

Replacement of velvet bean (*Muncuna pruriens*) with faba bean (*Vicia faba*) in crisp common carp (*Cyprinus carpio*) production

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Abstract. Crisp fish, a kind of tough firmness fish, is produced by using *Vicia faba* bean (VB) by many Asian countries including Vietnam. However, the production is depending strongly on the source of VB from China, which has a relatively high price and is unstable. The current experiment was conducted to examine the possibility of replacing VB with *Muncuna pruriens* bean (MB), which are an available and cheap bean source in Vietnam. 90 common carps (*Cyprinus carpio*) (1 kg fish⁻¹) were divided into 3 feeding groups, in triplicates: control (industrial feed), VB and MB. Feed was administered twice a day, at a rate of 2% of body weight for 90 days. The growth rate, survival rate, feed conversion ratio and flesh quality were determined. Results showed that fish fed control feed showed the highest growth rate, followed by those fed VB and MB diets. However, the firmness of fish fed with the MB diet was similar to those fed with the VB diet and significantly higher than that of control group. The yellow color and lipid content of fish fed with MB diet were lower than those from other groups, but other differences were not found. The results demonstrated that MB based diets can completely replace VB in crisp carp production.

Key Words: crisp fish, cyprinid, fillet, firmness, sole bean.

Introduction. Freshwater aquaculture plays a significant role in global aquaculture production. In 2014, it accounted for 47.1 million tons and contributed for approximately 63.8% of the global aquaculture production, in which carp (*Cyprinus carpio*) was one of the most important species (FAO 2016). In Asia, particularly in China and Vietnam, farmers aim to increase the production of value-added products in order to improve profitability by producing crisp fish (Zhu et al 2013; Yu et al 2014; Kieu 2011). Crisp fish is a term firstly used by Zhu et al (2013) to indicate the tough firmness of fish after being fed a long time with *Vicia faba* beans (VB). Several previous studies pointed out some changes in the structure and composition of the muscles of crisp fish compared to that of normal fish. Lin et al (2009a) and Lin et al (2009b) stated that crisp grass carp (*Ctenopharyngodon idella*) had a higher firmness of muscle and higher content of crude protein and amino acids than normal ones. Furthermore, Lin et al (2012) reported that crisp grass carp had a higher content of myofibrillar, sarcoplasmic, stromal proteins, sulfur amino acids and hydrophobic amino acids in comparison to normal fish. These characteristics of crisp fish are favored by local people, crisp fish becoming a special product, with a cost twice, even three times higher than that of normal fish (Kieu 2011; Nam 2018).

In Vietnam, crisp fish can be produced simply by feeding solely VB during the last 4-6 months, with a ration of 2% of the body weight, prior to selling the fish in the market. Thus, the crisp fish production in Vietnam is strongly dependent on the source of an ingredient from China. Due to the high cost of transportation, importing tax and difficulty in quality control for raw input materials, crisp fish production is unstable and vulnerable. Since growing this bean in Vietnam has not yet been feasible, finding local alternative beans do not only assist the development of aquaculture, but can also bring more benefits to farmers. *Muncuna pruriens* is multipurpose plant, which produces velvet beans (MB). It is cultivated to protect soil from erosion in the mountainous regions of North Vietnam. MB leaves showed a high potential as green fertilizer or feed for ruminants (Thi Thuy et al

2017; Ha 2008). Although the composition of MB is complex, it is similar to that of VB (Siddhuraju et al 1996; Siddhuraju & Becker 2003; Janardhanan & Vadivel 2000). If the MB can be used to produce crisp fish, the local aquaculture can develop independently from imported ingredients, and reduce the production costs. It can also promote the development of growing MB in mountainous regions, protect the soil from erosion and generate income for farmers in mountainous areas.

