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Pycnogenol and Its Nanoparticles: A Comparative Study of Their Effects on Growth, Gut Morphology, and mRNA Level of Lipid Metabolic Pathways and Antioxidant-Related Genes in Broilers

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Abstract

THIS STUDY aimed to find out how dietary supplementation with pycnogenol and its nanoparticles affected the growth performance, immune response, and antioxidant activity of broilers. In a 35-day feeding study, 150 one-day-old male avian chicks were randomly assigned to one of three nutrition regimens in three repetitions. The studied birds received a Pycnogenol free diet (Control), a Pycnogenol at 50 mg/kg diet (PYC), or a Nano-Pycnogenol at 50 mg/kg diet (Nano-PYC). At the end, Nano-Pycnogenol group demonstrated the highest growth performance among the groups followed by Pycnogenol group *($P < 0.05$)*. Concurrent with the improved duodenum and jejunum morphologies of the tested groups in comparison to control *($P < 0.05$)*. Additionally, there were significant decreases in triglyceride and very low-density lipoprotein levels (VLDL) and elevations in HDL cholesterol *($P < 0.05$)*. Furthermore, fatty acid synthetase (FAS), hormone-sensitive lipase (HSL), and lipoprotein lipase (LPL) levels and activities have been assessed, in addition to, super oxide dismutase (SOD), glutathione peroxidase (GPX), insulin like growth factor -1 (IGF-1), growth hormone (GHR) and myostatin mRNA expression levels and activities. All of these genes were found to be highly expressed in the Nano-PYC and PYC groups as compared to the control except FAS and Myostatin mRNA expression levels which demonstrated significantly lower levels, than the control group *($P < 0.05$)*. To sum up, giving a broiler a 50 mg/kg dose of Pycnogenol and its nanoparticles may improve the shape of its gut, and its immune system and antioxidant defences.

Keywords: Broilers, Pycnogenol, Nano- Pycnogenol, Growth performance, Health status.

Introduction

Over the past three decades, the poultry industry has been able to considerably boost both chicken production and consumption thanks to its capacity to deliver high-quality protein quickly while maintaining competitive prices. Broilers are therefore regarded as a crucial source of protein for humans [1].

Current massive poultry production, however, results in difficult rearing circumstances for broilers because of the temperature, high stocking density, immunological struggles, feed efficiency, shipping, and handling [2, 3]. Such stresses lead the chicken's body to produce more reactive oxygen species (ROS) and upset the balance of its antioxidant and oxidative defence mechanisms, resulting in oxidative stress and cellular damage, reducing farm animal production and development [4]. The negative effects of oxidative stress can be mitigated by giving enough

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antioxidants to chickens.

Medicinal herbs are often used as effective trace element supplements in animals' diet to promote feed stability as well as antioxidant's ability. Furthermore, they include bioactive chemicals with a wide spectrum of properties that could be applied in the pharmaceutical and food sectors [5-7]. They have recently gained popularity owing to the potential medicinal properties linked with their nutritional composition, which includes anthocyanins, flavonoids, vitamins, and polyphenols among other elements[8].

Pycnogenol® is a standardized compound of natural chemical derived from pine needles plants [9]. It includes a unique combination of procyanidins, a class of pharmacologically active biopolymers made up of catechin and epicatechin units, bioflavonoids, and phenolic acids, all of which have been demonstrated to have several health benefits [10], having complicated complimentary connections between its many constituents.

Over 40 years of research, Pycnogenol® which standardized to contain 70% procyanidins, has undergone significant investigation, with multiple scientific studies confirming its safety and effectiveness as a component of human alternative medicine and herbal medications. [11, 12]. In addition to procyanidins, Pycnogenol® contains catechin, taxifolin, and a number of phenolic compounds, including compounds of cinnamic and benzoic acids [13]. Two of the pharmacological advantages of Pycnogenol are its antioxidant and free radical scavenging activity, which results in ascorbic acid preservation and vitamin E recycling; along with inhibition of lipid peroxidation [14, 15].

Previous research has demonstrated that PYC's anti-inflammatory properties provide a positive preventive impact against atherosclerotic disease [16]. Additionally, PYC dramatically lowered cholesterol and triglyceride levels, according to clinical research. PYC supplementation reduced the triglyceride level in vitro by inducing lipolysis in adipocytes, suggesting that PYC may have an antiatherosclerotic impact via controlling lipid levels [17]. Nevertheless, the impact of PYC on lipid metabolic pathways has not received much attention in research.

Furthermore, nanotechnology is a novel technique that could be effective in nutraceutical research by improving the distribution and bioavailability of the investigated ingredient within the animal body. To the best of our knowledge, the effects of Nano-Pycnogenol in poultry have not yet been examined or compared to the organic form of Pycnogenol.

This study examined the effects of Pycnogenol, which is a pine needle procyanidin, and its nanoparticles as a natural feed additive on growth efficiency, blood biochemical parameters, liver enzyme activity, and lymphoid organ morphology. In addition, levels of mRNA expression were used to compare the effects of Pycnogenol and its nanoparticles on lipid metabolic pathways genes, anti-oxidant associated genes, and muscle growth-related genes in broilers to better understand the impact that Pycnogenol had on growth performance and antioxidant status. The current study could help the poultry industry enhance poultry production, anti-oxidant status, and general health.

Material and Methods

Ethical approval

The experimental procedures were authorised and supervised in accordance with the guidelines of the animal care and ethics committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

Materials

Pycnogenol® (PYC) is derived from the bark of maritime pine, a specific kind of pine tree growing primarily in southwest France (the Les Landes de Gascogne forest). In this study, PYC was bought from Horphag Research Co., Ltd. (Geneva, Switzerland). PYC is mostly composed of phenolic chemicals [18, 19] , with standardized procyanidins, a kind of flavonoid, accounting for around 65-75% [20]. Furthermore, it includes bioflavonoids, organic acids, and trace amounts of inorganic ions [21].

Graphite, tripolyphosphate (TPP) and ethanol were provided from Sigma-Aldrich. All of the compounds employed in the present study were at the reagent level, and none of them underwent any additional purification.

Nano-Pycnogenol preparation

Drop by drop, 3.2g of Pycnogenol® was immersed in a solution of acetic acid (1%) until a transparent solution appeared, then 150 mL of distilled water was incorporated with stirring and very little heat, The resulting liquid was passed through a filter, and 10 N of KOH was added drop by drop until the pH was adjusted to 4.7 to 5, and the resulting solution was sonicated for 20 minutes.

Characterization techniques

The dimension, structure, form, and crystal structure of Pycnogenol nano-particles were described using a variety of laboratory tools, including granular spectroscopy, a scanning electron microscope, and X-ray diffraction.

SEM analysis

Figure 1 gives the SEM Pycnogenol® composite. SEM at various magnification levels was used to confirm the loading of Pycnogenol® antioxidant on the outer layer of the nanosheets and chitosan

nanostructures, as shown in Figure 2. This confirms the loading of Pycnogenol on nanostructure materials.

Bird management, Experimental diets and Design

One day old male avian chicks (n=150) had been bought from a private breeder in Egypt's El-Gharbia Governorate. Each chick was weighed individually (mean body weight was 43±1.1 g) and checked for any health problems or deformities. Every group was subdivided into five replicates, each with ten chicks. For 35 days, the chicks were grown in an ecologically regulated space (10 chicks/m^2) at Kafrelsheikh University's chicken farm in Egypt. The room ambient temperature during the experiment was gradually decreased (3 °C each week) from 33 °C on day 1 to 21 °C at the 5th week of age.

Chicks were separated into three groups: The control group (Control) got a basal diet, the second group (PYC) got a basal diet supplemented with 50 PPM Pycnogenol® /kg diet, whereas the third group (Nano-PYC) group received a basal diet supplemented with 50 mg Nano-Pycnogenol /kg diet. The nutritional regimens were developed to match the dietary requirements for hens established by the National Research Council (NRC, 1994). All entire diets have been examined, and the data is shown in Table (1).

