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■ Keywords

Hot pepper waste powder, quails, growth performance, small intestine, gut health.



## Effects of Dietary Hot Pepper Waste Powder on Gut Health and Small Intestine Properties in Japanese Quails

### ABSTRACT

The present study was conducted to evaluate whether dietary hot pepper waste powder (HPWP) would affect the performance and small intestine histology parameters in Japanese quail chicks. A total of 160, one-day-old Japanese quail chicks were divided into 4 treatment groups of similar mean weight, comprising 4 subgroups of 10 chicks each. Chicks were fed on their basal diet supplemented by 0, 100, 200 or 400 mg/kg of dietary HPWP for each kg of starter (0 to 10 days), grower (11 to 24 days) and finisher (25 to 42 days) diets for 7 weeks. At the end of 42 days of age, 10 birds per subgroup were slaughtered and intestinal samples were taken to evaluate histomorphological analyses. The results showed that dietary HPWP supplementation did not affect performance parameters, but 400 mg/kg HPWP supplementation tended to increase the growth performance of the chicks. The villus length, submucosa layer ( $p < 0.05$ ), serosa, muscular layer, villus-crypt length ratio, and villus surface area increased with HPWP supplementation ( $p < 0.01$ ). The goblet cell numbers of the group receiving 200 mg/kg HPWP increased compared to the control and 400 mg/kg HPWP supplementation groups ( $p < 0.05$ ). It could be concluded that dietary HPWP supplementation could improve gut health in quails.

### INTRODUCTION

Worldwide, the equivalent of 1.2 billion tons of petroleum agricultural waste is generated each year (Biyoenerji 2018). In recent years, studies have looked at generating income from the possibility of using agricultural wastes in the field of animal nutrition. Research efforts are continuing to convert the waste residue produced by fruits and vegetables to new, alternative and cheap protein sources and to evaluate other nutritional ingredients (Liadakis *et al.*, 1995; Arogba 1997; Wang *et al.*, 1999; Moure *et al.*, 2002; Quanhong & Caili 2005; Wani *et al.*, 2006; Filik & Kutlu, 2018). Researchers have therefore been investigating whether these waste products can be used as feed sources in animal husbandry. For instance, Garau *et al.* (2007) reported that after drying, the orange pulp is a good source of crude cellulose and antioxidants. Spigno & Faveri (2007) reported that the antioxidant content increased after the processing of grape pomace. Roldan *et al.* (2008) reported that onion waste might be an important source of antioxidants for useful food production. Civaner & Ertürk (2009) determined that mushroom harvest waste could substitute for 25% of the crude protein source in place of soybean in quail rations. Although there are many agricultural wastes used in animal husbandry, there are some by-products not used in animal production or as useful animal food. One of them is *Capsicum annuum* L. waste; this is the most commonly grown species of capsicum-grown peppers that is



mainly grown for vegetables. According to the FAO (2017) there are 36 million tons of pepper production worldwide and approximately 28% of it is waste (Yurdagel *et al.*, 1997). Therefore, every year, there are 11 million tons of pepper waste. Pepper waste includes capsaicin, which is the active component (Sim & Sil, 2008). Depending on growing conditions and harvesting time, the total amount of capsaicinoids vary from 0.1 to 2.0% of dry matter (Korkmaz, 2016). *Capsicum annuum* L. has been reported to decrease abdominal fat pads in the intestinal area and stimulates the central nervous system, accelerates the elimination of metabolic waste products, enhances body heat, facilitates digestion and vasoconstriction (blood vessel contraction) as well as reduce blood cholesterol and abdominal fat accumulation (Özer *et al.*, 2006; Seviçin 2011; Puvača *et al.*, 2014; Arabacı, 2015). Besides, capsaicin exhibits anti-virulence activity and has been used against various pathological microorganisms such as *Salmonella enteritidis*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Staphylococcus aureus*, *Porphyromonas gingivalis* and others (McElroy *et al.*, 1994; Marini *et al.*, 2015). Capsaicin also has a positive effect on reproductive organs and hormones, on follicle-stimulating hormone (FSH) and luteinizing hormone (LH) cells (Erdost *et al.*, 2006) and an increase in the spermatogenic cell count (Özer *et al.*, 2006). Although these positive effects of capsaicin in pepper and pepper waste have already been determined, there has been no study about the use of pepper waste as a useful food or gut enhancer in animals in the present literature. Therefore, the question is whether the hot pepper waste powder (HPWP) could be used as a food additive in animal diets. This study aimed to determine the effects of dietary HPWP on gut health and histomorphological parameters of the ileum in quails.