Material and Method

Experiment set-up. Common carp weighing 1 kg were purchased from the Research Institute for Aquaculture N°1 (Bac Ninh province) and kept in a large net (45 m²) for acclimatization for 20 days before being assigned to the experiment at Vietnam National University of Agriculture, from May to July 2018. Fish were fed with industrial feed during acclimatization, as they were before. 90 fish were equally divided into 3 groups: control group, VB bean group and MB bean group. Totally, 9 hapa net cages (1.5x2x2 m) were set up in a 3000 m² pond with a depth of 1.2 m. Each cage had 10 fish, and each treatment was performed 3 times. The total weight of fish in each net was initially recorded.

The experimental procedure followed the protocols approved by the Ethical Committee of the Pibulsongkram Rajabhat University (PSRU-(AG)-2020-001).

Bean preparation and feeding regime. During the experiment, fish were fed twice a day (at 8 AM and 4 PM), with industrial feed (control feed, with 30% crude protein), 100% *Vicia faba* bean (VB) and 100% *Muncuna pruriens* bean (MB), in each lot. The VB originated from China and was purchased from LUCAVI farm (Bac Ninh province). The MB was collected from local farmers in Ha Giang province.

Whole seeds of VB and MB were soaked in warm water for 24 hours to soften them, remove antinutrients, and enhance the palatability for the fish. In the first month of the experiment, beans were chopped into a smaller size to ensure that all fish could ingest it easily. Fish were fed with 2% of live body weight (similar to the level farmers use for crisp fish production). Bean residue was collected by using a tray on the net bottom, 1 hour after feeding, and it was dried and deducted to determine more accurately the amount of consumed beans, as well as the FCR. The chemical compositions of the feed and beans were determined by following the guideline from AOAC (1990) and are presented in Table 1.

Table 1
Chemical composition of diets (%DM)

Feed	Control	VB	MB
Dry matter	89.93	90.44	91.03
Crude protein	26.02	34.31	35.24
Crude lipid	8.00	10.33	15.36
Crude ash	12.82	13.56	13.18

Note: DM - dry matter; VB - *Vicia faba* bean; MB - *Mucuna pruriens* bean.

Fish sampling. Weight of all the fish was determined every month after 24 hours of starvation and the amount of feed was adjusted afterwards. When the experiment was terminated, fish growth rate and survival rate were calculated. Three fish were randomly selected from each net for measuring hepatic and intestine somatic indexes. The fresh fillets of these fish were used for analyzing flesh quality. Three fish were randomly sampled for chemical composition analysis. The three samples were placed in an autoclave at 121°C for 15 minutes before being homogenized. The samples were dried by a freezer-drier before being analyzed for the chemical composition according to AOAC (1990). During the experiment, dissolved oxygen, pH and temperature were measured twice daily, whereas NH₃ and H₂S were monitored weekly with Sera test kits.

Measurement parameters and formulas

Weight Gain (g) = Final Weight – Initial Weight

Specific Growth Rate (SGR, % day⁻¹) = $\frac{\text{Ln (Final Weight)} - \text{Ln (Initial Weight)}}{\text{Cultured duration}}$

Feed Conversion Ratio (FCR) = $\frac{\text{Consummed feed}}{\text{Live fish weight gain}}$

Hepato Somatic Index (HSI) = $\frac{\text{Weight of Liver}}{\text{Body Weight}} \times 100\%$

Intestine Somatic Index (ISI) = $\frac{\text{Weight of Intestine}}{\text{Body Weight}} \times 100\%$

Relative Intestine Length (RIL) = $\frac{\text{Intestine length}}{\text{Body standard length}}$

Condition Factor (CF, %) = $\frac{\text{Body Weight}}{\text{Standard Length}} \times 100\%$

Suvival Rate (SR, %) = $\frac{\text{Number of fish at the end of the experiment}}{\text{Number of fish at begining}} \times 100\%$

Drip loss and processing drip loss. Drip loss was determined as a percentage of weight loss after 24 hours of storage at 4°C or processing after 10 minutes of cooking in a water-bath. A piece of fillet with the size of 5x2.5 cm was placed in a plastic bag and cooked for 10 minutes at 90°C. Weight of the flesh before and after 24 hours of cooking was determined (Figure 1).

