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in

Brufau J. (ed.).
Feed manufacturing in the Mediterranean region. Improving safety: From feed to food

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 54

2001
pages 141-150

Plasma protein antimicrobial substitution at negligible risk

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SUMMARY – The use of blood derived products has increased worldwide because of their wide functional characteristics. Food, feed, animal and human health are the main industries using those products. The main products used by the feed industry are plasma protein and red cell fraction. Those are especially important in swine and pet food. The production system is described. A risk assessment has been done that indicates that the risk of blood products usage is negligible. Plasma is a biosafe product as far as bacteriology, virology and BSE are concerned. The production benefits of plasma are well documented: increased feed intake, growth performance, decreased diarrhoea incidence, protected intestinal function and morphology. The most plausible modes of action for those effects are palatability, digestibility and intestinal protection. The fraction responsible for those effects is the high molecular fraction.

Key words: Plasma protein, biosafety, production and heath benefits, antimicrobial substitution.

RESUME *– "Substitution antimicrobienne des protéines de plasma, avec risque négligeable". L'utilisation de produits dérivés du sang a augmenté dans le monde en raison de leurs grandes propriétés fonctionnelles. L'alimentation humaine, animale, la santé humaine et animale sont les principales industries utilisant ces produits. Les principaux produits utilisés par l'alimentation animale sont les protéines de plasma et la fraction des cellules rouges. Ils sont spécialement importants pour les porcins et les animaux de compagnie. Le système de production est décrit. Une estimation du risque a été menée qui indique que le risque lors de l'utilisation des produits du sang est négligeable. Le plasma est un produit sans risque pour ce qui est de la bactériologie, la virologie et l'ESB. L'intérêt du plasma est très bien documenté et il augmente la prise alimentaire, les performances de croissance, il diminue l'incidence des diarrhées, il protège la fonction intestinale et la morphologie. Le mode d'action pour ces effets sont l'appétence, la digestibilité et la protection intestinale. La fraction responsable de ces effets est la fraction de haut poids moléculaire.*

Mots-clés : *Protéines de plasma, bio-sécurité, production et santé, substitution antimicrobienne.*

Objectives

The objectives of this paper are to discuss in detail the following topics:

(i) Introduction.
(ii) Plasma production.
(iii) Plasma and red cell composition.
(iv) Risk assessment.
(v) Biosafety.
(vi) Facts and figures.
(vii) Production.
(viii) Health benefits: antimicrobial substitution.

Introduction

Traditionally blood derivatives have been widely used worldwide and moreso due to their wide functionality. The most important industries using blood derivatives are the food industry, mainly in meats as gelling agents and natural colorants, it uses about 30% of them. The pet food industry uses about 30% mainly in the wet feed as a gel agent and water holding agents and in dry feed as ingredients. Feed industry, especially swine, has been the industry with the highest growth on the usage of this type of products in particular plasma and red cells and has experimented an important growth in the last 10 years in Asia, America and Europe. Briefly, there are 200 million pigs yearly

worldwide consuming spray-dried plasma in their diets of which 50% in America (North and South), 25% in Asia and finally 25% in Europe. Other industries that use blood-derived products are: pharmaceutical industry (nutraceuticals), animal health (colostrum substitutes), agriculture (fertilizers), cosmetics (foaming agents and gelling agent), diagnostics (bovine serum albumin and immunoglobins), paper industry (glue), biotechnology (reagents). All of those industries use about 10% of all blood-derived products, nonetheless they use small quantities of highly valued products.

Those ingredients are used for different properties, which mainly are:

(i) High biological value (meats).
(ii) Gelifying and water holding agents (pet food and food, cosmetics).
(iii) Immunological activity (human and animal health, feed).
(iv) Gluing agent (paper industry).
(v) Foaming agent (plywood industry, construction industry, cosmetics).
(vi) High nutritional value (animal and human nutrition, food).
(vii) Reagent (biotechnology, immunology, diagnostics).

Since the main focus of this meeting is on feed industry we will specifically target this sector and most specifically on plasma since it is the most interesting and value added ingredient.

Plasma production

As indicated blood has a high biological value and their proteins have interesting functional properties. Blood is collected from animals and abattoirs that have been veterinary inspected and health approved. Thus, they have a double inspection at the farm and at the abattoir before going to manufacturing.

