Tikrit Journal for Agricultural Sciences
ISSN:1813-1646 (Print); 2664-0597 (Online)
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq
DOI: https://doi.org/10.25130/tjas.26.2.10

TJAS
Tikrit Journal for Agricultural Sciences

Influence of Dietary Supplementation with Radish Seed Oil on Performance, Blood Biochemical Parameters, and Bacterial Count of Broiler Chickens

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KEY WORDS:
Performance, gut health, essential oil

Received: 16/10/2025
Revision: 28/12/2025
Proofreading: 09/01/2025
Accepted: 16/12/2025
Available online: 30/06/2026

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ABSTRACT
This study was conducted at the Poultry Farm belongs to the Animal Production Department, College of Agriculture Engineering Sciences, University of Baghdad, during the period from 8-5-2023 to 12-6-2023 to Investigate effect of supplementing radish seed oil to broiler diet on performance, blood biochemical and cecal bacterial count. One hundred and twenty unsexed commercial, one day initial average body wight (39-41) g of broiler chicks (Ross 308) were randomly distributed among four treatments, 30 birds per treatment, with three replicates and 10 birds per replicate. The chicks of the first treatment (T1) were fed a standard diet in addition to the radish seed oil at levels of 50, 100 and 150 ml/kg diet respectively. The results revealed that adding radish seed oil at three different levels to broiler diets led to higher significant ($P \leq 0.01$) average live body weight and accumulative feed conversion ratio compared to T1. Moreover, decreased ($P \leq 0.05$) serum glucose, cholesterol and Coliform count were found for all treatments compared to the control group. Higher significant ($P \leq 0.01$) Lactobacilli Count was noticed for supplementing radish seed oil compared with control group. In conclusion, supplementing radish seed oil to the diet improved the productive performance, blood biochemical and cecal bacterial count of broiler.

تأثير اضافة زيت بذور الفجل الى عليقة فروج اللحم في الاداء الانتاجي وبعض الصفات الكيموحيوية للدم والعد البكتيري

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الخلاصة
اجريت هذه الدراسة في حقل الطيور الداجنة ،التابع لقسم الانتاج الحيواني ، كلية علوم الهندسة الزراعية / جامعة بغداد للمدة من 2023/5/8 الى 2023/6/11 لبيان تأثير اضافة زيت بذور الفجل الى عليقة فروج اللحم في الاداء الانتاجي ، بعض الصفات الكيموحيوية للدم والعد البكتيري للأعورين. استعمل في هذه التجربة 120 فرخاً من فروج اللحم غير المجنس (Ross 308) بعمر يوم واحد و بمعدل وزن ابتدائي (39-41) غم وزعت عشوائياً على اربع معاملات احتوت كل معاملة 30 طير بواقع ثلاث مكررات و عشرة طيور لكل مكرر. غذيت افراخ المجموعه الاولى (T1 السيطرة) على عليقه قياسية خالية من الأضافات بينما غذيت الافراخ المعاملة T2، T3 و T4 على عليقة تحتوي على زيت بذور الفجل بنسبة 50 و 100 و 150 مل / 100 كغم علف على التوالي. اشارت النتائج الى ان اضافة زيت بذور الفجل بالمستويات الثلاثة المختلفة لعلائق فروج اللحم قد ادى الى تحسن عالي المعنوية ($P\leq0.01$) في معدل وزن الجسم الحي و معامل التحويل الغذائي التراكمي مقارنةً بمجموعة السيطرة. انخفض معنوياً ($P\leq0.05$) تركيز الكلوكوز والكوليسترول و Coliform للمجاميع T2، T3 و T4 مقارنةً بمجموعة السيطرة بينما حصل تفوقاً عالي المعنوية ($P\leq0.01$) لجميع المعاملات في اعداد البكتريا المفيدة *Lactobacilli* . يمكن الأستنتاج ان اضافة زيت بذور الفجل بالمستويات المختلفة قد حسن من الصفات الانتاجية وبعض الصفات الكيما حيوية والعد البكتيري للأعورين لفروج اللحم.

الكلمات المفتاحية: اداء النمو، صحة الامعاء، الزيوت الأساس.

