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Omega-3 profiles and chemical substances of chicken meat fed diets containing purslane (*Portulaca oleraceae*) meal rich in omega-3 fats

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Abstract

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Purslane (*Portulaca oleraceae*) is a plant rich in omega-3 (n-3) fatty acids which can be added to the diets of chicken to produce meat high in n-3 fatty acids. This research aimed to analyse the impact of the purslane meal addition into a basal diet on the quality and n-3 fatty acids of broiler meat. A total of 150 broilers were assigned randomly to five treatments with six replications. Each replication consisted of five broilers. The treatments were a basal diet supplemented with purslane meal at levels of 0% (Br-E0), 1.5% (Br-E1), 3% (Br-E2), 4.5% (Br-E3), and 6% (Br-E4). Meat samples were taken on day 42 for quality and n-3 fatty acid analysis. Results showed that the addition of purslane meal to the basal diets did not affect (P>0.05) the protein and moisture content of the meat but significantly reduced (P<0.05) the fat content. Increasing the levels of purslane meal rich in alpha-linolenic acid (ALA) in the diets increased the ALA, DHA, total n-3 PUFA, total n-6 PUFA, and total PUFA (P<0.05) in the broiler meat. The DHA level of the meat from chickens fed with 6% purslane meal increased by double compared to those fed with control diet. In conclusion, chickens fed ALA enriched diets up to a level of 6% produced meat higher in n-3 fats without negatively affecting the chemical composition of the meat.

1. Introduction

Broiler chicken meat is favored by many people as it contains high nutrition, good taste and aroma, soft texture, and is relatively cheap in price. The chemical composition of chicken meat consists of 21.53% protein, 5.21% fat, 73.61% water, and 1.07% ash (Bostami *et al.*, 2017). Chicken meat is a good source of nutritional food because it has highly digestible protein, B-group vitamins (mainly thiamin, vitamin B6, and pantothenic acid), minerals (iron, zinc, and copper), and unsaturated fatty acids (Marangoni *et al.*, 2015).

Currently, meat consumers are very concerned about quality in choosing broiler chicken meat. High fat is a source of cholesterol and tends to be a consumer consideration in consuming broiler chicken. Livestock products such as eggs, meat, milk can be upgraded to become functional foods. For more than two decades, efforts have been made to increase the content of omega-3 (n-3) fatty acids in livestock products including broiler chicken meat through the modification of animal feed. These efforts can produce good quality meat that is safe, healthy, and can meet human needs (Mandal *et al.*, 2014; Ibrahim *et al.*, 2018). Some researchers use marine feed ingredients (fish meal or fish oil) and plants (vegetable).

The supplementation of marine n-3 sources such as fish oil can increase the content of n-3 fatty acids (EPA and DHA) of chicken meat (Lopez-Ferrer *et al.*, 2001). However, the excessive use of products derived from fish rich in n-3 long chain polyunsaturated fatty acid (n-3 LCPUFA) such as EPA and DHA can cause oxidative damage, resulting in off-flavour and odour which adversely affects the sensory quality of chicken meat products and consumer acceptance (Alagawany *et al.*, 2019).

Therefore, it is necessary to find alternative sources of n-3 fatty acid-rich feed ingredients by using plants, flour or vegetable oils that contain high n-3 PUFA, alpha -linolenic acid (ALA). Research conducted by Kartikasari *et al.* (2012) found that the supplementation of plant oil rich in ALA can improve the level of n-3 fatty acids, EPA, DPA, and DHA of broiler breast and thigh meat while maintaining the chemical quality of the meat. Sources of n-3 fatty acids can be found in purslane or *Portulaca oleraceae* plants (Aydin and Dogan, 2010). Purslane has a high content of n-3 fatty acids compared to other types of plants, which is 4.05 mg/g (Simopoulus, 2004). Purslane plants also contain β -carotene, folic acid, vitamin C, potassium, calcium, and anti-oxidants (Irawan *et al.*, 2003). The high nutritional content and n-3 PUFA in purslane plants means it has the potential to be used as an alternative feed source of n-3 fatty acids.

Based on the description above, a study was conducted to determine the influence of supplementation of purslane meal rich in n-3 fats in the diet on the accumulation of n-3 fatty acids and the chemical quality of broiler meat.

