Buletin Peternakan 48 (2): 193-199, August 2024

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v48i3.91801

The Effect of Nanoencapsulated *Citrus sinensis* Peel Extract in Drinking Water on Growth Performance of Broiler Chicken

Hanggara Haidar Azmi[1], Zuprizal[1], Nanung Danar Dono[1], Ahmad M Abdel-Mageed[2], Heru Sasongko[3], and Bambang Ariyadi[3*]

[1]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, 55281, Indonesia
[2]Department of Immunology and Physiology, Faculty of Science, Minia University, 61519, Egypt
[3]Department of Animal Production, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, 55281, Indonesia

Article history
Submitted: 12 December 2023
Accepted: 22 March 2024

* Corresponding author:
E-mail: bambang.ariyadi@ugm.ac.id

ABSTRACT

This research aimed to investigate the characteristics of nanoencapsulated *Citrus sinensis* peel extract (NCSPE) applied in drinking water and its effect on broiler chickens' growth performance and jejunal histomorphology. The total of 192 Male Broiler chicken was distributed into six treatments with four replicates (Eight birds each replicate). The experimental treatments were control diet and drinking water added with no treatment (T0; negative control), tetracycline 50 mg/l (T1; positive control), CSPE (*Citrus sinensis* peel extract) 1,5%(T2), CSPE 3% (T3), NCSPE 1,5% (T4), and NCSPE 3% (T5). The variables evaluated in this research were characteristics of NCSPE, growth performance. Data were analyzed using ANOVA in a completely randomized design. All data with a significant difference were then testedagain using the Duncan Test with a probability of less than 5%. Results showed that the size of NEPM was 13,70 nm with a spherical shape and negative charges with zeta potentials of -13.37 mV. Supplementing 1,5% and 3% of NCSPE affects on feed intake, weight gain, and feed conversion ratio. It concluded that both 1,5% and 3% supplementation of NCSPE had a positive effect on growth performances.

Keywords: Broiler, Citrus sinensis peel extract, Nanoencapsulation

Introduction

Antibiotic Growth Promoters (AGP) have been widely known for their application to achieve higher production traits of broiler chicken. AGPs contain an antibacterial effect that helps minimize the damage done by the bacteria that affect the development of the small intestine during rearing. The use of AGP has been prohibited by the Ministry of Agriculture, the Republic of Indonesia, and most other country due to the residual effect that may potentially harms the consumer and also other potential health risk (Yakhkeshi *et al*., 2011).

The alternative resource has been studied in many research to replace the use of AGP such as phytobiotics, which uses natural ingredients. Plant material such as herbs, spices, and their extracted form (essential oil) may promote growth rate, feed intake, increase the secretion of endogenous enzymes, and display antibacterial effects (Lee *et al*., 2015; Kim *et al*., 2016; Gheisar and Kim, 2018; Aljumaah *et al*., 2020).

Citrus sinensis (sweet orange) is one of the tropical fruits, which is rich in polyphenols that serve as antioxidant and antimicrobial properties. The peel parts of the fruits are often discarded due to their non-edible parts, however, it contains more phenolic acids than their juice and pulp approximately 50-100% (Zhang *et al*., 2012; Zhu *et al*., 2020; Wang *et al*., 2023). The extraction process of *Citrus sinensis* peel bioactive compounds is necessary to draw out their antioxidant and antimicrobial properties known as polyphenols. The extracted polyphenols can be used as feed additives. The *Citrus sinensis* peel extract (CSPE) has low bioavailabilty when used for feed additives due to the sensitiveness to several factor such as temperature, acidic pH, oxygen,and microbiota in the intestinal (Enaru *et al*., 2021). Furthermore, a necessary technique were needed to increase the bioavailability of these compound to boost the efficacy when administered as feed additives.