Throughout the experiment, the area's humidity was maintained at close to 70%. The light program was 24-hour light during the experimental period. In addition, the diet and drinking water were both freely available, with a starting diet for the initial 15 days of age, a grower diet for the third and fourth weeks of age, and finally a finisher feed during the final 5th week of age [22]. Birds received the Newcastle disease vaccine on days 7, 17, and 28 in the water. The Gumboro disease vaccination was delivered to the drinking water on the 14th day of life. The bird's management was carried out according to the guidelines set forth by the Faculty of Veterinary Medicine at Kafrelsheikh University in Egypt's Animal Care and Ethics Committee.

Diet preparation:

Experimental diets (starter, grower ,and finisher) were manufactured at the Nutrition and Clinical Nutrition Department at the Faculty of Veterinary Medicine, Kafrelsheikh University (Table 1).After the grinding process, the dietary ingredients were thoroughly mixed in a feed mixer for 15 min. The mixture was then passed through a meat grinder (TORNADO Meat Grinder 2000 Watt, Stainless Discs, Turbo Speed, White MG-2000) with an appropriate diameter (1.6–2.1 mm) to prepare pellets at room temperature. The pellets were air-dried in a dry air mechanical oven (VENTICELL, Medcenter, Einrichtungen GmbH) at 50°C for 3 hours then stored in a freezer at –20°C until use.

Growth performance measures

Using a sensitive weighing device (0.0001 g, Fisher Scientific), The body weights and weight gains of the chicks in each replication were recorded individually on day one and at every week of age until the end of the trial. the body weight and weight gain means were then computed for each group. The following formulas were used to determine the final body weight, weight gain, and feed conversion ratio, for each chicken after 35 days of feeding;

Total body weight gain (g) = final body weight (g) − initial body weight (g)

Feed intake (g) = given feed (g) – remaining feed (g)

Feed conversion ratio = feed intake (g) / weight gain (g)

The diet consumption of each replicate was tracked daily from the first day of life until the last day of the experiment. The diet consumption mean had been subsequently determined for each replicate and group for each week of age.

Histopathological examination

The abdomen was dissected, and tissue specimens from the duodenum, jejunum, of three birds per replicate (control, pycnogenol, and nano-pycnogenol) were randomly selected directly after euthanasia and cervical dislocation. All samples were fixed in Bouin's solution for 18 hours. Following fixation, the tissue samples were dehydrated using ethyl alcohol in increasing concentrations (from 70% to 100% alcohol), twice rinsed with xylene (1 and 2 h), and then embedded in paraffin. Sections of 5-µm thickness were gathered and stained with haematoxylin and eosin (H&E). From each tissue, two cross-sectional slices were made. The tissue sections were then stained with H&E before being examined using Sigma Scan Pro 5 software and a light microscope (Leica DM500) and camera (Leica EC3, Leica GmbH, Wetzlar, Germany) [23]. Villi length, villi width, crypt depth and Musclaris thickness, were measured using ImageJ analysis software with magnifications of 100 × , 200 × , and 400 × .Ten measurements were taken for every tissue in accordance with an established technique [23].

Blood sampling

At the end of the study, pooled blood samples were obtained from 5 randomly chosen birds from every replicate using wing vein penetration while the birds were gently restrained without anaesthesia. Collected blood sample (3 mL) was taken into a vacutainer tube without anti-coagulant for biochemical parameters determination, liver and kidney functions, lipid, albumin and globulin profiles in serum. After centrifuging blood samples at 3000 rpm for 15 minutes, serum was stored at -20 °C till

the biochemical examination was done.

Liver and Kidney Functions

The GOT kit from (Diamond Diagnostics, Egypt) measured glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), confirming the approach previously published by [42]. creatinine was determined using the techniques of [2].

Tissue sampling

Three birds were randomly chosen from each replication after 35 days of study, and they were euthanized, followed by cervical dislocation manually. Liver tissue was dissected and kept at −80 °C in liquid nitrogen for gene expression [15].

Gene Expression

RT-PCR was used to assess the expression of hepatic genes. Invitrogen, Life Technologies, Carlsbad, California, USA, provided the TRIzol reagent, which was used to extract total RNA from about 100 mg of liver tissue. A cDNA synthesis package (Fermentas, Waltham, MA, USA) was utilised to synthesise DNA from RNA samples, and quantification was also carried out by Nanodrop (Uv-Vis spectrophotometer Q5000/Quawell, USA). Then, using the SensiFASTTM cDNA synthesis kit (Bioline, UK), cDNA was created in accordance with the manufacturer's instructions.

SYBR Green QuantiTect PCR kits from Qiagen company were used to perform quantitative real-time PCR using the Rotor-Gene Q cycler. Each gene underwent relative expression of mRNA levels. The primers' sequences are shown in Table 2 along with the addition of the internal control gene (GAPDH) for cDNA amplification. The 2x SYBR Green PCR Master Mix (12.5 l), 0.25 l of Revert Aid Reverse Transcriptase (200 U/l), 0.5 l of each primer (20 pmol concentration), 8.25 l of water, and 3 l of RNA template made up the reaction mixture. The real-time PCR machine was utilised to perform the reaction. The primers were annealed at 59°C for 1 minute for the IL-10 gene, 60°C for 1 minute for the IL-1 and GHR genes, and 65°C for 1 minute for IGF-1, before being extended at 72°C for 30 seconds. The thermal cycling settings were as follows: initial denaturation at 95°C for 15 minutes for a total of 40 cycles, followed by first heat activation at 94°C for 15 seconds for a total of 40 cycles. Calculations were made of the relative fold changes in the analysed genes' mRNA expression as recorded by [24] using the $2^{-\Delta\Delta}$ Ct methods (Ct: cycle threshold).

Statistical analysis

The data gathered from all groups was statistically examined using statistical software (IBM SPSS for Windows, version 22, USA) in order to compare means with the control group. One-way analysis of variance (ANOVA) with Duncan testing was employed to assess differences between the means of the experimental groups. In order to show the significance between all groups, *A p value < 0.05* was used.

Results

Growth performance

Table 3 demonstrates that the use of Nano-Pycnogenol considerably *(P < 0.05)* increased the final body weight (2416.7 g/bird), and Pycnogenol (2350.8 g/bird) compared to control group (2263.3 g/bird). Similar to above result, Weight gain also demonstrated significant improvement using Nano-Pycnogenol (2373.6 g/bird), and Pycnogenol (2307.7g/bird) compared to control group. Furthermore, feed intake significantly increased in Nano-Pycnogenol (3194.5 g/bird), and Pycnogenol (3193.9 g/bird) treated groups *(P < 0.05)* compared to control group. On the other hand, feed conversion ratio was significantly enhanced in Nano-Pycnogenol treated group (1.35) than control and Pycnogenol treated groups *(P < 0.05)*.

Histopathological examination

The histological analysis of chicks in control group revealed regular structures of the duodenum and jejunum with four layers: tunica mucosa, lamina propria submucosa, tunica muscularis, and tunica serosa (Figs. 3 and 4). The incorporation of Pycnogenol and Nano-Pycnogenol to broiler diet revealed a significant change in villi length in both duodenum and jejunum compared to the control group, as well as improved structural and branching appearance of the intestinal villi *(P < 0.05)* (Table 4) (Figs. 3 and 4, C, D, E, F).

Haemato-biochemical indices

As shown in Table 5, There were no significant differences between the groups tested in total cholesterol or low-density lipoprotein (LDL) *(P > 0.05)*, but triglycerides and very low-density lipoprotein (VLDL) did, and chickens supplemented with a control diet reported the highest values, followed by the Pycnogenol and Nano-Pycnogenol groups, respectively *(P < 0.05)*. However, compared to the control group, both the Pycnogenol and Nano-Pycnogenol groups showed significantly higher levels of high-density lipoprotein (HDL) *(P < 0.05)*.

