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Effect of silkworm (*Bombyx mori* L.) pupae oil dietary inclusion on growth performance, digestibility and carcase traits of growing rabbits

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ABSTRACT

The study aimed to evaluate the effect of the dietary inclusion of silkworm (*Bombyx mori*) oil (SWO) in rabbit diets as a total replacement of sunflower oil on growth performance, carcase traits, total tract apparent digestibility (TTAD) and nutritive value of the diets. A total of $n = 64$ mixed-sex weaned rabbits (5-week-old) were pair-housed in cages and fed with a commercial diet containing 1.3% sunflower oil (control). From 7 to 10 weeks of age, two experimental groups were formed: half of the rabbits received a control diet, and the other half received a diet where the sunflower oil was replaced by the SWO. During the trial, growth parameters and feed intake were weekly recorded to calculate productive parameters. At 10 weeks of age, rabbits were slaughtered and dissected to determine carcase traits. In parallel to the growth trial, another twenty-four 55-day-old rabbits were individually housed in digestibility cages and randomly assigned to one of the two experimental groups ($n = 12$ rabbits/group) to study the TTAD and nutritive value of the diets. Overall, the dietary inclusion of SWO did not affect the *in vivo* performance and carcase traits of rabbits. Additionally, the TTAD of rabbits was unaffected by SWO inclusion, although the SWO diet exhibited lower digestible energy (DE) compared to the control diet ($p < .05$). These findings emphasise the importance of further investigating the nutritive value of SWO-supplemented diets in future studies. In conclusion, SWO can be considered a promising energy source for growing rabbits, an alternative to conventional vegetable oils.

HIGHLIGHTS

- Silkworm oil (SWO) is a feasible feedstuff for rabbit diets to substitute conventional energy sources.
- Total tract apparent digestibility of nutrients was not altered by the inclusion of SWO in growing rabbit diets.
- Growing rabbits fed with SWO as complete replacement sunflower oil showed satisfactory growth performance and carcase traits.

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Introduction

Silkworm (*Bombyx mori* L.) pupae (SWP) are obtained in great quantities from the silk production process (60% of the cocoon weight) and, despite a quote of them being directly consumed as food in some areas of the world, they are mainly considered a by-product. For this reason, they are often discarded thus representing a loss of nutrients (Sheikh et al. 2018). In fact, pupae are a nutrient-rich product which is characterised by a remarkable content of high biological value protein, and a great proportion of oil which contains a notable amount of linolenic acid (C18:3 *n*-3). This confers to this oil an extremely healthy *n*-3 rich fatty acids (FA) profile (up to about 60% of *n*-3 FA), which can be recommended for any diet. But these are not the only interesting nutritional compounds present in silkworm pupae oil: in fact, it contains tocopherols of whom α-tocopherol is the main representative and ranges between 73 and 131 g/kg oil. Silkworm oil (SWO) also contains relevant amounts of carotenoids (neoxanthin, violaxanthin, and lutein) and polyphenols in the interval 42–80 mg gallic acid equivalent (GAE)/g oil (Yap et al. 2023).

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In Italy, sericulture has a long tradition and, after a long production stop linked to the greater economic convenience of producing silk in other countries, the national industrial production of valuable yarn is starting again. In this perspective, exploiting silkworm pupae as value-added products for other industrial compartments, including the feed industry, represents a concrete possibility to build-up a circular productive process avoiding the production of waste (Altomare et al. 2020).

Owing to this, it must be emphasised that research studying the application of SWP into food-producing animals' feed formulations is still in its infancy, as the almost totality of studies, albeit limited, was conducted on poultry species (Banday et al. 2023). From existing data, it appears that the mulberry silkworm inclusion level is a key factor for guaranteeing satisfactory growth performance: this insect species naturally contains anti-nutritional compounds, namely chitin and particularly 1-deoxynojirimycin (1- DNJ), the latter having a proven negative effect on the digestibility of diets since it significantly reduces starch absorption (Dalle Zotte et al. 2021). The oil fraction, instead, could be used as an energy source with a healthy FA profile, and without nutritional drawbacks. From the research conducted to date considering the possible utilisation of the mulberry SWO in different species, it emerged that the incorporation of SWO into the diet of Wistar rats (7% inclusion, 8-week trial) improved hyperlipidaemia and hyperglycaemia (Mentang et al. 2011). When tested into juvenile carp's diet (Chen et al. 2017; Xu et al. 2020), SWO improved growth performance without affecting the health status of the fish up to 22.5 g/kg. The interesting aspect of SWO is that in the EU it can be used in feed formulations for strict herbivore species too (Regulation EC No. 999/2001; EC 2001), thus opening further possibilities for promising feed applications.

