Competitive study to evaluate act of the Mycofix®5.0 and *Lactobacillus Plantarum* bacteria to prevent the Fumonisin B1 effect on some Ross-308 broilers production character

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ABSTRACT

The aim of this study was to investigate the efficiency of *Lactobacillus plantarum* or *Mycofix® 5.0* into decrease or prevents the negative influences of Fumonisin B1 in some broiler productive performance, one hundred and fifty broiler chicks 1-day-old (Ross 308) form both sexes were used. The chicks were randomly divided into five treatment groups with three replicates for each treatment and ten birds' cages each for 35 day, The experimental treatments were labeled as follows:- T1. Basil Diet (BD) no other addition (Control). T2. BD containing 300 mg FB1/kg diet; T3. BD containing 300 mg FB1/kg diet +2g/Kg diet *Mycofix® 5.0* ; T4. BD containing 300mg FB1/kg diet+1.5×10^8 *CUF Lb. plantarum*; T5. BD containing 300 mg FB1/kg diet +3.0×10^8 *CUF Lb. plantarum*. The results indicated that FB1 was significantly ($p\leq0.05$) decreased body weight gain(BW), feed intake(FI) and the birds fed with an FB1 contaminated diet showed significantly higher (FCR) value than the control group. The supplementation of *Lb. plantarum* and the Mycofix® 5.0 to FB1 treated birds significantly diminished the inhibitory effects of dietary FB1 on growth performance character with no differences compared to the control diet and there were no different between treatments three, four and five and there were improvements compare with treatment two for most characters' result. To sum it up both of the bacteria concentrations in live mode who added to drink water for reduced the effect of fumonisin B1 achieve in significant way improvement in diet consumption and feed conversion ratio compare to normality.

Key word: Fumonisin B1, *Lactobacillus Plantarum*, broiler, body weight, body weight gain, feed intake, feed conversion ratio.

دراسة مقارنة لتقييم فعل مركب ال ® 5.0Mycofix و بكتريا *Lb.plantarum* على الحد من تأثير Fumonisin 1B في بعض الصفات الانتاجية للفروج نوع 308-ROSS

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الخلاصة

إن الهدف من هذه الدراسة هو التحري عن كفاءة بكتريا *Lb.plantarum* أو مركب Mycofix®5.0 على تقليل أو منع التأثيرات السلبية للفيومنسين 1B في بعض الصفات الانتاجية لفروج اللحم , أستخدم في التجربة مئة وخمسون طير من الهجين 308-ROSS وبعمر يوم واحد بدون تجنيس قسمت الى خمسة معاملات ولكل معاملة ثلاث مكررات والمكرر الواحد يحوي عشرة طيور ولمدة خمسة وثلاثون يوم.

وزعت معاملات التجربة كالآتي:- المعاملة الاولى: عليقة أساسية بدون إضافة. المعاملة الثانية :عليقة أساسية ملوثة بالفيومنسين 1B 300 ملغم/كغم علف .المعاملة الثالثة: عليقة أساسية ملوثة بالفيومنسين 1B 300 ملغم/كغم علف+ Mycofix®5.0 2 غم/كغم علف. المعاملة الرابعة: عليقة أساسية ملوثة بالفيومنسين 1B مع أضافة بكتريا *Lb.plantarum* الى ماء الشرب .1.5×10^8 CFU المعاملة الخامسة: عليقة ملوثة بالفيومنسين 1B مع أضافة بكتريا*Lb. plantarum* الى ماء الشرب . 3.0×10^8 CFU

وقد أشرت النتائج أن تلوث العليقة بالفيومنسين 1B أحدث انخفاض معنوي في صفات وزن الجسم , الزيادة الوزنية الاسبوعية و استهلاك العلف مع ارتفاع معنوي في صفة كفاءة التحويل الغذائي كمؤشر سلبي للصفة. إن أضافة ال Mycofix® 5.0 أو تجريع البكتريا *Lb. plantarum* كمعاملات للطيور المتناولة للعليقة الملوثة بالفيومنسين 1B حسنت معنويا من قيم معدلات الصفات المدروسة مع عدم وجود فرق معنوي لبعضها مقارنة مع معاملة السيطرة وتسجل فروق معنوية في نتائج المعاملات الثالثة والرابعة و الخامسة مقارنة بالسيطرة قد حققت معدلات افضل مقارنة مع المعاملة الثانية. ومن ذلك نستنتج أن التركيزين المستخدمين من البكتريا بصورة حية في مياه الشرب للحد من تأثير 1FB نجحت وبصورة معنوية في إعادة معدلات إستهلاك العلف وكفاءة التحويل الغذائي الى معدلاتها الطبيعية.