Liver and kidney function test

Serum glutamate-pyruvate transaminase (SGPT), glutamic-oxaloacetic transaminase (GOT), and serum creatinine levels, as well as other indicators of liver and kidney function in broilers fed the studied diets, demonstrated normal physiological values and did not show significant variations *(P > 0.05)* between the groups (Table 6).

Gene expression

The mRNA expression profiles of the tested genes

(SOD 1, SOD2, GPX1, LPL, FAS, HSP70, GHR, IGF-1, myostatin, and JNK genes) in poultry fed experimental diets for 35 days are shown in (Figures 5). Pycnogenol and Nano-Pycnogenol considerably enhanced the expression of the genes SOD1, SOD2, GPx1, muscle JNK, and hepatic LPL when compared to the control group *(P < 0.05)*. Moreover, hepatic FAS and muscle myostatin gene expression was significantly down-regulated in both the Pycnogenol and Nano-Pycnogenol groups *(P < 0.05)*. Nevertheless, no significant variations in HSP70, IGFI, or muscle GHR expression were found between the control and experimental groups *(P > 0.05)*.

Discussion

Various herbs contain naturally occurring polyphenolic compounds, which have traditionally been used by humans as a nutritional supplement. However, there has been a shortage of data on the impact of Pycnogenol as a natural herb containing pine needle procyanidin on poultry performance; hence, in this study, we explored the effects of two alternative sources of Pycnogenol, namely, Pycnogenol and Nano-Pycnogenol, in chicken diets. Broiler chicks administered Pycnogenol and Nano-Pycnogenol-supplemented diets had considerably better growth characteristics; this improvement could be attributed to improved intestinal structure, resulting in greater effectiveness and rapid digesting. The acquired results are comparable to those stated in prior studies' findings [25, 26]. Furthermore, it could be attributed to intestinal cells producing more intracellular protein, which might enhance feed breakdown and absorption [27, 28]. Therefore, the chemical components of these Pycnogenol forms may be beneficial as a dietary supplement for poultry growth. However, other investigations on the use of procyanidin containing products as feed additives for broiler chicks found no growth-promoting effects [29, 30], which could be linked to variations in housing, stress levels, and performance levels, as well as the type and dosage of procyanidin added to the poultry feed. However, additional research is required to verify these possibilities.

The intestine, which serves as the primary barrier between the host's internal and external environments, is highly specialised in the breakdown and absorption of nutrients. The integration of the digestive tract's immunological, digestive, and absorptive activities, as well as the capacity to control these functions, is crucial. A key method in determining the effectiveness of feed digestion and utilisation in the gut of chickens is the evaluation of gut morphology, which takes into account the conditions of the intestinal villi and the quantity of goblet cells [31, 32]. The power of the intestinal epithelium to absorb nutrients can be significantly improved as a result of improved length, width, and depth of the intestinal villus, which will ultimately result in better nutritional absorption and greater development [33].

While both the duodenum and jejunum are integral parts of the small intestine, they have distinct morphological features and physiological functions. The duodenum is primarily involved in the continuation of digestion, while the jejunum is specialized for nutrient absorption. This specialization is reflected in their structural differences, with the duodenum focusing on enzymatic activity and the jejunum maximizing nutrient uptake. According to the results of the current study's intestinal morphometric analysis, the addition of Pycnogenol and Nano-Pycnogenol to the diet of broilers caused substantial alterations in the villi length, villi width, crypt depth, and muscle layer thickness in the duodenum and jejunum (where the nutrient absorption take place) compared to the control group. This finding may be explained by the significance of Pycnogenol's proanthocyanidin content as a mediator for the health-promoting effects of the gut and the augmentation of several digestive enzymes, which are essential for improving gut shape and digesting capacity [34]. The observed improvement in gut morphology may be a sign that the birds fed diets supplemented with Pycnogenol and its nano particles had a stronger capacity to absorb and use nutrients. Furthermore, the higher improvement in gut morphology in the Nano-Pycnogenol fed group may be attributable to the Nano-Pycnogenol form's higher efficiency, which results in a greater absorption effect in tissues through fine blood vessels in addition to epithelial lining bumps, and increasing the shelf-life of the gut contents.

Haematology indicators are frequently used to assess general health; however, they might change depending on animal feed, diseases, and stressful circumstances [35-37]. During the current investigation, the examined broiler chicks exhibited normal values for several haematological parameters. Furthermore, feeding on the experimental diets had no effect on SGPT, SGOT, or serum creatinine concentration, demonstrating that Pycnogenol and Nano-Pycnogenol included in the diet were safe for poultry.

In the current study, considerable upregulation of genes (Muscle JNK, Hepatic SOD1, Hepatic SOD2, Hepatic GPX1, and Hepatic LPL) was seen in broiler chicks that given a diet supplemented with various Pycnogenol sources. While significant downregulation was detected in cases of hepatic FAS, and myostatin expression, however, the expression of the HSP70, GH, and IGF1 genes demonstrated non-significant differences. Lipolysis and lipogenesis are two lipid metabolism pathways in animals that collaborate to control fat accumulation in vivo [38]. Lipoprotein lipase (LPL) and fatty acid synthetase (FAS) are two enzymes that catalyse

various lipid metabolic pathways[39]. LPL is a member of the lipase family whose major function serves to supply tissues with non-esterified fatty acids (NEFA) and 2-monoacylglycerol by disintegrating triacylglycerol (TAG) in lipoprotein molecules ,which include chylomicrons (CM) and very low density lipoprotein (VLDL) [40]. During the catalytic process, LPL binds to the luminal surface of capillary endothelial cells, gaining a connection to the TAG and acting as a major enzyme in the breakdown of triacylglycerol and the absorption of free fatty acids from the plasma [41]. Furthermore, LPL enhancement was linked to a lower mRNA level of FAS. Several variables, including hormones, diet, and micronutrients, might influence FAS activity and mRNA levels [42-44] .

Superoxide dismutase (SOD), the most essential basic defense enzyme, removes superoxide anion free radicals through altering highly reactive O2 to low reactive H2O2, SOD thus acts as a crucial antioxidant defence against oxidative damage [45, 46]. In mammals, SOD comes in three forms: intracellular superoxide dismutase (SOD1), nuclear superoxide dismutase (SOD2), and extracellular superoxide dismutase (SOD3)[47]. By significantly increasing SOD 1 and SOD 2 expression, the use of Pycnogenol and its nano-particles as a feed additive improved the broiler's anti-oxidative status in this study. Plant-based extracts are known to contain secondary metabolic compounds with potent antioxidant properties [48]. The improved antioxidant capacity of chicks treated with essential oils, which regulate SOD [49], can be used to explain this. In addition, when tested broilers were given Pycnogenol, the expression of antioxidant enzymes like GPx increased significantly. This study backs with previous research on Pycnogenol and demonstrates the compound's efficiency as an antioxidant [50, 51].

Various growth-related genes in mammals have been identified, including IGF, IGFR, GH, and GHR [52-54]. Our findings led to a considerable improvement in the levels of hepatic IGF-I and GHR mRNA in poultry given feeds enriched via two Pycnogenol sources. IGF-1 from the liver affects how well growth and nutrient hormones work [55]. The pituitary gland produces growth hormone (GH), and after activating GH receptors, it increases the hepatic synthesis of IGF-1[56]. Previous research has demonstrated that consuming Pycnogenol could influence GHR and IGF-1 mRNA expression, which could be used to build a growth index in poultry livers [57]. Comparable results were published by [58],who discovered that utilizing procyanidins feed additives in poultry's diet improves growth performance and immunological status enhancement by boosting IGF-1 gene expression in the liver. The molecular switch known as c-Jun N-terminal kinase (JNK), when triggered, stimulates muscle fibre growth, hence increasing muscular mass [59]. When muscle JNK activation is inhibited, a new remodelling process is initiated, which leads to smaller, more oxidative muscle fibers and increased aerobic fitness [26].

