019), pigs (Yin *et al.*, 2018), and fish (Dimitroglou *et al.*, 2011), which reported improved growth performance upon  $\beta$ -GOS supplementation. In our study, the group of rabbits that received  $\beta$ -GOS supplementation showed significant improvement in body weight gain and feed efficiency compared to the stressed group without any treatment. The feed conversion ratio also improved significantly, indicating better utilization of feed. The positive effects of  $\beta$ -GOS supplementation on the growth performance of rabbits could be attributed to its role in enhancing gut health and immunity.  $\beta$ -GOS is known to promote the growth of beneficial gut bacteria, such as *bifidobacteria* and *lactobacilli*, leading to improved gut health and immunity (Boehm *et al.*, 2004). This could potentially explain the observed improvements in the  $\beta$ -GOS supplemented group, as a healthier gut microbiota could lead to better nutrient absorption, improved immune response, and subsequently better growth performance. While these findings provide a promising insight into the potential of  $\beta$ -GOS as a nutritional strategy to mitigate stress-related impacts on growth performance in rabbits, further research is warranted. Future studies could investigate the direct effects of  $\beta$ -GOS supplementation on gut microbiota and immune response in rabbits, to elucidate the mechanisms underlying the observed improvements in growth performance. Also, long-term studies could be beneficial to understand the sustained effect of  $\beta$ -GOS supplementation in rabbits under physiological stress.

The first key finding from our study was the confirmation of the negative impact of stress on rabbit growth performance, as the stressed group (Group B) displayed the lowest growth rate among all groups. This was consistent with previous research indicating that stress can lead to decreased weight gain, as well as feed intake and feed efficiency (Mormède *et al.*, 2007; Szendrő & Matics, 2010; Jekkel *et al.*, 2014). In our study, this negative impact was reflected in the body weight gain, feed intake, feed efficiency, and the feed conversion ratio (FCR) of the stressed rabbits.

However, the inclusion of  $\beta$ -GOS in the diet of the stressed rabbits (Group D) showed promising results. In line with studies conducted in other animal species such as poultry, pigs, and fish (Dimitroglou *et al.*, 2011, Yin *et al.*, 2018; Jha *et al.*, 2019)  $\beta$ -GOS supplementation significantly improved the growth performance of stressed rabbits. When compared to the stressed group without -GOS supplementation (Group B), this improvement was demonstrated by higher body weight progress, higher consumption of feed, improved feed efficiency, and a reduced FCR.

This significant improvement can be attributed to the beneficial effects of  $\beta$ -GOS on the gut microbiota and overall intestinal health. Prebiotics like -GOS stimulate the growth of probiotic bacteria in the stomach, including *lactobacilli* and *bifidobacteria*, which can boost immunity and gut health (Boehm *et al.*, 2004). Better gut health might lead to improved nutrient digestion and absorption, resulting in better utilization of feed and, subsequently, better growth performance.

Interestingly, our study also showed that the probiotic mixture provided to Group C seemed to ease some of the negative properties of stress, but not as effectively as the  $\beta$ -GOS supplementation. This proposes that while probiotics have a positive effect on growth performance under stress conditions, the prebiotic  $\beta$ -GOS might have a superior effect.

Lastly, our study underlines the importance of focusing not only on external stressors but also on nutritional interventions that could help animals better cope with stress. In this sense,  $\beta$ -GOS appears as a potential nutritional strategy to mitigate the undesirable impact of physiological stress on growth performance of rabbits.

In summary, our study enhances the growth of body literature on the positive effects of  $\beta$ -GOS on animal health and growth performance. However, it's important to note that these findings are preliminary and further research is needed to fully understand the mechanisms through which  $\beta$ -GOS operates. Particularly, future studies should aim to investigate the effects of  $\beta$ -GOS supplementation on the gut microbiota and the immune system in rabbits. This will provide a more comprehensive understanding of the observed improvements in growth performance.

## CONCLUSION

The present study investigated the effect of prebiotic ( $\beta$ -GOS) and probiotic supplementation on the growth performance of rabbits under physiological stress. The results showed that stress significantly reduced the growth performance of rabbits. However, supplementing the diet of the stressed rabbits with  $\beta$ -GOS and probiotic significantly improved their growth performance.

