Effect of probiotics and Chinese medicine polysaccharides on meat quality, muscle fibre type and intramuscular fat deposition in lambs

Chuntao Nie\textsuperscript{a}, Yiqing Hu\textsuperscript{a}, Rongrong Chen\textsuperscript{b}, Beibei Guo\textsuperscript{a}, Lin Li\textsuperscript{a}, Huan Chen\textsuperscript{a}, Hao Chen\textsuperscript{a} and Xiaozhen Song\textsuperscript{a}

\textsuperscript{a}Jiangxi Province Key Laboratory of Animal Nutrition/Engineering Research Center of Feed Development, Jiangxi Agricultural University, Nanchang, China; \textsuperscript{b}College of Bioengineering, Jiangxi Agricultural University, Nanchang, China

\textbf{ABSTRACT}

This study was carried out to investigate the effects of probiotics and Chinese medicine polysaccharides (CMP) on carcase characteristics, meat quality, muscle fibre type and intramuscular fat deposition in lambs. Forty female lambs with 4-5 months old and 21.69 ± 0.46 kg were randomly divided into four groups as follows: control, probiotics, CMP and probiotics + CMP groups. The results showed that supplemental probiotics increased carcase yield, the percentage of abdominal fat and perirenal fat of lambs, the levels of moisture and intramuscular fat in Longissimus thoracis (LT) muscle and the mRNA expression of MyHC I but decreased the value of shear force, lightness (L\textsuperscript{*}) and yellowness (b\textsuperscript{*}) and the percentage of heneicosanoic acid (C21:0) in LT muscle compared with the control group (\(p < .05\)). Supplemental CMP declined the value of b\textsuperscript{*}, the percentage of C21:0 and mRNA expression of MyHC I but enhanced the percentage of arachidonic acid (C20:4N6) and the content of moisture in LT muscle compared with the control group (\(p < .05\)). The percentage of abdominal fat and perirenal fat, the value of meat redness (a\textsuperscript{*}) and the mRNA expression of MyHC Ix were higher, while pH\textsubscript{24h} value was lower in lambs fed probiotics + CMP diets than those fed control diets (\(p < .05\)). All groups increased muscle fibres density of lambs compared with the control group (\(p < .05\)). Current results suggested that supplemental probiotics can affect muscle fibre characteristics, increase intramuscular fat deposition and improve meat quality. Probiotics should be a promising feed additive for production of high-quality lamb meat.

\textbf{HIGHLIGHTS}

- Probiotics improved meat tenderness by promoting intramuscular fat deposition and increasing the mRNA expression of MyHC I (slow-twitch oxidative).
- Chinese medicine polysaccharides (CMP) could enhance the concentrations of polyunsaturated fatty acids in LT muscle.
- Probiotics + CMP improved meat redness and increased fat deposition in dorsal subcutaneous and perirenal of lambs but did not affect intramuscular fat deposition.

\textbf{Introduction}

Lamb is defined as a high-quality product and it is considered a delicacy in many countries (Vieira and Fernández 2014). It has been observed a growing demand in large urban centres in China for high-quality, green and safe mutton with the enhancement of health awareness. However, in the process of lamb breeding, the improper use of antibiotics may lead to drug residues in meat products, thus endangering human beings. Therefore, many investigators have focussed on alternative feed additives or supplements, such as probiotics, herbal extracts and plant bioactive (Redoy et al. 2020; Campbell et al. 2021; Tekce et al. 2021).

Chinese medicine polysaccharides (CMP) are secondary metabolites extracted from traditional Chinese herbs such as Lycium barbarum and Astragalus. It is usually used as a bioactive substance to improve animal performance. A study has reported the incorporation of 3% w/w of L. barbarum in the rabbit diet was able to decrease lipid oxidation and improve the sensory traits of rabbit meat (Castrica et al. 2020). Long et al. (2020) found that dietary supplementation with...
L. barbarum polysaccharides can increase the growth performance and antioxidant properties of broiler chickens. Another report showed that Astragalus and Wolfberry extract can improve growth performance and carcass traits of Tibetan fragrant pigs and improve the most of the nutritional indicators in pork (Hao et al. 2021). Zhong et al. (2012) indicated that dietary Astragalus polysaccharides and Astragalus membranaceus root supplementation improved antioxidant capacity and affected the rumen fermentation patterns of lambs.