Nanotechnology was used to increase polyphenol extract bioavailability (Sharif *et al*., 2017). Nanoencapsulation could be defined as the technology of packaging nanoparticles known as the core within the secondary material or coating agent as a matrix or shell to form nanocapsules (Cano-Sarabia and Maspoch, 2015). The biological polymer used as its material is usually made of albumin, agarose, alginate, chitosan, gelatin. Crosslinking agents such as Sodium tripolyphosphate (STPP) and formaldehyde were also used to increase the matrix stability, preventing aggregation. Chitosan and STPP are widely used material in nanoencapsulation due to its low toxicity and it is relatively safe to use (Augustia *et al*., 2018; Mora-huertas *et al*., 2010; Reis *et al*., 2006). Various methods can be used to create nanoparticles, The ionotropic gelation method was one of the simplest methods to obtain nanoencapsulation through a high-speed rotation as well as the interaction between chitosan and STPP (Shah *et al*., 2016; Debnath *et al*., 2011).

The research goal was to determine nanoencapsulation of *Citrus sinensis* peel extract characterization and its effect on Broiler chicken growth performance. Nanoencapsulation was supplemented through drinking water to increase CSPE consumption and their antibacterial effect. The antibacterial factor would decrease the risk of potential damage in the gastrointestinal tract and improve the growth perfomance of chickens.

Materials and Methods

Animal, diets, and experimental design

All experimental procedures applied in this research were approved by the Research Ethics Committee of Veterinary Medicine Faculty, Gadjah Mada University with number 038/EC-FKH/Eks./2022.

The research was conducted at the Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. Total of 192 Indian River male broiler chickens were distributed into six treatments and four replicates with eight birds in each replicate pen. Each pen was provided with a feeder and a drinking water trough. The basal diets were formulated to meet the nutrition recommendations of broiler chickens from NRC (1994). The basal diet consist of cornbean meal and soybean meal. The chemical composition of basal diet shown in Table 1.

The diets and drinking water were supplied in ad libitum from days 8 to 35. The first seven days of broiler chicks were considered a critical phase, as such the treatment was given after 7 days to ensure the gastrointestinal and digestive tract had developed and well functioned. The treatments used in this research consisted of the basal diet and drinking water with no treatment (T0; negative control), tetracycline 50 mg/L (T1; positive control), 1,5% CSPE (T2), 3% CSPE (T3), 1,5% NCSPE (Nanoencapsulated *Citrus sinensis* Peel Extract) (T4), and 3% NCSPE (T5).

Nanoencapsulation

The nanoencapsulation process of *Citrus sinensis* peel extract was formulated using the ionic gelation method (Shah *et al*., 2016; Debnath *et al*., 2011). The formulation of 5% CSPE, 2% Chitosan, and 0,5% STTP were the concentration used in this research. The extraction process begin by separating the peels from their pulps and content with only their flavedo and albedo part remains. The peels were dried in the oven at 55^{0}C temperature for one day. The dried peel was then sieved and macerated using ethanol 70% for three days. The filtrate was filtered and evaporated using a water bath. The extract was collected and used as part of the nanoencapsulation process.

The nanoencapsulation method used in this research was the ionotropic gelation method. The nanoencapsulation process began by dissolving 5% of aqueous extract of CSPE with 2% chitosan which has been dissolved using 2% acetic acid. Both extract and chitosan were stirred using a magnetic stirrer under 600 rpm speed for 30 min. 0,5% aqueous STTP was added to the solution and then stirred continuously for another 30 min.

Characterization of nanoencapsulation

Particle size and zeta potential. Particle size and zeta potential were determined using the Dynamic Scattering Light (DLS) method. Five mL of NCSPE was analyzed for particle size with a Particle Size Analyzer (PSA) (Horiba Scientific SZ – 100, Horiba, Kyoto, Japan). The evaluation was done using a scattering angle of 90°C with a temperature holder of 24.8°C according to Liang *et al*. (2017).

Nanoencapsulation morphology. Structure of NCSPE morphologically was evaluated using Transmission Electron Microscopy (TEM) (JEOL JEM 1400 Plus, Jeol, Peabody, USA). The samples were prepared by dropping solutions into Copper grids coated with carbon using the auto carbon coated for 5 min, prior to the samples drying. The samples were stayed in the copper network for 2–3 min. The samples were then immersed in 2% phosphotungstic acid stain and stained for 2–3 min. After natural drying, the samples were placed under TEM for observation according to Liang *et al*. (2017).

