

The Effects of Oregano Oil (*Origanum onites* L.) on the Growth Performance and Some Blood Parameters of Holstein Friesian Calves

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ABSTRACT

This study was conducted on 44 newborn Holstein Friesian calves raised in a private farm in Hatay province, Turkey. The calves were divided into two groups; the first group (control group) was fed only with milk replacer and the second group was fed with milk replacer added 893 mg oregano oil (oregano oil group) from the fourth day after calving. The amount of milk replacer offered to the calves was 10% of their weekly live weights. The calves were weaned when they consumed 800 g concentrate feed daily for three consecutive days. Weaning age and daily live weight gains for control and oregano oil group calves were found as 68.6 ± 4.45 , 67.7 ± 4.62 days and 0.300 ± 0.09 , 0.400 ± 0.011 kg, respectively. The mean consumption of milk replacer, hay and concentrate feed of calves for the control and oregano oil calves were found as 327.7 ± 42.17 , 331.0 ± 49.40 liters; 13.25 ± 3.025 , 15.23 ± 3.682 kg and 19.97 ± 4.186 , 22.37 ± 5.709 kg, respectively. The average initial hay, and concentrate feed consuming ages and diarrhea days for the control and oregano oil groups were determined as 10.6 ± 0.29 , 9.5 ± 0.28 and 3.1 ± 0.111 , 1.9 ± 0.073 days. The initial hay and concentrate feed consuming ages were the same in both groups. According to the results of the study, more research is needed to evaluate the optimal dosing of oregano oil.

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Siyah Alaca Buzağlarında Kekik Yağının (*Origanum onites* L.) Buzağların Gelişim Performansı ve Bazı Kan Parametreleri Üzerine Etkisi

ÖZET

Bu çalışma, Hatay ilinde özel bir işletmede yetiştirilen 44 baş yeni doğmuş Siyah Alaca ırkı buzağı üzerinde yürütülmüştür. Buzağlar 2 gruba ayrılmış olup, birinci gruba (kontrol grubu), 4. günden itibaren sadece buzağı maması, ikinci gruba (kekik yağı grubu) ise buzağı mamasına ilaveten 893 mg kekik yağı ilave edilmiştir. İçirilen mama miktarı, buzağların haftalık canlı ağırlıklarının % 10'u kadar hesaplanmıştır. Üç gün üst üste 800 gr kesif yem tüketen buzağlar mamadan kesilmiştir. Kontrol ve kekik yağı grubu buzağı gruplarında mamadan kesim yaşı ile günlük canlı ağırlık artışı sırasıyla; 68.6 ± 4.45 , 67.7 ± 4.62 gün ve 0.300 ± 0.09 , 0.400 ± 0.011 kg olarak belirlenmiştir. Toplam tüketilen mama, kaba ve kesif yem miktarları muamele gruplarına göre sırasıyla; 327.7 ± 42.17 , 331.0 ± 49.40 litre; 13.25 ± 3.025 , 15.23 ± 3.682 kg ve 19.97 ± 4.186 , 22.37 ± 5.709 kg olarak hesaplanmıştır. Buzağların kaba ve kesif yem tüketimine başlama yaşları ile ishali geçirdikleri gün sayısı ise sırasıyla; 10.6 ± 0.29 , 9.5 ± 0.28 gün ve 3.1 ± 0.111 ve 1.9 ± 0.073 gün olarak tespit edilmiştir. Buzağların kaba ve kesif yeme başlama yaşları benzer gerçekleşmiştir. Araştırma sonuçları, mamaya ilave edilen kekik yağının buzağlarda uygun dozunun ayarlanması amacıyla daha çok araştırma yapılması gerektiğini ortaya koymuştur.