Probiotics are mainly viable and non-pathogenic organisms, including yeast (Saccharomyces cerevisiae), Bacillus and Lactobacillus, their supplementation benefits the host health by competing with other pathogenic microbes (Kulkarni et al. 2022). A previous study revealed that probiotics consist of Pediococcus acidilactici and Pediococcus pentosaceus was beneficial to improve the dry matter intake, growth performance, feed conversion rate and nutrient digestibility of weaned lambs (Saleem et al. 2017). Moreover, probiotics (Bacillus subtilis) improved meat quality characteristics of breast muscle in broilers exposed to chronic heat stress (Cramer et al. 2018). Supplemental with Bacillus licheniformis improved the broiler’s weights, carcass traits, meat quality traits as well as some blood indices and caecal microbial load (Abd El-Hack et al. 2021). Additionally, Hou et al. (2021) found that probiotics and Achyranthes bidentata Polysaccharides could increase the average daily weight gain for improvement of the growth performance of piglets.

The above reports indicated that Chinese medicine extracts and probiotics as feed additives have proven to improve the antioxidant and meat quality of pigs and chicken; however, the research interest in the application of probiotics and CMP in ruminant production is growing because they help maintain the balance of intestinal microbiota and serve as a possible alternative to the use of traditional antibiotics. The purpose of the current study was to study the effects of probiotics and CMP on meat quality and to explore its mechanism from the types of muscle fibres and intramuscular fat deposition of lambs.

### Materials and methods

#### Animals, diets and experimental design

The experimental lamb’s feeding and management have been described in detail in our previous study (Chen et al. 2021). Forty Chuanzhong black lambs with four to five months old (21.69 ± 0.46 kg) were randomly divided into four groups (n = 10): control group, probiotics (the mixture of Bacillus licheniformis, B. subtilis and Lactobacillus plantarum in the ratio of 1:1:0.5), CMP (the mixture of A. membranaceus and L. barbarum in the ratio of 1:2) and probiotics + CMP by 10 g/kg in the feed concentrate, respectively. The composition and nutrient levels of the basal diet are shown in Table 1. The feeding trial lasted for 70 days including a 10-day adaptation period and another 60-day experimental period, and antibiotics were not used in the whole period.

### Carcass characteristics and sample collection

At the end of the experiment, five lambs were randomly selected from each group and fasted for 12 h, weighed and recorded as the pre-slaughter live weight, and then all lambs were slaughtered in a commercial meat plant according to normal procedures. At this moment, a sample (about 1 g) of Longissimus thoracis (LT) muscles taken at the last rib of the left carcase were immediately frozen in liquid nitrogen for the analysis of the expressions of gene mRNA. Another LT muscle sample of 0.5 cm × 0.5 cm × 1 cm was cut out, parallel to the muscle fibre direction, from the last rib of the left carcase with 1 h post-mortem, and frozen in liquid nitrogen and stored at −80°C for making frozen sections. After slaughtered, the hot carcases were weighed, and carcass yield was calculated as the ratio of hot carcase weight to pre-slaughter live weight. The fat thickness over the longissimus muscles (between the 12th and 13th ribs) was obtained by means (triplicate in each carcase) of a vernier calliper. The adipose tissues were collected from perirenal and abdominal and weighed, respectively, to calculate perirenal fat percentage and abdominal fat percentage. Thereafter, the LT muscle samples were taken by cross-section between the 12th and 13th

### Table 1. Composition and nutrient levels of the basal diet (air-dry basis) %.

| Ingredients             | Content | Nutrient levels | Content |
|-------------------------|---------|-----------------|---------|
| Peanut vine             | 50.00   | Dry matter      | 84.01   |
| Corn                    | 30.00   | Metabolisable energy, MJ / kg | 7.07   |
| Soybean meal            | 11.00   | Crude protein   | 12.53   |
| Wheat bran              | 4.00    | Ca              | 1.20    |
| CaHPO₄                  | 2.00    | P               | 0.64    |
| NaHCO₃                  | 1.50    | Neutral detergent fibre | 33.03   |
| NaCl                    | 0.50    | Acid detergent fibre | 22.24   |
| Premix                  | 1.00    |                 |         |
| Total                   | 100.00  |                 |         |