## MATERIALS AND METHOD

### Animals and Feeds

A total of 160, one-day-old Japanese quail chicks were divided into 4 treatment groups of similar mean weight, comprising 4 sub-groups (10 chicks of mixed sex) and each 5 cages including 2 birds. The Power Procedure Overall F test for One-Way Anova in the SAS Software (SAS 1996) statistical package program calculated the number of animals to be used in the experiment at 40 chickens per group to give a confidence interval of 99% (Cohen 1988). The chicks were fed on their basal diet supplemented with 0, 100, 200 or 400 mg/kg of dietary HPWP per kg of

a starter (23.32%, Crude Protein (CP); 3000 ME kg/diet), grower (21.50%, CP; 3100 kg/diet) or finisher (19.50%, CP; 3200 ME kg/diet) diet for 7 weeks (Table 1). The quail diets were prepared by a local company according to NRC (1994) recommendations. HPWP was obtained from a private red pepper paste factory in Şanlıurfa, Turkey. Feed and water were offered daily *ad libitum*.

**Table 1** – Composition of the starter, grower, and finisher diet (kg/t).

Ingredients	Starter Diet (0 to 10 days)	Grower Diet (11 to 24 days)	Finisher Diet (25 to 42 days)
Maize (7.5% CP)	467.101	544.228	584.749
Soybean meal (46% CP)	387.893	366.087	320.522
Sunflower seed meal (36% CP)	40.000	-	-
Soybean oil	59.803	49.446	59.944
DL-methionine (99%)	3.530	3.016	2.712
NaCl	2.640	2.313	2.873
Limestone	11.817	8.477	7.595
Dicalcium phosphate (18%)	20.269	18.300	16.252
Vitamin Premix*	2.000	2.000	2.000
Mineral Premix**	1.000	1.000	1.000
L-Lysine HCl	2.233	3.164	1.342
L-Threonine	0.944	0.598	0.331
Sodium sulphate	0.771	1.371	0.680
Total (kg)	1000.00	1000.00	1000.00
Calculated Analysis			
Dry Matter (%)	88.000	87.681	87.667
Crude Protein (%)	23.323	21.500	19.500
Metabolizable Energy (kcal/kg)	3000.00	3100.00	3200.00
Crude Fiber (%)	4.322	3.686	3.487
Ether Extract (%)	8.323	7.519	8.644
Ash (%)	6.419	5.684	5.215
Ca/P (%)	2.186	2.000	2.000
Ca (%)	1.049	0.870	0.780
p (%)	0.480	0.435	0.390
Lysine (Digestible) (%)	1.280	1.269	1.020
Methionine (Digestible) (%)	0.659	0.580	0.530

Premix provided per kg of diet: \* Vitamin A, 12,000 IU; Vitamin D3, 2,400 IU; Vitamin E, 30 mg; Vitamin K3, 4 mg; Vitamin B1, 3 mg; Vitamin B2, 7 mg; Vitamin B6, 5 mg; Vitamin B12, 15 µg; niacin, 25 mg; # Fe, 80 mg; folic acid, 1 mg; pantothenic acid, 10 mg; biotin, 45 mg; Choline, 125000 mg; Cu, 5 mg; Mn, 80 mg; Zn, 60 mg; Se, 150 µg.

### Experimental Conditions

The experiment was performed using sixteen group cages (sized 50×75 cm, having 10 birds each) within the animal section of the Quail Unit of the Agriculture Faculty of Kırşehir Ahi Evran University. Artificial illumination was provided in the experimental coop by white fluorescent lamps and a thermostatically controlled infrared electric heater for floor heating. The temperature of the coop was maintained at 33 °C during the first week of life and was then gradually reduced by 3 °C weekly according to age until it



reached 24 °C between 21 to 42 days. The relative humidity was maintained at 55% throughout the rearing period. During the trial period, the animals were given a 23-hour light/1-hour dark schedule for the first three days in case of a power interruption during the trial, and 24 hours for the other 39 days according to commercial conditions.

An ethics document for the trial was obtained from the Animal Experiments Local Ethics Committee of Kırşehir Ahi Evran University with a decision date and number: 07/11/2018-21-1.

### Experimental and Histomorphological Measurements

The body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were determined weekly. Histological samples were randomly taken from ten birds per subgroup. The gizzard weight, proventriculus weight, gastrointestinal tract weight (GITW), gastrointestinal tract length (GITL), hot carcass, and cold carcass yield were measured using 10 healthy birds of each group. Ileum samples were cut into 10mm pieces and placed into 10% formalin for histological processing. Tissue sections were inserted into tissue cassettes. After the dehydration process, the tissue sections were embedded in paraffin blocks, cut into 5 $\mu$ -thick pieces, and placed on a slide. The tissue on the slides was deparaffinized with xylene, and a Periodic acid solution (PAS) with Schiff's reagent staining procedure was applied, see Table 2.