Broiler growth performance

Feed intake, feed conversion ratio (FCR), and water intake were recorded daily. The body weight gain of broiler chicken was weighed weekly. Feed intake and water intake ratio (F:W) were also recorded. On day 35, a chicken from each different replicates was randomly selected to slaughter according to Islamic law.

Statistical analysis

The characteristic of NCSPE (Nanoencapsulated *Citrus sinensis* peel extract) was analyzed descriptively for particle size, zeta potential, and morphology. Data of growth performance were expressed as mean.The data were statistically analyzed by one-way ANOVA with a completely randomized design, using Statistical Package for Social Science or SPSS (SPSS GmbH, Munich, Germany). Data with a significant difference were then tested again using the Duncan Test. All indication of significance was based on a probability of less than 5%.

Table 1. Ingredients and chemical composition of the broiler chicken basal diet

Ingredients	Amount (%)	CP (%)	ME (kcal/kg)	Ca (%)	Pav (%)	Lys (%)	Met (%)	Thr (%)
Yellow corn	58.50	4.93	1926.18	0.01	0.13	0.15	0.10	0.17
Soybean meal	28.50	13.43	974.99	0.08	0.19	0.84	0.19	0.53
Meat bone meal	7.00	3.61	150.50	0.72	0.36	0.05	0.05	0.12
Rice brand	2.60	0.25	74.36	0.00	0.01	0.01	0.00	0.01
Crude palm oil	2.00	0.00	31.86	0.00	0.00	0.00	0.00	0.00
Vitamin premix*	0.25	0.00	0.00	0.08	0.00	0.00	0.00	0.00
L-lysine HCL	0.20	0.00	0.00	0.00	0.00	0.16	0.00	0.00
DL-methionine	0.20	0.00	0.00	0.00	0.00	0.00	0.18	0.00
CaCO3	1.00	0.00	0.00	0.38	0.00	0.00	0.00	0.00
NaCl	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	100.0	22.23	3157.89	1.27	0.72	1.35	0.53	0.84

CP= Crude protein; ME= Metabolizable Energy, Ca= Calcium; Pav= Available Phosphor; Lys= Lysine; Met= Methionine; Thr= Threonine. Chemical compositions of the basal diet determined in a laboratory analysis basedon AOAC (2005) and applied result for nutritious composition.*Vitamin premix chemical composition was Vit. A=12,500,000 IU; Vit. D3= 2,500,000 IU; Vit. E= 10000 mg; Vit. K3= 2000 mg; Vit. B1= 2000 mg; Vit. B2= 4000 mg; Vit. B6= 1000 mg; Vit. B12= 12000 mcg; Vit. C= 40000 mg; Niacin= 40000 mg; Biotin= 200 mg.

Results and Discussion

Nanoencapsulation characterization

The characteristic of NCSPE was obtained by using the ionic gelation method, the detailed value of NEPM characteriscs is shown at Table 2. DLS observation of NCSPE showed a particle size distribution of 12,40 nm. Nanoencapsulation of *Citrus sinensis* peel extract (NCSPE) particle size was observed at 13,70 nm, which met the criteria of nanoparticle size. Smaller particle size also leads to better dissolution behavior due to the increase in surface area to volume ratio of nanoparticles (Pateiro *et al*., 2021). Nanoparticle size of chitosan and STPP was influenced by chitosan and STPP concentrations, molecular weight of chitosan material, chitosan STPP mass ratio, the medium pH, and the stirring speed of medium (Oudih *et al*., 2022). The distribution of nanoparticle size shown in Figure 1.