2. Materials and methods

2.1 Birds, management, and diets

This study used 150 day-old unsexed broiler chickens of the Lohman strain (MB 202 Silver) that were treated with diets for 42 days. The broiler chicks were weighed, vaccinated, and randomly assigned to one of five dietary treatments. Each treatment was repeated six times and each replication consisted of five chickens. Feeding and drinking were adjusted to the stage of chicken growth and accessed ad libitum. Chicken rations were formulated based on the recommendation by SNI (2006). The formulation of the basal diet was conducted according to procedures by Kartikasari et al. (2018) consisting of rice bran, yellow corn, soybean meal, lime flour, DCP, DL-Methionine, L-Lysin, premix, salt, limestone, and palm oil. The nutrient content of the dietary treatments can be seen in Table 1. The dietary treatments consisted of levels of 0, 1.5, 3, 4.5, and 6% purslane meal as a source of n-3 fatty acid.

2.2 Meat preparation

A total of six chickens (30 chickens in total) at 42 days of age was taken randomly from each treatment and processed into carcasses at a commercial slaughterhouse. The broiler chickens were processed with scalding at 63° C for 45 s (Sams *et al.*, 2001). The samples of breast meat were separated from the carcass and followed by a

| Table 1. Nutrient content of the d | liets |
|------------------------------------|-------|
|------------------------------------|-------|

deboning process after chilling. The chicken breast fillets were airtight packed and stored at -20°C until the quality test was carried out.

2.3 Chemical composition test

Chemical composition of breast meat samples was using FoodScan determined Near-Infrared Spectrophotometer (FoodScan Lab, type 78800, Foss Analytical, Hillerød, Denmark) with the procedure of nutritional value analysis following the method described by Anderson et al. (2007) with some modifications. The meat samples were homogenized by grinding. The ground chicken meat weighing at 30 g was put on a round 140 mm petri dish. The dish was set in the FoodScan where the operator ID was inputted on the software and the meat sample profile was chosen. The scanning process was started by pressing the "start" button. The analysis results were displayed in percentage (g/100 g) for collagen, protein, fat, and moisture.

2.4 Fatty acid analysis

The total lipid (TL) in tissue samples was extracted by the method described by Folch et al. (1957) with methanol/chloroform (1:2, v/v). The meat sample was thoroughly mixed with methanol (3 mL) and then chloroform (6 mL) was added and the tube was shaken vigorously. The sample was then centrifuged to obtain a chloroform layer. The chloroform was transferred into a labelled 20 mL scintillation vial. Using a vacuum concentrator, the chloroform was evaporated to dryness. The total lipid extract was weighed to calculate the fat content of the meat. Fatty acid transmethylation was performed using the method described by Tu et al. (2010), the fatty acids were transmethylated with 1% H₂SO₄ in methanol for 3 hrs at 70°C. The FAME was then transferred to a gas chromatography (GC) vial which contained anhydrous sodium sulphate and then the sample was stored at 20°C for GC analysis.

| Table 1. Nutrient content of the diets | | | | | | | |
|--|------------|---------|---------|---------|---------|--|--|
| Nutriant contanta ¹ | Treatments | | | | | | |
| Nutrient contents | Br-E0 | Br-E1 | Br-E2 | Br-E3 | Br-E4 | | |
| Dry matter (%) | 86.97 | 87.07 | 87.17 | 87.27 | 87.37 | | |
| Crude fat (%) | 5.77 | 5.78 | 5.78 | 5.78 | 5.78 | | |
| Crude fiber (%) | 3.25 | 3.51 | 3.78 | 4.04 | 4.31 | | |
| Crude protein (%) | 23.10 | 23.03 | 22.96 | 22.89 | 22.82 | | |
| Ash (%) | 3.97 | 3.95 | 3.92 | 3.89 | 3.87 | | |
| ME (Kcal/kg) | 3090.79 | 3069.71 | 3048.62 | 3027.53 | 3006.45 | | |
| Calcium (%) | 1.07 | 1.12 | 1.18 | 1.23 | 1.29 | | |
| Pav (%) | 0.566 | 0.559 | 0.552 | 0.546 | 0.539 | | |
| Phosphor (%) | 0.87 | 0.86 | 0.86 | 0.85 | 0.84 | | |
| Lysin (%) | 1.22 | 1.21 | 1.20 | 1.20 | 1.19 | | |
| Methionine (%) | 0.65 | 0.66 | 0.66 | 0.67 | 0.68 | | |