Based on these premises, the present research aimed to evaluate for the first time the potential dietary inclusion of SWO (*Bombyx mori*) in rabbit diets as a total replacement of sunflower oil. This manuscript deals with the impact on the TTAD, nutritive value of diets, growth performance and carcase traits, while physico-chemical meat quality traits (including the FA profile and contents of meat, liver and perirenal fat), sensory characteristics and shelf-life have been published elsewhere (Cullere et al. 2022; Dalle Zotte et al. 2022).

Materials and methods

*Silkworm (*Bombyx mori *L.) pupae oil*

Silkworm cocoons were obtained from a local farmer and subsequently transported to the Department of Animal Medicine, Production, and Health (MAPS) of the University of Padova (Padova, Italy). The cocoons were cut to obtain the full-fat SWP, which were then subjected to oil extraction (cold pressing). The resulting product, SWO, was utilised in the formulation of the experimental diet for growing rabbits.

Animals and experimental design

The trial was conducted at the experimental rabbit farm located at the Kaposvár Campus, Hungarian University of Agriculture and Life Sciences (Gödöllő, Hungary), by using Pannon White rabbits. All rabbits were handled according to the principles stated in the European Directive 2010/63/EU regarding the protection of animals used for experimental and other scientific purposes (EC 2010), and according to the Hungarian legal requirements (32/1999./III 31/and 178/2009/XII 29/).

A total of $n = 64$ mixed-sex weaned rabbits (5 weeks of age) were pair-housed in wire mesh cages (0.61 m × 0.32 m; 10.2 rabbits/m^2). From 5 to 7 weeks of age, all rabbits received a control diet consisting of 1.3% sunflower oil (control). Then, from 7 to 10 weeks of age, rabbits were fed with the experimental diets ($n = 32$ rabbits/treatment): the first group was fed with the control diet (the same as the period of 5–7 weeks), while the second group (SWO) received a diet containing 1.3% SWO as a complete replacement of sunflower oil. Diets were formulated to meet the minimum requirements for growing rabbits (de Blas and Mateos 2020); they were isonitrogenous, isoenergy, and in pellet form. Feed and water were provided *ad libitum* throughout the experiment. During the experiment, the ambient temperature range was 15–18 °C, and a 16 light–8 dark lighting regime was applied. The ingredients and the chemical composition of experimental diets are shown in Tables 1 and 2, respectively. The FA profile of SWO and the experimental diets were presented elsewhere (Dalle Zotte et al. 2022).

The individual body weight (BW) of rabbits ($n = 32$ rabbits/group) and feed intake (FI) per cage ($n = 16$ cages/group) were measured weekly, and the daily weight gain (DWG) and feed conversion ratio (FCR) were calculated. Morbidity of animals was monitored weekly, whereas mortality was checked daily. At 10 weeks of age and after overnight fasting, all the rabbits were weighed (slaughter weight), slaughtered, and bled by cutting the carotid arteries and jugular veins. Then the skin, genitals, urinary bladder, gastro-intestinal tract and the distal part of the legs were removed. Carcases were chilled for 24 h at 4 °C, and

Table 1. Ingredients (g/kg) of the experimental diets.