Table 2. Nanoencapsulation characteristics

Parameter	Value
Particle size	12.40
Polidispersity Index	0.342
Zeta potential	-13.37

The zeta potential of NCSPE was negative charges with a value of -13,37 mV. The stability of the nanocapsule was deemed relatively sufficient (Honary and Zahir, 2013).This result was different with Collado-Gonzalez *et al*. (2017) statement that chitosan-based nanomaterial would yield a positive zeta due to their ability to stabilize negative zeta surface charges, however similar result with this research using chitosan-based nanomaterial also reported that both pH and substances inside nanoparticle plays a role in zeta potential surface charge, effectively changes the positive zeta value into negative zeta value (Iswanti *et al*., 2019; Ramanery *et al*., 2013). Ghasemi *et al*. (2017) also added that the zeta potential was affected by several factors such as ionic strength, pH, type and concentration of coating materials and the ratio between them, effective amount of surface charges, and electrophoretic mobility.

The polydispersity index was observed at 0,342. The polydispersity index value under 0,7 was potrayed as a narrowly and uniformly distributed nanoparticle, which implies higher nanoparticle stability (Danaei *et al*., 2018). The NCSPE of TEM image displayed a stable formulation with a spherical shape (Figure 2). Nanoparticle of chitosan-based material usually form a spherical shape (Di santo *et al*., 2021). Martien *et al*. (2012) added that non-spherical shape of nanoparticle tends to break down and easy to aggregate. These characteristics of nanoparticle with the formulation of 5% CSPE, 2% Chitosan, and 0,5% STTP was believed to be optimal and may improve the bioavailability of the CSPE when administered to broiler's chicken.

Growth performance

Feed intake. The analysis of variance results shown that there was a significance difference ($p<0.05$) on feed intake. The supplementation of NCSPE at 3% (T5) increases the feed intake of broiler chicken. Abdulameer (2018) reported a similar result using a dietary 2% dried *Citrus sinensis* peel to improve the feed intake

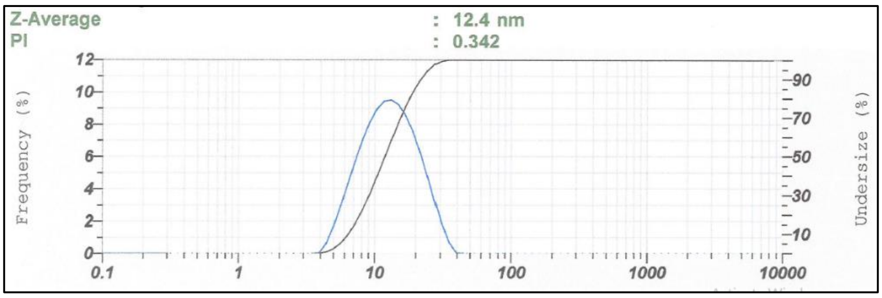

Figure 1. Graph of NCSPE nanoparticle size distribution.

intake of broiler chicken during the grower period (16-28 d). The flavonoid content within the CSPE plays a role on increasing the feed intake of broiler chicken's and the nanoencapsulation technique (NCSPE) was also evidently plays role as a carrier that increase bioavailability of the extract when administered. The flavonoid from plant based material such as peel has an effect on increasing feed intake of livestock (Goliomytis *et al*., 2014). The antioxidant effect of the flavonoid improve the body condition from oxidative stress due to the condition such as heat. The broiler chicken's under heat stress may exhibit a decreased feed intake and also increased the water intake to regulate their body heat. Heat stress also activates the chicken hypothalamus, pituitary adrenal axis, resulting in elevated serum corticosterone, which in turn decreases food intake, body weight gain, relative immune organ weight, and innate immunity (Mishra and Jha, 2019). The increased feed intake may have been affected by the antioxidant effect of CSPE which contain flavonoid.

Water intake and F:W ratio. The analysis of variance results shown that there was a significance difference ($p<0.05$) on Water. The feed intake and water intake ratio data however revealed that the supplementation of NCSPE decreased water intake per feed consumed with T0 having the highest value. The NCSPE which contains CSPE might be the reason for decreased water intake because of tannin contents inside the extract that made bitter taste in the drinking water (Sudarman *et al*., 2012).