**Table 2** – PAS staining procedure for histological properties.

Slide with histological specimen	
Distilled water	rinse
Algian Blue (Merck-1.01647.0500)	5 minutes
Periodic acid solution (Merck 202.646/1)*	5 minutes
Running tap water	3 minutes
Distilled water	rinse
Schiff's reagent (Merck 101.646/2)	15 minutes
Running tap water	3 minutes
Distilled water	rinse
Running tap water	3 minutes
Ethanol 70%	1 minute
Ethanol 70%	1 minute
Ethanol 96%	1 minute
Ethanol 100%	1 minute
Ethanol 100%	1 minute
Xylene or NEO-CLEAR®	5 minutes
Xylene or NEO-CLEAR®	5 minutes

Mouth the NEO-CLEAR®-wet slides with Neo-Mount® or the xylene-wet slides with C Balsam and cover glass.

\*To enhance and optimize the specificity of the stain, the tissue should be treated with sulfite water after the reaction with periodic acid (3\*2 min). Prepare sulfite water by first mixing 10 ml of sodium disulfide solution (10%) and 10 ml of hydrochloric acid (1 mol/l), and then mix this solution with 200 ml of tap water.

The tissue samples with PAS staining were illustrated by following the manufacturer's instructions for tissue incubation conditions (Merck). After the embedding process, the ileum villus length and width, serosa, muscular layer, submucosa layer, crypt length, and the number of goblet cells per villus were evaluated by using an image processing and analysis system (ZEN 2012 SP2) for the Zeiss Primo Star HD Light Microscope. The villus surface area (M Value) calculation was performed according to the method of Kisielinski *et al.* (2002). The villus-crypt length ratio (VCR) calculation was performed according to the method of Wilson *et al.* (2018).

### Statistical Analysis

The data obtained in the experiment were analyzed using General Linear Models (GLM), Duncan's multiple range test procedures, and orthogonal polynomials using SAS Software (SAS 1996). The linear, quadratic, and cubic effects were determined by orthogonal polynomial contrasts (Düzgüneş *et al.*, 1987). Means differences were considered significant at  $p < 0.05$ .

## RESULTS

In this study, initial and final BW, FI, and FCR are given in Table 3. Dietary supplemental HPWP had no significant ( $p > 0.05$ ) effects on BW, FI, and FCR throughout the study. The differences in final BW, FI, and FCR were not significant among the groups ( $p > 0.05$ ). The differences in the gizzard and proventriculus weight, the gastrointestinal tract weight and length, and the hot and cold carcass yield were also not significant ( $p > 0.05$ ).

However, dietary supplemental HPWP had significant effects on some intestinal histomorphological parameters. The results concerning intestinal samples taken at the end of the current study showed that dietary 100 and 200 mg/kg HPWP supplementation increased the villus length compared to the control group ( $p < 0.05$ ) while villus length did not change by 400 mg/kg of dietary supplemental HPWP. Villus width increased with 100 mg/kg to 200 mg/kg HPWP supplementation ( $p > 0.05$ ). The dietary HPWP supplementation also increased the M value ( $p < 0.01$ ). Linear and cubic effects were found with the M value ( $p < 0.01$ ). Crypt length decreased more in the 400 mg/kg group than in the 200 mg/kg HPWP supplement group. Goblet cell number per villus increased more in the 200 mg/kg group than in the control and 400 mg/kg HPWP supplementation groups ( $p < 0.05$ ).


**Table 3** – Hot pepper waste powder effects of Japanese quail performance, carcass, and small intestine histology parameters.