INTRODUCTION

In recent years, plants have played a role in modulating the immune response and the activity of digestive enzymes, which has helped to improve the internal environment of the intestine by converting into an environment with a higher number of *Lacto bacili* than *Coliform* bacteria, thus directly affecting the productive and immune traits of birds (Chowdhury *et al.*, 2018). Herbal plants, herbs, spices and their extracts including essential oils have been used in poultry feed as alternatives to antibiotics, the trend has been towards the use of essential oils due to their effective impact (Movahedi *et al.*, 2024). Since ancient times, using of spices have been common as food additives, regardless of their enhancement of taste and flavor, because it was believed that they have a role in stimulating the digestive system in addition to their medicinal properties, which also have a role in expelling worms, being diuretic, antispasmodic, and relieving digestive disorders (Jin *et. al.*, 2020; Majekodumi *et al.*, 2024; Al-Habsi *et. al.*, 2025). Some of these plants and their oils include Chamomile oils, propolis, eucalyptus leaf powder and mentha, avocado, chia oil and there mixture, grape seed powder and mixture of anise and thyme oil (Al-Mashhadani *et. al.*, 2013; Qassim *et. al.*, 2024). Radish (*Armoracia rusticana*) belongs to the *Brassicaceae* family and the reason for its pungent taste is the active substance isothiocyanate (Manivannan *et. al.*, 2019). Radish is characterized by its different colors in Asian countries due to the concentration of chlorophylls, carotenes and proanthocyanidin (*Kim et al.*, 2023), while in European countries, it is characterized by its red color due to the presence of ascorbic acid, anthocyanin and phenolic compounds, therefore the active substances differ according to the edible parts and the percentage of anthocyanin accumulation diuretic and treatment for colds, asthma, cough, colic, rheumatism, atherosclerosis and an anti-mutant, it also has a role against bacterial and viral infections, aging and cancer (Xu *et. al.*, 2017; Manivannan *et. al.*, 2019; Manzoor *et. al.*, 2021).

The radish seeds contain alkaloids such as coumarin, saponins, flavonoids and anthocyanins. The latter has proven its effective role in radish seeds in treating or reducing inflammation and free radical reactions by acting as a natural antioxidant through protecting

body cells from oxidative damage caused by free radicals formed in the body, which gives the bird immunity against a wide spectrum of different diseases (EL-Sayed, 2001; Ibrahim *et. al.*, 2024). In addition to containing dietary fiber and a group of mineral elements such as calcium, magnesium, manganese, copper, zinc, potassium, sodium and folic acid (Kang *et. al.*, 2020). Radish oil is highly content with unsaturated fatty acids including oleic and α-linolenic acids, these kinds of acids being critical for poultry well-being and being echoed in enhanced performance as well as productivity (Aly, 2023; Oketch *et al*., 2023). Additionally, supplementation of the diet with radish oil was reduced as shown by decreased loads of intestinal microbes and increased intestinal well-being in broiler chicks (Jang *et al.*, 2024). Due to the scarcity of scientific research on the application of radish seed oil in broiler nutrition, this experiment was created to critically evaluate its effect on growth productivity, biochemical parameters in the blood, and bacterial content in the guts, thus providing new findings on its value as a natural feed supplement to promote poultry health and production productivity.

MATERIAL AND METHODS

Ethical Approval Statement:

The study protocol was approved by the Research Ethics Committee at the College of Agricultural Engineering Sciences, University of Baghdad (Ref. No. 21, dated 28 March 2023). All experimental procedures were conducted in strict compliance with the guidelines for sound scientific conduct and animal welfare.

Diets and management:

This experiment was conducted at the Poultry Farm, Animal Production Department, Collage of Agricultural Engineering Sciences, University of Baghdad, Abu - Ghraib. From 8/5/2023 to 12/6/2023 (35 days) using 120 one day unsexed broiler chicks (Ross 308), were randomly distributed into four treatments, with three replicates, 10 chicks per replicate. The experimental groups have been referred to as follows: control treatment T1 (without addition); treatment T2, T3 and T4 include adding radish seed oil at a levels of 50, 100 and 150 ml/ 100 kg diet. Feed and water were *Ad libitum* during the experimental period. The chicks were fed starter diet during 1 – 28 days containing 3128.1 kcal / kg diet, and 21.89% crude protein, then the finisher diet during 28-35 days as 3218.54 kcal/kg diet and 20.17% crude protein. The basal diet was formulated for broiler chickens according to the provisions of the NRC (NRC, 1994) to meet the nutrients requirements of broilers (Table 1).