Consequently, the upward regulation of hepatic GHR, IGF-I, and c-Jun N-terminal kinase (JNK) mRNA and downregulation of myostatin expression levels in birds fed Pycnogenol nourished treatments could be attributed to the growth-promoting effects of the bioactive components included in Pycnogenol, particularly thymol and phytol [60]. Furthermore, Procyanidins have the ability to connect with cell plasma membranes, protecting Caco-2 cells from bile acid-induced cytotoxicity, rises in cell oxidants, and alterations in tight junction protein distribution as well as barriers strength [61, 62].

Conclusions

In conclusion, it has been demonstrated that Pycnogenol and its nanoparticles participate in the cellular antioxidant system and have the ability to alter the expression of genes that are controlled by cell redox status. Our research also suggests these feed additives, especially nano-forms, may help broiler chicks enhance their growth performance, and gut health. Therefore, our findings provide strong support for the use of Pycnogenol and its nano-form as a feed supplement to enhance growth, protect gut health, and improve oxidative status in broilers.

Conflict of Interest

The authors had no conflict of interest in this study.

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Author`s contributions:

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Aya Elattar, Amr I. Zaineldin, Sahar Elnaggar, Mostafa Shukry, Foad A. Farrag, Eldsoky Nassef, Abdelnasser Bakr, and Sayed Hegazi. The first draft of the manuscript was written by Aya Elattar, Amr Zaineldin, and Sayed Hegazi. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

TABLE 1.Ingredients and chemical composition of the experimental diets (as fed basis)*

Ingredients %	**Starter**	**Grower**	**Finisher**
Ground yellow corn (8% CP)	56.52	60.13	64.72
Soya bean meal (46% CP)	32.57	28.87	24.16
Corn gluten (60% CP)	4.8	4.8	4.5
Soybean oil	1.8	2.26	3
L-Lysine (purity 99%)	0.4	0.34	0.34
DL-methionine (purity 99%)	0.15	0.12	0.11
Limestone (38% Ca)	1	0.8	0.7
Di-calcium-phosphate (22% Ca and 19% P)	1.955	1.925	1.765
Iodized sodium chloride	0.5	0.45	0.4
Vitamin and mineral premix**	0. 3	0.3	0.3
Pycnogenol	0.005	0.005	0.005
Nutrients %			
Metabolizable energy (kcal/kg diet)	3024	3100	3196
Crude protein (%)	23	21.5	19.5
Calorie/protein ratio (*C/P*)	131.48	144.19	163.9
Lysine %	1.44	1.29	1.17
Methionine %	0.56	0.51	0.47
Calcium %	1	0.91	0.82
Available phosphorus	0.46	0.44	0.4
Sodium	0.23	0.20	0.18

*Formulated according to Avian nutrition specifications [22], and Chemical analysis was performed according to AOAC (AOAC 1994).

**Premix Supplied per kg of premix: *trans*-retinol(A), 12,500,000 IU; cholecalciferol(D3), 500,000 IU; α-tocopherol acetate(E), 75,000 mg; thiamine(B1), 4500 mg; riboflavin(B2), 8000 mg; pyridoxine(B6), 5000 mg; vitamin B12, 22,000 mg; pantothenic acid, 20,000 mg; folic acid, 2000 mg; biotin, 200,000 µg; Fe, 100,000 mg; Co,250 mg; Mn, 100 mg; Cu, 10,000 mg; Zn, 80,000 mg; I, 1000 mg; Se, 300 mg; Mo, 0.5 mg; Ca, 7.7%; P, 0.01%; Na, 0.18%; Ash, 97%.

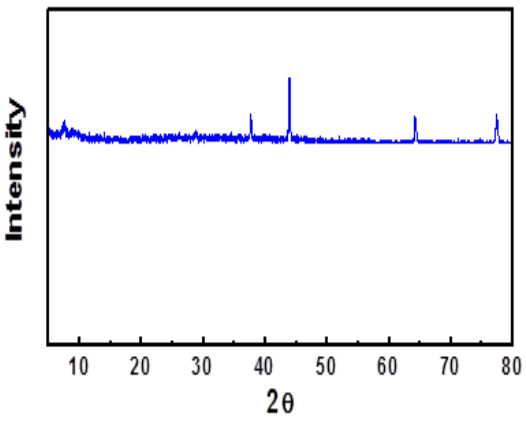

Fig. 1. XRD pattern of Pycnogenol

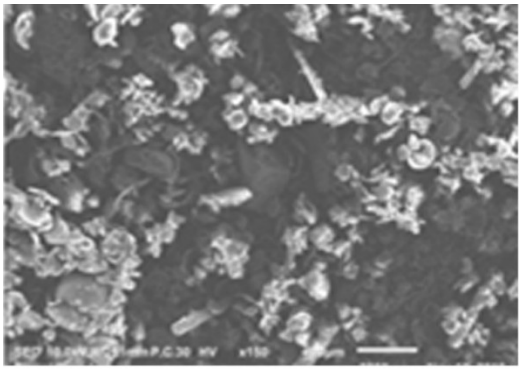

Fig. 2. SEM images of NPs Pycnogenol

TABLE 2.Primers used for cloning and q-PCR *

Primer name	Primer sequences (5′ to 3′)	Accession Number
GAPDH	F: GGTGAAAGTCGGAGTCAACGG	NM_204305
	R: CGATGAAGGGATCATTGATGGC	
SOD1	F: TGGCTTCCATGTGCATGAAT	NM_205064
	R: AGCACCTGCGCTGGTACAC	
SOD2	F: GCTGGAGCCCCACATCAGT	NM_204211
	R: GGTGGCGTGGTGTTTGCT	
GPX1	F: TCCCCTGCAACCAATTCG	NM_001277853
	R: AGCGCAGGATCTCCTCGTT	
LPL	F: TTGGTGACCTGCTTATGCTA	NM_205282
	R: TGCTGCCTCTTCTCCTTTAC	
FAS	F: CCAACGATTACCCGTCTCAA	J03860
	R: CAGGCTCTGTATGCTGTCCAA	
IGF-1	F: CATTTCTTCTACCTTGGC	M32791
	R: TCATCCACTATTCCCTTG	
Myostatin	F: GGGACGTTATTAAGCAGC	NM 001001461
	R: ACTCCGTAGGCATTGTGA	
GHr	F: GTTCTCCCCCTGAGAACAGT	AJ506750
	R: GTGCCCAAGGGCATATAGCA	
HSP70	F: GGGAGAGGGTTGGGCTAGAG	JO2579
	R: TTGCCTCCTGCCCAATCA	
JNK1	F: GCCGATGATCAGCCAGGAT	NM_205095
	R: GGCCCAATGGAAGCAAGAG	

*Internal reference gene (GAPDH, house-keeping gene), SOD 1, superoxide dismutase type 1; SOD2, superoxide dismutase type 2; GPX 1, glutathione peroxidase type 1; LPL, lipoprotein lipase; FAS, fatty acid synthase; IGF, insulin-like growth factor type 1; GHr, growth hormone receptors; HSP70,heat shock protein 70; JNK1, C-Jun N-terminal kinase type 1.

TABLE 3.Growth performance parameters of broiler chickens fed test diets for 35 days*

Parameters	Test diets		
	Control	**Pycnogenol**	**NanoPycnogenol**
Initial Body Weight (g)	42.99±0.04	43.04±0.03	43.1±0.05
Final Body Weight (g)	2263.3±26.7 ^{a}	2350.8±8.0 ^{b}	2416.7±20.1 ^{b}
Body Weight Gain (g/bird)	2220.3±26.7 ^{a}	2307.7±8.0 ^{b}	2373.6±20.1^{b}
Feed Intake (g/bird)	3084.6±14.1 ^{a}	3193.9±8.1 ^{b}	3194.5±16.2 ^{b}
Feed conversion ratio	1.39±0.01 ^{b}	1.38±0.01 ^{b}	1.35±0.007 ^{a}

* Values are means of five replicates(n=5) ± S.E.M. Values with different superscript (a, b, c) differ significantly *(P < 0.05)*. Within a row, means with the same superscripts are not significantly different *(P > 0.05)*.

