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Efficacy of Glucans on Growth Variables, Antioxidant Potential, and Immunity of Growing Rabbits

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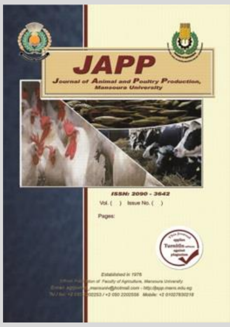

ABSTRACT

The goal of this study was to see how dietary -glucan (BG) supplementation affected growth performance, blood biochemicals, and antioxidant capacity in growing NZWrabbits. 75 NZW rabbit females, 6 weeks old, with an average live body weight of 845.3735.23 g. Rabbits were distributed into five experimental groups at random (fifteen rabbits in each group). The control group (G1) received only the basal diet, while the G2, G3, G4, and G5 groups received the basal diet supplemented with BG at 0.5,1.0,1.5, and 2.0 g/kg diet, respectively. Growth performance parameters, blood biochemical, and antioxidant enzymes, and immunity markers in the hepatic tissues were determined. Results show that all BG supplements developed (P<0.05) final weight, and gain without any effects on feed efficiency, and viability, and decreased (P<0.05) concentration of glucose, total cholesterol, and triglycerides. The effect of BG on serum activity of ALT and AST was not significant. Dietary BG supplementation increased (P<0.05) SOD, CAT, and GPx levels, and decreased (P<0.05) MDA levels in hepatic tissues. Percentage of NBT and ACP level increased (P<0.05) BG groups (G2-G5) in comparing with G1.The current study concludes that β-glucans represent a promising factor for improving antioxidant capacity, immunity, and lipid profile as well as promoting growth performance of growing rabbits, without adverse effects on liver and kidney function and protein metabolism.

Keywords: Rabbit, β-glucan, growth, antioxidant enzymes, serum biochemical, immunity.

INTRODUCTION

Meat production from rabbits is reasonable solution to the developing countries' rising protein shortages, so the rabbit production is a suitable task because of its high fertility, low production costs, short generation intervals, and capacity to use a variety of forages. to help in resolving the global protein shortage problem (Ebeid *et al*., 2013).

Antibiotics are utilised as growth boosters as well as as feed additives for the treatment of bacterial illnesses. Antibiotic overuse, on the other hand, has resulted in a slew of issues, including resistant bacteria, drug residual in pig meat, and pollution, all of which are dangerous to animal and human health. As a result, several techniques, mainly the using of high-condition animals, feeding tactics, and nutrition, exist to deal with this issue. As a result, the usage of probiotics and prebiotics has become increasingly popular. Prebiotics are non-digestible food components that promote saprophyte bacteria growth. (Du *et al*., 2015). European legislation claims to drastically reduce antibiotics used in livestock production, favoring innovative techniques to maintain strong immune responses in the face of breeding stress.

Carbohydrate structures are beleived to perform a central role in the potentiation of immunity, and they could open up new possibilities for the development of novel types of immunological adjuvants that are both safe and well-tolerated . Glucans (-D-glucans, hereafter referred to as "glucans") are among the most promising carbohydrates. These compounds are indeed the basic building unit of cell walls in higher plants, algae, fungus, yeast, and a variety of bacterial species. They are mainly comprised of repeated D-glucose bonded with glycosidic bonds in a wide range of formats. (Vetvicka *et al*., 2014).

Beta-glucan (BG), one of the most common prebiotics is a type of efficient polysaccharide found in the cell walls of fungus, bacteria, and cereals. It has a variety of natural activities, including immune role enhancement, anti-infection, and glucose regulating (Xiong *et al*., 2015). Also, due to its beneficial impacts on human and animal wellbeing, BG is being believed as a biological response modulator by the pharmaceutical and functional food industries. The degree of branching, molecule mass, and tertiary structure all influence their biological effects (Oliveira *et al* 2009). Many recent research have suggested that glucans are the most promising and practical choices for such safe immunostimulants. As a result, BG administration could be beneficial in animal sectors that require less antibiotic treatment while keeping an alert immunological condition to effectively combat pathogens, such as rabbits(Crespo *et al*., 2017).