Table 1. Composition and calculated chemical analysis of experimental diets.

Gredient%	Starter diet (1-28)	Finisher diet (29-35)
Yellow corn	48	48
Wheat	12.8	15.6
Soyabean meal (48%)	29.1	25.3
Protein conc. (1)	5	5
Sunflower oil	3.1	4.3
Dicalcium phosphate (DCP)	0.5	0.4
Salt	0.2	0.2
A mixture of Vitamins and minerals	0.2	0.2
Limestone	1.1	1
Chemical composition		
Crude protein (%)	21.89	20.17
Metabolized energy (kcal/kg)	3128.1	3218.54
Lysine (%)	1.27	1.15
Cyst. +Methionine (%)	0.90	0.85
Calcium (%)	0.81	0.74
Available phosphorus (%)	0.49	0.41

(1) protein concentrate, Koudij's type, each kg of which contains: 40% crude protein, 2390 kilocalories/kg energy, 2.4% crude fat, 4.40% crude Fiber, 3% Sodium 4.5% digestible Lysine, 4.6% digestible methionine, 5.1% digestible Meth + Cyst., 2% digestible threonine,3.6% calcium ,5.5% available phosphorus ,200000IU vitamin A ,60000IU vitamin D3 ,500mgs vitamin E.

The artificial light was used in addition to normal daylight to provide a light period of 23 hours/day and 1 hour of darkness. The room temperature was controlled using the gas incubator and electric heaters to obtain a temperature of 34 °C during the first three days of life, after which it was reduced to 33-32 °C until the end of the first week, and then gradually reduced until the end of the experiment period to settle at a temperature of 20-22 °C.The experiment lasted for 35 days and productive performance including the average body weight, average weight gain, feed consumption and feed conversion ratio (FCR).
Three birds were randomly selected from each replicate for blood sampling, and the internal contents of the ceca were collected.

Radish seed oil

Radish seed oil purchased from local market and from a single source and prepared for analysis to quantify its fatty acid composition with soxholet (Banihani *et. al.*, 2013; Table 2).

Table 2. Chemical composition for fatty acids of radish seed oil. fatty acid content (1)

Fatty acid content (1)	(%)
Oleic	25.25
Linoleic	17.58
Palmatic	5.12
Stearic	3.25
Linolenic	1.56

(1) Percentage of fatty acid methyl esters of total fatty acid methyl esters.

Coliform bacteria and Lacto bacili

Escherichia coli was isolated on MacConkey Agar (Oxoid, UK), and the plates were incubated at 37°C for 24 hours. The total coliform count was determined using Violet Red Bile Agar (VRBA), with incubation at 37°C for 24–48 hours. The results were expressed as CFU/g (colony forming units per gram)

Statistical analysis

The experiment data were analyzed using complete randomized design (CRD) to determine the effect of treatments with different levels of radish seed oil on traits studied by SAS (2018). The significant means were compared using multiple range test and used statistical analysis system (Duncan, 1955).

RESULTS AND DISSCUSION

Table (3) shows the effect of adding radish seed oil for 1 – 35 days on mean body weight to the higher significant ($P \leq 0.01$) in the T4, T3 compared with T1 and T2 at 10 days, while T4 recorded a significantly higher ($P \leq 0.01$) value (1083.80 g) compared with T1, T2, T3 at 28 days. There was higher significant in T4 compared with T1, while there was no difference between T2, T3 with T4 at 35 days.

Table (4) illustrates the effect of adding radish seed oil for 1 - 5 weeks on wight gain. There were higher ($P \leq 0.01$) wight gain in T3 and T4 compared with T1, while T2 didn't differ significantly with T1 at 10 days. T4 was increased significant ($P \leq 0.05$) compared with T1, T2 and T3 at 28 days. In 35 days, the no significant differences between all treatments compared with T1. The results revealed higher ($p \leq 0.01$) feed consumption rate in T2, T3 and T4 compared to the control group at 10 and 24 days. In 35 days, there were significant increase ($P \leq 0.05$) in T2 and T4 compared to the T1, while T3 didn't differ significantly with T2. No significant differences were observed among the experimental treatments compared to the T1 in the amount of accumulative feed consumption during weeks 1 - 5 (Tabel 5).