TABLE 4. Micromorphology of the intestine of broiler chickens fed test diets for 35 days*

Items	Test diets			
	Parameters	**Control**	**Pycnogenol**	**NanoPycnogenol**
Duodenum	Villus height (µm)	785.1±18.6[a]	814.8±6.7[ab]	892.8±46.1[b]
	Villus width (µm)	198.2±7.5[a]	283.4±20.3[b]	323.3±35.2[b]
	Crypt depth (µm)	105.1±11.3[a]	155.8±10.1[b]	156.7±1.5[b]
	Musclaris thickness (µm)	211.9±3.4[a]	330.8±6.4[b]	314.7±16.05[b]
Jejunum	Villus height (µm)	1021.9±11.1	1026.18±14.7	1047.3±50.7
	Villus width (µm)	188.18±15.2[a]	297.8±36.6[ab]	319.7±20.3[b]
	Crypt depth (µm)	120.6±18.6[a]	137.7±8.1[a]	201.8±14.35[b]
	Musclaris thickness (µm)	193.6±7.3[a]	192.3±1.3[a]	276.1±17.3[b]

* Data represent means±pooled SEM. Values with different superscript (a, b, c) differ significantly (P < 0.05). Values with the same letter are not significantly different *(P > 0.05)*.

TABLE 5. Blood biochemical indices of broiler chickens fed test diets for 35 days*

Parameters	Test diets		
	Control	**Pycnogenol**	**NanoPycnogenol**
Triglyceride (mg/dL)	80.75 ±11.9 [b]	61.2 ±5.1[ab]	43.2 ±5.4 [a]
Total Cholesterol (mg/dL)	92.25 ±10.5	96.64 ±11.1	89.28 ±12.3
HDL (mg/dL) **	26 ±1.1 [a]	29 ±0.6 [b]	29.6 ±0.6 [b]
LDL (mg/dL) ***	54.1 ±7.9	54.4 ±7.9	51.04 ±4.15
VLDL (mg/dL) ****	16.15 ±2.4 [b]	12.24 ±1.01 [ab]	8.64 ±1.1 [a]
Total Protein (g/dL)	2.7±0.06 [a]	3.52±0.13 [b]	3.52±0.04 [b]
Albumin (g/dL)	0.85±0.03	1.08±0.05	1.04±0.05
Globulin (g/dL)	1.85±0.04 [a]	2.44±0.12 [b]	2.48±0.06 [b]

*Values are means of five replicates(n=5) ± S.E.M. Different lowercase letters refer to significant differences (*P < 0.05*). Within a row, means with the same superscripts are not significantly different (*P* > 0.05).
** HDL: High density lipoprotein
*** LDL: Low density lipoprotein
**** VLDL: very low-density lipoprotein.

TABLE 6. Liver and kidneys functions tests of broiler chickens fed test diets for 35 days*

Parameters	Test diets		
	Control	**Pycnogenol**	**NanoPycnogenol**
SGOT**(U/L)	138.7±14.7	124.3±31.1	136±6.7
SGPT***(U/L)	15.3±2.8	19.3±3.4	17.7±3.4
Serum Creatinine (mg/dL)	0.91±0.04	0.93±0.02	0.88±0.05

*Values are means of five replicates(n=5) ± S.E.M. Different lowercase letters refer to significant differences *(P < 0.05)*. Within a row, means with the same superscripts are not significantly different *(P > 0.05)*. ** SGOT: Serum glutamic oxaloacetic transaminase. ***SGPT: Serum glutamic pyruvic transaminase.

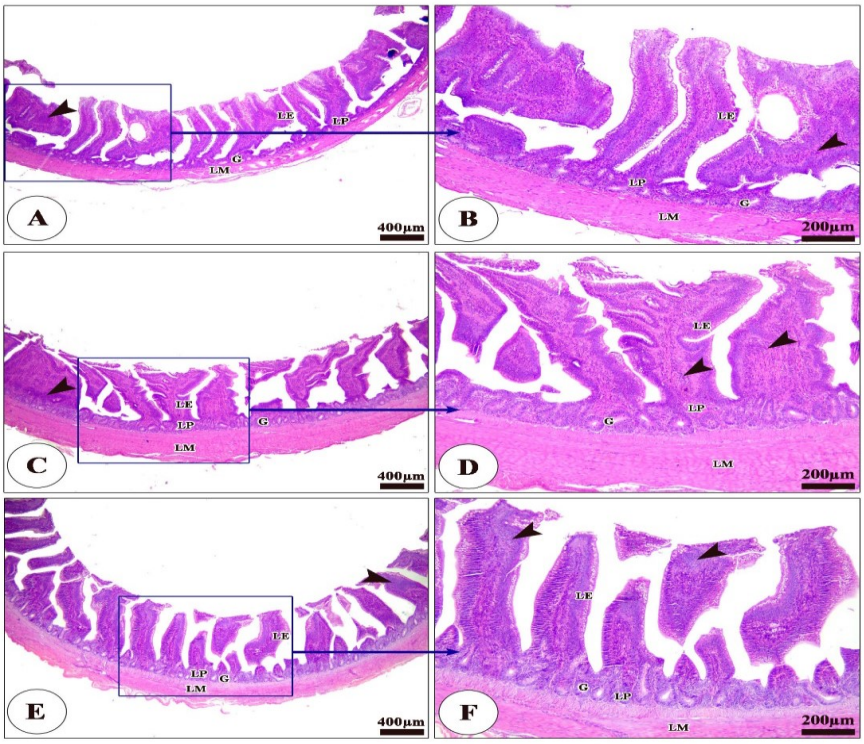

Fig. 3. Photomicrographs of histological H&E-stained duodenum sections of chickens; Control (A&B), Pycnogenol (C&D) and NanoPycnogenol (E&F) showing intact lamina epithelialis of intestinal villi (LE), lamina propria (LP), intestinal mucosal glands (G), tunica muscularis (LM), diffuse (arrow heads) and localized (arrows) lymphoid elements. Scale bar = 400 and 200 µm.

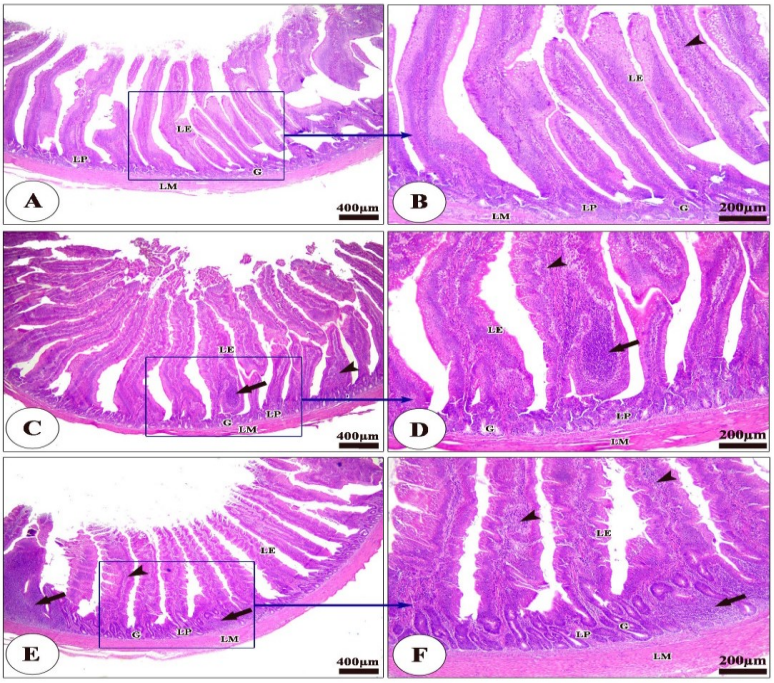

Fig. 4. Photomicrographs of histological H&E-stained jejunum sections of chickens; Control (A&B), Pycnogenol (C&D) and NanoPycnogenol (E&F) showing intact lamina epithelialis of intestinal villi (LE), lamina propria (LP), intestinal mucosal glands (G), tunica muscularis (LM) and diffuse lymphoid elements (arrow heads). Scale bar = 400 and 200 µm.