By encouraging the synthesis of antibodies,β -glucan speeds up the creation of lymphocytes in the biological system and enhances the body's capacity to withstand infection (Neamat-Allah *et al*., 2020). Furthermore, BG can be employed as an antioxidant to protect animals' body tissues from oxidative impairment by eliminating free radicals from the system (Pietretti *et al*., 2013). Via the modulation of liver enzyme ectivities, lipid profile, and total protein levels, the BG has a hepatoprotective impact (Dawood *et al*., 2020).

In earlier researches, BG supplementation in the feed has been shown to improve poultry growth and

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immunity (Soliman *et al*., 2014; Tian *et al*., 2016), pigs (Wang *et al*., 2008; Luo *et al*., 2019), rats (Belobrajdic *et al*., 2015), cattle calves (Tao *et al* 2015), fish (Cao *et al*., 2019; Dawood *et al*., 2020), rabbits (García-Ruiz *et al*., 2008; Abo Ghanima *et al*., 2020). Contrary, Supplementing rabbit diets with yeast-glucans has no effect on growth or digestibility (Guenaoui, 2015). As a result, the goal of this research was to see the influence of the dietary implementation of β-glucan on growth parameters, blood biochemicals profile, antioxidant capacity, and immunity of growing NZW rabbits.

MATERIALS AND METHODS

Animals and system:

The current work was conducted on a commercial farm in Mansoura, Egypt, employing 75 NZW rabbit females aged 6 weeks and weighing an average of 845.3735.23 g as their initial weight. All rabbits were raised in galvanised wire batteries with typical sizes (60 x 35 x 35 cm) and under equivalent managerial and environmental circumstances . Galvanized steel feeding containers and automated drinkers (nipple) were included in every cage. Rabbits were fed a commercial diet (crude protein= 16%, fiber =10%, DE = 2200 kcal) as a basal diet supplemented with different levels of BG. Every morning, the urine and faeces that had accumulated under the batteries were removed.

The experimental design

The experimental rabbits (n=75) were classified randomly into five experimental groups (fifteen rabbits in each group). The basal group (G1) fed only the control diet, while the G2, G3, G4, and G5 groups provided the basal diet augmented with BG at 0.5, 1.0, 1.5, and 2.0 g/kg diet, respectively. β-glucan, which was utilized in this research, is a marketable product comprising 85% BG and made by Daigon do Co., Tokyo, Japan.

Experimental procedures

Throughout the experimental period of 8 weeks, rabbits were individually weighed as initial and final to calculate the total and percentage of weight gain relative to initial weight for the whole period. Before receiving any feed or water, rabbits were weighted first early in the morning.

Every day, the amount of feed consumed by each individual was reported. The feed conversion ratio (FCR) was assessed by weighing residues and waste feeds daily and subtracting them from the provided amounts to get the total amount of feed consumed. The survival rate of rabbits was determined by tracking their deaths on a daily basis.

Blood profile

Blood samples were gathered from five slaughtered rabbits per group at the end of the experiment. Blood samples were taken in sterile tubes that did not contain heparin. After allowing the samples to coagulate at room temperature (30 minutes), serum was obtained by centrifugation at 3500 rpm for 15 minutes. –20oC was used to keep serum samples for further analysis. Total proteins, albumin, glucose, total cholesterol, total triglycerides, creatinine, and urea levels, along with alanine (ALT) and aspartate aminotransferases (AST) activities, were measured in the serum using commercial kits following the manufacturer's protocol (Bio-diagnostic, Giza, Egypt). Globulin contents were computed by subtracting albumin % from total protein %.