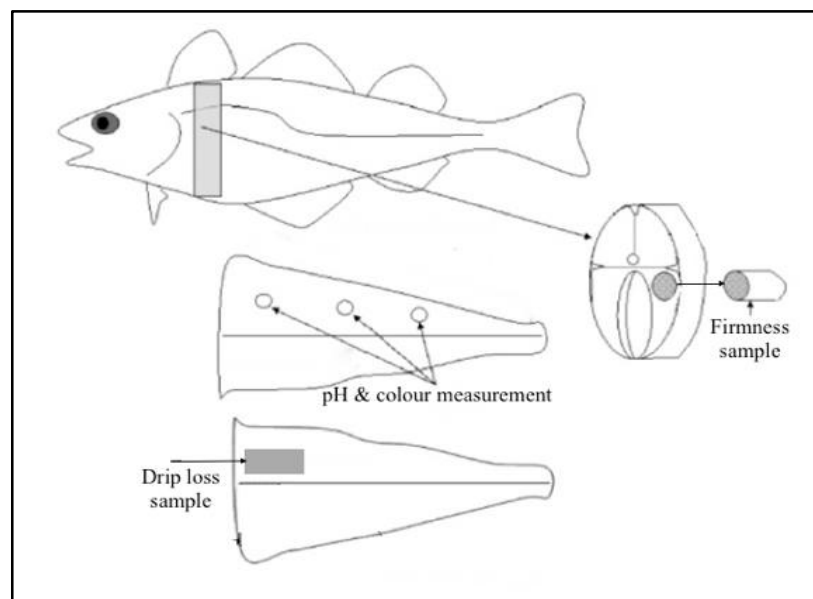


Figure 1. Morphological sampling. Source: modified after Kieu (2011).

Firmness (Shear force, $N\ mm^{-1}$). Instrumental texture analysis was performed using a Texture-Analyser, model Warner-Bratzler 2000D (USA). Texture analyses using the Warner-Bratzler blade were performed by cutting through a 0.5 cm diameter cylindrical longitudinal muscle sample removed by a borer from the lower part of the fillet, under the lateral line (Figure 1).

The pH of fillet. The pH was measured at three different *places* across the fillet surface with a pH meter *Star CPU* Matthaus (Germany). The *mean* of the six pH readings was used to represent the ultimate pH value.

Color. Color reflectance (L^*) was measured one hour after the bloom period with a Minolta Chromameter (Nippon Denshoker Handy Colorimeter NR-300, Japan). Six readings of L^* were also obtained and averaged for each sample across the surface of the Longissimus muscle. The averaged L^* value was used as a color indicator of the sample. The tristimulus L^*a^*b measurement mode was used, as this relates to the human eye response to color. The L^* variable represents lightness ($L^*=0$ for black, $L^*=100$ for white), the a^* scale represents the red/green, $+a^*$ intensity in red and $-a^*$ intensity in green and the b^* scale represents the yellow/blue, $+b^*$ intensity in yellow and $-b^*$ intensity in blue.

Data analysis. Data were analyzed with ANOVA and the significant differences between treatment means were determined by Tukey's test, using the SAS computer software. Percent data is normalized using an arcsine transformation before analysis. Significant levels for all analysis were set at $P<0.05$.

Results and Discussion

Water quality parameters. In general, all water quality parameters were not in optimum levels, but in an acceptable range for aquaculture. pH ranged from 7 to 8.5 and temperature fluctuated from $26.9\pm 0.5^\circ\text{C}$ (in the morning) to $28.4\pm 0.6^\circ\text{C}$ (in the afternoon). The lowest temperature was $20.8\pm 0.7^\circ\text{C}$. The dissolved oxygen (DO) of water ranged from 2 to 6 $\text{mg}\ \text{L}^{-1}$ for morning and afternoon, respectively. Daily average DO was above 4 $\text{mg}\ \text{L}^{-1}$.