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INTRODUCTION

In cases where the prices of raw milk are high and the number of calves are higher, dairy cattle farms can be given milk replacer after drinking colostrum for the first 3 days. Milk replacer are mainly obtained by drying milk and milk products with special processes. In addition to milk and milk products, various plant sources are used to obtain milk replacer. Although calves fed with milk replacer have a lower body weight gain than calves fed with normal milk, this difference closes with compensatory growth after the weaning. Milk replacer should be taken at body temperature and be sure of their quality. Cold drinking or poor quality calf foods can lead to diarrhea and development disorders (Türkmen, 2011; Akyüz *et al.*, 2017). The case of diarrhea caused by infection or feeding is defined as the loss of too much liquid and mineral in the body due to the deterioration of normal fluid movement in the digestive tract. Diarrhea can cause the loss of the body weight with excessive fluid loss by disrupting the chemistry of the body, causing loss of developmental performance and death in more advanced cases (Costello, 2005). The high level of loss of calves both endangers the future of breeding herds and reduces the amount of milk and meat to be obtained from cattle and causes enormous economic losses of the enterprises. In the neonatal period, calf loss is between 1% and 10% in developed countries, while this rate can reach up to 10% and 15% level in Turkey (Civelek, 2018). In the neonatal period, 60 % and 62.5 % of calf deaths are caused from diarrhea. Calves are very sensitive and vulnerable in the first month after birth and they are faced with many types of harmful bacteria threat. Pathogenic bacteria such as *Escherichia coli*, *Salmonella spp.*, and *Campylobacter spp.* cause diarrhea in calves, and in more severe cases, it may result in coccidiosis and death (Tüzemen and Yanar, 2013; Akyüz *et al.*, 2017). Albeit cattle breeders take numerous measures to prevent calf deaths, pathogenic bacteria are abundant in digestive systems in the early days of calves and diarrhea cases persist and calf deaths cannot be fully prevented (Tüzemen and Yanar, 2013). Due to the widespread use of antibiotic feed additives in recent years, the resistance of human and animal pathogens has increased as result of renewing and improving themselves. These pathogens have passed on to humans by leaving residues in animal products, and it has been a concern that antibiotics used for the treatment of these diseases do not work. Due to these concerns, antibiotics were banned in Sweden in 1986 and later in 2005 in EU countries as a feed additive which promotes growth in animal nutrition (Ünlü and Erkek, 2013). The use of antibiotics as a growth factor in animal feed was prohibited in Turkey in January 1, 2006. Due to the antibacterial and antifungal effects of oregano oil, carvacrol and thymol have a lethal effect on

microorganisms such as *Erwinia amylovora*, *Bacillus subtilis*, *Escherichia coli*, *Hafnia alvei*, *Micrococcus luteus*, *Proteus vulgaris*, *Staphylococcus aureus* and *Streptococcus faecalis* (Souza *et al.*, 2007). Furthermore, due to the effect of essential oils on the intestinal microflora, there are many studies that report feed and daily body weight gain increase and prevent diarrhea and some diseases (Busquet *et al.*, 2006; Calsamiglia *et al.*, 2007; Jouany and Morgavi, 2007; Rusenova and Paronova, 2009; Uetaka, 2013; Ünlü and Erkek, 2013). The aim of this study was to determine the effect of the dietary addition of oregano oil (*Origanum onites* L.) on growth performance, blood parameters and diarrhea of calves in order to increase the operating income of high milk prices in neonatal life.

MATERIAL and METHODS

This study was conducted in a private dairy cattle farm in Akcurun district of Antakya, Hatay province. In the study, total 44 of Holstein Friesian calves (24 males, 20 females) were allocated into two groups. All calves were born from the primiparous cows that imported from Hungary. After calving, calves were allowed to stay for 8 hours with their mothers. At the end of this period, calves were taken to individual calf pens and 5 liters colostrum was given for consecutive three days in the morning and evening. From the fourth day on, liquid milk replacer were given to the calves. The milk replacer contained lactoferrin, lactoperoxidase, prebiotics, organic acids, organic selenium, sorbitol, choline and methionine. While the first group (n: 21) was given only milk replacer (n: 23), the second group of calves were given 893 mg oregano oil in addition to milk replacer. Milk replacer was prepared by adding per kg of powdered milk replacer to 8 liters of water. Liquid milk replacer was given to the calves at 35.5-36.5 °C temperature twice a day (morning-evening). The amount of milk replacer to be given to the calves was calculated as 10% of the weekly live weight of the calves. Quality alfalfa and calf starter were given to the calves as *ad libitum* after the 4th day. Calf starter consists of 30% barley, 20% corn, 10% wheat bran, 25% soybean meal, 5% cottonseed meal, 8% molasses and 2% vitamin-mineral mixture, the amount of protein was 196 g/kg and the amount of crude fiber was 53 g/kg. Each kg alfalfa hay contained as 850 g/kg of dry matter, 1530 kcal/kg metabolizable energy, 150 g/kg crude protein and 290 g/kg of crude cellulose. Clean and fresh water at 20-22 °C temperature was provided in front of the calves for 24 hours. Calves were weaned when they consumed 800 g/day of concentrate feed for three consecutive days. Oregano oil used in the study was obtained from a commercial enterprise and content analyzes were determined by Hewlett Packard 6890 model gas chromatography in Hatay Mustafa Kemal University Central Laboratory. In this study,