Antioxidant capacity

Samples (liver and intestine) were obtained after the animals were slaughtered, homogenised (Homogenizer VEVOR, FSH-2A) in iced NaCl (0.86%), and centrifuged (10 min, 4 °C, 12,000 rpm). The supernatant was maintained for colorimetric analyses at 550, 280, and 412 nm for superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) according to the manufacturer's protocol, using JianCheng Co., Nanjing, China detection kits.

Statistical analysis

The IBM® SPSS® Statistics software version 22 (SPSS Inc., IL, USA) was run to statistically analyse the data, which was presented as means and standard errors (S.E.) of triplicates. At a 5% probability level, Shapiro-Wilk and Levene tests were performed to check for normality and homogeneity, followed by one-way model of ANOVA and Duncan's post hoc testing.

RESULTS AND DISCUSSION

Results

Growth performance parameters and survival rate:

The effect of BG on growth parameters was significant (P<0.05) on final body weight, total gain, and weight gain percentage while BG administration had no influence on feed utilization (intake and conversion ratio), and survival rate . Results showed that BG supplementation at all levels significantly (P<0.05) improved final weight, and gain without any effects on feed intake, feed conversion, and viability of growing rabbits. Dietary supplementation of BG at a level of 1 g/kg gave the best growth performance parameters in comparison with other BG levels and control (Table 1).

Table 1. Efficiency of β-glucan on growth variables and survival rate of growing rabbits.

Parameter	Experimental group				
	G1 (Control)	G2 (0.50 g BG)	G3 (1.0 g BG)	G4 (1.50 g BG)	G5 (2.0 g BG)
Initial weight (g)	851.9±31.20	836.8±25.13	842.7±30.25	848.6±33.64	846.8±35.23
Final weight (g)	2041.8±50.39^{b}	2213.6±37.18^{a}	2271.4±47.88^{a}	2238.6±60.91^{a}	2229.3±37.82^{a}
Total gain (g)	1189.9±42.01^{b}	1376.8±39.58^{a}	1428.7±52.78^{a}	1390.0±48.69^{a}	1382.5±46.71^{a}
Weight gain (%)	141.12±3.59^{b}	161.40±2.71^{a}	168.23±3.54^{a}	164.36±4.46^{a}	163.25±2.76^{a}
Total intake (g/h)	4481.2±113.82	4492.1±92.19	4465.9±139.37	4509.2±204.37	4397.4±97.33
Feed conversion	3.77±0.34	3.26±0.18	3.13±0.17	3.24±0.22	3.18±0.21
Survival (%)	100	100	100	100	100

Means within the same line with various superscripts are different at P<0.05.

Serum biochemicals profile:

The influence of BG on serum biochemicals was significant (P<0.05) only on glucose, total cholesterol, and triglycerides, while serum total proteins, albumin, globulin, creatinine, and urea were not affected by BG treatment. Results revealed that BG supplementation at all levels notably (P<0.05) decreased the concentrations of glucose, total cholesterol, and triglycerides, being the lowest in G5, G4, and G3, respectively (Table 2).

Enzyme activity:

The effect of BG on serum activity of ALT and AST was not significant (Table 3).

Table 2. Impact of β-glucan on blood serum biochemicals profile of growing rabbits.

Blood biochemical	Experimental group				
	G1 (Control)	G2 (0.50 g BG)	G3 (1.0 g BG)	G4 (1.50 g BG)	G5 (2.0 g BG)
Total proteins (g/dl)	6.99±0.31	6.65±0.18	7.31±0.88	6.89±0.51	6.93±0.82
Albumin (g/dl)	4.12±0.13	3.94±0.71	4.23±0.58	3.97±0.33	3.92±0.47
Globulin (g/dl)	2.87±0.09	2.71±0.19	3.08±0.30	2.92±0.24	3.01±0.53
Glucose (mg/dl)	88.17±1.08^{a}	73.19±1.85^{b}	74.33±2.17^{b}	78.02±3.02^{b}	72.13±2.52^{b}
Cholesterol (mg/dl)	152.4±4.04^{a}	134.0±2.11^{b}	131.9±3.11^{b}	129.8±4.12^{b}	136.2±3.12^{b}
Triglycerides (mg/dl)	104.80±3.01^{a}	94.47±2.31^{b}	92.71±1.47^{b}	95.37±2.41^{b}	94.02±1.22^{b}
Creatinine (mg/dl)	1.49±0.26	1.51±0.17	1.48±0.24	1.52±0.29	1.47±0.31
Urea (mg/dl)	41.34	38.66±3.29	39.57±2.87	40.22±5.16	38.99±1.22

