

Effects of Dietary Arginine and Methionine Levels on Broiler Carcass Traits and Meat Quality

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Abstract: The current trial with 4×2 factorial arrangement was carried out to determine the effects of dietary arginine and methionine levels on broiler carcass traits and meat quality. Four dietary levels of arginine were 80, 100, 120 and 140% of NRC recommendation and dietary levels of methionine were 100 and 120% of NRC recommendation. On day 42 of age, chickens from each group were slaughtered to evaluate carcass traits, meat quality and muscle characteristics. Arginine supplementation significantly improved muscle growth ($p_L < 0.01$), especially breast muscle growth while methionine showed no effect. For meat quality, arginine deficient diet resulted in decreased lightness (L^* value) while methionine supplementation increased yellowness (b^* value). Except for the arginine deficient group, shear force values were decreased with arginine supplementation. Total collagen was not affected by the levels of arginine and methionine while heat-soluble collagen was increased with arginine ($p_L < 0.05$) and methionine supplementation ($p < 0.05$). The result of the present study indicated that dietary arginine and methionine could affect collagen property and myoglobin status in the final formation of meat characteristic and a level of dietary arginine up to 120% of the recommendation by NRC should be suggested for better lean meat deposition and meat tenderness.

Key words: Arginine, methionine, carcass traits, meat quality, myoglobin, collagen content, China

INTRODUCTION

Both arginine and methionine are essential amino acids for chicken. The requirement of chicken for dietary arginine on both dietary concentration (1.10-1.25 g/100 g) and a percentage of CP basis (5.4-5.5%) is among the highest of any other species studied and this results from lackness of endogenous synthesis and high rate of protein deposition because of the rapid growth rate of broilers (Ball *et al.*, 2007). Arginine serves as a precursor for synthesis of ornithine, creatine, agmatine, NO, etc. (Cynober *et al.*, 1995; Morris, 2007). Dietary L-arginine stimulates protein synthesis and reduces fat mass in rats and pigs (Fu *et al.*, 2005; Tan *et al.*, 2009; Yao *et al.*, 2008). Ornithine produced by arginase can serve as a precursor for synthesis of proline which is crucial for collagen synthesis, wound healing and tissue remodelling (Flynn *et al.*, 2002) and polyamines which are essential for cell proliferation, differentiation and function (Thomas and Thomas, 2001; Igarashi and Kashiwagi, 2000).

Collagen as a connective tissue constituent present in all tissues is the most abundant mammalian and avian protein. Another pathway for arginine is to produce

creatine when it is condensed with glycine and methionine. De Boo *et al.* (2007) reported that arginine infusion increased the percentages of hydroxyproline, citrulline and proline in both control and embolised animals. Gigure *et al.* (2008) reported that methionine supplementation improved growth performance but showed similar effects on oxidative stability of the fresh meat.

The colour of meat is an important aspect of consumer acceptability. Amount of work has been published about metmyoglobin (MetMb) reducing system for the importance of its role in maintaining meat colour. Myoglobin is an intracellular globular haem protein found in skeletal and cardiac muscle (Giddings and Solberg, 1977) with three common forms: oxymyoglobin (MbO_2), deoxymyoglobin (DeoxyMb) and metmyoglobin and the relative proportions of these forms determine the colour of fresh meat. Metmyoglobin is an undesirable physiologically form for meat quality because of the brownish colour it imparts when present in the surface of fresh meat (Bekhit and Faustman, 2005).

Due to the biological roles of arginine, methionine and their metabolites, we hypothesized that arginine and methionine might affect muscle growth and meat conformation. The objectives of this study were to

examine the effects of dietary levels of arginine and methionine on carcass traits and meat quality and the possible mechanisms.

MATERIALS AND METHODS

Animals and diets: About 480 days old Arbor Acre male broiler chicks were obtained from a local hatchery and randomly allocated into eight groups with five replicates each, 12 chicks per replicate (cage). A 4×2 factorial arrangement was employed with four levels of arginine (80, 100, 120 and 140% arginine of NRC recommendation) and two levels of methionine (100 and 120% methionine of NRC recommendation) in the diet during the experiment. Crude protein, amino acid contents, dry matter, metabolizable energy of the feed ingredients including corn, soybean, corn gluten meal, rapeseed meal were analyzed before used in the design of formulation (Table 1).