كلمات مفتاحية / فيومنسين *Lb. plantarum* , B1 , فروج اللحم , وزن الجسم , الزيادة الوزنية الاسبوعية, استهلاك العلف,كفاءة التحويل الغذائي.

INTRODUCTION

Mycotoxins are secondary metabolites of some filamentous fungi, mainly those belonging to *Aspergillus, Penicilliu ,Fusarium, Alternaria*, and the Ergot Alkaloid Groups, these toxigenic fungi are a major problem in cereal crops as they produce a multitude of toxic metabolites contaminating plants and food products (1,2).They can be found in raw materials and products of food industry as well as in feedstuffs (3,4).

High incidence of mycotoxin infections in cereals have been observed worldwide, in different crops and regions(5,6).Fumonisins are fungal secondary metabolites produced by species of Fusarium, mainly *Fusarium verticillioides* and *Fusarium proliferatum* (7,8). There are several identified fumonisins, but fumonisin B1 (FB1) and fumonisin B2 (FB2) are the most important and constitute up to 70% of the fumonisins found in naturally contaminated foods (9–11).

A decrease in dietary intake and body weight gain of chickens fed up fumonisin was observed on maize-based feeds has been reported to cause poor performance increased organ weights decreased immune responses and organ lesions (12–14).

Lactic acid bacteria (LAB) encompass a heterogeneous group of Gram-positive, non sporeforming, non-motile, aerotolerant, rod and coccus-shaped organisms, which produce lactic acid as a major end product during carbohydrate fermentation. (15).

These microorganisms play an important role in the manufacture and storage processes by enhancing the shelf-life, microbial safety, texture, sensory characteristics, nutritional value for many product (16,17) (18)(19). Several reports have been published about AFB1 reduction by some LAB such as *Lactobacillus, Bifidobacterium, Propionibacterium* and *Lactococcus* were found to be active in removing AFB1 primarily by the adhesion method (20–22).

(23) study the ability of *Lactobacillus acidophilus* to remove fumonisin B1 the results obtained indicated that it was able to remove FB1 from liquid medium. stated that cell wall structural integrity of the microorganisms was required for fumonisin B1 removal and cell envelope was the binding site.

Therefore, a promising alternative is the use of microorganisms as FB1 chelating agents. Inclusion of such microbes in the diet may reduce the toxic effects of mycotoxins on humans, as a FB1–microorganism complex may decrease availability of the mycotoxin and consequently its absorption in the gastrointestinal tract.

MATERIAL AND METHOD

This study was carried out at the poultry farm of the animal resources department College of Agriculture, University of Tikrit for period (26/2/2020) to (1/4/2020). For experiments, one hundred fifty unvaccinated broiler chicks (Ross-308) were obtained from a commercial hatchery the birds were placed in batteries of identical size. Each experiment consisted 5 treatments, 3 replicates and 30 chicks of each, individually weighed chicks 41 g were divided into groups at 1 day of age. Birds assigned at random to 15 cages (10 birds / cage) treatment groups. In an environmentally controlled room with 24-h day fluorescent lighting room temperature was maintained at 32ºC during the first week and gradually decreased to 22º C by the end of 3th week.

Birds allowed to consume feed and water *ad libitum* that were fed rations containing know concentration of fumonisin. The *L. Plantarum* suspension prepared by directly suspending bacteria in normal saline (NaCl) 0.9% and skim milk powder 2% (inhibitor-free), final pH 7.0±0.2 (20ºC) and then give it to the birds to drink after one hour of taking away the normal

The experimental treatments received a corn – soybean meal basal diet (BD) and depending on the addition were labeled as follows (that depend on previous studies): -

T1. BD no other addition (Control).