	Experimental diets	
Ingredients	Control	SWO
Dehydrated alfalfa meal (17.5 g CP/100 g)	150	150
Meadow grass hay (11 g CP/100 g; 30.5 g CF/100 g)	150	150
Sunflower meal (36 g CP/100 g)	148	148
Dried sugar beet pulp	120	120
Ground wheat	100	100
Wheat bran	100	100
Oat meal (11.5 g CP/100 g)	55.0	55.0
Ground barley	40.0	40.0
Full-fat soybean meal (34 g CP/100 g)	35.0	35.0
Arbocel®[a]	35.0	35.0
Vitamin-mineral premix[b]	30.0	30.0
Corn cob meal	20.0	20.0
Sunflower oil	13.0	0.00
Silkworm oil	0.00	13.0
Lignobond DD®[c]	2.00	2.00
Limestone	1.50	1.50
DL-methionine	0.70	0.70

SWO: silkworm oil; CP: crude protein.
[a]Crude fibre concentrate based on lignocellulose.
[b]Vitamin and mineral premix provided the following per kg of diet: vitamin A, 325000 IU; vitamin D, 45000 IU; vitamin E, 1375 IU; crude protein, 13.85%; lysine, 7.4%; methionine, 2.7%; Zn, 2600 mg/kg; Cu, 320 mg/kg.
[c]100% calcium lignosulphonate.

Table 2. Chemical composition (g/kg as fed) and gross energy (MJ/kg) content of the pre-weaning diet and of the experimental diets.

		Experimental diets	
	Pre-weaning diet	Control	SWO
Dry matter	923	918	916
Crude protein	180	186	190
Ether extract	45.5	37.2	39.7
Ash	77.5	79.8	83.7
Nitrogen-free extracts[a]	403	351	353
Crude fibre	160	186	182
NDF	285	328	323
ADF	179	204	201
ADL	44.4	47.7	50.5
Starch	201	167	170
Gross energy[b]	16.2	17.5	17.2

SWO: silkworm oil.
[a]Calculated: 100 − (water + crude protein + ether extract + crude fibre + ash).
[b]Calculated: [(nitrogen-free extracts × 4.11) + (crude protein × 5.64) + (ether extract × 9.44) + (crude fibre × 4.78)] × 10.

then the dressing out percentage was determined. Rabbit carcases were dissected according to the recommendations of the World Rabbit Science Association (Blasco and Ouhayoun 2010). After dissection, the reference carcase (RC) weight and parts, as well as fat depots, were weighed and used to compute the ratios to the RC.

Digestibility trial

A separate *in vivo* digestibility trial was conducted at the facilities of the farm 'Il Tramonto' (Casalserugo, Padova, Italy). The farm has a scientific agreement with the MAPS Department, University of Padova. The digestibility trial was conducted according to the European standardised method, as described by Pérez et al. (1995). A total of $n = 24$ fifty-five-day-old Martini (Budrio di Longiano, Italy) rabbits ($n = 12$ rabbits/treatment) were individually housed in digestibility cages and subjected to an adaptation period of 10 days during which they were fed with a commercial feed. Subsequently, they were randomly assigned to one of the two experimental dietary treatments (control or SWO) and subjected to a 4-day collection period of faeces.During this period, overall individual FI was recorded. At the end of the digestibility, two rabbits per treatment were excluded based on injuries or aberrant feed waste behaviour.

The digestibility trial focused on determining the total tract apparent digestibility (TTAD) of various nutritional components, including dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), ash, crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), starch and energy. Subsequently, the nutritive value of the experimental diets was calculated based on the obtained digestibility data.

Chemical analyses

The chemical analyses of the experimental diets and faeces were carried out following the Association of Official Analytical Chemists (AOAC 2000) methods to determine DM (method no. 934.01), CP (method no. 2001.11), CF (method no. 978.10), ash (method no. 967.05) and starch (amyloglucosidase-α-amylase, method no. 996.11). The EE was determined after acid hydrolysis (EC 1998). The energy content of diets and faeces was measured using an adiabatic bomb calorimeter (ISO 1998). Fibre fractions: NDF, ADF and ADL were analysed using a sequential procedure according to Van Soest et al. (1991). Hemicellulose and cellulose were calculated as NDF-ADF and ADF-ADL, respectively.

Statistical analysis

The normality of the data distribution was assessed using the Shapiro–Wilk statistic (PROC UNIVARIATE). Subsequently, data conforming to a normal distribution were subjected to a one-way analysis of variance (ANOVA) with the experimental diet (control *vs*. SWO) considered as a fixed effect, following the general linear model (PROC GLM) procedures of SAS 9.1.3 statistical analysis software for Windows (SAS Inc., Cary, NC) (SAS 2008). In the context of performance assessment, the cage was designated as the experimental unit, while for digestibility data and carcase traits, the individual rabbit served as the experimental unit. Least square means were obtained using the Bonferroni test, and the significance was calculated at a 5% confidence level.