¹Results of proximate analysis of diet ingredients was performed in the Nutrition and Animal Feed Laboratory, Gajah Mada University, Yogyakarta

Br-E0: basal diet + 0% purslane meal; Br-E1: basal diet + 1.5% purslane meal; Br-E2: basal diet + 3% purslane meal; Br-E3: basal diet + 4.5% purslane meal; Br-E4: basal diet + 6% purslane meal.

The fatty acid composition of broiler meat was determined by the procedure described by Kartikasari *et al.* (2012) and measured using a Hewlett-Packard 6890 GC (CA, USA) equipped with a flame ionization detection and a capillary column. The FAME was separated using carrier gas (helium). The percentage of each fatty acid was calculated by comparison of retention times with the original lipid standard using the Agilent GC Chemstation software package provided by Agilent Technologies Inc., Palo Alto, CA, USA. Each peak of the trace is expressed as a relative percentage of the total FAME in the sample, with the detection limit of each fatty acid being 0.05% of the total fatty acids.

2.5 Statistical analysis

All the data were analysed for normality (the Shapiro -Wilk test) and homogeneity of variances (Levene's test). Next, the data were analysed using one-way ANOVA. The following test used the Tukey post-hoc analysis if there are significant mean differences between the treatments at P < 0.05.

3. Results and discussion

3.1 Chemical composition of breast meat

The results showed that there was a very significant difference (P<0.01) in the collagen content of meat with feed containing purslane flour (Table 2). There was a significant decrease in the administration of 6% purslane flour to the control. This study is not in accordance with Kartikasari et al. (2021) who reported that purslane flour did not affect the collagen content of 35-day-old broilers. This difference may be caused by the difference in the age of the chickens used. In this study, the age of the chickens was 42 days. The collagen content in breast meat linearly decreases with age (Dias et al., 2020). Collagen is formed from tropocollagen which contains glycoproteins (Soeparno, 2015). Glycoproteins and glycolipids in cells are formed from glycans that are covalently bound to proteins or lipids (Shajahan et al., 2017). Glycolipids in the cell membrane system play an important role in protein binding (Evans and MacKenzie,

1999). Protein binding is mainly on the hydrophobic side with certain amino acids in mammalian cells (Tuuf and Mattjus, 2014). With the decrease in fat content in meat in this study, it was possible to decrease the glycolipid content, so that the collagen content also decreased at the level of 6% purslane flour (P4).

According to Schulz (2013), fat is also used as energy in the beta-oxidation process by utilizing saturated and unsaturated fats. The process of energy formation is better in saturated fat than unsaturated, because an additional reaction process is needed to form energy in the unsaturated fat. It was also reported that the energy formation reaction would be shorter if n-3 fatty acids were used compared to n-6 and n-9, and were also more effective in the form of trans rather than cis. Thus, the reduced source of trans fatty acids in feed sourced from purslane flour can cause higher energy utilization using a glucose biosynthesis system compared to using fat. This can cause a decrease in the glycoprotein used as a constituent of collagen, causing a decrease in the collagen content at the level of 6% purslane flour.

The results showed that there was a very significant difference (P < 0.01) in the fat content of the meat with purslane flour as a source of n-3 fatty acids in the feed (Table 2). The decrease was significant in the administration of purslane flour 3 (P2) and 6% (P4) compared to the control. Dillak et al. (2020) reported that the use of purslane flour at levels of 5 and 10% as n-3 fatty acid sources in commercial diets can reduce the fat content of broiler meat. This decrease was caused by a decrease in the MUFA and C16:0 fatty acid content of chicken meat in this study where these fatty acids were the constituents of fat in meat (Table 3). These results are in accordance with the research of Zhaleh et al. (2020) who found that broiler chickens fed up to 15% flaxseed had decreased MUFA and C16:0 content in the thigh meat. The composition of the fatty acid content in broiler chicken meat is determined by the fatty acid content in the feed, because the fatty acid content of the meat is determined by the biosynthesis of fat sources in the feed after digestion of the feed ingredients occurs