T2. BD containing a 300 mg FB1/kg diet.

T3. BD containing a 300 mg FB1/kg diet +*Mycofix® 5.0* 2g/Kg diet

T4. BD containing a 300 mg FB1/kg diet + *Lactobacillus Plantarum* CFU(1.5×10^{8} live cell /ml) .

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T5. BD containing a 300 mg FB1/kg diet+ *Lactobacillus Plantarum* CFU(3×10^8 live cell /ml).

The basal diet was also tested for possible residual fumonisin B1 before feeding(24). Diet Preparation mycotoxin test concentrations were obtained using Sun flower oil as the diluent and appropriate amounts of these solutions were added to the basal diet to obtain the required levels FB1 to guarantee a balanced diet for all treatments. Concentrations FB1 in final mixtures were confirmed by analyzing 1-kg samples following procedures proposed by (Shenzhen Lvshiyuan Biotechnology Co., Ltd. Guangdong, China) sensitivity: 0.1ppb.

Table. 1 Composition of experiment diet %

Ingredient	Starter diet 1-14 day	Grower diet 15-28 day	Finisher diet 29-42 day
Corn	51.9	58.6	62
Soybean meal	36	30	27
Premix[1]	5	5	5
Dicalcium phosphate	0.4	0.4	0.5
Limestone	1.4	1.2	0.9
NaCl	0.3	0.3	0.3
Sunflower oil	5	4.5	4.3
Total	100	100	100
Approximate analysis(calculated)			
Crude protein%	22.25	20.18	19.15
Metabolizable energy%	3098.95	3144.6	3173.06
Crude fiber%	3.761	3.489	3.354
Lysine%	1.295	1.151	1.08
Methionnine%	0.501	0.476	0.464
Lysine+ Methionnine%	0.80	0.76	0.72
Calcium%	0.994	0.906	0.814
Available phosphate%	0.443	0.432	0.445

[1] Each kilogram contains calcium, 196 g; phosphorous, 64 g; sodium, 30 g; magnesium, 6 g; copper, 400 mg; zinc, 1,200 mg; iron, 2,000 mg; manganese, 1,200 mg; cobalt, 20mg; iodine, 40 mg; selenium, 8 mg; vitamin A, 200,000 IU; vitamin D3, 80,000 IU; vitamin E, 1,600 mg; vitamin K3, 34 mg; vitamin C, 1,300 mg; vitamin B1, 35 mg; vitamin B2, 135 mg; vitamin B6, 100 mg; vitamin B12, 670 µg; nicotinic acid, 1,340 mg; calcium pantothenic acid, 235 mg; choline chloride, 8,400 mg; folic acid, 34 mg; biotin, 3,350 µg; and methionine, 30 g.

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Body Weight of the birds each repeater collectively using the digital balance and then extracted the overall rate. Body weight gain: Calculated increase the weight each week according to the following equation: -
Body weight gain (g) = Body weight at the end of the week (g) - Body weight at the beginning of week (g).
Feed intake (g): calculated feed intake each week by weighting the amount of feed remaining at the end of the week, and subtracted from the total amount provided in the beginning of the week. consideration the feed intake by dead birds using the following formula:-
Average daily feed intake (g / bird) = Total amount provided in the week (g)/ (The account of live birds in the end of the week × 7) +Total days' birds eat.
Feed conversion efficiency: was calculated per week according to the following equation.
Feed conversion efficiency= Average of feed intake (g/week) / Average of body weight gain (g/week).

Data of the experiment were analyzed using the Complete Randomized Design (CRD) model and the (SAS,2001) statistical program to study the effect of the transactions studied in different qualities and compared the differences morale among the averages using the test Duncan (1955) according to the mathematical model the following:

$Yij = \mu + Ti + eij$

Yij = value attributable to the treatment of viewing I
μ = the overall average for the recipe studied
Ti = effect of treatment I
eij = random error which is normally distributed with an average equal to zero and variance equal amount² *eσ*.

RESULTS AND DISCUSSION

The effects of *lactobacillus Plantarum* on growth performance (body weight, body weight gain, feed intake and feed conversion ratio) of broilers fed basal diets contaminated with fumonisin B1(FB1) illustrate in flowing tables.