The experimental basal diet (Table 2) was formulated based on the amino acid pattern recommended by the NRC except for a level of 80% arginine (10 g kg⁻¹ for starter diet and 8.8 g kg⁻¹ for grower diet) and then supplemented with four levels of L-arginine (0, 2.5, 5.0 and 7.5 g kg⁻¹ for starter diet and 0, 2.2, 4.4 and 6.6 g kg⁻¹ for finisher diet) and with two levels of DL-methionine (0, 1 g kg⁻¹ for starter diet and 0.76 g kg⁻¹ for finisher diet). To equalize other nutrient concentrations, cellulose was added to make the sum of the ingredients in every treatment up to 100%. Samples of the feeds produced were analyzed to verify the amino acids and crude protein contents were the same as designed. The composition and nutrient levels of the basal diet are shown in Table 2. The broilers were placed in cages for 42 days with free access to feed and water. All procedures were approved by China Agriculture University committee on Laboratory Animal Care and Use.

Carcass traits measurement: After a fasting period of 8 h, the chicks were individually weighed and the electrically stunned. The carcasses were defeathered and

eviscerated following exsanguination. Breast muscle, leg muscle and abdominal fat content were stripped and measured. Abdominal fat, skinless and boneless breast muscle (pectoralis major and pectoralis minor) and thigh muscle were dissected and measured. Muscle and fat yield were calculated as their weights divided by body weight.

Meat quality: The meat quality data were collected including the pH value, meat colour, water-holding capacity and tenderness. At 45 min after slaughter, shear force of the breast muscles (pectoralis major) was measured in triplicate using a TA.XT Plus/50 texture analyser (Stable Micro Systems, UK). Strips (1.0 cm width ×1.0 cm thickness ×3.0 cm length) parallel to the muscle fiber were prepared from the medial portion of the meat and sheared vertically. Shear force was expressed in kilograms.

At 45 min and 24 h after slaughter, the breast meat pH was tested at a depth of 2.5 cm below the surface of pectoralis major. The colour measurement was evaluated at 48 h post slaughter by a spectrophotometer (model WSC-S, Shanghai Shengguang Ltd., China) using the CIELAB system (L* = Lightness; a* = redness; b* = yellowness).

Total collagen and heat-solubility of collagen were determined by the method of Hill (1966) modified by Liu *et al.* (1996). The content of collagen was expressed as mg of collagen per g of skeletal muscle. Total myoglobin content and of the proportions of oxymyoglobin, myoglobin and metmyoglobin in meat were determined by the method of Krzywicki (1982) based on the different absorbance spectra of these molecules when in solution.

Statistical analysis: Data were analyzed by SPSS 11.0 program for windows. Results were expressed as treatment means with their pooled standard error of the mean. ANOVA, GLM and Duncan's Test were used. Significance was declared at p<0.05, unless otherwise indicated.

Table 1: Analyzed composition of the feed ingredient

Composition	Corn	Soybean meal	Corn gluten meal	Rapeseed meal
Dry matter (g kg ⁻¹)	860.20	901.40	900.90	880.10
Metabolizable Energy (MJ kg ⁻¹)	13.47	9.83	16.23	7.41
Crude protein (g kg ⁻¹)	780.20	497.00	566.00	387.00
Amino acid contents^a (g kg⁻¹)				
Arginine	3.50	37.80	18.00	16.90
Lysine	2.20	30.70	8.80	9.60
Methionine	1.20	6.10	14.80	9.40
Cystine	2.60	7.10	11.40	9.90
Threonine	2.90	20.30	20.50	14.90
Tryptophan	0.50	4.90	3.00	3.60

^aAmino acids were analyzed by ion-exchange chromatography done with an HPLC following the National Standard of China by the Ministry of Agriculture Feed Safety and Efficiency Evaluation Center of China

Table 2: Ingredient and composition of basal diets (g kg⁻¹)