 Table 2. Chemical composition of breast meat at day 42

| Parameters (%) - | Treatments | | | | | D value |
|------------------|----------------------|-----------------------|-----------------------|-----------------------|----------------------|---------|
| | Br-E0 | Br-E1 | Br-E2 | Br-E3 | Br-E4 | r value |
| Collagen | $1.49{\pm}0.130^{a}$ | $1.34{\pm}0.114^{ab}$ | $1.31{\pm}0.066^{ab}$ | $1.36{\pm}0.212^{ab}$ | 1.12 ± 0.180^{b} | 0.005 |
| Fat | $3.44{\pm}0.408^{a}$ | $2.60{\pm}0.886^{ab}$ | 2.02 ± 0.716^{b} | $2.14{\pm}0.531^{ab}$ | $1.71{\pm}1.174^{b}$ | 0.008 |
| Moisture | 72.11±0.244 | 73.10±0.911 | 73.13±0.742 | 72.82 ± 0.856 | 73.42±1.208 | 0.117 |
| Protein | 22.06 ± 0.334 | 22.21±1.120 | 22.09±0.416 | 22.56 ± 0.754 | 22.73±0.461 | 0.355 |

Values are presented as mean \pm SD of five replicates per treatment. Values with different superscripts within the same row are significantly different (P<0.05).

Br-E0: basal diet + 0% purslane meal; Br-E1: basal diet + 1.5% purslane meal; Br-E2: basal diet + 3% purslane meal; Br-E3: basal diet + 4.5% purslane meal; Br-E4: basal diet + 6% purslane meal.

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(Suchy et al., 2016).

The results showed that there was no significant difference (P>0.05) in the water content of the meat with feeding containing purslane flour. The addition of purslane flour up to 6% had the same water content as the control. Dillak et al. (2020) reported that the administration of 5% and 10% purslane flour in commercial diets had no effect on the water content of broiler meat. The result of this study was supported by Wang et al. (2020) who found that the addition of a source of n-3 fatty acids in the feed had no significant effect on the water content of broiler breast meat in the starter and grower phases. Fats as well as carbohydrates and proteins play a role in the energy metabolism system that produces CO₂ and H₂O as end products (Soeparno, 2015). Fat tissue has a function to regulate intracellular and extracellular water, whereas fat cells in tissue cells have a function to regulate excess and lack of water in cells (Engelking, 2011). This may be why the water content in meat is no different from the feed containing purslane flour because the water in the cells that make up the meat are in equilibrium.

The results showed that there was no significant difference (P>0.05) in the protein content of the broiler chicken of the Lohman strain (MB 202 Silver) with purslane flour. The addition of purslane flour up to 6% had the same protein content as the control. This study is in accordance with Dillak et al. (2020), who reported that broiler chicken strain CP 707 with purslane flour 5 and 10% in commercial rations resulted in protein content that was not different from the control. Kartikasari et al. (2021) found that the protein content did not differ in the breast meat of 35-day-old Lohman strain broiler chicken fed with purslane flour addition of up to 6%. Proteins are hydrophilic because of the presence of polar amino acid bonds with water in the hydroxyl and amino acid groups (Schnepf, 1992). Myosin is the main protein in postmortem muscle with polar (70%) amino acids (Whiting, 1988). In this study, the protein content may not be different because the water content is also not different. This is due to the hydrophilic nature of meat protein which binds water to the myosin. Myosin makes up the majority of muscle protein, so the amount of protein is positively related to water.