Means within the same line with various superscripts are different at P<0.05.

Table 3. Influence of β-glucan on AST and ALT in blood serum of growing rabbits.

Enzyme activity	Experimental group				
	G1 (Control)	G2 (0.50 g BG)	G3 (1.0 g BG)	G4 (1.50 g BG)	G5 (2.0 g BG)
ALT (IU/ l)	82.22±2.48	79.83±1.64	82.94±1.39	81.15±2.08	80.34±1.92
AST (IU/ l)	41.68±6.24	39.09±5.88	38.99±3.07	38.95±5.39	41.99±2.08

Antioxidant status

Dietary BG supplementation significantly affected antioxidant capacity markers including the level of SOD, CAT, and GPx as well as lipid-peroxidation marker including the level of MDA in the tissue of the liver. (P<0.05). In comparing with the monitor group (G1), the level of CAT and GPx remarkably (P<0.05) increased in G2. The increasing level of BG in G3 significantly (P<0.05) increased the level of SOD, CAT, and GPx to the maximal values, while the MDA level significantly (P<0.05) decreased to the lowest values. On the other hand, increasing BG in G4 and G5 significantly (P<0.05) increased the level of CAT, and GPx, and decreased MDA, except SOD level was increased considerably (P<0.05) only in G4 (Fig. 1).

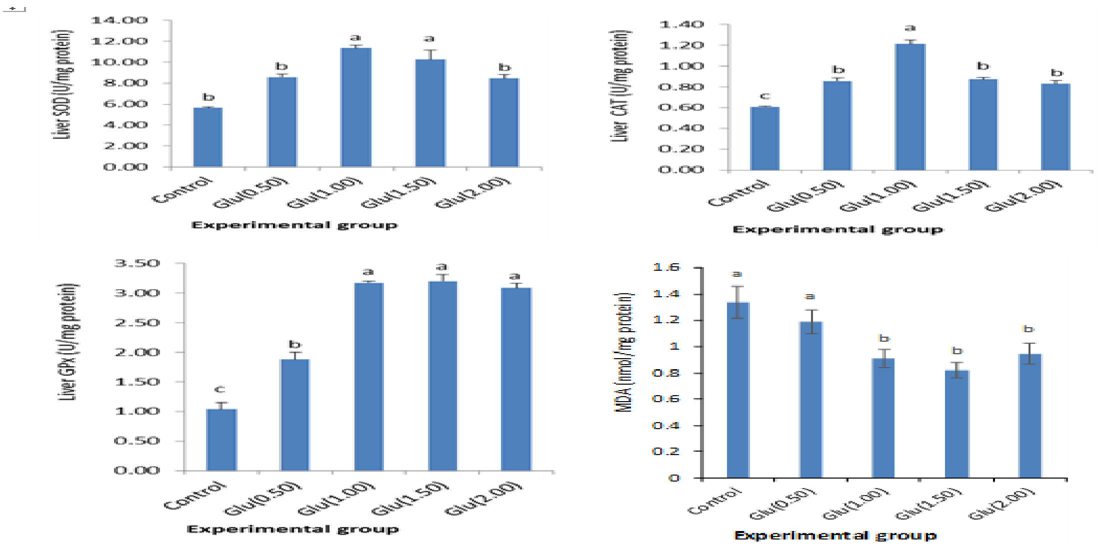

Fig. 1. Antioxidant status responses to β-glucan

Nitroblue tetrazolium and acid phosphatase:

Dietary BG supplementation significantly affected the NBT percentage and activity of ACP in liver tissues. Percentage of NBT and ACP level meaningfully (P<0.05) enhanced in all BG groups (G2-G5) in comparing with the control group (G1, Fig, 2)

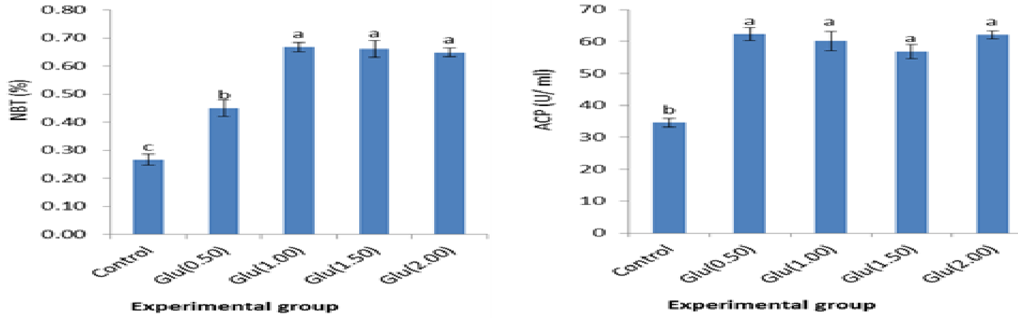

Fig. 2. Impact of β-glucan on level of NTB and activity of ACP in liver tissue of growing rabbits.

Discussion

BG treatment in diets has been shown to benefit animal development and health status (ElSawy, *et al*., 2015– Khanna *et al*., 2014). BG is also thought to be a viable alternate to antibiotics, as it increases broiler survival and performance (Moon, *et al*., 2016). The target of this work was to see how dietary -glucan supplementation affected growth performance , blood biochemicals, antioxidant capacity, and immunity of growing rabbits. The obtained results indicated beneficial effects of BG supplementation, especially at a level of 1 g/kg, on the growth performance parameters of rabbits. Consistent with these results, Abo Ghanima *et al*. (2020) revealed that oral BG at doses of 0.25 and 0.5 ml/L water considerably (P 0.05) sped up body weight gain and improved feed conversion ratio in rabbits when compared to control, with the dose of 0.5 ml BG per one-liter drinking water being the best. Likewise, multiple publications have documented improvements in body weight gain and feed conversion ratio in rabbits given S. cerevisiae and a probiotic (Shehata *et al*., 2012; Ezema and Eze, 2012). Furthermore, utilising varying quantities of a commercial product called Fibosel (0, 50, 100, 150, 200, and 250 ppm) resulted in considerably higher body weight and daily weight increase in rabbits fed Fibosel-containing diets than rabbits on the control diet. Rabbits consumed diets containing 50, 200, and 250 ppm Fibosel achieved intermediate results (García-Ruiz *et al*., 2008). In broiler, Soliman *et al*. (2014) found that the addition of different levels of mannan and β- Glucan as a prebiotic to broilers diets contaminated with aflatoxin might be reduced the effects of aflatoxin toxicity on body weight. In pigsBG has a considerable effect on fishing pig growth, according to Luo *et al*. (2019). At the early stage and throughout the fattening period, the supplementation of 100 mg/kg BG considerably boosted the daily gain and improved the feed to gain relative amount. Tao *et al*. (2015) discovered that calves given 75 mg yeast BG/kg feed gained more weight throughout the course of the feeding period than any other group. Dietary supplementation had no effect on feed intake throughout the study (P>0.05), while calves fed with 75 mg yeast β-glucan /kg diet had a better feed intake from days 28 to 56 (P<0.05) than the reference group. During the whole experiment period, calves fed 75 mg yeast BG/kg feed had a considerably greater gain-to-feed ratio than the other groups. In fish, Cao *et al*. (2019) found that fish given diets treated with BG had significantly higher final weight and specific growth rates, along with a greater feed efficiency ratio. Guenaoui (2015), on the other hand, found that dietary treatment with BG reduced growth rate in rabbits from 28 to 38 days of age when compared to a control ration, with no impact on feed intake or efficiency. During the fattening phase, dietary supplementation with chito-oligosaccharides at various dosages had no effect on rabbit growth.