Parameters (g per bird)	HPWP Doses (mg/kg)				SED	P	L	Effects	
	0	100	200	400				C	Q
<b>Performance</b>									
Initial BW (g)	9.95	9.96	9.98	10.00	0.04	0.962	0.596	0.993	0.997
Final BW (g)	275.39	276.91	276.15	285.92	2.30	0.333	0.139	0.531	0.370
FI (g)	887.13	907.33	890.47	936.29	7.45	0.094	0.056	0.140	0.390
FCR	3.22	3.28	3.23	3.28	0.02	0.716	0.611	0.304	0.952
<b>Carcass</b>									
Gizzard Weight (*)	1.11	0.98	1.15	1.24	0.07	0.589	0.380	0.540	0.435
Proventriculus Weight (*)	0.34	0.34	0.35	0.43	0.02	0.431	0.199	0.752	0.378
GITW (*)	4.21	4.36	4.56	4.58	0.46	0.987	0.751	0.955	0.944
GITL (**)	20.12	20.50	18.99	23.40	1.11	0.545	0.420	0.445	0.385
Hot Carcass Yield (%)	0.67	0.63	0.62	0.63	0.03	0.908	0.631	0.961	0.642
Cold Carcass Yield (%)	0.59	0.56	0.55	0.57	0.02	0.928	0.720	0.972	0.621
<b>Small Intestine Histology</b>									
Villus length (µm)	165.46b	182.65a	181.19a	178.25ab	0.01	0.047	0.077	0.431	0.037
Villus width (µm)	37.14ab	39.01a	38.49ab	35.15b	0.01	0.106	0.223	0.938	0.034
Serosa (µm)	12.32cb	13.25b	15.79a	11.30c	0.29	0.001	0.084	0.001	0.001
Muscular layer (µm)	23.59a	19.82b	24.82a	17.60c	0.39	0.001	0.001	0.000	0.027
Submucosa layer (µm)	9.06ab	9.57a	9.76a	8.61b	0.16	0.038	0.406	0.465	0.009
Crypt length (µm)	15.09ab	14.05ab	16.23a	13.16b	0.01	0.039	0.330	0.033	0.238
VCR	11.23d	12.45b	13.52c	14.41a	0.16	0.001	0.001	0.576	0.001
M Value (µm <sup>2</sup> )	9.53c	10.85b	10.50b	11.51a	0.00	0.001	0.001	0.001	0.435
Goblet cell number for per villus	86.39b	92.75ab	96.02a	86.05b	0.01	0.032	0.864	0.422	0.005

SED: The standard error of the difference between 2 means; P: Probability; L: Linear effect; C: Cubic effect; Q: Quadratic effect; BW: Body Weight; FI: Feed Intake; FCR: Feed Conversion Ratio; GITW: Gastro intestinal tract weight; GITL: Gastro intestinal tract length; VCR: Villus-Crypt length ratio; M Value: Villus surface area (µm<sup>2</sup>); \*: g/100g Live Weight; \*\*: cm/100g Live Weight.

VCR increased with dietary HPWP supplementation compared to the control group, and the highest VCR was found in the 400 mg/kg HPWP supplementation group ( $p < 0.01$ ). Linear and quadratic effects were found to be significant ( $p < 0.01$ ) for VCR.

## DISCUSSION

The results of the study showed that dietary HPWP supplementation of the basal diet did not change the chicks' performance. In addition, rising amounts of HPWP tended to increase feed intake and appetite in the animals, although there was no statistical difference in feed intake. The highest feed intake was observed in the 400 mg/kg HPWP group ( $p > 0.05$ ), where the supplementation of HPWP numerically increased the body weight in a dose-related manner ( $p > 0.05$ ). As the current study did not investigate any stress factors, the growth performance did not change. If any stress factors had been used in the trial, growth performance would have been changed. It has been reported that growth in the chick depends on the same genetic, environmental and stress factors (Ozturk & Yildirim 2004). This means that, in this study, there were no stress factors in the environment affecting the chicks' genetic capacity for growth.

In our study, we found that FI, GITW, and GITL tended to increase ( $p > 0.05$ ). It can be said that dietary HPWP supplementations did not have a detrimental effect on growth, internal organ development, or GITL development. The 400 mg/kg HPWP supplementation increased the GITL compared to the control group. The feed may have remained in the gastrointestinal tract (GIT) and better digestion may have occurred because growth in the GITL in chicks increased in the 400 mg/kg HPWP supplementation group ( $p > 0.05$ ). Dietary HPWP supplementation also increased the gizzard and proventriculus weight (Table 3), which may have been caused by the increased digestion time, but they were not important statistically ( $p > 0.05$ ). The increase in the development of the gizzard and proventriculus indicates that the feeds can be digested in the digestive tract. Feed waiting to be digested in the GIT may have increased the GITW (Gariel *et al.*, 2003).