The premix provided the following nutrients per kg of diet: Vitamin A,12000IU; Vitamin D, 5000 IU; Vitamin E,50 mg; Fe, 40 mg; Cu, 16 mg; Zn,70 mg; Mn,80 mg; Co, 0.3 mg; I, 0.8 mg; Se, 0.3 mg. Metabolisable energy was calculated according to the standards for raising mutton sheep (NY/T 816-2004), while the other nutrient levels were measured values.
ribs, 24 h after refrigeration in a cold chamber at 4°C to determine meat quality.

**Meat quality**

The pH values, meat colour, cooking loss and shear force were measured according to the method of Zhao et al. (2015). Muscle pH45min and pH24h were measured by using a pH electrode (HI99163N, Hanna, Padova, Italy). Meat colour (L*,a* and b*) was measured using a spectrocolourimeter (WSC-S, Shanghai, China) at 24 h post-mortem, the aperture was 8 mm, the illuminant was D65, 10° standard observer, and an open cone was used, three measurements were taken each time and the average value was calculated. And samples of LT muscle (3 cm x 4 cm x 5 cm) were vacuum-packed and cooked in a water bath at 80°C to reach an internal temperature of 70°C for measuring the cooking loss. Then, the shear force was measured using a C-LM3B shear apparatus (Northeast Agricultural University, Harbin, China). Drip loss was measured immediately after slaughter, muscle samples (3 cm x 4 cm x 5 cm) were weighed and packed in individual ziplock bag filled with air and hung with an iron wire hang for 24 h in 4°C, and then, the samples were wiped with absorbent paper to remove residual moisture and reweighed to calculate the drip loss. Moreover, the contents of moisture, crude protein and fat in LT muscle samples were according to the Association of Official Analytical Chemists (AOAC).

**Intramuscular fat deposition and muscle fibre types**

A sample of 0.5 cm x 0.5 cm x 1 cm was cut out, parallel to the muscle fibre direction, from the last rib of the left carcass with 1 h post-mortem. The cut sample was frozen in liquid nitrogen and stored at −80°C. The samples were sectioned into 10 μm slices with a cryostat microtome (CM1900, Leica, Germany) at −27°C. These sections were dried half an hour in air and divided into two parts, a part was stained with 60% oil red O for 7 min at room temperature, washed three times with PBS and then re-stained with haematoxylin for 30 s. Other sections were stained for mATPase activity after both acid (pH = 4.6 and 4.3) and alkaline (pH = 9.4) preincubation as described by Brooke and Kaiser (1970) and assisted staining for succinic dehydrogenase (SDH) activity. The samples were examined to differentiate type I (slow, red muscle, oxidative), type IIa (fast, red muscle, oxidative) and type IIb (fast, white muscle, glycolytic) fibres. Finally, these sections were taken photographs with Nikon eclipse 80i microscope (Nikon Corporation, Japan) under the microscope in 100 and 400 equipped with the NIS-Elements F 3.00 imaging software. Image-Pro Plus version 6.0 software (Media Cybernetics, Inc., USA) was used to analyse pictures including lipid accumulation was measured with Oil Red O staining, and the quantity and proportion of muscle fibre types with mATPase activity staining. Five views were captured in each section.

**Fatty acid composition**

The fatty acid profile of skeletal muscle was analysed as described by Zhang (2021); 150 mg of lyophilised muscle sample was extracted using chloracetyl methanol and treated with n-hexane and internal standard FA solution. The mixtures were centrifuged, and the supernatant was analysed by gas chromatography (HP 6890 series, Hewlett Packard, Avondale, PA, USA) using a DB-23 capillary column and a flame ionisation detector.