the growth performance characteristics of calves such as daily live weight gain, the amounts of daily hay and concentrated feed consumption, feed conversion ratio, weaning age, weaning weight, total milk replacer consumption and fecal scores were emphasized. In addition a fecal scoring was performed according to Larson *et al.* (1977). In fecal scoring, normal consistency was evaluated as 1 point; soft but could not be handled was 2 points, easily and easily dispersible was 3 points and no solids in the consistency was evaluated as 4 points. Blood samples from calves were collected from the vena jugularis by the veterinarian, giving importance to the necessary hygiene and cleanliness with the decision of the Board of Ethics Committee No. 2014-8 / 1 of the Animal Experiments Local Ethics Committee of Hatay Mustafa Kemal University. Albumin, AST (SGOT), phosphorus, GGT, glucose, calcium, creatinine, cholesterol, triglyceride, total T3 and T4, globulin, insulin and growth hormone (growth hormone) levels of blood samples were analyzed. The analyzes were carried out in a commercial laboratory with procurement of services. Colorimetric method was used for albumin, creatinine, cholesterol and globulin analyzes; Kinetic method was used for AST (Aspartate aminotransferase) and G-GT (Gamma Glutamyl Transferase); Radioimmunoassay method was used for insulin, total T3 and T4 levels; ELISA (Enzyme Linked Immunosorbent Assay) method was used for the growth hormone (GH) analysis; Glycerol Phosphate Dehydrogenase method was used for triglyceride analysis; flame photometer method was used in calcium analysis and the inorganic phosphate method was used for phosphorus analysis (Karagül *et al.*, 2000). In this study, SPSS (2015) package program was used for the statistical analysis. While General Linear Model "REPEATED MEASURES" test used for weekly live weights and weaning weights, General Linear Model "UNIVARIATE" test was used for daily live weight gain, feed intakes, age of weaning ages, ages of initial consuming hay and concentrate feed, fecal scores, blood parameters and number of days with diarrhea characteristics of calves.

Table 1. Growth performance values of group

| Characteristics | Calf Groups | | P-values |
|--------------------------------------------------------|----------------|--------------------|----------|
| | Control (n=21) | Oregano oil (n=23) | |
| Live weights | | | |
| Birth (kg) | 38.7 ± 5.24 | 38.6 ± 4.27 | 0.918 |
| Week 3 (kg) | 41.4 ± 4.53 | 42.3 ± 4.75 | 0.514 |
| Week 6 (kg) | 47.5 ± 6.80 | 47.9 ± 5.26 | 0.830 |
| Week 9 (kg) | 58.1 ± 8.27 | 59.6 ± 7.95 | 0.538 |
| Weaning weight (kg) | 61.0 ± 7.65 | 63.2 ± 9.38 | 0.398 |
| Weaning age (d) | 68.6 ± 4.45 | 67.7 ± 4.62 | 0.547 |
| Initial consuming ages of hay and concentrate feed (d) | 10.6 ± 0.29 | 9.5 ± 0.28 | 0.012 |
| Daily live weight gain (kg) | 0.300 ± 0.09 | 0.400 ± 0.11 | 0.242 |
| Total body weight gain (kg) | 22.3 ± 6.37 | 24.7 ± 8.05 | 0.289 |