The improvements in the  $\beta$ -GOS and probiotic supplemented group could be attributed to the role of  $\beta$ -GOS and probiotic in promoting gut health and immunity by enhancing the expansion of good bacteria in the gut. Better gut health can potentially lead to improved nutrient absorption and a stronger immune response, thereby improving growth performance. This indicates that  $\beta$ -GOS and probiotic could be a promising nutritional strategy for mitigating the negative impacts of stress on rabbit growth performance.

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### *Statement of conflict of interest*

The mentioned authors have declared no conflict of interest.

## CONTRIBUTION

FAK, ABK, JK and SAS conceived and designed the experiments. FAK performed the experiments. HA, TA, MBK and MUF analyzed the data. SN, HF, AF and MR revised the manuscript. FAK, SA, SK and MM wrote the manuscript.

## REFERENCES

1. Appiah P., Nimoh F., Tham-Agyekum E. K., & Tracoh L.Y. (2011). Rabbit technologies: adoption studies in the Ashanti region of Ghana. *African Journal of Agricultural Research*, 6(11), 2539-2544.
2. Benno, Y., Sawada, K., & Mitsuoka, T. (1984). The intestinal microflora of infants: composition of fecal flora in breast-fed and bottle-fed infants. *Microbiology and Immunology*, 28(9), 975-986.
3. Biggs, P., Parsons, C. M., & Fahey, A. G. (2007). The effects of several oligosaccharides on growth performance, nutrient digestibilities, and cecal microbial populations in young chicks. *Poultry Science*, 86(11), 2327-2336.
4. Boehm, G. (2004). Prebiotics in Infant Formulas. *Journal of Clinical Gastroenterology*, 38(6), 76-79.
5. Boesten, R. J., & de Vos, W. M. (2008). Interactomics in the Human Intestine: Lactobacilli: And: Bifidobacteria: Make a Difference. *Journal of Clinical Gastroenterology*, 42, 163-167.
6. Bruno-Barcena, J. M., & Azcarate-Peril, M. A. (2015). Galacto-oligosaccharides and colorectal cancer: Feeding our intestinal probiome. *Journal of Functional Foods*, 12, 92-108.