Weight gain. The analysis of variance results shown that there was a significance difference ($p<0.05$) on weight gain parameter. The NCSPE supplementation both T4 and T5 shown a significance difference compared to T0 (control). Majekodunmi *et al*. (2021) also found a similar result where supplementing sweet citrus peel powder in drinking water increase the body weight gain of broiler chickens. The polyphenols contents such as flavonoid and phenolic compound in *Citrus sinensis* peel has an antibacterial effect that reduce microbial activity by inhibiting bacterial cell wall synthesis, exert a change in cell membrane permeability, inhibition of bacterial metabolism, and inhibition of nucleic acid and protein synthesis of the bacteria. Phytochemicals of polyphenol also contain antioxidant, which scavenge free radicals caused by ROS (Reactive Oxygen Species) under oxidative stress and may potentially damage the cell tissue (Marin *et al*., 2015; Li *et al*., 2021). The NCSPE supplementation improve the chicken's gastrointestinal health from pathogen bacteria and oxidative stress due to their antibacterial and antioxidant. Improved feed nutrients absorption in gastrointestinal leads to a better weight gain of Broiler chicken's.

Feed conversion ratio. The analysis of variance results shown that there was a significance difference ($p<0.05$) on Feed conversion ratio (FCR). The supplementation of NCSPE yields a significant result at T4, thus supplementation of 1,5% NCSPE decrease feed conversion ratio which indicates a better feed efficiency. Previous research found that adding a flavonoid-based diet has improved the feed conversion ratio of broiler chicken (Batista *et al*., 2007). Hassan *et al*. (2019) also found that polyphenol-based diet has a positive impact on feed conversion ratio. Majekodunmi *et al*. (2021) also reported an improvement of broiler chicken's feed conversion ratio after supplementation 6 g of sweet citrus peel powder through drinking water. Nanoencapsulation increases bioavailability of CSPE, the CSPE antibacterial effect hinder the growth and reduce the pathogen bacteria thus

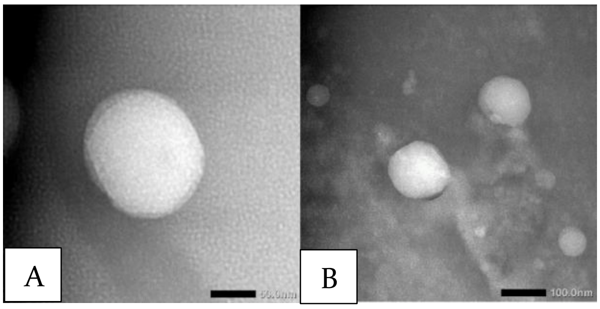

Figure 2. Nanoparticle Morphology of NCSPE under TEM Analysis (A: TEM under 50.00 nm Magnification; B: TEM under 100.00 nm magnification).

Table 3. The performance of broiler chickens supplemented with treatments

Variables	Treatments						SEM	P-Value
	T0	T1	T2	T3	T4	T5		
Feed intake*	3114.78^a	3047.46^a	3016.43^a	3128.46^a	3013.55^a	3337.06^b	32.98	0.02
Final body weight*	2026.25	2143.75	2085.25	2090.00	2090.00	2188.75	19.52	0.236
Weight gain*	1878.12^a	2024.23^b	2037.69^b	2044.12^b	2043.58^b	2143.68^b	22.73	0.017
Feed conversion ratio**	1.66^b	1.51^a	1.48^a	1.53^a	1.48^a	1.56^{ab}	0.019	0.022
Water intake	8151.50^b	7473.07^a	7386.08^a	7109.75^a	7440.87^a	8185.39^b	1.02	0.001
F:W ratio***	2.62^b	2.45^{ab}	2.45^{ab}	2.27^a	2.47^b	2.45^{ab}	0.03	0.016

T0= Negative control without feed additive, T1= Positive control with antibiotic tetracycline 50 mg/l, T2= CSPE 1.5%, T3= CSPE 3%, T4= NCSPE 1.5%, T5= NCSPE 3%.