P<0.05

RESULTS and DISCUSSION

Live Weights, Initial Hay and Concentrate Feed Consuming Ages of Calves

Birth weights, weekly live weights and weaning weights, weaning ages, initial consumption ages of hay and calf starter, daily live weight and total body weight gains of calves are shown in Table 1 and Figure 1. The oregano oil has a positive effect on all growth performance characteristics of calves. The differences between control and oregano oil used groups for the initial consumption ages of hay and concentrate feed were found statistically significant (P<0.05), while other characteristics were not found significant (P>0.05). Calves used Oregano oil have earlier weaning age, higher daily live weight and total weight gains and weaning weights (Table 1). It was observed that calves used oregano oil consumed more 3.3 kg milk replacer, 1.98 kg hay and 2.4 kg concentrate feed than the control groups. Similar studies were conducted on different animal species (Hernandez *et al.*, 2009; Chaves *et al.*, 2008; Simitzis *et al.*, 2008; Abdel-Wareth *et al.*, 2012; Ionescu *et al.*, 2013; Van der Vliet and Cardozo, 2013; Özalpaydın, 2014; Silper *et al.*, 2014; Cardinali *et al.*, 2015). Oregano oil group began to consume hay and concentrate feed at an earlier age of 1.1 days than control group calves. This due to that the oregano oil may have increased feed consumption and appetite, rumen development and regulated rumen pH at an earlier age.

The Hay, Concentrate Feed and Milk Replacer Consumption Amounts of Calves

The amounts of milk replacer, feed conversion ratio, hay and concentrate feed consumption of control and oregano oil groups are shown in Table 2. The differences between control and oregano oil groups were not significant (P>0.05) for the total milk replacer, hay, concentrate feed intake and feed conversion ratio.

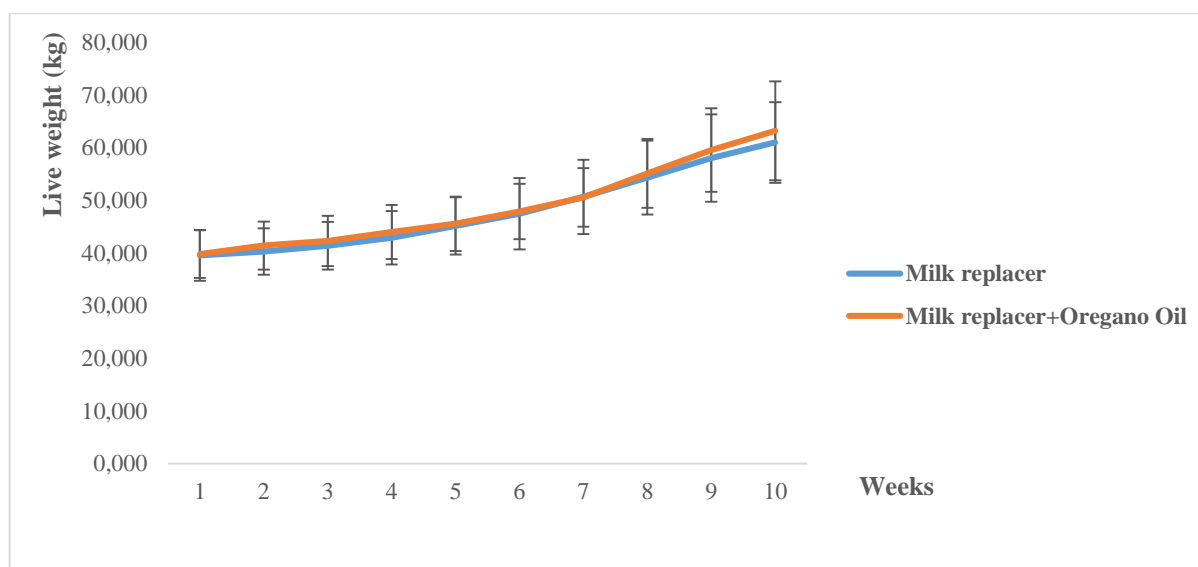


Figure 1. Average weekly live weights of calves (kg)