Blood is collected in an hygienic way and an anticoagulant is added by automatically operated devices. The anticoagulants are typically citrates and phosphates. Blood is immediately refrigerated at 4°C and goes to stainless storage tanks at the abattoir level. Blood is continuously stirred and isothermal stainless steel trucks collect the blood similarly as is done by the milk industry to transport it to a manufacturing plant. Trucks are only used for blood transportation. They are routinely cleaned automatically by Cleaning in Process System (CIP) before they leave the plants. Once the blood arrives to the plant it is unloaded and Quality Control and Quality Assurance (QA&QC) is done on temperature, color, pH, clotting. If QA&QC is passed then blood is unloaded. The blood that does not pass QA&QC goes to blood meal production in other facilities with different heat and pressure treatment (133°C, 20 min, 3 atm in batch system or 100°C for 150 min in continuous systems). If QA&QC is passed then trucks can unload the blood and it goes through closed systems to refrigerated and stirred stainless steel tanks. It is important to notice that from collection in the abattoir to the final products blood and its products are always in enclosed systems with two transfers the first from tank at abattoir to a truck and the second from a truck to the plant and once in the plant is a totally closed system as well.

Blood is then centrifuged with industrial centrifuges that separate plasma from cells. Blood consists of 60% plasma and 40% cells in volume. Cells are heavier than plasma thus they tend in a dish centrifuge to stay down whereas plasma goes to the top of the centrifuge. They are both collected then with different tubing systems. The cell fraction goes directly to the spray dryer and the plasma fraction is then submitted to a process of ultrafiltration or reverse osmosis to remove water and concentrate and remove ash content, in the case of ultrafiltration, which has low nutritional value. Finally, the plasma is spray-dried. Briefly, this process consists in micronization of plasma in small droplets (10-100 μm), achieved by using very high pressure (200 atm), in a tower with very high temperatures during a short time (Filková, 1987). The inlet temperatures are 240°C and the outlet temperatures are 90°C. This drying system allows the proteins to remain functional and yet it hygienizes the plasma. However, the biosafety of plasma protein is due to the whole production process and not only due to spray drying. Finally, plasma is dried with hot warm air in order to reduce the humidity then bagged in new polylined paper bags and stored.

There is a plasma with standardized and optimized immunoglobin content in the market (***APPETEIN***®) which after spray drying undergoes another process yet consisting in analyzing the

globulin fraction during the production process by an automated Cobas method (Etzel *et al.*, 1997) and then it is mixed to achieve a homogeneous content on the globulin fraction and finally it is chelsinated, which is a process of microgranulation with pressure and not with temperature.

Other products may be obtained from by fractionation from plasma proteins or cell fraction. They normally consist of reactions with specific reagents and pH adjustments and further separations, then they are finally spray-dried. Those are immunoglobins, albumin, globin and fibrin all available in the market. Additionally, further refining can be done to extract transferrin, transforming growth factor, DNA, iron and other molecules.

All these production systems render different types of plasma that are equally safe, but could be functionally different. Thus, in order to consider plasma as a feed ingredient one needs to take into account the following points: protein and ash content, in principle, the higher the protein and the lower the ash the better. The fact that a plasma is food grade does not guarantee an optimal function as a feed ingredient, since for both pet food industry and food industry gel strength is the required function. The species of origin has no effect on functionality (Gatnau and Zimmerman, 1994; Rantanen *et al.*, 1994; Russell, 1994; Pierce *et al.*, 1996). Recently, it has been reported in the scientific literature that the high molecular weight fraction is the responsible for the plasma effect (globulin), thus a plasma was developed to render this fraction high and homogeneous. Additionally, it is well known in the scientific community that the globulin fraction has several and diverse activities such as virus, bacteria and toxin binding, immune system activation and they can generate active biopeptides.

Plasma and red cells composition

Protein is the most important fraction in plasma and red cell products. Obviously, it contains as well minerals, fat, carbohydrates and water. There is a substantial amount of literature describing those values (Delaney, 1975; Howell and Lawrie, 1983; Jobling, 1986; Young and Lawrie, 1994). Nowadays commercially it can be said that for plasma protein values for crude protein oscillate between 65 and 80%. Inversely, their ash content oscillates between 5% to 20%. Plasma proteins have a high lysine and threonine content whereas their methionine content is low (Delaney, 1975; Graham, 1978). The amino acid profile of that protein is very similar to the ideal protein ratio. The red cell fraction on the other hand has crude protein values of around 90%, with lysine content of 9%.