Results and discussion

Due to their potential for sustainability and thanks to their interesting nutritional composition, different insect species and the derived products, i.e. protein and fat fractions, are steadily emerging as novel ingredients for different food-producing animal species, mainly as an alternative to conventional feedstuffs such as fishmeal, soybean meal, vegetable oils, etc. (van Huis and Oonincx 2017). In the case of rabbit, the possible use of insects as feed ingredient in the EU is limited to the fat fraction, since the protein fraction of an animal-origin food cannot be fed to a strict herbivore. For this reason, and also because rabbits represent a niche meat species, research on the possible use of insects as alternative feed for their rations is still limited to a few studies, as it will be shown later in this section.

Table 3. Effect of the dietary inclusion either with 1.3% sunflower oil (control) or 1.3% silkworm (*Bombyx mori* L.) oil (SWO) on the live performance of growing rabbits.

	Experimental diets			
	Control	SWO[a]	SEM[b]	*p* Value
No. of rabbits	32	32		
Body weight, g				
5th week	881	881	8.50	.971
6th week	1226	1242	11.7	.482
7th week	1598	1615	12.3	.426
8th week	1924	1959	12.4	.161
9th week	2250	2291	14.1	.147
10th week	2506	2544	15.8	.234
Daily weight gain, g				
5–6 weeks	49.3	51.6	0.93	.219
6–7 weeks	53.1	53.3	0.73	.918
7–8 weeks	46.6	49.0	0.60	.040
8–9 weeks	46.7	47.5	0.82	.594
9–10 weeks	36.6	36.1	0.83	.783
Daily feed intake, g				
5–6 weeks	85.0	86.0	0.93	.704
6–7 weeks	113	115	1.28	.541
7–8 weeks	123	126	1.30	.230
8–9 weeks	144	147	1.80	.413
9–10 weeks	142	142	1.60	.942
FCR[c]				
5–6 weeks	1.74	1.67	0.02	.091
6–7 weeks	2.15	2.16	0.03	.840
7–8 weeks	2.64	2.57	0.03	.286
8–9 weeks	3.10	3.10	0.03	.992
9–10 weeks	3.91	3.95	0.06	.740

[a]SWO rabbits received the control diet until 7 weeks of age, then the SWO diets between 7 and 10 weeks of age.
[b]SEM: standard error of the means.
[c]FCR: feed conversion ratio.

The growth performance of rabbits from 5 to 10 weeks of age is presented in Table 3. From 5 to 7 weeks of age rabbits received the same sunflower oil-based diet, thus no differences between the two groups were expected which was confirmed by the results. This feeding strategy was chosen in the perspective of a cost-benefit utilisation of the SWO: since the SWO is a relatively expensive source of *n*-3 FA and the period of 7–10 weeks is sufficient to incorporate those FA into rabbit meat (Dalle Zotte et al. 2022), it was chosen to provide the experimental feedstuff only in the finishing phase. Starting from week 8 and throughout the experiment, the complete substitution of sunflower oil with SWO resulted in comparable performance of control and SWO rabbits. Specifically, rabbits of the two dietary groups exhibited similar BW (2506 and 2544 g for control and SWO rabbits at 10 weeks of age, respectively), DWG (46.4 and 47.5 g/day for control and SWO rabbits in the period 5–10 weeks, respectively), as well as daily FI (121 and 123 g/day for control and SWO rabbits in the period 5–10 weeks, respectively) and FCR (2.61 and 2.59 for control and SWO rabbits in the period 5–10 weeks, respectively). The only exception regarded the DWG in the period 7–8 week, which showed a value 5% higher in SWO rabbits compared to control ones ($p < .05$).