Table 2 Performance of calves according to measurement time in treatment groups

| Feed Intake (kg) | Calf Groups | | P-values |
|-----------------------------------------------|--------------------------|--------------------|----------|
| | Control (n=21) | Oregano oil (n=23) | |
| Milk Replacer | Live weights, kg/calf | | |
| Week 3 | 29.0 ± 3.17 | 29.6 ± 3.33 | 0.663 |
| Week 6 | 33.2 ± 4.76 | 33.5 ± 3.68 | 0.962 |
| Week 9 | 74.5 ± 27.45 | 73.2 ± 35.35 | 0.538 |
| At the weaning | 33.8 ± 26.78 | 32.6 ± 31.89 | 0.398 |
| Total intake | 327.7 ± 42.17 | 331.0 ± 49.40 | 0.815 |
| Alfalfa Hay | Feed intakes, kg | | |
| Week 3 | 0.17 ± 0.053 | 0.20 ± 0.067 | 0.262 |
| Week 6 | 1.21 ± 0.330 | 1.53 ± 0.466 | 0.703 |
| Week 9 | 2.80 ± 0.512 | 3.44 ± 0.645 | 0.589 |
| At the weaning | 3.43 ± 0.462 | 3.60 ± 0.573 | 0.537 |
| Total consumption | 13.25 ± 3.025 | 15.23 ± 3.682 | 0.881 |
| Daily intake | 0.200 ± 0.075 | 0.220 ± 0.062 | 0.196 |
| Calf Starter | Calf starter intakes, kg | | |
| Week 3 | 0.14 ± 0.051 | 0.18 ± 0.062 | 0.685 |
| Week 6 | 1.09 ± 0.408 | 1.59 ± 0.723 | 0.395 |
| Week 9 | 5.32 ± 0.986 | 5.53 ± 1.264 | 0.425 |
| At the weaning | 4.52 ± 1.020 | 4.62 ± 1.004 | 0.387 |
| Total intake | 19.97 ± 4.186 | 22.37 ± 5.709 | 0.164 |
| Daily intake | 0.290 ± 0.088 | 0.330 ± 0.104 | 0.225 |
| Feed conversion ratio (g feed/ g gain) | 3.1 ± 0.16 | 2.8 ± 0.16 | 0.346 |

P<0.05

It was observed that oregano oil group calves consumed more 3.3 kg milk replacer, 1.98 kg hay and 2.4 kg concentrate feed than the control group (Table 2). Similar results were reported by Morrison *et al.* (2010), Ünlü and Erkek, (2013), Özalpaydin, (2014), Silper *et al.* (2014), and Seifzadeh *et al.* (2017), while Santos *et al.* (2015) stated that oregano oil decreased the amount of consumption of milk replacer.

These differences can be thought to be caused by the fact that essential oils are given to animals in different

doses. Because calves initially do not like the taste of essential oils, feed intake was lower. The oregano oil group calves consumed 1.98 kg more hay than the control group calves. These results can be explained that the oregano oil caused increasing feed consumption due to the positive effect of the calves on the rumen microflora, digestive system and the increasing appetite of calves. Many studies conducted in this subject support the results of current research (Montoro *et al.*, 2011; Vakili *et al.*, 2013; Seifzadeh *et al.*

al., 2017). The calves used oregano oil was consumed 2.40 kg more concentrate feed than the control group calves (Table 2). These results are similar to Ünlü & Erkek (2013), and İzzaddeen & Kaygısız (2018). These results indicated that oregano oil increases calves' appetite and concentrate feed consumption, and starts consuming at earlier ages. However, some studies emphasized that essential oils reduce the number of proteolytic rumen bacteria and decrease the rumen deamination and thus reduce the consumption of concentrate feed (Santos *et al.* (2015). It may be stated that the differences between studies are based on use

of different animal species and essential oils, use of essential oils in different doses and forms and feeding methods. The feed conversion ratio was better in oregano oil group calves (Table 2).

Fecal Scores and Diarrhea Incidence of Calves

The diarrhea days, fecal scores of calves and the results of statistical analysis are given in Table 3. The differences between control and oregano oil groups for the diarrhea days and the fecal scores were not statistically significant ($P>0.05$). Oregano oil group calves had 1.2 diarrhea days less than control group.