Feed acceptance, survival and body condition factors. Fish did not accept the beans in the first day. However, they actively started to ingest beans if no standard feed was supplied. The adaptation to beans was clearly observed and the amount of bean intake was increased in the following days until it reached 2% of live body weight in the second week and was maintained in a constant level. Feed residue was not found on the tray, suggesting that fish ingested all administered beans.

During the experiment, neither disease nor mortalities were observed. The fish condition factor, hepato and intestine somatic indexes showed that the different feed did not affect the weight and length of intestine, but beans could have increased the liver weight (Table 2). Both HSI for VB and MB groups were higher than that of the control, especially the HSI from VB, which was $2.83\pm 0.32\%$, significantly higher in comparison to that of the control group ($1.44\pm 0.28\%$).

Fish growth. Although fish actively fed on all the feed in the experiment, growth rate was low. The highest weight gain was $548.33\pm 53.46\ \text{g}$, in the control group, followed by VB ($470\pm 30\ \text{g}$) and MB ($445\pm 55\ \text{g}$). Similarly, the results of the SGR analysis showed that the highest value was for the control group (0.42 ± 0.04), followed by VB and MB, with 0.38 ± 0.02 and 0.36 ± 0.04 , respectively. The FCR values were low in all groups, ranging from 3.17 ± 0.23 in the control group to 3.71 ± 0.49 in the MB group. However, no significant difference was detected between groups ($P>0.05$).

Table 2

Intestine, hepato somatic index, condition factor, weight gain and feed conversion ratio at the end of experiment

<i>Feed</i>	<i>Control</i>	<i>VB</i>	<i>MB</i>
ISI (%)	1.39±0.30	1.91±0.24	1.35±0.07
HSI (%)	1.44±0.28 ^a	2.83±0.32 ^b	2.04±0.09 ^b
RIL	2.19±0.27	2.58±0.06	2.12±0.25
CF (%)	2.79±0.01	2.79±0.01	3.0±0.01
WG (g)	548.33±53.46	470.00±30.00	445.00±55.00
SGR (% day ⁻¹)	0.42±0.04	0.38±0.02	0.36±0.04
FCR	3.17±0.23	3.62±0.21	3.71±0.49

Note: different superscript letters on the same row show significant difference $P < 0.05$. VB - *Vicia faba* bean group; MB - *Mucuna pruriens* bean group; ISI - Intestine Somatic Index; HSI - Hepato Somatic Index; RIL - Relative Intestine Length; CF - Condition Factor; WG - Weight Gain; SGR - Specific Growth Rate; FCR - Feed Conversion Ratio.

pH of fish flesh. The pH values measured from flesh at the moment of death and 24 hours post-mortem did not show any difference between fish from the control group and fish fed beans. The same results were observed for drip loss.

Fish color and flesh color. There was no difference in all color reflectance of fish at marketable sizes between fish in the control group and fish fed beans. The same results were recorded for the brightness and redness of flesh. However, the differences of yellow color (b^*) was visible. The lowest level of yellow color was observed in the fish fed control feed (1 ± 0.2), whereas the fish fed VB had the highest level (5.4 ± 0.6), followed by the MB group (3.3 ± 0.6). The values of yellow color of flesh 24 hours post-mortem in all groups were higher than those at the time of death, reaching 7.9 ± 0.4 , 8.9 ± 0.6 and 5.4 ± 0.7 for control, VB and MB groups, respectively (Table 3).

Table 3

Chemical composition (% of DM), pH and color of fish flesh at the end of the experiment