**Total RNA isolation and mRNA expressions analyses**

Total RNA was isolated from the LT sample using the phenol and guanidine isothiocyanate based Trizol reagent (Invitrogen, USA), according to the manufacturer’s instruction. The purity and quantity of total
RNA was measured by a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and the 260/280 ratios at 1.9–2.0 and the 260/230 ratios at 2.0–2.2 were used for subsequent PCR reactions. Total RNA was treated with DNaseI (Takara Biotechnology Co. Ltd., Dalian, China) to remove DNA and transcribed to cDNA using a PrimeScript RT Master Mix kit (Takara Biotechnology Co. Ltd., China) following the manufacturer’s instructions. Relative gene expression was calculated using the 2−ΔΔCt method, Primers used for mRNA expression were presented in Table 2. The quantitative real-time PCR cycling conditions were as follows: pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing temperature at 60°C for 15 s and extension at 72°C for 15 s (40 cycles from denaturation to annealing). A dissociation curve was constructed at the end of the reaction to validate the specificity of the reactions.

### Statistical analysis

All data analyses were statistically analysed by one-way ANOVA with SPSS statistical software (Ver.20 for windows, SPSS). Tukey–Kramer HSD (Honestly Significant Difference) test was used to compare differences among the groups. *p* Value < .05 was considered to be significant and .05 ≤ *p* < .10 was considered as a tendency. Values were expressed as mean ± SE.

### Results

#### Carcase trait and meat quality

As shown in Table 3, compared with the control group, the carcase yield in probiotics group was enhanced, and the percentage of abdominal fat and perirenal fat in probiotics and probiotics + CMP groups were raised (*p* < .05). Moreover, the percentage of abdominal fat in the probiotics + CMP group was higher than that in the CMP group (*p* < .05). There were no differences in pre-slaughter live weight, hot carcase weight and backfat thickness of lambs among all groups (*p* > .05). CMP group decreased the value of **b**<sup>−</sup>, probiotics group decreased the value of **L**<sup>+</sup>, **b**<sup>−</sup> and shear force in LT muscle of lambs and probiotics + CMP group increased the value of **a**<sup>+</sup> but declined the value of **pH**<sub>24h</sub> compared with the control group (*p* < .05). The moisture contents of LT muscle in probiotic and CMP groups were significantly higher than those in control group (*p* < .05). No difference was noticed about the value of **pH**<sub>45 min</sub>, drip loss, cooking loss and the levels of crude protein and ether extract among all groups (*p* > .05).

#### Intramuscular fat deposition

Oil Red O staining was performed to evaluate fat deposition in LT muscles in Figure 1. The content of intramuscular fat in LT muscle of lambs and probiotics + CMP group increased the value of **a**<sup>+</sup> but declined the value of **pH**<sub>24h</sub> compared with the control group (*p* < .05). The moisture contents of LT muscle in probiotic and CMP groups were significantly higher than those in control group (*p* < .05). No difference was noticed about the value of **pH**<sub>45 min</sub>, drip loss, cooking loss and the levels of crude protein and ether extract among all groups (*p* > .05).

#### Fatty acid composition

Table 4 shows the fatty acid composition of the IMF, there are a decrease of the proportion of heneicosanoic acid (C21:0) in probiotics and CMP groups compared with the control group (*p* < .05). In contrast, the
The proportion of arachidonic acid (C20:4N6) in the CMP group were higher than that in the control group \((p < .05)\). Supplemental CMP tended to increase the proportion of polyunsaturated fatty acids (PUFA) and PUFA/SFA comparing with the control \((.05 < p < .10)\). Moreover, the proportion of MyHC I in the probiotics group was higher than those in both CMP and probiotics + CMP groups \((p < .05)\).

**The morphology and types of muscle fibre**

Figure 2 shows the quantity and proportion of muscle fibre types, all groups enhanced the density of muscle fibre \((p < .05)\), and supplemental probiotics tend to decrease the proportion of MyHC IIb \((.05 < p < .10)\), but no difference was noticed about the proportion of MyHC I and MyHC IIa compared with the control \((p > .05)\). Moreover, the proportion of MyHC I in the probiotics group was higher than those in both CMP and probiotics + CMP groups \((p < .05)\).

**Genes expressions**

As shown in Figure 3, dietary supplemental with probiotics group increased the mRNA expression of MyHC
I (p < .05), and probiotics + CMP enhanced the mRNA expression of MyHC I (p < .05), but supplemental CMP declined the mRNA expression of MyHC I compared with the control group (p < .05). No difference was noticed about the mRNA expressions of MyHC Ila and MyHC IIb among groups (p > .05).