Table 3. Effect of supplementing radish seed oil to broiler diet on the live body weight (Mean ± SE)

Average live body weight (g)			
Treatments (1)	0-10 days	11-24 days	25-35 days
T1	223.33±6.07^{b}	952.51±20.22^{b}	1891.60±18.61^{c}
T2	235.63±1.94^{ab}	989.56±3.70^{b}	2017.46±26.80^{b}
T3	242.78±1.64^{a}	993.59±12.87^{b}	2062.96±28.29^{ab}
T4	244.88±4.61^{a}	1083.8±31.04^{a}	2133.90±27.03^{a}
Sig.	P≤0.01	P≤0.01	P≤0.01

(1) Treatments= T1, means control treatment, T2, T3 and T4 means 50-, 100- and 150-ml radish seed oil addition to 100 kg diets. Means with different letters within each column indicate significance (P≤0.01)

Table 4. Effect of supplementing radish seed oil to broiler diet on the weight gain (Mean ± SE)

Average weight gain (g)				
Treatments (1)	0-10 days	11-24 days	25-35 days	0-35 accumulative
T1	184.33±6.07^{b}	729.18±24.04^{b}	939.09±30.51^{b}	1852.67±18.65^{ab}
T2	195.63±1.94^{ab}	753.93±2.54^{b}	1027.90±26.64^{a}	1977.46±26.80^{ab}
T3	201.78±1.64^{a}	750.82±13.34^{b}	1069.36±24.87^{a}	2021.96±24.43^{b}
T4	204.88±4.61^{a}	838.92±35.02^{a}	1050.08±20.96^{a}	2093.88±27.04^{a}
Sig.	P≤0.01	P≤0.05	P≤0.01	P≤0.05

(1). Treatments= T1, means control treatment, T2, T3 and T4 means (50, 100 and 150) ml radish seed oil addition 100 kg diets. Means with differen within each column indicate significance (P≤0.001) and (P≤0.05)

Table 5. Effect of supplementing radish seed oil to broiler diet on the feed consumption rate (Mean ± SE)

Feed consumption rate (g/bird)				
Treatments (1)	0-10 days	11-24 days	25-35 days	0-35 accumulative
T1	208.09±3.95^{b}	1014.59±7.61^{b}	1328.81±13.34^{b}	2551.49±19.56
T2	221.05±1.71^{a}	1041.55±7.78^{a}	1358.65±10.76^{a}	2621.25±20.5
T3	221.69±0.92^{a}	1041.54±4.05^{a}	1353.73±4.98^{ab}	2616.96±9.78
T4	224.21±2.60^{a}	1049.14±4.86^{a}	1359.38±4.55^{a}	2632.73±11.53
Sig.	P≤0.01	P≤0.01	P≤0.05	N. S

(1). Treatments= T1, means control treatment, T2, T3 and T means (50, 100 and 150) ml radish seed oil addition 100 kg diets. Means with different letters within each column indicate significance (P≤0.001) and(P≤0.05).
N.S. Non-significant.

Table (6) shows the influence of adding different levels of radish seed oil to broiler diets on the feed conversion ratio. There were significantly improvement (P≤0.05) in T3

and T4 groups compared with T1, while T2 didn't differ from T1 at 10 days. In 24 days, the T4 exhibited significant (P≤0.05) improve than T1. No significant variation was found among the experimental treatments compared to T1 in 35 days (Table 6). Feed conversion ratio accumulative (1 – 35 days) were increase (P≤0.05) for T2, T3 and T4 compared with T1.