In the present trial, dietary BG incorporation improved the growth performance of growing NZW rabbits. Higher digestibility and absorption of feed ingredients may explain the increased efficiency of BG dietry supplementation in growing rabbits. (Resta-Lenert *et al*., 2003; Shehata *et al*., 2012). Furthermore, enhanced intestinal health was found to result in an increase in villus height, indicating increased growth efficiency. (Zhang *et al* 2005). Some research confirms the hypothesis of employing prebiotics to lengthen the intestinal villus and boost immunity (Abd El-Hack *et al*., 2018; Teng and Kim, 2018). Furthermore, this improves nutrient absorption and, as a result, body weight (Chen, 2003). The increased villi height / crypt depth ratio, which would support for more nutrient uptake, could explain the improved growth efficiency in response to BG (Chen *et al*., 2008; Tsukada *et al*., 2003).

As such, the present experiment showed an insignificant effect of BG on total proteins, albumin, globulin, urea, and creatinine, but significantly improved lipid profile and reduced glucose concentration in the blood serum of growing rabbits. Incomparable with the effect of BG supplementation on blood biochemical of growing rabbits in our study, Abo Ghanima *et al*. (2020) found that administrating rabbits with BG (0.5 ml /L drinking water) raised total proteins and globulin concentrations, which is in contrast to the obtained results, whereas BG supplementation did not alter total proteins and their fractions in blood serum of growing rabbits. This may be linked to the method of treatment used, dietary supplementation, or oral administration. The later researchers noted that blood urea and creatinine levels were non-significantly affected by dietary supplementation, which is consistent with the current findings. Parallel with the current outcomes, Tao *et al*. (2015) discovered that yeast BG supplementation had no effect on blood concentrations of total proteins or albumin, but had a negative result on serum contents of total cholesterol and triglycerides when compared to the control group, claiming that supplementing pre-ruminant calves with BG had no negative consequences for their metabolism. In contrast, the later authors found that yeast BG supplementation did not influence the serum levels of glucose. Comparing to control chicks, ElSawy *et al*. (2015) found that oral administration of yeast BG had no consequence on serum protein, albumin, or globulin levels. The observed reduction in the level of total cholesterol and triglycerides in the current study due to dietary BG was also concluded in rats (Jonker *et al*., 2010), hamsters (Wilson *et al*., 2004), and humans (Keenan *et al*., 2007). β-Glucan is a type of fibre that slows down digestion and assimilation of nutrients (Huth *et al*. 2000). The viscosity of BG was the most important element impacting its biological roles, such as cholesterol lowering and bile acid and fat binding capability (Wood *et al*., 1994). Usage of BG as dietary supplementation in our study indicated normal liver and kidney function in terms of the unchanged activity of ALT and AST in the blood serum of growing rabbits. In fish, although Cao *et al*. (2019) found no significant difference in AST, Dawood *et al*. (2020) found that BG treatment decreased ALT compared to controls. In this respect, Soliman *et al*. (2014) found that the addition of different levels of mannan and BG as a prebiotic to broilers diets contaminated with aflatoxin might be reduced the effects of aflatoxin toxicity on liver and kidney function