Diet	Starter (1-21 day)	Finisher (22-42)
Ingredients (CP)	20.5%	18.5%
Corn	631.60	682.30
Soybean meal	125.80	97.00
Corn gluten meal	112.20	96.00
Rape seed meal	60.00	60.00
Soybean oil	15.00	17.00
Limestone	13.30	14.50
Dicalcium phosphate	17.30	11.90
L-lysine	5.40	5.30
DL-methionine	1.30	0.40
L-threonine	0.40	0.60
L-tryptophan	0.50	0.50
Mineral premix ^a	2.00	2.00
Vitamin premix ^b	0.20	0.20
Choline chloride 50%	1.60	1.20
Cellulose ^c	8.80	7.80
Calculated composition		
Metabolizable energy (Mcal kg ⁻¹)	3.00	3.05
Crude protein (g kg ⁻¹)	205.00	185.00
Ca (g kg ⁻¹)	10.00	9.00
Available P (g kg ⁻¹)	4.50	3.50
Arginine (g kg ⁻¹)	10.00	8.80
Methionine (g kg ⁻¹)	5.00	3.80
Methionine+Cystine (g kg ⁻¹)	9.40	8.00
Lysine (g kg ⁻¹)	11.00	10.00
Threonine (g kg ⁻¹)	8.00	7.40
Tryptophan (g kg ⁻¹)	2.00	1.80
Analyzed composition^d		
Arginine (g kg ⁻¹)	10.00	8.80
Lysine (g kg ⁻¹)	11.10	10.00
Methionine (g kg ⁻¹)	5.00	3.90

^aThe mineral premix supplied the followings per kilogram of complete feed: Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0.30 mg; I, 0.35 mg.

^bThe vitamin premix supplied the followings per kilogram of complete feed: vitamin A, 12, 500 IU; vitamin D3, 2,500 IU; vitamin K3, 2.65 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; vitamin B12, 0.025 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid 12 mg; niacin 50 mg; ^cCellulose was added with this amount only in the basal diet. In the other groups, L-arginine (in the form of L-arginine, Shine Star (Hubei) Biological Engineering Co., Ltd. China) and DL-methionine (Adisseo Life Science (Shanghai) Co., Ltd. China) were supplemented with the corresponding level to attain the total arginine level and methionine level as designed and cellulose was added to make the total sum of ingredients up to 100%. ^dAmino acids were analyzed by the same method as stated in the footnote of Table 1. All the other diets were manufacture based on the basal diet, except for the supplemented amount of L-arginine, DL-methionine and cellulose. The contents of arginine and methionine in other diets were also analyzed to verify they were the level as designed

RESULTS AND DISCUSSION

The Breast Meat Weight (BRW), Breast Meat Yield (BRY), Leg Meat Weight (LEW) and Leg Meat Yield (LEY) were significantly affected by dietary arginine level. The effects of dietary arginine and methionine levels on broiler carcass are shown in Table 3. Addition of graded levels of arginine to the arginine-deficient diet increased breast muscle weight ($p_L < 0.001$) and yield ($p_L < 0.001$), leg muscle weight ($p_L < 0.001$) and yield ($p_L < 0.01$) and linearly decreased abdominal fat yield ($p_L < 0.01$). The increases in the breast muscle weight and yield, leg muscle weight and yield also contained quadratic components

($p_Q < 0.05-0.001$). Breast and leg muscle weight increased up to 120% arginine level of NRC recommendation and then declined with further addition ($p_Q < 0.001$). Abdominal Fat Weight (FAW) was not affected by dietary arginine. Dietary methionine level showed no effect on carcass characteristic. Interaction between arginine and methionine was observed for BRY, LEW and LEY ($p < 0.05$). The growth response of BRW, LEW and LEY were more dependent on arginine level when fed with methionine of NRC recommendation than with methionine 120% of NRC recommendation. There was no interaction between dietary arginine and methionine level on BRW, FAW and FAY.

The effects of dietary arginine and methionine levels on meat quality are shown in Table 4. Arginine supplementation linearly increased L* value and cooking loss ($p_L < 0.05$). pH 45 min was linearly decreased with arginine supplementation ($p_L < 0.05$). Supplementation of arginine to NRC recommendation level could decrease shear force value. Arginine level showed no effect on a* and b* value. Methionine supplementation significantly increased b* values but showed no effect on water loss, pH and tenderness of the meat. The interaction between arginine and methionine on cooking loss was observed ($p < 0.05$).