Body weight presented in tables (2) shows that T2 result from 2 to 5 weeks (248.5, 442, 713.5 and 1093 g) they did show significant differences between the (T2) and control groups control group in broiler for this character, compared to it, body weight significantly reduced ($p \leq 0.05$) as observed.(25) studied the effects of 200 mg/kg of FB1as incorporated into the diets of broiler chicks, which significantly diminished body weight gain that effects of FB1 observed on all performance results measured in the experiment were significantly affected by the presence of FB1 in broilers feed after 28 days.
Birds fed a diet containing no FB1 have a tendency to have the greatest BW gain among the treatment groups followed by treatment contain *lactobacillus Plantarum* and Mycofix® 5.0 respectively. In general, the positive effects of probiotic additive tested on BW gain are in agreement with the results reported by several researchers (26,27). It has been demonstrated that probiotic inhibit the negative effect of FB1 on body weight in treatments three, four and five that

Table. 2 Effects of contaminated diet and detoxification ability of *Lb. plantarum* on broiler body weight in broiler(BW)(g).

Age/ Week	Control	T2	T3	T4	T5
1	118.500 [a] ±0.645	119.000 [a] ±2.041	116.750 [a]± 1.376	115.500 [a]± 1.108	116.250 [a]± 1.108
2	292.000 [a] ±1.471	248.500 [c] ±3.617	273.00 [b]± 7.842	268.00 [b]± 2.041	266.250 [b]± 3.145
3	598.000 [a] ±6.480	442.00 [c] ±10.931	568.250 [ab] ±17.427	540.000 [b]± 4.760	547.750 [b]± 3.520
4	1081.000 [a] ±8.225	713.500 [c] ±17.965	969.750 [b] ±52.199	921.500 [b]± 21.152	913.250 [b]± 22.276
5	1651.00 [a] ±11.908	1093.250 [c] ±17.787	1462.500 [b]± 109.192	1358.00 [b]± 52.300	1357.50 [b]± 53.948

T1. BD no other addition (Control) . T2. BD containing a 300 mg FB1/kg diet.T3 BD containing a 300 mg FB1/kg + Mycofix® 5.0 2g/Kg diet. T4 BD containing a 300 mg FB1/kg diet +*Lb. plantarum*(1.5×10^8 cfu/ml) .T5. BD containing a 300 mg FB1/kg diet+ *Lb. plantarum*(3.0×10^8 cfu/ml).within the same row means with different letter are significantly different p≤0.05 .Results are reported as means ±SEM.

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shows improvement compare to T2 even T3 shows no significant differences in age three-week (568.250) g compere to T1(598) g respectively.

Body weight gain (BWG) for birds fed on rations contaminated with FB1 (300mg/kg) showed the poorest BW-gain during 2 to 5 and 1-5 weeks of the experiment periods compared with other treatments as table 3 shows.

This delay of growth inhibition in broiler suggests that the length of exposure to fumonisin as well as the level of concentration can influence an animal's response in terms of performance. Meanwhile, the birds in the treatment *L. Plantarum* or Mycofix® 5.0 plus FB1 treatments recorded a higher BWG compare birds dietary FB1 without adding during 1-6 weeks. In the current study, supplementing a contaminated diet with FB1, probiotic significantly ameliorated the toxic effects of fumonisin on broilers growth performance but with significant differences, while at week three, T3 shows no deferent from control group. The basic mechanism appeared to be that *L. plantarum* germinated in the animal digestive tract and which then secretes the active substance that degrades fumonisin thus, alleviating the effects of FB1.

Table 4 shows Feed intake (FI), the lowest value of feed intake was recorded for broiler fed a diet contaminated with FB1 (T2) start from third week (459.5) g compare with T1 (529.75) g. However, the results from the current trial showed that the supplementing of *L. plantarum* and Mycofix® 5.0 to the contaminated diet with 300mg FB1/kg resulted in a significant increase in feed intake at different periods of the experiment, when compared with the (T1).