In the present study, BG dietry treatment significantly enhanced the antioxidant status of growing rabbits in terms of increasing the level of SOD, CAT, and GPx and reducing MDA levels in the hepatic tissues. Immunity markers including NBT and ACP levels in the hepatic tissues were also improved as affected by BG supplementation. In accordance with these results, Sharma *et al*. (2012) discovered that BG supplementation dramatically boosted the intestine's antioxidant capacity in terms of GPx, decreased glutathione (GSH), CAT, and total -SOD activities, and boosted the immunological response in the intestine as evaluated by ACP and alkaline phosphatase

(AKP) activities. All of these antioxidant tools are contributed in scavenging reactive oxygen species (ROS), the excessive exposure of which causes oxidative stress in the host's cells, and then produce a lot of MDAm, which is one of the most important lipid-oxidation products and an important indicator of peroxidative tissue injury (Janero, 1990). A substantial reduction in MDA was seen in the fish-fed BG diet, which agrees with our findings (Kim *et al*., 2009; Zeng *et al*., 2016; Zhu and Wu, 2018). corollary, the current data could point to BG supplementation having a positive impact on liver function and tissue peroxidation. Measurments related to immunity and antioxidant potential may also verify the beneficial impacts of BG on the health of growing rabbits that have been observed in fish (Pionnier *et al*., 2013; Ghaedi *et al*., 2015; Do Huu *et al*., 2016).

CONCLUSION

It could be concluded that β-glucans represent a promising factor for improving antioxidant capacity, immunity, and lipid profile as well as promoting growth performance of growing rabbits, without adverse effects on liver and kidney function and protein metabolism.

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فعالية الجلوكان في أداء النمو وحالة مضادات الأكسدة ومناعة الأرانب المتنامية

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هدفت هذه الدراسة إلى تحديد مدى تأثير الاضافة الغذائية لمركب بيتا جلوكان BG على كفائة النمو والمكونات البيوكيميائية للدم و كفائة مضادات اللأكسدة فى أرانب النيوزيلندي الابيض (NZW) النامية . تم استخدام إجمالي ٧٥ من إناث الأرانب بمتوسط وزن حي يبلغ ٨٤٥,٣٧ ± ٣٥,٢٣ جم وعمر ٦ أسابيع. تم تقسيم الأرانب عشوائياً إلى خمس مجموعات تجريبية (خمسة عشر أرنباً في كل مجموعة). تم تغذية المجموعة الضابطة (G1) بالنظام الغذائي الأساسي دون أي اضافات ، بينما تم تغذية المجموعات الاخرى (G2 و G3 و G4 و G5) بالنظام الغذائي الأساسي مع اضافة البيتا جلوكان بمستويات ٠,٥ و ١,٠ و ١,٥ و ٢,٠ جم / كجم. على التوالى. تم تقدير قياسات كفائة النمو والمكونات البيوكيميائية في الدم وإنزيمات مضادات الأكسدة ، وعلامات المناعة في أنسجة الكبد. أظهرت النتائج أن جميع مستويات اضافة البيتاجلوكان قد ادت الى تحسن الوزن النهائي والزيادة فى الوزن دون أي تأثير على استهلاك العلف ، والتحويل الغذائى ، والحيوية ، كما ادت الى انخفاض تركيز الجلوكوز والكوليسترول الكلي والدهون الثلاثية.كما اظهرت الدراسة عدم معنوية تاثير البيتا جلوكان على ال ALT و AST. بينما ادت اضافة البيتا جلوكان الى تحسن مستويات SOD و CAT و GPx ، وانخفضت مستويات MDA في أنسجة الكبد. و زادت النسبة المئوية لمستوى NBT و ACP فى مجموعات (G2-G5) مقارنة بالمجموعة الضابطة (G1). خلصت الدراسة الحالية إلى أن البيتا جلوكان يمثل عاملاً واعدًا لتحسين قدرة مضادات الأكسدة ، والمناعة ، ومستوى الدهون بالإضافة إلى تعزيز كفائة النمو للأرانب النامية ، دون آثار سلبية على وظائف الكبد والكلى وتمثيل البروتين.