<i>Feed</i>	<i>Control</i>	<i>VB</i>	<i>MB</i>
Crude protein	20.76±0.42	19.52±0.53	19.34±0.47
Crude lipid	2.06±0.04 ^a	1.21±0.03 ^b	1.14±0.04 ^b
Crude ash	3.16±0.09	3.36±0.21	3.29±0.28
Water	73.87±1.02 ^a	75.67±0.99 ^b	75.86±0.89 ^b
pH T ₀	6.68±0.07	6.54±0.07	6.57±0.09
pH T ₂₄	6.04±0.05	5.93±0.07	5.75±0.02
Skin L*	63.42±2.16	62.07±1.68	63.42±2.16
Skin a*	0.62±0.30	0.19±0.74	0.62±0.30
Skin b*	12.68±0.48	15.57±1.62	12.68±0.48
Flesh L*	46.61±0.78	49.21±1.44	46.26±1.42
Flesh a*	13.10±0.31	11.95±0.54	12.94±0.5
Flesh b*	3.34±0.61	5.387±0.61	0.99±0.22
Firmness T ₀ (N mm ⁻¹)	9.33±0.46	14.15±2.03	13.73±1.15
Firmness T ₂₄ (N mm ⁻¹)	5.16±0.35	6.57±0.49	7.01±0.58
Drip loss T ₀	9.32±3.48	12.68±1.52	14.38±5.90
Storage drip loss T ₂₄	2.39±0.08	2.4±0.17	2.95±0.35
Processing drip loss T ₂₄	9.53±0.47	9.23±1.1	10.88±1.09

Note: different superscript letters on the same row show significant difference $P < 0.05$. DM - dry matter; VB - *Vicia faba* bean group; MB - *Mucuna pruriens* bean group; T₀ - time at dissection; T₂₄ - time at 24 h after mortem; L* - lightness; a* - red-green component; b* - yellow-blue component.

Firmness. At death, the firmness of fish fed VB was $14.15 \pm 2.03 \text{ N mm}^{-1}$, in the same range with that from the MB group ($13.73 \pm 1.15 \text{ N mm}^{-1}$), but significantly higher than that of fish fed standard feed. After 24 hours of storage at 4°C , the firmness of fish fed MB was $7.01 \pm 0.58 \text{ N mm}^{-1}$, in the same range with that of VB ($6.57 \pm 0.49 \text{ N mm}^{-1}$), but higher than that from the control group ($5.16 \pm 0.35 \text{ N mm}^{-1}$).

The chemical composition of the fish. The chemical composition of the fish carcass at the end of experiment showed that fish fed standard feed had the highest crude protein content, followed by fish in VB and MB groups (Table 3). However, significant differences were not found. The lipid content of the fish from the control group was significantly higher than those of the other groups. The lowest value of the water content belonged to the control group.

Growth and FCR. Comparison of fish growth was not the main purpose of this study, and the experiment was not designed for it. Thus, nutrient values such as protein, lipid and other components were not compatible between the control feed (standard feed) and beans. The standard feed was normally produced with an adequate formulation to ensure the nutritional requirements of carp. The nutritional components from sole bean are probably unbalanced and did not meet all the requirements of fish, which resulted in low growth rates for the fish fed beans. It was not unexpected that the bean groups showed a significant decrease in growth rate compared to the control group. This phenomenon was similarly reported in several previous studies. For example, Peng et al (2012) and Li et al (2007) demonstrated that fish fed broad beans (*V. faba*) or sprouted beans showed lower growth and higher FCR than those fed industrial feeds. Fish in the control group could ingest the standard feed immediately, while the other fish probably needed more time to adapt and accept a new feed, like beans. However, fish growth in the experiment, including in the control group, was slower than that observed in many other studies, and especially in comparison to that of carp fish from a farm nearby. In other farms, common carp was either stocked in earthen ponds in lower densities or in cages on rivers with higher water exchange. In the current study, the high stocking density could be a factor inhibiting fish growth. The density in the current study was much higher than in another farm (0.7 fish m^{-2}), as reported by Nam (2018). On the other hand, the growth rate of the fish in this experiment could be influenced by the development of their reproductive systems, because the dissection at the end of the experiment showed that both male and female fish contained mature gametes. This could be eliminated if mono-sex fish were selected.