Productive data found confirmation in the results of the digestibility trial (Table 4), since rabbits of the two dietary treatments showed similar DM intake, as well as comparable DM, CP, EE, ash, CF and fibre fractions (NDF, ADF, hemicelluloses and cellulose), starch and energy digestibility. Despite this, it was observed that the inclusion of SWO affected the nutritive value of the diets, as it lowered the digestible energy (DE) compared to the control diet (11.3 *vs.* 11.7 MJ/kg for SWO and control diet, respectively; $p < .05$). As a result, the DP to DE ratio was also different in the two dietary treatments with the SWO diet having a higher value than the control one (12.4 *vs.* 11.9, respectively; $p < .001$). No matter the treatment, diets showed suboptimal DP and DE: in fact, nutritional recommendations for intensively farmed growing rabbits indicate 9.7–11.5 MJ/kg of DE to avoid negative effects on rabbit performance, while for DP 100–110 g/kg feed are recommended to prevent digestive disorders (de Blas and Mateos 2020). Despite this apparent issue, the productive performance of growing rabbits was satisfactory and no morbidity and/or mortality episodes were observed throughout the trial. The present result on DE, and thus DP/DE, was unexpected since the two diets had identical formulations and chemical composition. Furthermore, despite sunflower and SWO oils

Table 4. Effect of the dietary inclusion either with 1.3% sunflower oil (control) or 1.3% silkworm (*Bombyx mori* L.) oil (SWO) on the total tract apparent digestibility (TTAD) of growing rabbits and nutritive value of the experimental diets.

	Experimental diets			
Traits	Control	SWO	SEM[a]	*p* Value
No. of rabbits	10	10		
Live weight, g	2160	2154	148	.924
Dry matter (DM) intake, g/d	141	147	17.6	.450
TTAD, %				
DM	60.3	60.2	1.77	.980
Organic matter	61.6	61.5	1.76	.879
Crude protein	75.2	76.3	2.37	.299
Ether extract	76.0	74.8	3.02	.384
Ash	44.9	46.9	2.56	.097
Crude fibre	16.1	17.0	3.14	.560
Neutral detergent fibre (NDF)	28.4	30.0	2.70	.181
Acid detergent fibre (ADF)	21.1	20.6	5.59	.840
Hemicelluloses (NDF-ADF)	41.2	48.8	8.60	.061
Cellulose (ADF-ADL)	28.9	28.2	5.08	.752
Starch	98.8	98.7	0.23	.179
Energy	61.5	60.6	1.73	.256
Nutritive value				
Digestible protein (DP, g/kg)	138	140	4.42	.299
Digestible energy (DE, MJ/kg)	11.7	11.3	0.33	.022
DP to DE ratio (g/MJ)	11.9	12.4	0.13	<.001

[a]SEM: standard error of the means.

Table 5. Effect of the dietary inclusion either with 1.3% sunflower oil (control) or 1.3% silkworm (*Bombyx mori* L.) oil (SWO) on rabbit carcase traits.

	Experimental diets			
Traits	Control	SWO	SEM[a]	*p* Value
No. of rabbits	32	32		
Slaughter weight (SW), g	2547	2593	15.5	.135
Chilled carcase (CC), g	1550	1564	11.0	.530
Reference carcase (RC)[b], g	1335	1346	9.81	.573
Dressing out percentage, % SW	60.8	60.3	0.19	.153
RC yield, % CC	86.1	86.1	0.11	.879
Drip loss, %	3.01	3.10	0.06	.415
Ratio to CC, %:				
Head	8.02	8.20	0.06	.123
HLTTO[c]	1.32	1.28	0.02	.360
Liver	3.60	3.63	0.04	.701
Kidneys	0.97	0.99	0.01	.348
Ratio to RC, %				
Fore part	28.4	28.3	0.12	.546
Intermediate part	30.9	30.9	0.14	.773
Hind part	39.0	39.2	0.10	.442
Perirenal fat	1.28	1.21	0.05	.424
Scapular fat	0.39	0.37	0.02	.673
Dissectible fat	1.67	1.58	0.06	.429

[a]SEM: standard error of the means.
[b]Reference carcase: without head, and visceral organs.
[c]HLTTO: heart, lung, thymus, trachea and oesophagus.

have diverse FA profiles, being the second rich in linolenic acid (C18:3 *n*-3) and thus *n*-3 PUFA (Dalle Zotte et al. 2022), SWO is considered an energy source nutritionally equivalent to commonly used vegetable oils, including sunflower oil (Tangsanthatkun et al. 2022). Therefore, given the present result on the DE, the nutritional value of SWO for growing rabbits should probably be investigated more accurately.