Table 6. Effect of supplementing radish seed oil to broiler diet on the of feed conversion ratio (Mean ± SE)

	feed conversion ratio (g feed /g weight gain)			
Treatments (1)	0-10 days	11-24 days	25-35 days	0-35 accumulative
T1	0.932±0.017[a]	1.39±0.036[a]	1.415±0.058	1.312±0.013[a]
T2	0.938±0.008[ab]	1.377±0.012[a]	1.242±0.092	1.250±0.031[b]
T3	0.913±0.008[b]	1.385±0.026[a]	1.262±0.031	1.245±0.015[b]
T4	0.915±0.012[b]	1.255±0.060[b]	1.290±0.024	1.215±0.016[b]
Sig.	P≤0.05	P≤0.05	N. S	P≤0.05

(1) Treatments= T1, means control treatment, T2, T3 and T4 means (50, 100 and 150) ml radish seed oil addition 100 kg diets. Means with different letters within each column indicate significance (P≤0.01) and (P≤0.05). N.S. Non-significant.

The increase in live body weight averages is predominantly attributed to the statistically significant weight gain observed in animals subjected to the T2, T3, and T4 treatments. Adding dietary radish seed oil at 50, 100 and 150 ml/kg at 35 days of the age causing obvious increase in body wight gain to This may be due to the presence of phenolic compounds and alkaloids, such as coumarin, synocin and anthocyanin, in radish seed oil contributes to increased nutrient absorption (Bento *et. al.*, 2013; Eugenio *et. al.*, 2017; Al-Rubaya *et. al*., 2023). Furthermore, radish seed oil is also a source of vitamin C and phenolics, and these molecules represent natural antioxidants (Fernandes *et al*., 2013; Eugenio *et al.*, 2017). The active moieties suppress the action of free radicals and oxidative stress by combining with hydrogen in the form of H_2O_2, an action that results in oxidative damage to the cell as well as inducing apoptosis (Gamba *et al.*, 2021). As such, the bioactive constituents of radish seed oil can lead to the repair of the membrane of the mitochondrion (Jang *et al.*, 2024). Besides the high content of essential fatty acids and phenolic components that induce digestive enzyme activities (Aly, 2023), radish seed oil also occurs with strong antioxidant activities that can decrease oxidative stress (Jang *et al.*, 2024). Its functional properties make it also a suitable candidate to replace, in broilers' nutrition, the natural but chemical antibiotics, as shown by enhanced physiological responses as well as by health status after supplementation with radishes (Majekodumi *et al.*, 2024). Radish oil seeds could significantly contribute to bile acid secretion stimulation, thereby regulating the stomach and pancreas digestive enzymes (Barbarestani *et al.*, 2020). Stimulating drug action on the gastrointestinal secretions promotes the digestion of nutrients, thus improving mucosal membrane effectiveness and villi adsorption rate,

eventually helping in body weight gain (Al-Rubaya *et al.*, 2023). Active ingredients have a direct impact on stimulating the olfactory nerve centers due to the presence of pungent substances that promote salivation and gastric juice secretion, thereby enhancing the digestive system (Sharifi *et al.*, 2013). Radish root extract plays a role in gastrointestinal motility by activating acetylcholine, which in turn stimulates the motility of the small intestine through acetylcholine receptors (Farahat *et al.*, 2020).

Higher decrease (P≤0.01) in glucose, cholesterol and uric acid levels was noticed in T2, T3 and T4 than control group. No significant differences among the experimental treatments compared to T1, in triglyceride, protein, albumin, globulins, uric acid, AST and ALT (Table 7).

Table 7. Effect of supplementing radish seed oil to broiler diet on some blood biochemical traits (Mean ± SE)

	Mean ± standard error				
Treatments (1)	**T1**	**T2**	**T3**	**T4**	**Sig.**
Glucose(mg/dl)	162.00±3.83[a]	152.42±1.74[b]	147.4±1.51[b]	148.48±1.54[b]	P≤0.01
Cholesterol(mg/dl)	132.47±2.09[a]	119.14±3.18[b]	116.75±2.16[b]	116.18±4.29[b]	P≤0.01
Triglyceride(mg/dl)	148.95±3.92	141.42±2.19	141.94±2.21	142.41±2.60	N. S
Protein(g/dl)	6.33±0.18	6.32±0.40	6.31±0.59	6.36±0.29	N. S
Albumin(g/dl)	4.36±0.28	4.45±0.49	4.35±0.52	4.40±0.18	N. S
Globulin (g/dl)	1.96±0.33	2.12±0.37	1.95±0.16	1.96±0.41	N. S
Uric acid (mg/dl)	6.30±0.37 [a]	6.37±0.45 [b]	6.35±0.34 [b]	6.39±0.51 [b]	P≤0.01
AST(U/L)	132.42±2.44	132.73±1.75	132.96±1.77	132.69±1.67	N. S
ALT(U/L)	5.85±0.58	5.84±0.53	5.99±0.50	5.72±0.42	N. S