The results in study concur with(28) who reported that chicks fed dietary FB1 at 80 mg/kg had a significantly low value feed intake. The addition of probiotic to the broiler diet had benefit on feed intake at 1 to 5 weeks. The results of this experiment clearly showed that the supplemented diet with probiotic and Mycofix® 5.0 enhanced feed consumption during the whole of the experiment (T3 and T5).

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Table. 3 Effects of contaminated diet and detoxification ability of *Lb. Plantarum* on broiler weekly weight gain (g).

Age/ Week	Control	T5	T4	T3	T2
1	77.500 [a] ± 0.645	78.000 [a]± 2.041	75.750 [a]± 1.376	74.500 [a]± 1.554	75.250 [a]± 1.018
2	173.500 [a] ±0.957	129.500 [c]± 5.515	156.250 [b]± 7.498	152. 50 [b]± 1.108	150.000 [b]± 3.719
3	306.000 [a] ±6.467	193.500 [b]± 7.632	295.250 [ab]± 10.184	272.000 [c]± 4.222	281.500 [bc]± 4.518
4	483.000 [a] ±6.027	271.500 [c]± 10.586	401.500 [b]± 35.039	381.250 [b]± 19.669	365.300 [b]± 25.517
5	570.000 [a] ±4.564	379.750 [c]± 7.498	492.750 [ab]± 57.692	436.500 [bc]± 32.039	444.200 [bc]± 33.467
1-5	1610.94 [a] ± 18.59	1052.25 [c]± 28.91	1421.50 [b]± 62.89	1317.36 [b]± 41.11	1316.51 [b]± 61.81

T1. BD no other addition (Control) . T2. BD containing a 300 mg FB1/kg diet .T3 BD containing a 300 mg FB1/kg + Mycofix® 5.0 2g/Kg diet. T4 BD containing a 300 mg FB1/kg diet +*Lb. plantarum*(1.5×10^{8} cfu/ml) .T5. BD containing a 300 mg FB1/kg diet+ *Lb. plantarum*(3.0×10^{8} cfu/ml).within the same row means with different letter are significantly different p≤0.05 .Results are reported as means ±SEM.

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Generally, it has been suggested that in animals, the effectiveness for most probiotics could be demonstrated with a daily intake of 10^8 to 10^9 microorganisms per broiler established a probiotic efficacy in improving broiler growth and feed conversion ratio. However, it is very difficult to directly compare several studies using different probiotics and different administration levels because probiotic application additionally depends on many other factors stated in the introduction section.

The mean FCR values given in table (5) the result was affected by the presence of FB1 (T2) in the feed at all the experiment period (2.01, 2.38, 2.41, 2.39 and 2.29) compare to control group.

when the FCR values increased in the birds receiving FB1 contaminated diet, similar results were described in broilers given FB1 in concentrate 200 mg/kg of feed(14).

Result during 1 to 5 weeks of the experiment period, and were not noticeably different. The results of this experiment showed that the supplemented with probiotic did have a no significant difference in the treated group (T4) and (T5) for FCR value compared with the T1 group from 1- 5 week except T3 in week 5 (1.92) and T4 in week 4 (1.84) show different result. A similar effect due to probiotic supplementation in broiler chickens was observed by (29)(Shlej *et al* 2016). In addition, the beneficial effects of probiotic products on broiler performance, including FCR have been reported by (30,31).

In this study, diet contaminated with fumonisin at level 300mg/kg marked increased FCR values between 2 to 5 weeks (2.01, 2.38, 2.41, 2.39 and 2.29) compared with the control group (T1) (1.55 , 1.73 , 1.71 , 1.81 and 1.71) respectably.

The mechanics that the fumonisin effect the bird's cells happen by inhibited or distribute the Sphingolipid metabolism due to the simulates in Sa ( ) and So ( ) to FB1 structure that included two base from Sphingoid so it linked to these bases and reduced their availability causing inhabit the Ceramide compound who is very important to build the cells walls(32,33). The fumonisin effect negatively most metabolism activates that included carbohydrate, protein and fat in bird's bodies and in the end effect body weight body weight gain and diet consumption(34)(35).

In digestive tract the lactic acid bacteria work by bind with the hydroxyl groups in fumonisin B1 structure to destroy it, this ability really depends on pH of each part of digestive system (36).