Discussion

The current results showed that supplemental probiotics, which were consist of *Bacillus licheniformis*, *B. subtilis* and *L. plantarum*, enhanced carcase yield, and the carcase yield is a key index to reflect meat production of livestock. Although some studies have indicated that the effects of *B. subtilis* supplementation on carcase characteristics of crossbred beef steers and lambs were small and non-appreciable (Rabelo et al. 2018;
Smith et al. 2021), a report from Melegy et al. (2011) pointed that carcase yields were significantly increased in broilers fed B. subtilis supplementation. Another study in pigs found that dietary addition of B. subtilis KN-42 can improve the growth performance and gastrointestinal health of piglets (Hu et al. 2014). Our previous results showed that dietary supplemental with probiotics can promote rumen microbial protein synthesis in lambs (Chen et al. 2021). We speculate that the improvement of carcase traits of probiotics group may be due to the enhance of MCP fed probiotics diets.

Meat colours and tenderness are extremely important indicators of mutton sensory qualities and consumer acceptability. In general, myoglobin pigments are responsible for the redness (a\(^*\)), and the value of lightness (L\(^*\)) and yellowness (b\(^*\)), which reflects water-holding capacity in raw meat (Allen et al. 1997). Meat colour is determined by the amount of myoglobin and haemoglobin and the level of lipid oxidation in muscle tissue. Some reports pointed that the oxidation of myoglobin to metmyoglobin might cause a decrease in muscle tissue. Some reports pointed that the oxidation of myoglobin to metmyoglobin might cause a decrease of a\(^*\) value, and a lower value of L\(^*\) or b\(^*\) of meat indicated less pale meat (Higgins et al. 1998; Fernandez-Lopez et al. 2005). The current results showed the value of L\(^*\) and b\(^*\), and the shear force in the probiotics group were declined, but water content was significantly increased, which suggested that supplemental probiotics could improve muscle colour and tenderness. Similar result was observed by Wu et al. (2018), who reported that lambs fed B. subtilis demonstrated reduced shear force and moisture content, and improved meat quality.

The content of muscle fat is one of the determinants of juiciness, so meat can be juicier and tender if the content of intramuscular fat is high (Jalloul Guimarães et al. 2020). In the present study, lambs fed probiotics demonstrated an increasing of intramuscular fat deposition, which were agreed with the improvement of meat tenderness. Coincidently, a report pointed by Oliveira et al. (2016) that feedlot cattle supplemented with B. subtilis had a greater intramuscular fat accumulation compared to control. Regrettably, the percentage of abdominal fat and perirenal fat in lambs fed probiotics diets were raised, which is not conducive to the fattening of lambs.

Muscle fibre is an important part of the muscle, and the quantity and type of muscle fibre are the keys to determine meat quality (Lee et al. 2010). Generally, the tenderness of meat would better if the fibre of muscle was thinner and denser (Gwartney et al. 1992). Fortunately, the current study showed that all groups enhanced the density of muscle fibre of LT muscle, which imply that both polysaccharides and probiotics as feed additives can be advantageous to the tenderness of muscle in lambs. Moreover, muscle fibre is classified into four categories according to the diversity of myosin heavy chain subtypes: MyHC I (slow-twitch oxidative), MyHC IIa (fast-twitch oxidative), MyHC IIx (fast-twitch oxidative-glycolytic) and MyHC IIb (fast-twitch glycolytic), and its transformation way is I→→IIa→→IIx→→IIb (Lee et al. 2010). Previous study reported that slow-twitch oxidative myofibre type (MyHC I) contained a higher content of fat (Hwang et al. 2010) and greater tenderness of meat was expected. The current study indicated supplemental probiotics increased the mRNA expression of MyHC I, but tended to decrease the percentage of MyHC IIb, which suggested that probiotics promote the transformation of MyHC IIb to MyHC I. This result is consistent with the change of muscle tenderness and intramuscular fat accumulation. Studies have shown that there is a high myoglobin content in MyHC I, which has a positive effect on the redness of meat, while MyHC IIb were the opposite (Su et al. 2013). Surprisingly, probiotics had no positive effect on the redness but decreased significantly the lightness and yellowness of LT muscle.