(1). Treatments: T1, means control treatment, T2, T3 and T4 means (50, 100 and 150) ml radish seed oil addition 100 kg diets. Means with different letters within each column indicate significance (P≤0.01). N.S. Non-significant.

This may owe to the antidiabetic and antioxidant phytochemicals in radish seed, established to enhance glucose homeostasis and attenuate insulin resistance (Wang *et al.*, 2025). Radish seeds were established to lower hyperglycemia by reducing intestinal glucose absorption, increasing peripheral glucose uptake, and enhancing insulin sensitivity (Kajszczak *et al.*, 2024). As such, bioactive metabolites in radish seed or oil could exert a modulating action in the energy metabolite and glycemic controls. The decrease in cholesterol levels may could attribute to the fact that the fatty acids in radish seed oil have improved HDL-C levels and reduced LDL-C levels (Arisha *et al.*, 2024; Jang *et al.*, 2024). This has directly reflected in a decrease in overall serum cholesterol levels through its anti-atherosclerotic effects (Geng *et al.*, 2024). The decrease in cholesterol levels could be attributed to its conversion into bile acids (Zhu *et al.*, 2024). This process is facilitated by low-density lipoprotein (LDL-C) receptors, which play a crucial role in reducing the risk of cardiovascular diseases (Jin *et al.*, 2020). Bile acids are production in the liver and secreted into the gallbladder (Majekodumi *et. al.*, 2024).

The digestive stimulating potential of active ingredients could be attributed to their ability to induce the liver to produce bile and release it into the gallbladder, this process may divert cholesterol from being produced in the bloodstream (Du *et. al.*, 2015). There is a higher significant (P≤0.01) Lacto bacili bacteria for all treatment groups compared with control groups, while there was a decrease significant (p≤0.05) in number of Coliform bacteria for all treatment groups (Table 8). The immune - enhancing properties and antimicrobial activity of specific substance may play a role in increasing the secretion of pancreatic enzymes (Barbarestani *et al.*, 2020), which in turn aid in the digestion of nutrients in the intestines, this suggests that gut bacteria have a role in protecting and maintaining the integrity of the gastrointestinal mucosal lining (Muzaffer *et al.*, 2013; Kpomasse *et al.*, 2021).

Table 8. Effect of supplementing radish seed oil to broiler diet on the ceca microbial count of broiler chickens (Mean ± SE)

Microbial counting of coliform bacteria and *Lacto bacili* (CFU/g)		
Treatments [(1)]	Coliform	*Lacto bacilli*
T1	7.92±0.56[a]	6.12±0.14[b]
T2	6.52±0.30[b]	7.08±0.08[a]
T3	6.25±0.50[b]	7.13±0.09[a]
T4	6.43±0.21[b]	7.04±0.14[a]
Significance Levels	P≤0.05	P≤0.01

(1). Treatments= T1, means control treatment, T2, T3 and T4 means (50, 100 and 150) ml radish seed oil addition 100 kg diets. Means with different letters within each column indicate significance (P≤0.01) and (P≤0.05).

CONCLUSION

Research shows that supplementing broiler feed with radish seed oil can help birds digest their food better and perform better. This is because it increases the activity of digestive enzymes and improves the balance of intestinal microflora, which in turn affects the absorption of nutrients and birds' growth. Aside from improving blood parameters and reducing the pathogenic bacterial load in the intestine, the results also showed that the active ingredients in radish seed oil possess antioxidant and antimicrobial properties. This supports its role as a natural alternative to antibiotics in poultry nutrition. To learn how much radish seed oil is best and what physiological pathways it works through, especially in relation to the effects on liver bile production and lipid metabolism, more research is needed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the staff of the [Department, College or University] for their technical and general support.

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