^{a,b} Means with different superscript in the same row differ significantly. *g/bird/35 d, **L/birds/35 d; ***Feed intake : Water intake ratio.

improving intestinal health and also improving nutrient absorption of broiler chicken (Rashidinejad and Jafari, 2020).

Final body weight. The analysis of variance results shown that there was no significance difference ($p<0.05$) on final body weight. The supplementation of NCSPE did not improve final body weight in this research compared to other treatment. Several previous studies with *Citrus sinensis* peel supplementation as feed additive in broiler chicken reported similar results (Agu *et al*., 2010; Akbarian *et al*., 2013; Ebrahimi *et al*., 2014). The growth response of chickens to feed additives might be attributed by several other factors, such as diet feed, animal age, hygiene, and environmental factors (Amad *et al*., 2011).

Conclusion

In conclusion, the results shown that NCSPE could be beneficially used as analternative AGP for broiler chickens.The supplementation of 1,5% and 3% NCSPE could significantly increase feed intake, weight gain, and improve feed conversion ratio.

Conflict of interest

The researchers state that no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

Funding statement

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Abdulameer, Y. S. 2018. The effects of dietary vitamin C and *Citrus sinensis* peel on growth, hematological characteristics, immune competence, and carcass characteristics in broilers exposed to heat stress. Iraqi J. Vet. Sci. 32: 253-260.

Agu, P. N., O. Oluremi, and C. D. Tuleun. 2010. Nutritional Evaluation of Sweet Orange (*Citrus sinensis*) Fruit Peel as a Feed Resource in Broiler Production. Int. J. Poult. Sci. 9: 684-688.

Akbarian, A., A. Golian, A. Gilani, H. Kermanshal, S. Zhaleh, A. Akhavan, S. De Smet, and J. Michaels. 2013. Effect of feeding citrus peel extracts on growth performance, serum components, and intestinal morphology of broilers exposed to high ambient temperature during the finisher phase. Livest. Sci. 157: 490-497.

Aljumaah, M. R., G. M. Suliman, A. A. Abdullatif, and A. M. Abudabos. 2020. Effects of phytobiotic feed additives on growth traits, blood biochemistry, and meat characteristics of broiler chickens exposed to Salmonella typhimurium. J. Poult. Sci. 11: 5744-5751.

Amad, A. A., K. Männer, K. R. Wendler, K. Neumann, and J. Zentek. 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poult. Sci. 90: 2811-2816.

Augustia, V. A. S., M. Musdzalifah, D. A. Lestari, and A. Chafidz. 2018. Effect of Sodium Tripolyphosphate on The Characteristics of Anthocyanin Microcapsules Extracted from Purple Sweet Potato (*Ipomoea batatas* L.). Pages 020056-1 – 020056-6 in AIP Conference Proceedings 2049. Universitas Islam Indonesia (UII), Indonesia.

Batista, L. S., E. A. Garcia, A. B. G. Faitarone, M. R. Sherer, C. Mori, K. Pelicia, and C. C. Pizzolante. 2007. Flavonoids and Mannanoligosaccharides in Broiler Diets. Braz. Rev. J. Poult. Sci. 9: 33-37.

Cano-Sarabia, M. and D. Maspoch. 2015. Nanoencapsulation. In: Bhushan, B. (eds) Encyclopedia of Nanotechnology. Springer, Dordrecht.

Collado-gonzalez, M., M. G. Montalban, J. Pena-Garcia, and H. Perez-Sanchez. 2017. Chitosan as stabilizing agent for negatively charged nanoparticles. Carbohydr. Polym. 161: 63-70.

Danaei, M., M. M. Dehghankhold, S. Ataei, F. Hasanzadeh Davarani, R. Javanmard, A. Dokhani, S. Khorasani, and M. R. Mozafari. 2018. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics 10: 57.