In the current study, supplementing a contaminated diet with (*L. Plantarum* 1.5×10^8 cell/ml) significantly decreased FCR ratio values compared with the T2 group during the experimental period. Therefore, as a feed additive for biodegradation of fumonisin, the addition of *L.Plantarum* to broiler diets is believed to be a potentially viable approach to the biodetoxification of fumonisin naturally occurring in mouldy feed. In our study the results corroborated the hypothesis that the protective effects of *L. Plantarum* against FB1 might be due to its capability to effect a specific biotransformation of it in the intestinal tracts of animals. The image also revealed that FB1 binding produces structural changes that modify the bacterial cell surface.

Table. 4 Effects of contaminated diet and detoxification ability of *Lb. Plantarum* on broiler weekly feed intake(FI) (g).

Age/ Week	Control				
		T5	T4	T3	T2
1	113.00 ab ±0.912	117.500 a± 1.892	114.500 ab± 2.02	110.500 b± 2.387	113.750 ab± 1.376
2	269.250 a ±4.888	271.750 a± 4.989	273.500 a± 5.181	264.250 a± 2.096	259.750 a± 7.586
3	529.750 ab ±15.553	459.500 c± 4.369	571.500 a± 4.787	500.750 bc± 17.084	537.500 ab± 21.929
4	827.500 a ±19.632	654.250 c± 23.449	769.250 ab± 39.69	700.750 bc± 12.844	753.00 ab± 26.465
5	1032.00 a ±28.059	908.500 b± 12.533	1036.250 a± 27.86	984.00 a± 26.851	986.000 a± 22.343
1-5 week	2771.50 a ±23.47	2411.50 b± 37.14	2765.00 a± 17.26	2560.25 b± 22.76	2650.00 a± 19.28

T1. BD no other addition (Control) . T2. BD containing a 300 mg FB1/kg diet .T3 BD containing a 300 mg FB1/kg + Mycofix® 5.0 2g/Kg diet. T4 BD containing a 300 mg FB1/kg diet +*Lb. plantarum*(1.5×10^8 cfu/ml) .T5. BD containing a 300 mg FB1/kg diet+ *Lb. plantarum*(3.0×10^8 cfu/ml).Within the same row means with different letter are significantly different p≤0.05 .Results are reported as means ±SEM.

Table. 5 Effects of contaminated diet and detoxification ability of *Lb. Plantarum* on feed conversion ration broiler(FCR).

Age/ Week	Control				
		T5	T4	T3	T2
1	1.46 ^{a}± 0.035	1.51 ^{a} ±0.031	1.51 ^{a}± 0.043	1.48 ^{a}± 0.017	1.51 ^{a}± 0.011
2	1.55 ^{b}± 0.045	2.01 ^{a} ±0.104	1.75 ^{b}± 0.050	1.73 ^{b}± 0.016	1.73 ^{b}± 0.066
3	1.730 ^{b} ±0.036	2.38 ^{a} ±0.108	1.94 ^{b}± 0.060	1.84 ^{b}± 0.066	1.91 ^{b}± 0.059
4	1.71 ^{c} ±0.029	2.41 ^{a} ±0.089	1.92 ^{bc}± 0.075	1.84 ^{bc}± 0.052	2.06 ^{b}± 0.107
5	1.81 ^{b} ± 0.055	2.39 ^{a} ±0.043	2.10 ^{ab}± 0.161	2.25 ^{a}± 0.189	2.22 ^{ab}± 0.182
1-5	1.72 ^{b} ± 0.081	2.29 ^{a} ±0.060	1.95 ^{b}± 0.07	1.94 ^{b}± 0.05	2.01 ^{b}± 0.06

T1. BD no other addition (Control). T2. BD containing a 300 mg FB1/kg diet .T3 BD containing a 300 mg FB1/kg + Mycofix® 5.0 2g/Kg diet. T4 BD containing a 300 mg FB1/kg diet +*Lb. plantarum*(1.5×10^{8} cfu/ml) .T5. BD containing a 300 mg FB1/kg diet+ *Lb. plantarum*(3.0×10^{8} cfu/ml).Within the same row means with different letter are significantly different p≤0.05 .Results are reported as means ±SEM.

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