Although the present results showed that the expression of MyHC I in the CMP group was significantly decreased, there was no negative effect in the tenderness of mutton. And also, there was a clear enhance of moisture content and a decrease of the value of b\(^*\) in LT muscle. A similar result of Orzuna-Orzuna et al. (2021) observed that supplementation with a polyherbal mixture (HM) containing polysaccharides did not affect the values of L\(^*\) and a\(^*\) but b\(^*\) decreased. Yusuf et al. (2018) reported that dietary supplementation of Andrographis paniculata leaves enhanced the juiciness and tenderness of the Longissimus thoraciset lumborum (LTL) muscle in goats. Previous study has shown that antioxidants can improve pork colour, tenderness and water-holding capacity (Swigert et al. 2004). Therefore, we speculated that the effects of CMP supplementation on meat quality may be related to the antioxidant properties of L. barbarum and Astragalus polysaccharides (Zhong et al. 2012). In addition, the present study showed CMP group decreased significantly the proportion of C21:0, but the proportion of arachidonic acid (C20:4N6) was increased by CMP supplementation. The composition of fatty acids is not only the key factor to determine the flavour of the meat, but also has a close relationship with human health. PUFA plays an
important role in reducing triglycerides, cholesterol and low-density lipoprotein and promoting growth and development (Sacks and Campos 2006). As an essential fatty acid for infants, C20:4N6 promotes the continuous development of brain intelligence and the body. The current results indicated that CMP could be considered healthy for humans. Similar to this experiment, Redoy et al. (2020) found that dietary supplementation of herbal led to an increasing of serum antioxidant and polyunsaturated fatty acid (PUFA) content in mutton. Another coincident report indicated that Astragalus by-product (ABP) supplementation in sheep fed diets significantly increased the content of C15:1 and C18:3n6 in the LT muscle (Abdallah et al. 2020).

To further explore the compound effect of probiotics and CMP, a mixture containing an equal proportion of probiotics and CMP was used as feed additives in lambs. The current results showed that supplemental with probiotics plus CMP in diets had no significant effect on intramuscular fat and fatty acid composition, but significantly increased the percentage of abdominal fat and perirenal fat of lambs, which suggested that probiotics plus CMP is not conducive to the fattening of lambs. The final pH of the carcase is associated with the tenderness and colour of meat (Rossi et al. 2016), and abnormal pH may cause PSE or DFD meat. Although the value of pH24 in the probiotics + CMP group was lower than that in other groups, the values of all groups were in the normal range. Fortunately, our data showed that the probiotics plus CMP group enhanced meat redness and the contents of moisture in LT muscle, which suggested probiotics and CMP have a synergistic effect on improving meat colour and juiciness. Similarly, Benamirouche et al. (2020) found that broilers fed diets supplemented with probiotics and Yucca schidigera extract could regulate lipid metabolism and improve meat quality of broilers. However, another report showed that supplementation of the diet with green tea plus probiotics positively affected carcase composition and oxidative stability of loin meat, but moisture contents and juiciness were decreased (Hossain et al. 2012).

Conclusions

In conclusion, supplemental probiotics had beneficial effects on meat tenderness by promoting intramuscular fat deposition and regulating muscle fibre characteristics, and Chinese medicine polysaccharides could enhance the concentrations of polyunsaturated fatty acids in LT muscle. Dietary supplemental with probiotics plus CMP improved meat redness and moisture but increased fat deposition in dorsal subcutaneous and perirenal of lambs. These results indicated probiotics should be a promising feed additive for production of high-quality lamb meat.

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Ethical approval

This experiment was approved by the Committee for the Care and Use of Experimental Animals at Jiangxi Agricultural University (JXAULL-20190015).

Author contributions

X.S., C.N. and Y.H. designed the overall study. C.N., Y.H., H.C., B.G., H.C. and M.Q. performed the animal feeding experiment and sample analysis. X.S. and C.T. wrote the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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ORCID

Chuntao Nie http://orcid.org/0000-0002-1405-3184
Yiqing Hu http://orcid.org/0000-0002-4147-3950
Xiaozhen Song http://orcid.org/0000-0002-7194-053X

Data availability statement

The data that support the findings of this study are available from the corresponding author (Song X.) upon reasonable request.

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