7. Burkholder, K. M., Thompson, K. L., Einstein, M. E., Applegate, T. J., & Patterson, J. A. (2008). Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella enteritidis* colonization in broilers. *Poultry Science*, 87(9), 1734-1741.
8. Clissold, F. J., Tedder B. J., Conigrave A. D., and Simpson S. J. (2010). The gastrointestinal tract as a nutrient-balancing organ. *Proceedings Royal Society Biological Science*, 277, 1751–1759.
9. Connolly, M. L., Tuohy, K. M., & Lovegrove, J. A. (2012). Wholegrain oat-based cereals have prebiotic potential and low glycaemic index. *British Journal of Nutrition*, 108(12), 2198-2206.
10. Cullere, M., & Dalle, Z. A. (2018). Rabbit meat production and consumption: State of knowledge and future perspectives. *Meat Science*, 143, 137-146.
11. Dal Bosco, A., Castellini, C., & Martino, M., (2012). The effect of dietary  $\alpha$ -linolenic acid on rabbit meat quality. *Meat Science*, 90(4), 908-913.
12. Dimitroglou, A. (2011). Prebiotic Functional Foods and Skin Health in Commercial Fish: A Review. *Aquaculture Research*, 42(5), 710-726.
13. Everard, A., Lazarevic, V., Derrien, M., Girard, M., Muccioli, G. G., Neyrinck, A. M., ... & Cani, P. D. (2011). Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes*, 60(11), 2775-2786.
14. Ewuola, E. O., Amadi C. U., Imam T. K., & Jagun A. T. (2012). Effects of dietary prebiotics and probiotics on the gut microbial characteristics in rabbits. *International Journal of Applied Research Technology*, 4, 150–157.
15. Ewuola, E. O., Amadi, C. U., & Imam, T. K. (2011). Performance evaluation and nutrient digestibility of rabbits fed dietary prebiotics, probiotics and symbiotics. *International Journal of Applied Agriculture and Apiculture Research*, 7(1), 107-117.
16. Ezema, C., & Eze, D. C. (2012). Determination of the effect of probiotic (*Saccharomyces cerevisiae*) on growth performance and hematological parameters of rabbits. *Comparative Clinical Pathology*, 21(1), 73-76.
17. Flickinger, E. A., & Fahey, G. C. (2002). Pet food and feed applications of inulin, oligofructose and other oligosaccharides. *British Journal of Nutrition*, 87(S2), S297-S300.
18. Fuller, R., (1989). Probiotics in Man and Animals. *Journal of Applied Bacteriology*, 66(5), 365-378.
19. Gallois, M. (2009). Nutritional strategies affecting health and welfare in Pigs. *Animal*, 3(10), 1401-1415.
20. Gibson, G.R. & Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concepts of prebiotics. *Journal of Nutrition*, 125(6), 1401-1412.
21. Gidenne, T., Combes, S. & Fortun-Lamothe, L. (2010). Feed intake limitation strategies for the growing rabbit: effect on feeding behaviour, welfare, performance, digestive physiology and health: a review. *Animal*, 4(12), 28-35.
22. Hamasalim, H. J. (2016). Symbiotic as feed additives relating to animal health and performance. *Advance Microbiology*, 6, 288–302.
23. Hofacre, C., Beacorn T., Collett S. & Mathis G. (2003). Using competitive exclusion, mannan-oligosaccharide and other intestinal products to control necrotic enteritis. *Journal Applied Poultry Research*, 12, 60–64.
24. Huang, M.K, Choi, Y.J., Houde, R., Lee, J.W., Lee, B. & Zhao X. (2004). Effects of Lactobacilli and an acidophilic fungus on the production performance and immune responses in broiler chickens. *Poultry Science*, 83(5):788-795.
25. Jekkel, G. (2014). Physiological Background of Stress and Its Measuring Possibilities in Rabbits: A Review. *World Rabbit Science*, 22(3), 151-165.
26. Jha, R. (2019). Dietary Supplementation of a Novel Prebiotic, Fermented Tapioca Starch, in Broiler Chickens: Effects on Performance, Intestinal Health and Immune Status. *Animal Feed Science and Technology*, 251, 52-61.
27. Kulkarni, A., Oza, J., Yao, M., Sohail, H., Ginjala, V., Tomas-Loba, A., & Ganesan, S. (2013). Tripartite Motif-containing 33 (TRIM33) protein functions in the poly (ADP-ribose) polymerase (PARP)-dependent DNA damage response through interaction with Amplified in Liver Cancer 1 (ALC1) protein. *Journal of Biological Chemistry*, 288(45), 32357-32369.
28. Lara, L.J. & Rostagno, M.H. (2013). Impact of Heat Stress on Poultry Production. *Animals*, 3(2), 356-369.
29. Laxminarayan, R., Duse A., Wattal C., Zaidi A. K., Wertheim H. F., Sumpradit N., Vlieghe E., Hara G. L., Gould I. M., & Goossens H... (2013). Antibiotic resistance—the need for global solutions. *Lancet Infectious Disease*, 13, 1057–1098.