Table 3 Day and fecal scores for diarrhea according to measurement time in treatment groups

| Diarrhea days (d) | Groups | | P-values |
|-----------------------------------|----------------|-------------------|----------|
| | Control (n=21) | Oregano oil(n=23) | |
| Week 3 | 0.9 ± 0.39 | 0.2 ± 0.15 | 0.124 |
| Week 6 | 0.1 ± 0.10 | 0.0 ± 0.00 | 0.159 |
| Week 9 | 0.0 ± 0.00 | 0.0 ± 0.00 | - |
| At the weaning | 0.0 ± 0.00 | 0.0 ± 0.00 | - |
| Total number of diarrhea days (d) | 3.1 ± 0.11 | 1.9 ± 0.07 | 0.370 |
| Average of diarrhea days (d) | 0.3 ± 0.01 | 0.2 ± 0.08 | 0.134 |
| Fecal Scores | | | |
| Week 3 | 1.57 ± 0.098 | 1.26 ± 0.062 | 0.211 |
| Week 6 | 1.29 ± 0.078 | 1.13 ± 0.046 | 0.422 |
| Week 9 | 1.06 ± 0.038 | 1.00 ± 0.000 | 0.301 |
| At the weaning | 1.05 ± 0.022 | 1.00 ± 0.000 | 0.286 |
| Average of fecal score | 1.26 ± 0.034 | 1.16 ± 0.028 | 0.313 |

$P<0.05$

This result can be explained by decreasing the activities of the harmful microorganisms which cause diarrhea by regulation of the rumen metabolism of the animals and increasing the rate of feed utilization. These results agree with Bambidis (2006); Ünlü and Erkek, (2013); Özalpaydın (2014); Ammar *et al.*,

(2017); Seifzadeh *et al.*, (2017) and Katsoulos *et al.* (2017).

Blood Parameters of Calves

The results of the statistical analysis of the blood parameters of the calf groups are shown in Table 4.

Table 4 Blood analysis according to the measurement time in treatment groups

| Blood Parameters | Calf Groups | | P-values |
|--------------------------|----------------|--------------------|----------|
| | Control (n=21) | Oregano oil (n=23) | |
| Albumin (g/dL) | 3.3 ± 0.11 | 3.1 ± 0.07 | 0.172 |
| AST (U/L) | 108.5 ± 8.55 | 86.5 ± 2.62 | 0.006 |
| Phosphorus (mg/Dl) | 8.1 ± 0.64 | 8.4 ± 0.28 | 0.603 |
| G-GT (U/L) | 17.4 ± 1.59 | 23.9 ± 2.50 | 0.086 |
| Glucose (mg/dL) | 92.5 ± 8.10 | 97.0 ± 6.55 | 0.680 |
| Calcium (mg/dL) | 9.9 ± 0.30 | 10.8 ± 0.19 | 0.010 |
| Creatinine (mg/dL) | 0.9 ± 0.05 | 0.91 ± 0.04 | 0.831 |
| Cholesterol (mg/dL) | 101.1 ± 5.94 | 126.6 ± 12.93 | 0.181 |
| Triacylglyceride (mg/dL) | 31.6 ± 3.25 | 36.9 ± 2.30 | 0.193 |
| T4 (nmol/L) | 68.6 ± 11.14 | 73.8 ± 6.91 | 0.679 |
| Globulin (g/dL) | 3.3 ± 0.20 | 3.4 ± 0.16 | 0.518 |
| T3 (nmol/L) | 2.0 ± 0.37 | 1.6 ± 0.21 | 0.273 |
| Insulin (pmol/L) | 114.9 ± 17.10 | 121.6 ± 11.78 | 0.747 |
| GH (ng/mL) | 6.6 ± 0.30 | 6.0 ± 0.33 | 0.232 |

$P<0.05$

The differences between control and added oregano oil calves for the AST and calcium were found significant ($P<0.05$), while the differences in all other blood parameters were not found significant ($P>0.05$). The phosphorus, glucose and triglyceride were not found within normal level range. The level of calcium in the blood was found to be higher in oregano oil added milk fed calves than the control group calves. It can be expressed that oregano oil increases the amount of calcium in the blood as a result of accelerating the absorption of calcium in the small intestine. These results were similar to Karagül *et al.* (2000) reports.

CONCLUSION

The results of the study showed that the addition of oregano oil to milk replacer was less important for calves for initializing of consuming hay and concentrated feed. In this study, it has been shown that it was effective in decreasing the age of onset of consumption of coarse and concentrated feed. In the present study, only one dose of oregano oil was used in calves. Since there have been not sufficient studies on the use of essential oils on calves, new studies are required for the effect of different doses and delivery times of essential oils of different plants.

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