The effects of dietary arginine and methionine levels on myoglobin status and collagen contents are shown in Table 5. DeoxyMb and MetMb showed quadratic responses to the increase of dietary arginine level ($p_Q < 0.05$). Groups with arginine at 120% NRC and methionine at 120% of NRC showed relatively high level of deoxyMB and low level MetMb ratio. Heat-soluble collagen content was linearly increased by arginine level ($p_L < 0.05$). Neither arginine nor methionine showed any effect on total collagen content.

It is generally known that amino acid deficiency would result in suppressed growth. In the present study, arginine deficient diet resulted in lower body and muscle weight compared to the standard diet. During the recent years, there were several studies focusing on effects of additional arginine supplementation on muscle growth and fat deposition.

Fu *et al.* (2005) observed dietary L-arginine supplementation could enhance NO synthesis and reduce fat mass in zucker diabetic fatty rats. Tan *et al.* (2009) reported dietary L-arginine supplementation up to 1.0% to a corn and soybean meal-based diet increased skeletal muscle content and decreased fat content of swine. Jobgen *et al.* (2009) reported 1.51% L-arginine-HCl in drinking water enhanced skeletal muscle mass and reduced white fat gain in diet-induced obese rats.

Table 3: Effects of dietary arginine and methionine level (expressed as percentage of NRC recommendation) on carcass characteristics

Arginine	Methionine (%)	Breast muscle (g)		Leg muscle (g)		Abdominal fat	
		BRW (g)	BRY (%)	LEW (g)	LEY (%)	FAW (g)	FAY (%)
80%	100	184.900	12.500 ^f	263.300 ^d	17.800 ^f	28.700	1.950
100%	100	240.200	14.600 ^d	343.800 ^{bc}	20.900 ^{ab}	30.000	1.810
120%	100	295.500	16.100 ^{bc}	407.200 ^a	22.200 ^a	31.200	1.720
140%	100	275.600	16.800 ^a	332.900 ^{bc}	20.300 ^b	25.000	1.550
80%	120	199.900	13.700 ^e	304.200 ^f	20.900 ^{ab}	27.500	1.880
100%	120	252.800	14.900 ^d	346.300 ^{bc}	20.400 ^b	27.500	1.610
120%	120	278.400	15.700 ^c	361.000 ^b	20.300 ^b	25.600	1.440
140%	120	275.500	16.600 ^{ab}	354.300 ^b	21.400	23.100	1.390
SEM		6.500	0.200	7.700	0.300	1.100	0.060
Main effect							
Arginine	80	192.400 ^e	13.100 ^d	283.800 ^f	19.300 ^b	28.100	1.920 ^a
	100	246.500 ^b	14.700 ^e	345.000 ^b	20.600 ^a	28.700	1.710 ^{ab}
	120	287.000 ^a	15.900 ^b	384.100 ^a	21.300 ^a	28.400	1.580 ^b
	140	275.500 ^a	16.400 ^a	343.600 ^b	20.800 ^a	24.100	1.470 ^b
Methionine	100	249.000	15.000	336.800	20.300	28.700	1.760
	120	251.600	15.100	341.500	20.700	25.900	1.580
ANOVA p-value							
Arginine	-	<0.001	<0.001	<0.001	0.009	0.263	0.059
Methionine	-	0.955	0.189	0.645	0.255	0.144	0.142
Interaction	-	0.199	0.011	0.027	0.001	0.842	0.931
Contrasts	-	-	-	-	-	-	-
Arg linear, P _L	-	<0.001	<0.001	<0.001	0.006	0.143	0.008
Arg quadratic, P _Q	-	<0.001	0.027	<0.001	0.030	0.190	0.674

Means in the same column without same superscripts differ significantly (p<0.05)

Table 4: Effects of dietary arginine and methionine level (expressed as percentage of NRC recommendation) on meat quality