Risk assessment

APC has intensively worked on a risk assessment program to investigate the risk of spreading known and unknown diseases by utilization of blood derived proteins (APC, 2000). This assessment usually adopts standards accepted by all OIE member countries and thus has international validity. It is frequently done in the pharmaceutical industry to quantify the risk that the usage of a product has.

The factors that in the case of plasma contribute to risk reduction are:

(i) Blood is collected only from healthy animals that had been veterinary inspected.
(ii) Blood is only recovered from approved abattoirs in which animals undergo veterinary inspection.
(iii) Blood manufacturing system is always closed.
(iv) All system is stainless steel.
(v) Blood is refrigerated and transported in isothermal trucks.
(vi) Trucks are cleaned and only used for this purpose.
(vii) Blood is pooled in the plants and reduces drastically the risk.
(viii) Ultrafiltration or reverse osmosis are used.
(ix) Spray-drying with pressure (200 atm) and inlet temperature of 240°C and outlet of 90°C.
(x) Plasma is bagged in new polylined paper bags.
(xi) Storage due to the low humidity content of plasma.
(xii) Dry plasma pooling.
(xiii) Quality control in the abattoir, at the plant and in the finished product.
(xiv) All machinery is subjected to regular cleaning schedules.
(xv) Plasma is included at low dosis in feed (5%) during 15 days.

(xvi) Mixing and pelleting.
(xvii) Route of administration always oral.
(xviii) Total consumption of 150 grams that is 10 grams per pig per day or 2.5 g/kg $BW^{0.75}$.

All this processes make the risk for known and unknown disease spread *negligible*.

Biosafety

There are two levels at which those ingredients are checked: one at the bacteriological level and at virological level.

Bacteriology

It has been controlled quite well since those industries are really food based industries and have the capabilities to study it. The total bacterial count, enterobacteriaceae and salmonella in 25 g are the criteria mostly used. Likewise there are results reported in the scientific literature that indicate the bacteriological safety of plasma proteins (Howell and Lawrie, 1983; Jobling, 1986).

Virology

Virus analysis is more complex and requires different facilities. Thus, external laboratories have been used for virus determination such as those from Universities or Research Institutes. There are 15 viruses that are regularly analyzed. Those are:

Bovine Adenovirus	PRRS
Bovine Respiratory and Vesicular Virus	Transmissible Gastro-enteritis
Bovine Viral Diarrhoea	Swine Influenza
Bovine Parvovirus	Rabies Virus
Bovine Reovirus	Blue Tongue
Infectious Bovine Respiratory Virus	Porcine Parvovirus
Aujezsky	Parainfluenza 3
Classical Swine Fever	African Swine Fever

Additionally, APC has developed with Iowa State University, University of Georgia as well as that with Health Department from Generalitat in Catalonia research project to demonstrate that experimental liquid plasma infection with several viruses did not have infective impact after the drying process. In all cases plasma was infected with viral concentrations up to 10^4 and then subjected to the whole spray drying process in a pilot plant and finally studied its infectivity in cell culture with 4 passages. The experiments were done with Pseudorrabies Virus, PRRS (Lukert and Drew, 1993, University of Georgia) and Parvovirus (Hill *et al.*, 1994, Iowa State University).

It is also important to mention that during the PPC outbreak in Catalonia in 1997 more than 200 production batches were analyzed and none of them had viral activity.

Bovine Spongiform Encephalopathy (BSE)

As milk, blood is a low risk material. Thus the risk for Transmissible Spongiform Encephalopathies (TSE) transmission is negligible. Also the fact that pigs are not known to develop natural TSE contributes to render the risk negligible. Blood has never been reported to be infected by naturally occurring TSE. Moreover, blood has never been reported to transmit infectivity in humans (via blood transfusion) and blood did not transmit Creuzfeldt Jakob Disease (CJD) from human subjects affected by the disease to chimpanzees. Blood, blood clot, buffy coat, serum and fecal calf serum did not transmit CJD to mice (used as model for bioassay). Even spleen, known to work as blood filter, from infected cattle has never been reported to be infective in cattle bioassay. Collectively, all these and other reports showing the lack of evidence of detectable infectivity in blood in natural cases of animal

TSE have enabled all authorities such as WHO, (WHO, 1997), OIE, (OIE, 1998) and EC, (SSC, 1997) to conclude that blood is a no risk tissue in regard to TSE infectivity.