Debnath, S., R. S. Kumar, and M. N. Babu. 2011.Ionotropic Gelation – A Novel Method to Prepare Chitosan Nanoparticles. Research J. Pharm. Tech. 4: 492-495.

Di Santo, M. C., C. L. D' Antoni, A. P. Domínguez Rubio, A. Alaimo, and O. E. Pérez. 2021. Chitosan-tripolyphosphate nanoparticles designed to encapsulate polyphenolic compounds for biomedical and pharmaceutical applications - A review. Biomed. Pharmacother. 142: 1-24.

Ebrahimi, A., A. A. A. Qotbi, A. Seidavi, and B. Bahar. 2014. The effects of dietary supplementation of Citrus sinensis peel extract on production and quality parameters of broiler chicken. J. Appl. Anim. Res. 42: 445-450.

Enaru, B., S. Socaci, A. Farcas, C. Socaciu, C. Danciu, A. Stanila, and Z. Diaconeasa. 2021. Novel delivery systems of polyphenols and their potential health benefits. Pharmaceuticals (Basel) 14: 946.

Ghasemi, S., S. M. Jafari, E. Assadpour, and M. Khomeiri. 2017. Production of pectin-whey protein nano-complexes as carriers of orange peel oil. Carbohydr. Polym. Food Hydrocoll. 177: 152–162.

Gheisar, M. M. and I. H. Kim. 2018. Phytobiotics in poultry and swine nutrition. Ital. J. Anim. Sci. 17: 92-99.

Goliomytis, M., D. Tsoureki, P. E. Simitzis, M. A. Charismiadou, A. L. Hager Theodorides, and S. G. Deligeorgis. 2014. The effects of quercetin dietary supplementation on broiler growth performance, meat quality, and oxidative stability. J. Poult. Sci. 93: 1957-1962.

Hassan, F. M., E. M. Roushdy, A. T. W. Kishawy, A. W. Ziglool, H. A. Tukur, and I. M. Saadeldin. 2019. Growth performance, antioxidant capacity, lipid- related transcript expression and the economics of broiler chickens fed different levels of rutin. Animals 9: 7-11.

Honary, S. and F. Zahir. 2013. Effect of zeta potential on the properties of nano-drug delivery systems - A Review (Part 1). Trop. J. Pharm. Res. 12: 255-264.

Iswanti, F. C., I. Nurulita, S. Djauzi, M. Sadikin, A. B. Witarto, and T. Yamazaki. 2019. Preparation, characterization, and evaluation of chitosan-based nanoparticles as CpG ODN carriers. Biotechnol. Biotechnol. Equip. 33: 390-396.

Kim, S., K. Lee, C. Kang, and B. An. 2016. Growth performance; relative meat and organ weights; cecal microflora and blood characteristics in broiler chickens fed diets containing different nutrient density with or without essential oils. Asian- Australas. J. Anim. Sci. 29: 549-554.

Lee, K., J. Kim, S. Oh. C. Kang, and B. An. 2015. Effects of dietary sanguinarine on growth performance; relative organ weight; cecal microflora; serum cholesterol level and meat quality in broiler chickens. J. Poult. Sci. 52: 15-22.

Liang, J., H. Yan, X. Wang, Y. Zhou, X. Gao, P. Puligundla, and X. Wan. 2017. Encapsulation of Epigallocatechin Gallate in Zein/Chitosan Nanoparticles for Controlled Application in Food Systems. Food Chem. 231: 19-24.

Li, L., X. Sun, D. Zhao, and H. Dai. Pharmacological Applications and Action Mechanisms of Phytochemicals as Alternatives to Antibiotics in Pig Production. Front. Immunol. 12: 1-18.

Majekodunmi, B. C., M. O. Lugunleko, E. O. Adekunie, M. O. Abioja, O. F. Akinjunte, T. O. Owalabi, and J. O. Daramola. 2021. Evaluation of sweet citrus peel supplement in water on performance and ileal microbial count of broiler chicken. Trop. Health. Anim. Prod. 53: 405.