Arginine	Methionine (%)	Meat colour			WHC		pH		Tenderness
		L*	a*	b*	Drip loss	Cooking loss	45 min	24 h	Shear force value (kg)
80%	100	48.470	3.940	11.800	2.980	15.900 ^a	5.510	5.160	3291
100%	100	52.800	4.830	11.620	4.060	21.700 ^b	5.440	5.010	3767
120%	100	51.050	4.610	12.000	3.610	21.180 ^b	5.500	5.000	3491
140%	100	54.150	4.240	14.560	3.920	25.230 ^b	5.340	5.010	3075
80%	120	48.430	3.690	14.700	4.320	22.350 ^b	5.420	5.130	2794
100%	120	56.620	5.870	13.340 ^f	3.780	19.520 ^{ab}	5.630	5.010	3512
120%	120	55.440	4.930	15.150	5.350	23.440 ^b	5.360	4.980	3248
140%	120	53.130 ^f	5.150	14.100	3.740	23.670 ^b	5.190	5.030	2922
SEM	-	0.700	0.240	0.340	0.220	0.530	0.040	0.020	88
Main effect									
Arginine	80	48.450 ^b	3.810	13.250	3.650	19.130 ^a	5.470	5.150	3043 ^b
	100	54.710 ^a	5.350	12.480	3.920	20.610 ^a	5.540	5.010	3640 ^a
	120	52.250 ^a	4.780	13.580	4.480	22.610 ^{ab}	5.430	5.000	3370 ^{ab}
	140	53.640 ^a	4.700	14.330 ^a	3.830	24.450 ^b	5.260	5.020	2999 ^b
Methionine	100	51.620	4.410	12.500	3.640	21.000	5.450	5.050	3406
	120	53.400	4.910	14.320	4.300	22.250	5.400	5.040	3119
ANOVA p-value									
Arginine	-	0.003	0.095	0.178	0.557	0.019	0.067	0.004	0.026
Methionine	-	0.136	0.233	0.004	0.135	0.295	0.503	0.776	0.083
Interaction	-	0.277	0.685	0.133	0.242	0.049	0.318	0.927	0.889
Contrasts	-	-	-	-	-	-	-	-	-
Arg linear, P _L	-	0.011	0.275	0.105	0.574	0.002	0.033	0.006	0.579
Arg quadratic, P _Q	0.018	0.062	0.200	0.288	0.780	0.107	0.010	0.005	-

Data in the same column without same superscripts differ significantly (p<0.05)

In the present study, It found that appropriate supplemental level of arginine (120% of NRC recommendation) could enhance both breast and thigh muscle growth compare to standard treatments (100% of NRC recommendation). On the other side, arginine supplementation did not influence abdominal fat weight but decreased abdominal fat yield. These results suggested that moderate arginine supplementation would

steer nutrient deposition away from fat to muscle in chicks which was similar with the conclusions found in rats and pigs of the reports above.

Chamruspollert *et al.* (2002) demonstrated the interaction between methionine and arginine for broiler chick growth and suggested that high level of arginine depressed feed intake and growth and the depression of arginine could be alleviated by methionine

Table 5: Effects of dietary arginine and methionine level (expressed as percentage of NRC recommendation) on biochemical parameters

Arginine	Methionine (%)	Total MB (mg g ⁻¹)	DeoxyMb (%)	MbO ₂ (%)	MetMb (%)	Total collagen	Heat-soluble collagen
80%	100	1.130	38.400 ^d	5.100	42.900	3.630	1.130
100%	100	1.060	39.300 ^{ab}	5.800	40.700	3.640	1.460
120%	100	1.390	38.700 ^{cd}	5.500	41.800	3.780	1.680
140%	100	0.880	38.400 ^d	5.600	42.000	3.870	1.920
80%	120	1.280	39.600 ^a	5.000	41.400	3.660	1.650
100%	120	1.160	39.600 ^a	6.300	39.700	3.950	2.080
120%	120	1.230	39.100 ^{abc}	5.300	41.600	3.810	2.190
140%	120	1.140	39.000 ^{bc}	5.400	41.500	3.790	2.010
SEM	-	0.050	0.100	0.100	0.200	0.090	0.090
Main effect							
Arginine	80	1.210	39.000 ^b	5.000 ^b	42.100 ^a	3.650	1.390
	100	1.110	39.500 ^a	6.000 ^a	40.200 ^b	3.800	1.770
	120	1.310	38.900 ^b	5.400 ^{ab}	41.700 ^a	3.800	1.940
	140	1.010	38.800 ^b	5.500 ^{ab}	41.800 ^a	3.830	1.970
Methionine	100	1.110	38.700	5.500	41.900	3.730	1.550
	120	1.200	39.400	5.500	41.000	3.800	1.980
ANOVA p-value							
Arginine	-	0.160	0.001	0.037	0.006	0.825	0.090
Methionine	-	0.343	0.000	0.965	0.038	0.622	0.016
Interaction	-	0.455	0.039	0.751	0.687	0.805	0.705
Contrasts	-	-	-	-	-	-	-
Arg linear, P _L	-	0.141	0.014	0.358	0.755	0.518	0.020
Arg quadratic, P _Q	0.572	0.010	0.085	0.013	0.379	0.313	-