Insect fats/oils can have a very diverse FA composition, which is linked to the species biological characteristics, including feeding substrate (Riekkinen et al. 2022). For instance: black soldier fly (*Hermetia illucens*) fat is particularly rich in saturated FA (45–83% of total FA), and yellow mealworm (*Tenebrio molitor*) fat in monounsaturated FA (45–53% of total FA), whereas SWO in *n*-3 polyunsaturated FA (28–60% of total FA) (Yap et al. 2023). FA digestibility is known to possibly vary depending on the fat source, proportion of FA in diet and presence of microbial population in caecum, potentially leading to differences in the DE of diets among rabbits fed varied fat sources (Fernández et al. 1994; de Blas and Wiseman 2010). This can explain why research conducted up to now testing the insect fat/oil fraction in meat-producing rabbit diets highlighted different effects on the TTAD. When growing rabbits were fed either with 30 g/kg or 60 g/kg black soldier fly fat as a replacement of linseed oil (Martins et al. 2018), the digestibility of the EE worsened compared to that of rabbits fed linseed oil. The possible negative effect was attributable to the composition of triacylglycerols and the regiospecific distribution of lauric acid (C12:0) within the triacylglycerols of black soldier fly fat. Despite this, the productive performance of rabbits was similar in all treatment groups. Conversely, a partial (50%) or total substitution of soybean oil either with black soldier fly or yellow mealworm fats, did not affect growing rabbits' digestibility and thus performance traits (Gasco et al. 2019).

A different scenario pertains to the use of insect meal in rabbit nutrition, as at certain species-specific inclusion levels the antinutritional factors such as chitin and/or other bioactive compounds (i.e. 1-DNJ in the case of silkworm meal; Dalle Zotte et al. 2021), demonstrated to negatively affect digestibility (Gugołek et al. 2021). This was mainly attributable to a depression in the large intestinal microbiota metabolism activity, leading to lesser enzymatic activity and lower short-chain FA concentration (Strychalski et al. 2021), thus leading to reduced growth performance (Gugołek et al. 2019).

The carcase traits of rabbits fed with control and SWO diets are shown in Table 5. Similar to that observed for growth traits, rabbits of the two groups coherently showed comparable outcomes ($p > .05$). Specifically, control and SWO rabbits had similar slaughter weight (2547 and 2593 g for control and SWO rabbits, respectively), RC weight (1335 and 1346 g for control and SWO rabbits, respectively), dressing out percentage (60.8 and 60.3% for control and SWO rabbits, respectively), and incidence of the meat cuts (% RC), namely fore, intermediate and hind part, as well as fat depots ($p > .05$). Research studies considering the possible use of insect fat in rabbit nutrition are limited but, consistently with the present findings, Martins et al. (2018) and Gasco et al. (2019) also did not observe any differences in carcase traits of rabbits fed with fat obtained either from black soldier fly or yellow mealworm. Even if it is known that dietary fat supplementation often increases the dressing out percentage and fat deposits in rabbits (Fernández-Carmona et al. 2000), the results of the present research were justified by the fact that the fat content of the insect-containing diets was the same, only the fat source was different.

Conclusions

The 1.3% incorporation of oil derived from the silkworm (*Bombyx mori*) pupae in the diet of growing rabbits demonstrated to be feasible, as a complete replacement of sunflower oil in the last phase of the production cycle (7–10 weeks of age) led to satisfactory digestibility, *in vivo* performance, and carcase traits. Special attention is worth dedicating to the nutritive value of the diets, since it was observed that SWO lowered the DE of rabbit diet, despite existing literature consider it to be nutritionally equivalent to commonly used vegetable oils. The latter suggests that the nutritional value of this emerging fat source needs to be better assessed. This, however, did not generate any undesirable effects on rabbit morbidity, mortality, and on the traits considered in the present study.

Disclosure statement

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Data availability statement

The data that support the findings of this study are available upon reasonable request.

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