Overall, there is a very low risk of blood being infected in live, native born animals that have passed ante-mortem inspection and which have come from regions, zones and farms that are not under restriction of movement for BSE. Strict ante-mortem procedures are very highly recommended to avoid risk. In cattle it is recommended to use stunning methods that do not penetrate the brain cavity.

Following collection and pooling of blood, and presuming that all the production systems described before in this paper are followed, there is no risk of increasing any infectivity by BSE. The main risk reducing factors are:

(i) Dilution and pooling, which would likely dilute the contamination risk.

(ii) Centrifugation, that would most likely decrease the risk to plasma fraction since BSE agents are associated to cells.

(iii) Ultrafiltration and reverse osmosis would also very likely decrease risk since they would remove cellular debris.

(iv) Spray drying is very likely also to have a decreasing effect over risk.

(v) Oral administration of plasma proteins as part of a total feed formula.

In conclusion, any infectivity in blood either resulting from the agents of BSE is more likely to partition with the cells, or to be filtered out from the plasma. The net result would be that the cells would be likely to have a greater infectivity concentration than the original blood and plasma proportionately less. Spray drying is likely to have very little effect on any BSE infectivity present. However, the oral route in order to be infective would require substantial quantities of prions and pigs are not known to develop the disease. Moreover, since the ingredients being described and in particular plasma protein are part of a diet the total quantity would be very low and the risk considerably mitigated.

Finally, following are recommendations to render any risk totally negligible:

(i) Animals from free areas or free countries.
(ii) Animals not developing the disease.
(iii) Quality of veterinary services in control of the diseases.
(iv) Ante-mortem inspection always recommended.
(v) Slaughter procedure, best not invasive in cattle.

Dioxin

Last year dioxin analysis were performed on European plasma. PCB were analyzed from several batches and in none of them were detected. Analysis were performed by CSIC (Centro Superior de Investigaciones Científicas, Barcelona, Spain). It is interesting to consider that dioxins are accumulated in fat, and blood has very low fat content (<0.2-0.4%), being the fat content in dried product <4%, therefore the risk is negligible.

Additional safety measures that could be applied on plasma could be irradiation (2.5 Mrads), pasteurization (50°C, 30 min, pH 10), hot box, quarantine should a problem arise.

Legislation

Council Directive 90/667/EEC of 27 November 1990 lays down the veterinary ruling for disposal and processing of animal waste, for its placing on the market and for the prevention of pathogens in feedstuffs of animal or fish origin and amending Directive 90/425/EEC.

This directive classifies blood in two categories:

(i) Low risk is the blood coming from healthy animals that pass an official ante-mortem inspection and any serious and transmissible diseases for man or animals was detected. This blood can be used to feed animals.

(ii) High risk is the blood coming from animals that not pass ante-mortem inspection or from animals that suffer any serious and transmissible diseases for man or animals. This blood needs to be processed as high risk tissue and need to be processed for incineration or at 133°C, 3 atm during 20 minutes.

Council Directive 92/118/EEC of 17 December 1992 laying down animal health and public health requirements governing trade in and imports into the Community of products not subject to the said requirements laid down in specific Community rules referred to in Annex A (I) to Directive 89/662/EEC and as regards. This directive and its amendment (Directive 96/405/EEC) establishes the requirements that needs to appear in the Veterinary Health Certificate to be used for imports from low risk blood products from third countries.

Facts and figures

Annually there are 200 million pigs receiving an average of 150 g plasma in 50 countries. During the last 15 years with an increasing rate of usage no disease problem has been detected or reported. On the contrary there has not been a single scientific correlation to disease. Conversely, in most of the cases health status has been improved. If these numbers are brought to number of consumers that means that yearly 500 million people consume pork products from pigs fed plasma protein considering a pork consumption of 30 kg per person per year.