3.2 Fatty acid analysis

The results showed that the addition of purslane flour as a source of n-3 fatty acids in feed had a significant effect (P<0.05) on the levels of n-3 PUFA, ALA (Table 3). The use of purslane flour at the level of 6% significantly increased the n-3 PUFA (ALA) content with an increase of up to 31.2% compared to the control

diet. This is possible because purslane flour is a feed ingredient that is rich in n-3 PUFA (18.5%) which will be deposited in broiler chicken meat. This indicates that the content of n-3 PUFA in meat can be regulated by modifying the composition of feed ingredients, in order to obtain meat that is more in line with human dietary needs for n-3 PUFA. Nutritional regulation through modification of feed formula is an acceptable strategy to safely increase fatty acid composition (Nong *et al.*, 2020). The results of this study support those reported by Kartikasari *et al.* (2012) and Carragher *et al.* (2016) which found that the increase in n-3 PUFA (ALA) feed through supplementation of plant oils rich in n-3 fatty acids can increase the ALA content of meat.

The content of EPA and DPA as a result of the n-3 PUFA metabolic pathway was not significantly different with an increase in the ALA content of the feed to a level of 6% but had a significant effect on the DHA content of meat, which increased by 88.9%. These results are in accordance with the study conducted by Ibrahim et al. (2018) who found that reducing the ratio of n-6 to n-3 PUFA through the addition of fish oil or linseed oil (Kartikasari et al., 2021) could increase n-3 LCPUFA. The absence of a significant increase in EPA and DPA deposition may be due to the relatively high content of n-6 PUFA (linoleic acid, LA) fatty acids in the dietary treatments. That condition may cause competition in the use of desaturase and elongase enzymes between n-3 PUFA (ALA) and n-6 PUFA (LA). Desaturase and elongase enzymes play an important role in converting ALA to EPA, DPA and DHA, but these enzymes are also used in metabolic pathways to convert LA to Arachidonic acid, AA (Palmquist, 2009). This indicates that the high LA content of the feed can reduce the production of n-3 LCPUFA fatty acids (EPA, DPA and DHA). These results support those reported by Mandal et al. (2014) which found that the consumption of feed containing high n-6 fatty acids can be a limiting factor in the formation of n-3 PUFA to n-3 LCPUFA because 6desaturation is the rate-limiting step in the metabolic pathway. This confirms that to maximize the n-3 LCPUFA content in meat, the ratio of LA to LA content is an important factor that needs to be considered. Kartikasari et al. (2012) reported that the strategy of giving broiler chicken feed formula with an increase in ALA content along with efforts to reduce the n-6 to n-3 ratio of broiler chicken feed was able to increase the EPA and DHA content of broiler chicken meat by 4 and 5 times, respectively. A study conducted by Zelenka et al. (2008) found that when chickens were fed with an ALA-rich diet, all n-3 PUFA content in the meat was significantly higher and n-6 PUFAs lower than when chickens were fed a diet containing high levels of n-6 PUFA. These results cause a decrease in the ratio of n-6/

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Table 3. Fatty acid composition of broiler meat fed diets enrich with ALA level from purslane meal