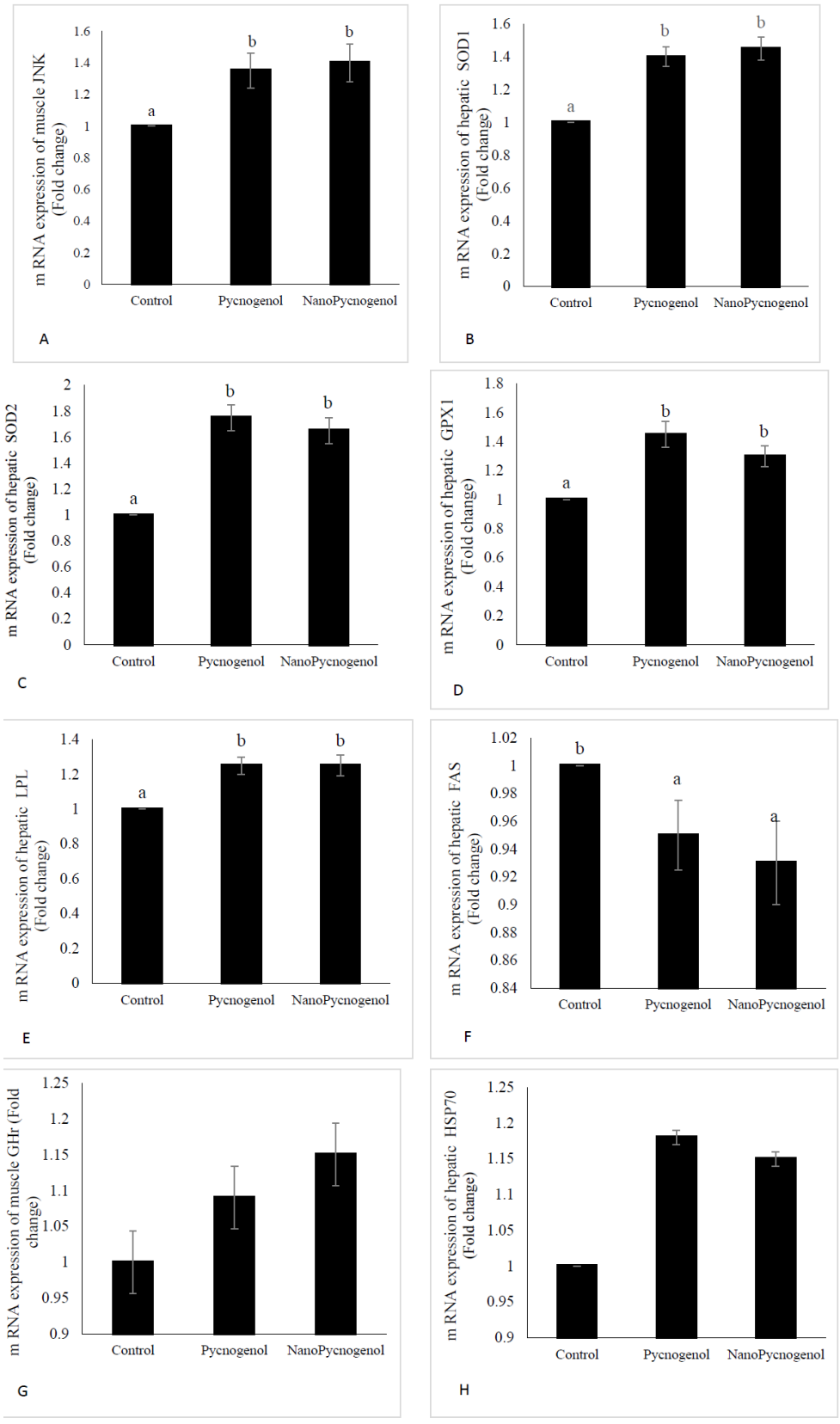

Fig. 5. fold change of muscle c-jun N-terminal kinase 1 (A), SOD1 superoxide dismutase 1 (B), SOD2 superoxide dismutase 2 (C), glutathione peroxidase (gpx1) (D), lipoprotein lipase (LPL) (E), fatty acid synthase (FAS) (F), growth hormone receptors (GHr) (G), HSP70 (H), Myostatin (I), and insulin-like growth factor (IGF-1) (J) in broilers, after supplementation with Pycnognol and NanoPycnognol. Data represent means ± pooled SEM. Values with different letters are significantly different (*P*<0.05). Values with the same letter are not significantly different (*P*>0.05).

References

1. Abdel-Moneim, A.-M.E., Shehata, A.M., Khidr, R.E., Paswan, V.K., Ibrahim, N.S., El-Ghoul, A.A., Aldhumri, S.A., Gabr, S.A., Mesalam, N.M. and Elbaz, A.M. Nutritional manipulation to combat heat stress in poultry–A comprehensive review. *Journal of Thermal Biology* ,**98**, 102915 (2021).
2. Bartle, K. D. Identification of polychlorobiphenyls by high resolution proton magnetic resonance. *J. Assoc. Off. Anal. Chem.,* 1101-1103 55 (1972).
3. Brandtzaeg, P. Mucosal Immunity: Induction, Dissemination, and Effector Functions. *Scandinavian Journal of Immunology,* **70**, 505-515 (2009).
4. Brenes, A., Montoro, A.V., Cambrodón, I.G., Centeno, C., Calixto, F.S. and Arija, I. Effect grape seed extract on growth performance, protein and polyphenol digestibilities, and antioxidant activity in chickens. *Spanish Journal of Agricultural Research,* 326-333(2010).
5. Chang, C.L. Lipoprotein lipase: new roles for an 'old' enzyme. *Current Opinion in Clinical Nutrition and Metabolism Care,* **22**, 111-115 (2019).
6. Chen, Q.L., Luo, Z., Wu, K., Huang, C., Zhuo, M.Q., Song, Y.F. and Hu, W. Differential effects of dietary copper deficiency and excess on lipid metabolism in yellow catfish Pelteobagrus fulvidraco. *Comparative Biochemistry and Physiology, part B Biochemical and Molecular Biology*,**184**, 19-28 (2015).
7. Cires, M.J., Wong, X., Carrasco-Pozo, C. and Gotteland, M. The Gastrointestinal Tract as a Key Target Organ for the Health-Promoting Effects of Dietary Proanthocyanidins. *Frontiers in Nutrition*, **3**(3), 57 (2017).
8. D'Andrea, G. Pycnogenol: A blend of procyanidins with multifaceted therapeutic applications?. *Fitoterapia*, **81(7)**, 724-736 (2010).
9. Da Silva, M., Jaggers, G.K., Verstraeten, S.V., Erlejman, A.G., Fraga, C.G. and Oteiza, P.I. Large procyanidins prevent bile-acid-induced oxidant production and membrane-initiated ERK1/2, p38, and Akt activation in Caco-2 cells. *Free Radical Biology and Medicine,* **52**, 151-159 (2012).
10. De-Santis, C. and Jerry, D.R. Candidate growth genes in finfish—Where should we be looking? *Aquaculture,* **272**, 22-38 (2007).
11. Devlin, R.H., Sakhrani, D., Tymchuk, W.E., Rise, M.L. and Goh, B. Domestication and growth hormone transgenesis cause similar changes in gene expression in coho salmon (Oncorhynchus kisutch). *Proceedings of the National Academy of Sciences,* **106**, 3047-3052 (2009).
12. Erlejman, A.G., Fraga, C.G. and Oteiza, P.I. Procyanidins protect Caco-2 cells from bile acid- and oxidant-induced damage. *Free Radical and Biological Medicine*, **41**, 1247-1256 (2006).
13. Fukai, T. and Ushio-Fukai, M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxidant Redox Signal*, **15**, 1583-1606 (2011).
14. Ghaffari, T., Kafil, H.S., Asnaashari, S., Farajnia, S., Delazar, A., Baek, S.C., Hamishehkar, H. and Kim, K.H. Chemical composition and antimicrobial activity of essential oils from the aerial parts of Pinus eldarica grown in Northwestern Iran. *Molecules,* **24**, 3203 (2019).
15. Gharib-Naseri, K., de Las Heras-Saldana, S., Kheravii, S., Qin, L., Wang, J. and Wu, S.B. Necrotic enteritis challenge regulates peroxisome proliferator-1 activated receptors signaling and β-oxidation pathways in broiler chickens. Anim Nutr., **1**,239-251(2021). doi: 10.1016/j.aninu.2020.08.003. Epub.
16. González-Quilen, C., Rodríguez-Gallego, E., Beltrán-Debón, R., Pinent, M., Ardévol, A., Blay, M.T. and Terra, X. Health-Promoting Properties of Proanthocyanidins for Intestinal Dysfunction. *Nutrients*, **12,**130 (2020).
17. Ho, J.N., Kim, O.K., Nam, D.E., Jun, W. and Lee, J. Pycnogenol supplementation promotes lipolysis via activation of cAMP-dependent PKA in ob/ob mice and primary-cultured adipocytes. *Journal of Nutritional Science and Vitaminology* (Tokyo), **60**, 429-435 (2014).
18. Ighodaro, O.M. and Akinloye, O.A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, **54**, 287-293 (2018).
19. Ishtiaq, S., Hanif, U., Shaheen, S., Bahadur, S., Liaqat, I., Awan, U.F., Shahid, M.G., Shuaib, M., Zaman, W. and Meo, M., Antioxidant potential and chemical characterization of bioactive compounds from a medicinal plant Colebrokea oppositifolia Sm. *Anais da Academia Brasileira de Ciências,* **92**(2), e20190387 (2020).
20. Kareem, K.Y., Loh, T.C., Foo, H.L., Akit, H. and Samsudin, A.A. Effects of dietary postbiotic and inulin on growth performance, IGF1 and GHR mRNA expression, faecal microbiota and volatile fatty acids in broilers. *BMC Veterinary Research*, **12**, 163 (2016).
21. Kim, B., Lee, T.K., Park, C.W., Kim, D.W., Ahn, J.H., Sim, H., Lee, J.C., Yang, G.E., Kim, J.D., Shin, M.C., Cho, J.H., Ryoo, S., Kim, Y.M., Won, M.H. and Park, J.H. Pycnogenol $^{(®)}$ Supplementation Attenuates Memory Deficits and Protects Hippocampal CA1 Pyramidal Neurons via Antioxidative Role in a Gerbil Model of Transient Forebrain Ischemia. *Nutrients,* **12**(15), 4592 (2020).