The first trials were conducted in 1987 at Iowa State University by Gatnau and Zimmerman (1990) and from then its usage has been expanded worldwide to Europe, Asia and South America. Of those 200 million pigs 50% are in America (Canada, Mexico, US and South America) and 25% in Europe and 25% in Asia. Previously plasma had been used by the meat industry for 25 years.

The price of plasma is 3 times that of spray dried skim milk whereas its protein content is only double.

A total of 37 Universities and Research Institutes in 15 different countries have carried out research on plasma with benefits that are summarized as increased feed intake, increased growth rate, diarrhoea reduction, increased or maintained health and welfare status, improved gut morphology and function and finally recent and original research shows how the inclusion of plasma in piglet diets is an alternative to antibiotic usage.

Plasma as an ingredient has been introduced in the new NRC (1998) tables and also FEDNA (1999) tables. Additionally the reference book in swine pathology swine diseases includes a chapter in piglet nutrition in which it is clearly stated that the ingredient is not only required but is the only indispensable one.

Production benefits

Since it is not the main objective of this article we will not emphasize this point. We will mention however that it is well documented in peer reviewed papers that a total of 34 trials have been reported using plasma in diets for weanling pigs in Europe as well as the US (Gatnau *et al.*, 1995a,b). A total of 5013 piglets have been used in those trials with an average inclusion rate of 6.5%, an average weanling age of 23.5 days. Results from those trials clearly indicate that plasma inclusion on these diets increased average daily feed intake 20%, average daily gain 37.5% and finally conversion index by 12.5%. All those trials had as a control diets either: skim milk, whey protein concentrate, dried whey, fish meal, potato protein, soybean meal, soy isolates, soy concentrates, casein or egg protein. Plasma is especially effective 15 days after weaning regardless of the weaning age. In particular it is interesting to mention that plasma fed piglets had better performances that pigs fed milk proteins such

as skimmed milk and whey protein concentrates as reported by Hansen *et al.* (1993). It is well documented that piglets have a digestive system at weaning that is not totally developed and proteases in particular do not reach full activity until eight weeks of age (Corring *et al.*, 1978).

Even there is some evidence that immunoglobins given at birth can increase growth in piglets as reported by Polo *et al.* (1995).

Interestingly, the red cell fraction has shown to be a very interesting protein for weanling pigs comparable to the most traditional protein sources for piglets in particular fish meal (Tokach *et al.*, 1991; Kats *et al.*, 1992; Richert *et al.*, 1994; Smith *et al.*, 1994).

Health benefits

Initial studies

Once the production benefits were well established it remained to answer the mode of action and some of those trials had the objective to compare then with antimicrobials. Studies done at Iowa State University conducted by Zimmerman (1999) showed that plasma had similar effects with and without the use of antimicrobials. However, a closer look to those experiments clearly indicated that plasma could be a good substitute to antimicrobials. These data were later supported by trials conducted at University of Kentucky by Coffey and Cromwell (1995). Previous studies had shown clearly that plasma fed pigs had a better response in immune challenging environments as was documented by Gatnau (1990), Stahly *et al.* (1994) and Coffey and Cromwell (1995).

Diarrhoea protection effects

Initial studies conducted by Gatnau and Zimmerman (1990), clearly demonstrated that inclusion of plasma in weanling pig diets significantly decreased diarrhoea incidence. Those were later supported by data from trials done at Rosmalen in Holland by Van der Peet-Schwering and Binnendijk (1995, 1997) comparing also vegetable protein diets with animal protein diets and both aquous and soft feces. Medication and labor costs were also reduced. These results were reviewed by Campbell *et al.* (1998).

Intestinal function and morphology

Plasma protein has a protective action at intestinal level reflected in a higher enzymatic function and morphology improvement as reported by Cain (1995) and Allee and Touchette (1999). Results from those authors show how piglets fed plasma protein at weaning have a higher maltase and lactase activity during the first two weeks of weaning than pigs fed control diets also pigs fed plasma protein have longer villi and higher relation villi height: crypt depth. Recent results published by Jiang *et al.* (2000) show how early weaned pigs fed plasma proteins had lower cellularity of lamina propria and higher efficiency of dietary protein utilization as jejunal and ileal protein and DNA masses of plasma protein fed piglets were lower than those of control diet fed piglets.