Marin, L., E. M. Miguelez, C. J. Villar, and F. Lombo. 2015. Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. BioMed Res. Int. 2015: 1-18.

Martien, R., A. Adhyatmika, V. Farid and D. P. Sari. 2012. Technology developments nanoparticles as drug delivery systems. Majalah Farmaseutik 8: 133-144.

Mishra, B. and R. Jha. 2019. Oxidative stress in the poultry gut: potential challenges and interventions. Front. Vet. Sci. 6: 1-5.

Mora-huertas, C. E., H. Fessi, and A. Elaissari. 2010. Polymer-based nanocapsules for drug delivery. Int. J. Pharm. 385: 113-142.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry: Ninth Revised Edition. The National Academies Press. Washington, DC.

Oudih, S. B., D. Tahtat, A. N. Khodja, M. Mahlous, Y. Hammache, Abd-Errahim Guittoum, and S. K. Ghana. 2022. Chitosan nanoparticles with controlled size and zeta potential. Polym. Eng. Sci. 63: 1-11.

Pateiro, M., B. Gomez, P. E. S. Munekata, F. J. Barba, P. Putnik, D. B. Kovacevi, and J. M. Lorenzo. 2021. Nanoencapsulation of promising bioactive compounds to improve their absorption, stability, functionality and the appearance of the final food products. Molecules 26: 1547.

Ramanery, F. P., A. A. P. Mansur, and H. S. Mansur. 2013. One-step colloidal synthesis of biocompatible water- soluble ZnS quantum dot / chitosan nanoconjugates. Nanoscale Res. Letters 8: 1-13.

Rashidinejad, A. and S. M. Jafari. 2020. Nanoencapsulation of Bioactive food Ingredients. In: Handbook of Food Nanotechnology: Application and Approaches. S.M. Jafari (ed). Academic Press, Cambridge, United States, pp. 279-344

Reis, C. P., R. K. Neufeld, A. J. Ribeiro, and F. Veiga. 2006. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomed.: Nanotechnol. Biol. Med. 2: 8-21.

Shah, B. R., L. Yan, W. J. Weiping, A. Yaping, H. Lei, L. Zhenshun, X. Wei, and L. Bin. 2016. Preparation and optimization of pickering emulsion stabilized by chitosan tripolyphosphate nanoparticles for curcumin encapsulation. Food Hydrocoll. 52: 369-377.

Sharif, M. K., Faiz-ul-Hassan Shah, M. S. Butt, and H. R. Sharif. 2017. Role of Nanotechnology. In: Enhancing Bioavailability and Delivery of Dietary Factors. In: Nutrient Delivery. Academic Press, Cambridge, pp. 587-618.

Sudarman, A., Sumiati and R. Kanidewi. 2012. Performance of broiler chickens offered drinking water contained water extracted beluntas (*Pluchea indica* L.) leaf and sugar cane. Media Peternakan 35: 117-122.

Wang, Z., X. Mei, X. Chen, S. Rao, T. Ju, J. Li, and Z. Yang. 2023. Extraction and recovery of bioactive soluble phenolic compounds from brocade orange (*Citrus sinensis*) peels:

Effect of different extraction methods thereon. Food Sci. Technol. 173: 1-11.

Yakhkeshi, S., S. Rahimi and K. Gharib Naseri 2011. The effects of comparison of herbal extracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. J. Med. Plants 10: 80-95.

Zhang, J. K., C. D. Sun, Y. Y. Yan, Q. J. Chen, F. L. Luo, X. Y. Zhu, X. Li, and K. S. Chen. 2012. Purification of naringin and neohesperidin from Huyou (*Citrus changshanensis*) fruit and their effects on glucose consumption in human HepG2 cells. Food Chem. 135: 1471-1478.

Zhu, C. H., X. Y. Zhou, C. R. Long, Y. X. Du, Li, J. J. Yue and S. Y. Pan. 2020. Variations of flavonoid composition and antioxidant properties among different cultivars, fruit tissues and developmental stages of citrus fruits. Chem. Biodivers. 17, Article e1900690.