22. Kim, J., Chehade, J., Pinnas, J.L. and Mooradian, A.D. Effect of select antioxidants on malondialdehyde modification of proteins. *Nutrition*, **16**, 1079-1081(2000).

23. Laron, Z. Insulin-like growth factor 1 (IGF-1): a growth hormone. *Molecular Pathology*, **54**, 311-316 (2001).

24. Laudadio, V., Passantino, L., Perillo, A., Lopresti, G., Passantino, A., Khan, R. and Tufarelli, V. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. *Poultry Science,* **91**, 265-270 (2012).

25. Lauriano, E., Pergolizzi, S., Capillo, G., Kuciel, M., Alesci, A. and Faggio, C. Immunohistochemical characterization of Toll-like receptor 2 in gut epithelial cells and macrophages of goldfish Carassius auratus fed with a high-cholesterol diet. *Fish & Shellfish Immunology*, **59**, 250-255 (2016).

26. Lee, J., Kang, H.K., Cheong, H. and Park, Y. A Novel Antimicrobial Peptides from Pine Needles of Pinus densiflora Sieb. et Zucc. Against Foodborne Bacteria. *Frontiers in microbiology*, **12**, 662462-662462 (2021).

27. Lee, O.-H., Seo, M.-J., Choi, H.-S. and Lee, B.-Y. Pycnogenol® Inhibits Lipid Accumulation in 3T3-L1 Adipocytes with the Modulation of Reactive Oxygen Species (ROS) Production Associated with Antioxidant Enzyme Responses. *Phytotherapy Research*, **26**, 403-411 (2012).

28. Lessard, S.J., MacDonald, T.L., Pathak, P., Han, M.S., Coffey, V.G., Edge, J., Rivas, D.A., Hirshman, M.F., Davis, R.J. and Goodyear, L.J. JNK regulates muscle remodeling via myostatin/SMAD inhibition. *Nature Communication*, **9**, 3030 (2018).

29. Li, X. and Bi, X. Integrated Control of Fatty Acid Metabolism in Heart Failure. *Metabolites*, **13**, 615 (2023).

30. Lourenço, S.C., Moldão-Martins, M. and Alves, V.D. Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules* (Basel, Switzerland), **24**, 4132 (2019).

31. Maharjan, P., Martinez, D.A., Weil, J., Suesuttajit, N., Umberson, C., Mullenix, G., Hilton, K.M., Beitia, A. and Coon, C.N. Review: Physiological growth trend of current meat broilers and dietary protein and energy management approaches for sustainable broiler production. *Animal*, **15**, 100284 (2021).

32. Mead, J.R., Irvine, S.A. and Ramji, D.P. Lipoprotein lipase: structure, function, regulation, and role in disease. *Journal of Molecular Medicine* (Berl), **80**, 753-769 (2002).

33. Michalak, M. Plant-Derived Antioxidants: Significance in Skin Health and the Ageing Process. *International Journal of Molecular Science,* **23**(2), 585 (2022).

34. Mishra, B. and Jha, R. Oxidative stress in the poultry gut: potential challenges and interventions. *Frontiers in Veterinary Science*, **6**, 60 (2019).

35. Mülek, M., Seefried, L., Genest, F. and Högger, P. Distribution of Constituents and Metabolites of Maritime Pine Bark Extract (Pycnogenol®) into Serum, Blood Cells, and Synovial Fluid of Patients with Severe Osteoarthritis: A Randomized Controlled Trial. *Nutrients*, **9**, 443 (2017).

36. NRC, -. N.R.C., 1994. Nutrient requirements of poultry. *National Academy Press Washington,* DC.

37. Ortega-Ramirez, L.A., Rodriguez-Garcia, I., Leyva, J.M., Cruz-Valenzuela, M.R., Silva-Espinoza, B.A., Gonzalez-Aguilar, G.A., Siddiqui, M.W. and Ayala-Zavala, J.F. Potential of medicinal plants as antimicrobial and antioxidant agents in food industry: a hypothesis. *Journal of Food Science*, **79**, R129-R137 (2014).

38. Packer, L., Rimbach, G. and Virgili, F. Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (pinus maritima) bark, pycnogenol. *Free Radical Biology and Medicine,* **27**, 704-724 (1999).

39. Park, J.C., Lee, S.H., Hong, J.K., Cho, J.H., Kim, I.H. and Park, S.K. Effect of dietary supplementation of procyanidin on growth performance and immune response in pigs. *Asian-Australas Journal of Animal Science,* **27**, 131-139 (2014).

40. Poznyak, A.V., Bharadwaj, D., Prasad, G., Grechko, A.V., Sazonova, M.A. and Orekhov, A.N. Anti-Inflammatory Therapy for Atherosclerosis: Focusing on Cytokines. Int *Journal of Molecular Science,* **22** (13),7061(2021).

41. Qu, D., Zhou, X., Yang, F., Tian, S., Zhang, X., Ma, L. and Han, J. Development of class model based on blood biochemical parameters as a diagnostic tool of PSE meat. *Meat Science*, **128**, 24-29 (2017).