| | | Т | reatments | | | | |
|-------------------|---------------------|---------------------|---------------------|----------------------|---------------------|---------|------|
| Purslane meal (%) | Br-E0 | Br-E1 | Br-E2 | Br-E3 | Br-E4 | P-Value | Sig. |
| (% fatty acids) | | | | | | | |
| 14:0 | 9.13 | 7.82 | 12.11 | 10.91 | 12.53 | 0.055 | NS |
| 16:0 | 25.06 ^a | 23.44 ^{ab} | 23.02 ^b | 23.21 ^b | 22.44 ^b | 0.002 | ** |
| 18:0 | 10.52 ^a | 7.76 ^b | 9.67 ^{ab} | 9.57^{ab} | 9.96^{ab} | 0.018 | * |
| Total SFA | 47.65 ^{ab} | 41.74 ^b | 48.34 ^a | 47.47^{ab} | 48.34^{a} | 0.027 | * |
| 18:1n-9 | 26.17 ^{ab} | 29.62 ^a | 21.81 ^b | 23.00 ^{ab} | 21.67 ^b | 0.015 | * |
| 18:1n-9 | 1.76^{ab} | 2.10 ^a | 1.35 ^b | 1.64 ^{ab} | 1.36 ^b | 0.023 | * |
| Total MUFA | 32.10 ^{ab} | 36.95 ^a | 25.28 ^b | 27.23 ^{ab} | 24.73 ^b | 0.010 | * |
| 18:3n-3 (ALA) | 0.72 ^b | 0.74 ^b | 0.92^{ab} | 0.88^{ab} | 1.03 ^a | 0.005 | ** |
| 20:5n-3 (EPA) | 0.35 | 0.29 | 0.35 | 0.29 | 0.31 | 0.614 | NS |
| 22:5n-3 (DPA) | 0.43 | 0.41 | 0.57 | 0.55 | 0.57 | 0.278 | NS |
| 22:6n-3 (DHA) | 0.45 ^a | 0.46^{a} | 0.46^{a} | 0.78^{a} | 0.85^{a} | 0.018 | * |
| n-3 LCPUFA | 1.22 | 1.17 | 1.39 | 1.63 | 1.73 | 0.073 | NS |
| Total Omega-3 | 1.94 ^b | 1.91 ^b | 2.32 ^{ab} | 2.50^{ab} | 2.76^{a} | 0.004 | ** |
| 18:2n-6 (LA) | 14.68 ^b | 15.94 ^{ab} | 18.95 ^a | 17.85 ^{ab} | 18.99 ^a | 0.005 | ** |
| 20:4n-6 (AA) | 1.85 | 1.77 | 2.84 | 2.80 | 2.94 | 0.060 | NS |
| Total Omega-6 | 18.01 ^b | 19.09 ^b | 23.78 ^a | 22.56 ^{ab} | 23.83 ^a | 0.002 | ** |
| PUFA | 19.95 ^c | 21.01 ^{bc} | 26.09 ^{ab} | 25.06 ^{abc} | 26.59ª | 0.002 | ** |

Values are presented as mean \pm SD of five replicates per treatment. Values with different superscripts within the same row are significantly different (P<0.05).

Br-E0: basal diet + 0% purslane meal; Br-E1: basal diet + 1.5% purslane meal; Br-E2: basal diet + 3% purslane meal; Br-E3: basal diet + 4.5% purslane meal; Br-E4: basal diet + 6% purslane meal, SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, ALA: alpha-linolenic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid, n-3 LCPUFA: omega-3 long chain polyunsaturated fatty acid, LA: linoleic acid, PUFA: polyunsaturated fatty acid, AA: arachidonic acid, NS: not significant.

*P<0.05, **P<0.01.

n-3 PUFA in meat. In addition, studies in pigs also found that the low dietary n-6 to n-3 ratio improved the accumulation of n-3 PUFA (Nong *et al.*, 2020). The total n-3 PUFA increased mainly due to the accumulation of ALA and n-3 LCPUFA (EPA, DPA and DHA). Total n-3 PUFA increased by 43.0%. The results obtained indicated that the broiler feed formula supplemented with ingredients rich in n-3 fatty acid can increase the total concentration of n-3 PUFA (Mandal *et al.*, 2014; Ibrahim *et al.*, 2018).

The results showed that the content of arachidonic acid (AA, C20:4n-6) in meat tends to increase. This is possible because n-6 PUFA, LA which is a precursor of AA also increased. The results obtained are probably due to the relatively high LA content of the feed. This supports the research by Mandal *et al.* (2014) who found that the AA content of chicken meat did not change because the fatty acid content of n-6 PUFA (LA) also did not differ significantly.

Total MUFA tended to decrease with the addition of purslane flour and the difference in MUFA accumulation was significant when using 6% purslane flour. This could be caused by an increase in PUFA levels which reached 33.3%. The results obtained are supported by the previous study of Kartikasari *et al.* (2012) which found that the MUFA concentration decreased with an increase in the total PUFA content. However, the PUFA composition in the meat is different between these studies. In this current study, PUFA content is mainly in the form of total n-6 PUFA while in the previous study, the PUFA content was in the form of n-3 PUFA. This difference is probably due to the formulation of the feed composition in the previous study by increasing the levels of n-3 PUFA while maintaining a relatively constant n-6 PUFA content.

4. Conclusion

The use of purslane meal up to a level of 6% in the broiler feed formula can maintain the chemical quality and also increase the level of ALA, DHA and total n-3 PUFA of the meat. This strategy can produce meat that is rich in n-3 fatty acids so that it can help provide quality and healthy food for consumers.

Conflict of interest

The authors declare no conflict of interest.

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