Infection protection

Recently several studies have demonstrated that plasma addition to weanling pig diets decreased *E. coli* shedding in feces when piglets have been infected. Studies conducted at Iowa State University (Cain and Zimmerman, 1999) demonstrated that weanling pigs shed rotavirus less time. Additionally, Bosi *et al.* (1999) in trials conducted at Reggio Emilia showed that addition of plasma to semisynthetic diets in pigs infected with *E. coli* had better performance than those fed a control diet with hydrolized food grade casein. Concluding that plasma is a good substitutive. They compared several plasmas and concluded that the plasma with standardized globulins (***APPETEIN***®) was the one that produced better performance effects.

Studies conducted in France (Brogniard and Gatnau, unpublished data) using plasma to study its role as alternative to antimicrobials clearly indicate that antimicrobials have a positive effect on performance data and that ***APPETEIN***® is a good alternative to medication. Likewise studies conducted in Catalonia at IRTA by Conde *et al.* (unpublished data) show how in piglets sensible to colistin action, ***APPETEIN***® was a plausible alternative.

Mode of action

Once the plasma effects were well established, the scientific community turned their attention to investigate the mode of action of such an interesting ingredient. However, this has not been easy and it is still an ongoing process. Following is a list of the most plausible mechanisms and areas of study:

(i) *Digestibility*. This was the first area been looked at. The results obtained did show good digestibilities, however not enough to justify the effects against other protein sources Knabe (1994).

(ii) *Palatability*. Ermer *et al.* (1994) compared the palatability of plasma with that of skimmed milk and their results show that plasma was more palatable than milk in an experiment where pigs had free choice of diets and their position was changed daily. This palatability was maintained thought 21 days after weaning. Pigs fed plasma had more meals, more time eating and more grams per meal than their counterparts feed skim milk protein diets.

(iii) *Protein fraction*. Plasma contains 100 well characterized proteins (Putnam, 1984). Several authors have demonstrated the main fractions in plasma (Donnelly and Delaney, 1977; Howell and Lawrie, 1983) by precipitation with polyethyline glycol and also with ion exchange chromatography. Those main fractions are: albumin (50%), alpha-globulins (15%), beta-globulins (15%), gamma-globulins (15%) and fibrin 5%. Thus the aim was to investigate which of those could possibly have the plasma effect. Several studies (Cain *et al.*, 1995; Gatnau *et al.*, 1995a,b; Owen *et al.*, 1995; Pierce *et al.*, 1995; Weaver *et al.*, 1995) have shown that the plasma effect is due to the high molecular weight fraction (globulins). Those effects of the high molecular fraction (150,000 dalton) are shown on growth performance, intestinal functionality and intestinal morphology. Results have been even more conclusive when very specific techniques of separation have been used. Previous studies showed that plasma proteins contain active immunoglobins that are active and functional and can be absorbed by newborn colostrum free pigs (Gatnau *et al.*, 1989). The effects of the immunoglobin fraction have also been seen in mice (Thomson *et al.*, 1994).

(iv) *Others*. Other mechanisms have been proposed but conversely to those mentioned above we can just say they are hypothesis. The case of glutamic acid could be one of them. Since plasma has a high content in glutamic acid some researchers have hypothesized if it could have an effect on intestinal health since there is some human data that shows benefits from using glutamic acid. Other authors (De Rodas *et al.*, 1995) however have investigated the effects of plasma on piglet's blood levels of IGF-1, growth hormone and insulin. Those results showed that pigs fed plasma proteins had higher growth hormone concentrations, equal IGF-1 and lower insulin plasma levels that pigs fed soybean proteins.

Conclusions

It can be concluded from this paper that plasma protein production yield a product that has a negligible risk. Its micobiological and virological safety is sound and also the probability for an unknown contamination is negligible. The use of plasma in diets for weanling pigs improve feed intake, growth rate, intestinal function and morphology whilst decreasing diarrhoea incidence. Recent and older data clearly demonstrate that addition of plasma protein to weanling pig diets is a suitable alternative to antimicrobial usage and can maintain health. The most plausible modes of action of plasma are digestibility, intestinal protection by immunoglobin fraction and increased palatability.

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