42. Reitman, S. and Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.,* **28**, 56-63 (1957).

43. Rehman, Z.u., Chand, N. and Khan, R.U. The effect of vitamin E, L-carnitine, and ginger on production traits, immune response, and antioxidant status in two broiler strains exposed to chronic heat stress. *Environmental Science and Pollution Research*, **24**, 26851-26857 (2017).

44. Rubió, L., Motilva, M.-J. and Romero, M.-P. Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles. *Critical Reviews in Food Science and Nutrition*, **53**, 943-953 (2013).

45. Ruff, J., Tellez, G., Jr., Forga, A.J., Señas-Cuesta, R., Vuong, C.N., Greene, E.S., Hernandez-Velasco, X., Uribe Á, J., Martínez, B.C., Angel-Isaza, J.A., Dridi, S., Maynard, C.J., Owens, C.M., Hargis, B.M. and Tellez-Isaias, G. Evaluation of Three Formulations of Essential Oils in Broiler Chickens under Cyclic Heat Stress. *Animals (Basel)*, **11**(4), 1084(2021).

46. Saponaro, C., Gaggini, M., Carli, F. and Gastaldelli, A. The Subtle Balance between Lipolysis and Lipogenesis: A Critical Point in Metabolic Homeostasis. *Nutrients,* **7**, 9453-9474 (2015).

47. Shamblott, M.J., Cheng, C.M., Bolt, D. and Chen, T.T. Appearance of insulin-like growth factor mRNA in the liver and pyloric ceca of a teleost in response to exogenous growth hormone. *Proceedings of the National Academy of Sciences*, **92**, 6943-6946 (1995).

48. Simpson, T., Kure, C. and Stough, C. Assessing the Efficacy and Mechanisms of Pycnogenol(®) on Cognitive Aging From In Vitro Animal and Human Studies. *Frontiers in Pharmacology*, **10**, 694-694 (2019a).

49. Simpson, T., Kure, C. and Stough, C. Assessing the Efficacy and Mechanisms of Pycnogenol(®) on Cognitive Aging From In Vitro Animal and Human Studies. *Frontiers in Pharmacology*, **10**, 694 (2019b).

50. Solis de los Santos, F., Donoghue, A.M., Farnell, M.B., Huff, G.R., Huff, W.E. and Donoghue, D.J. Gastrointestinal maturation is accelerated in turkey poults supplemented with a mannan-oligosaccharide yeast extract (Alphamune). *Poultry Science,* **86**, 921-930 (2007).

51. Swallah, M.S., Sun, H., Affoh, R., Fu, H. and Yu, H. Antioxidant Potential Overviews of Secondary Metabolites (Polyphenols) in Fruits. *International Journal of Food Science,* **2020**, 9081686 (2020).

52. Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Emwas, A.-H. and Jaremko, M. Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules (Basel, Switzerland),* **25**, 5243 (2020).

53. Verlaet, A., van der Bolt, N., Meijer, B., Breynaert, A., Naessens, T., Konstanti, P., Smidt, H., Hermans, N., Savelkoul, H.F.J. and Teodorowicz, M. Toll-Like Receptor-Dependent Immunomodulatory Activity of Pycnogenol®. *Nutrients,* **11**, 214 (2019).

54. Yang, J.Y., Zhang, H.J., Wang, J., Wu, S.G., Yue, H.Y., Jiang, X.R. and Qi, G.H. Effects of dietary grape proanthocyanidins on the growth performance, jejunum morphology and plasma biochemical indices of broiler chicks. *Animal,* **11**, 762-770 (2017).

55. Younus, H. Therapeutic potentials of superoxide dismutase. *International Journal of Health Science (Qassim)*, **12**, 88-93 (2018).

56. Yuan, S., Wang, Y., Zhang, C., He, H. and Yu, S. Genetic dissection of seed dormancy using chromosome segment substitution lines in rice (Oryza sativa L.). *International Journal of Molecular Sciences,* **21**, 1344 (2020).

57. Zhang, H., Xu, L., Yue, H., Wu, S., Pan, Y., Wei, S. and Wang, J. Effect of grape procyanidins on growth performance and immune function in broilers. *China Animal Husbandry & Veterinary Medicine*, **39**, 99-103 (2012).

58. Zhang, Z., Tong, X., Wei, Y.-L., Zhao, L., Xu, J.-Y. and Qin, L.-Q. Effect of Pycnogenol Supplementation on Blood Pressure: A Systematic Review and Meta-analysis. *Iranian Journal of Public Health*, **47**, 779-787 (2018).

59. Zhu, X., Tian, X., Yang, M., Yu, Y., Zhou, Y., Gao, Y., Zhang, L., Li, Z., Xiao, Y., Moses, R.E., Li, X. and Zhang, B. Procyanidin B2 Promotes Intestinal Injury Repair and Attenuates Colitis-Associated Tumorigenesis via Suppression of Oxidative Stress in Mice. *Antioxidant Redox Signal*, **35**, 75-92 (2021).

البيكنوجينول وجزيئاته النانوية: دراسة تأثيرها على النمو وصحة الأمعاء ومستوى mRNA لمسارات التمثيل الغذائي للدهون والجينات المرتبطة بمضادات الأكسدة في دجاج التسمين

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الملخص

هدفت هذه الدراسة إلى معرفة كيف أثرت المكملات الغذائية التي تحتوي على البيكنوجينول وجزيئاته النانوية على أداء النمو والاستجابة المناعية ونشاط مضادات الأكسدة لدى دجاج التسمين. في دراسة تغذية استمرت 35 يومًا، تم توزيع 150 فرخًا ذكرًا من الطيور بعمر يوم واحد بشكل عشوائي على أحد ثلاثة أنظمة غذائية في ثلاث تكرارات. تلقت الطيور محل الدراسة نظامًا غذائيًا خاليًا من البيكنوجينول (مجموعة التحكم)، أو البيكنوجينول بتركيز 50 جزء في المليون/كجم (مجموعة PYC)، أو البيكنوجينول النانو بتركيز 50 جزء في المليون/كجم (مجموعة Nano-PYC). في النهاية، أظهرت مجموعة البيكنوجينول النانو أعلى أداء للنمو بين المجموعات تليها مجموعة البيكنوجينول. بالتزامن مع تحسن مورفولوجيا الاثني عشر والصائم للمجموعات المختبرة مقارنةً بالمجموعة الضابطة. بالإضافة إلى ذلك، كانت هناك انخفاضات كبيرة في مستويات الدهون الثلاثية والبروتين الدهني منخفض الكثافة للغاية (VLDL) وارتفاعات في كوليسترول البروتين الدهني عالي الكثافة. وعلاوة على ذلك، قمنا بتقييم مستويات وأنشطة إنزيم تخليق الأحماض الدهنية (FAS)، والليباز الحساس للهرمون (HSL)، وليبوبروتين الليباز (LPL)، بالإضافة إلى مستويات وأنشطة إنزيم أكسيداز الفائق (SOD)، والجلوتاثيون بيروكسيديز (GPX)، وعامل النمو المشابه للأنسولين -1 (IGF-1)، وهرمون النمو (GHR) ومستويات وأنشطة التعبير عن mRNA للميوستاتين. وقد وجد أن كل هذه الجينات معبر عنها بشكل كبير في مجموعتي Nano-PYC وPYC مقارنة بالمجموعة الضابطة باستثناء مستويات التعبير عن mRNA للميوستاتين وFAS والتي أظهرت مستويات أقل بكثير من المجموعة الضابطة ($P < 0.05$). باختصار، إن إعطاء الدجاج اللاحم جرعة مقدارها 50 جزء في المليون/كجم من البيكنوجينول وجزيئاته النانوية قد يحسن شكل أمعائه، ووظائف أعضائه الليمفاوية، ونظامه المناعي ودفاعاته المضادة للأكسدة.

الكلمات الدالة: الدجاج اللاحم؛ البيكنوجينول؛ نانو- بيكنوجينول؛ أداء النمو؛ الحالة الصحية.