Data in the same column without same superscripts differ significantly (p<0.05)

supplementation. The arginine level in that study were 15.2, 20.2, 25.2, 35.2 g kg⁻¹ for 14 day chicks which were equal to 121.6, 161, 201.6 and 281.6% of NRC recommendation. The current study also showed that the retardation of arginine deficiency (80% of NRC recommendation) on muscle growth could partially be alleviated by methionine supplementation (120% of NRC recommendation).

Metmyoglobin which impart brownish colour when present in the surface of fresh meat could be reduced enzymatically by metmyoglobin reductase. Many factors such as temperature, storage time, muscle type, exercise, diet etc., could affect the MetMb reducing activity (Bekhit and Faustman, 2005) and where upon induce the transformation of meat colour. In the study, meat of arginine deficiency group showed the lowest L* value and a* value. This could be partially explained with the high ratio of MetMb and low ratio of deoxyMb. Methionine supplementation increased the deoxyMb ratio while decreased the MetMb ratio which means the brownish colour from MetMb could decreased. This result was in accordance with the fact that methionine supplementation significantly increased b* value.

Arginine condensed with glycine and methionine could lead to the formation of creatine. It has been reported that muscle creatine phosphate could increase maximum and total work capacity (Casey and Greenhaff, 2000). It has also been supposed that increased creatine phosphate may delay lactate formation in the muscle and consequently postpone the pH decline postmortem (Young *et al.*, 2005). Young *et al.* (2005) concluded that creatine monohydrate supplementation increased pH and

water-holding capacity of the meat from Duroc pigs but showed no effect on the meat of Landrace pigs. However, in the current study, arginine and methionine supplementations did not postpone the pH decline or increase water-holding capacity. Arginine deficient diet group showed the lowest cooking loss. While no difference of drip loss was found among treatments, cooking loss increased with arginine and methionine supplementation.

Liu *et al.* (1996) reported a significant correlation (r² = 0.94) between total amount of collagen and toughness of the meat. Greater fraction of heat-soluble collagen is indicative of more youthful, labile, less crosslinked collagen and greater tenderness of the meat (McCormick, 1999).

De Boo *et al.* (2007) reported a phenomenon of reduced collagen synthetic rates in the embolised animals. In the current study, high arginine supplementation group increased heat-soluble collagen content but showed no effect on total collagen content compared to arginine deficient group. About meat tenderness, arginine deficient group showed relatively lower shear force value.

Except for the arginine deficient group, arginine and methionine supplementation reduced shear force value (which means higher tenderness), increased heat-soluble collagen content and cooking loss but showed no effect on drip loss and total collagen content. There are cross-bridges between the collagen molecule units and also between the collagen molecules. The cross-bridges are supposed to determine the physical strength and heat stability of intramuscular connective tissue. The result of

current study suggested that arginine and methionine might act through retarding the growth of cross-bridges which subsequently resulted in higher heat-soluble collagen content, lower water-holding capacity after cooking and decreased shear force value. On the other hand, arginine and methionine supplementation would not affect water-holding capacity at normal temperature which might be explained by the unchanged amount of total collagen.

CONCLUSION

Dietary arginine could enhance muscle deposition, increase meat lightness while methionine could increase meat yellowness but showed limited effects on carcass traits and meat quality. The interaction of dietary arginine and methionine was not apparent on meat quality. Dietary arginine and methionine could affect collagen property and myoglobin status in the final formation of meat characteristic. A higher level of dietary arginine up to 120% of the recommendation by NRC is necessary for better lean meat deposition and meat tenderness.

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