Flesh quality. The effects of bean on flesh quality were reported in several previous studies. For example, muscle firmness was demonstrated to be increased for European seabass (*Dicentrarchus labrax*) and channel catfish (*Ictalurus punctatus*) fed on VB (Adamidou et al 2009b; Adamidou et al 2009a; Zhu et al 2012). However, this was one of the first studies on firmness of fish flesh using shear force methods. The used method in this study was modified from Kieu (2011). The position and size of the samples were adjusted to avoid systematic errors. The firmness of the fish in the study was much lower compared to those reported by Kieu (2011). However, many factors could have affected the firmness. First, the flesh was sampled below the lateral line and was thinner (0.5 cm) compared to the sampling conducted by Kieu (2011). Secondly, the dorsal flesh samples (above lateral line) could have potentially contained small pin bones (Y shape). The number of bones and the direction of the shear force could have lead to variable results regarding firmness. In this case, the effects of pin bones on firmness were not eliminated. Nevertheless, after 3 months of stocking, the results of firmness were already significantly different. If fish would have been stocked for a longer time, higher values for flesh firmness should have been expected.

At beginning of the experiment, fish did not feed on neither VB and MB, indicating that beans were not palatable for the fish. The beans contain antinutritional factors that lead to the reduction of palatability and reduce feed intake (Vadivel & Pugalenthil 2008).

The fish fed VB showed significantly higher values of HSI. These results were in agreement with those reported by Li et al (2007) and Qin (2010), who pointed out that

sole feeding on broad beans and sprouted beans could increase liver weight and change flesh quality. The results indicated that VB may still contain antinutritional factors after soaking. Francis et al (2001) and Hajra et al (2013) showed that antinutrients in the diet could result in adverse physiological effects, including the reduction of blood glucose levels, pancreatic hypertrophy, liver damage and other pathological lesions. Obviously, diet containing antinutrients could reduce food conversion efficiency and consequently decrease growth and health. Vadivel & Pugalenti (2008) reported that soaking and autoclaving could remove 73 to 87% of antinutrients in MB. Siddhuraju & Becker (2001; 2003) found that autoclaving could effectively remove trypsin and chymotrypsin inhibitors or lectin activity, resulting in better growth performance of tilapia (*Oreochromis niloticus*). However, cooking beans beforehand seems to not be feasible and practical in farm conditions. In addition, some of the other antinutrients, such as total phenolic compounds, tannins and phytates still remained after moisture heating. Francis et al (2001) have reviewed several methods to deactivate antinutritional factors from plant ingredients. However, the effectiveness depended much on the technique used. Even though no analysis for antinutrient content was carried out neither before nor after soaking in the study, it could be assumed that a certain amount of antinutrients still existed in the beans and these could have a negative impact on fish growth.

The increase of firmness was recorded for both VB and MB, related to the chemical composition of the fish flesh. Although fish fed beans did not show significant differences of firmness compared to the control, the lipid contents in these groups were significantly lower than that of control. These results were similar to many previous studies (Li et al 2007; Peng et al 2012; Lin et al 2016; Lin et al 2009a), which reported that sole feeding on VB could result in body changes and different flesh quality. Li et al (2007) reported that allogynogenetic crucian carp (*Carassius carassius*) fed on VB showed a change of protease activity and a decrease of 40 to 45% in body fat. Similarly, Gan et al (2017) reported that VB could replace soybean in the diets of grass carp up to 42% without any negative effect, level above which it could lead to the reduction of growth and health. In another study, Lin et al (2012) pointed out that fish fed solely on VB showed higher amounts of calcium and some specific collagens than the control fish. Furthermore, Li et al (2007) showed that the fiber diameter and myofibril length in fish fed beans were larger than those of control fish. These could be reasons to explain the increase of firmness at the end of the experiment after a long time of bean feeding.

Conclusions. Fish fed both beans (*M. pruriens* and *V. faba*) showed the same growth performances with fish fed a standard diet, but had significantly higher firmness of flesh. This indicates that the local *M. pruriens* bean could replace *V. faba* bean to produce crisp fish. However, further studies on the antinutrient content of beans and methods to deactivate them are needed to improve fish growth, flesh quality and use of both *M. pruriens* and *V. faba* beans more effectively.

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