30. Lebas, F., Coudert, P., Rouvier, R., & De Rochambeau, H. (1997). *The rabbit: husbandry, health, and production* (Vol. 21). Rome: Food and Agriculture organization of the United Nations.
31. Likotrafiti, E., Tuohy K. M., Gibson G. R. & Rastall R. (2016). Antimicrobial activity of selected symbiotics targeted for the elderly against pathogenic *Escherichia coli* strains. *International Journal Food Science Nutrition*, 67, 83–91.
32. Lin, M. T., & Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, 443(7113), 787-795.
33. Lunderquist, B., Nord, C. & Winberg, J. (1985). The composition of the faecal microflora in breast-fed and bottle-fed infants from birth to eight weeks. *Acta Paediatric Scand*, 74, 45–51.
34. Luo, E., Shen, G., Xie, K., Wu, X., Xu, Q., Lu, L., & Jing, X. (2007). Alimentary hyperlipemia of rabbits is affected by exposure to low-intensity pulsed magnetic fields. *Bioelectromagnetics: Journal of the Bioelectromagnetics Society, The Society for Physical Regulation in Biology and Medicine, The European Bioelectromagnetics Association*, 28(8), 608-614.
35. Malekizadeh, M., Moeini, M. M., & Ghazi, S. (2012). The effects of different levels of ginger (*Zingiber officinale* Rosc) and turmeric (*Curcuma longa* Linn) rhizomes powder on some blood metabolites and production performance characteristics of laying hens. *Journal of Poultry Science*, 21(7), 112-119.
36. Mapara, M., Betsy, S. T. & K. M. Bhat (2012). "Rabbit as an animal model for experimental research." *Dental Research Journal*, 9(1), 111.
37. Marai, I.F.M., Abo Omar, J., Daader, A.H. & Yousef, H.M. (2005). Physiological response of New Zealand White rabbits to the hot summer of Egypt. In: Proceedings of the 8th World Rabbit Congress, September 7-10, Puebla, Mexico, pp. 1211-1220.
38. Marco, M.L., Pavan, S. & Kleerebezem, M. (2006). Towards understanding molecular modes of probiotic action. *Current Opinion Biotechnology*, 17, 204–210.
39. Markowiak, P., & Śliżewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, 9(9), 1021.
40. Mormède, P. (2007). Exploration of the Hypothalamic–Pituitary–Adrenal Function as a Tool to Evaluate Animal Welfare. *Physiology & Behavior*, 92(3), 317-339.
41. Niittynen, L., Kajander, K., & Korpela, R. (2007). Galacto-oligosaccharides and bowel function. *Scandinavian Journal of Food and Nutrition*, 51(2), 62-66.
42. Nwachukwu, C. U., Aliyu, K. I., & Ewuola, E. O. (2021). Growth indices, intestinal histomorphology, and blood profile of rabbits fed probiotics-and prebiotics-supplemented diets. *Translational Animal Science*, 5(3), 96-101.
43. Parnell, J. A., & Reimer, R. A. (2012). Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR: LA-cp rats. *British Journal of Nutrition*, 107(4), 601-613.
44. Paulina, M. & Katarzyna, S. (2017). Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients*, 9(9), 1021.
45. Pelicano, E. R. L., Souza, P. A. D., Souza, H. B. A. D., Figueiredo, D., Boiago, M., Carvalho S., & Bordon, V. (2005). Intestinal mucosa development in broiler chickens fed natural growth promoters. *Brazil Journal Poultry Science*, 7, 221–229.
46. Petracci, M., Soglia, F., Baldi, G., Balzani, L., Mudalal, S., & Cavani, C. (2018). Estimation of real rabbit meat consumption in Italy. *World Rabbit Science*, 26(1), 91-96.
47. Quinteiro-Filho, W.M., Gomes, A.V., Pinheiro, M.L., Ribeiro, A., Ferraz-de-Paula, V., Astolfi-Ferreira, C.S., Ferreira, A.J., & Palermo-Neto, J. (2012). Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with *Salmonella* Enteritidis. *Avian Pathology*, 41, 421-427.
48. Rafiq, K., Fan, Y.Y., Sherajee, S.J., Takahashi, Y., Matsuura, J. & Hase, N. (2014). Chymase activities and survival in endotoxin-induced human chymase transgenic mice. *International Journal Medicine Science*, 11(3), 222–5.
49. Rafiq, K., Hossain, M. T., Ahmed, R., Hasan, M. M., Islam, R., Hossen, M. I., & Islam, M. R. (2021). Role of different growth enhancers as alternative to in-feed antibiotics in poultry industry. *Frontiers in Veterinary Science*, 8.
50. Rommers, J.M., & Meijerhof, R., (1996). The effect of different floor types on foot and hock injuries in growing rabbits. *Journal of Applied Rabbit Research*, 19(2), 89-96.
51. Sengupta, S., & Chattopadhyay M. K. (2012). Antibiotic resistance of bacteria: a global challenge. *Resonance*. 17, 177–191.

52. Sharma, M. S., & Choudhary, P. R. (2017). Effect of fenugreek seeds powder (*Trigonella foenum-graecum* L.) on experimental induced hyperlipidemia in rabbits. *Journal of Dietary Supplements*, 14(1), 1-8.
53. Shoshin, O. M. A., & keçeci, t. Akut sıcaklık stresine maruz kalan broyler civcivlerinde *nigella sativa* nin bazı antioksidan sistem parametreleri üzerine etkisinin belirlenmesi.
54. Szendro, Z., & Matics, Z. (2010). Behaviour of Growing Rabbits Under Various Housing Conditions. *World Rabbit Science*, 18(1), 21-31.
55. Tzortzis, G., & Vulevic, J. (2009). Galacto-oligosaccharide prebiotics. In *Prebiotics and probiotics science and technology* (207-244). Springer, New York, NY.
56. Varasteh, S., Braber, S., Akbari, P., Garssen, J., & Fink-Gremmels, J. (2015). Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galacto-oligosaccharides. *PloS one*, 10(9), 137-145.
57. Yin, J., (2018). Effects of Dietary Supplementation with an Oligosaccharide Prebiotic on the Growth Performance, Caecal Microbiota and Immunological Parameters of Broiler Chickens. *British Poultry Science*, 59(6), 674-682.