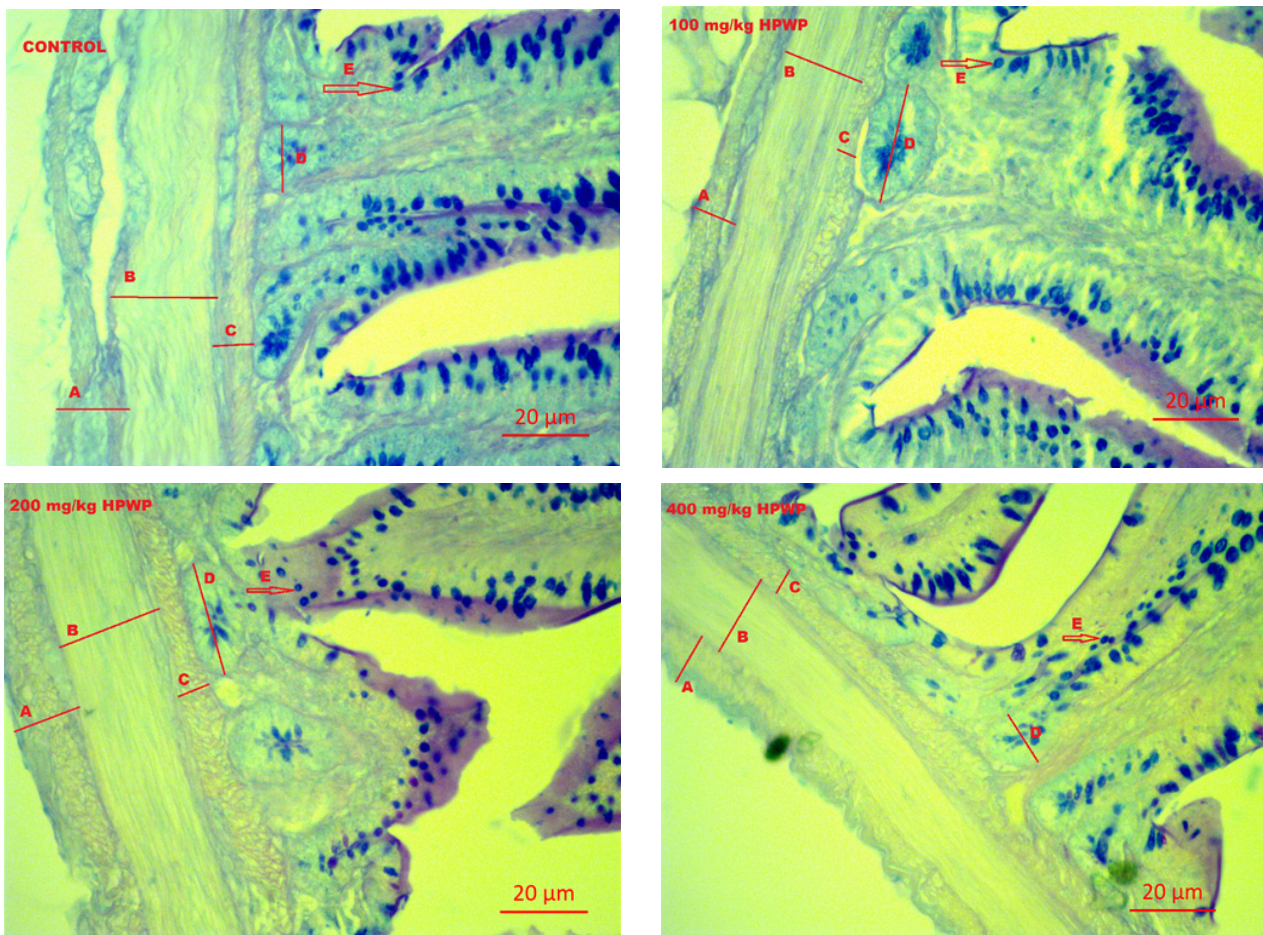
Dietary supplementation with HPWP had a positive impact on the development of the villus, which is responsible for the digestion of feed. Villus length was affected by the supplementation of HPWP ( $p < 0.05$ ), so the VCR and the M value increased with the dietary HPWP supplementation groups compared to the control group, and the highest VCR and M value were in the 400 mg/kg HPWP supplemented group ( $p < 0.01$ ).



In this study, the 200mg/kg HPWP supplementation group had an increased number of goblet cells per villus ( $p < 0.05$ ), and these results may be an indicator that 200mg/kg of HPWP supplementation increased the ileum epithelial health. It was reported that the deterioration of the mucosa structure due to the decrease in the number of goblet cells shows that the intestine is exposed to prolonged irritation (Kaur *et al.*, 2017). These results showed that raised doses in HPWP supplementation groups improved gastrointestinal health and digestion. Villus length decreased when the digestive system surface area deteriorated. The stem cells in the crypt are responsible for the recovery of the villus and the renewal of epithelial cells. It was reported that increased VCR proves there is no cell disruption and the intestinal cell mucosa is healed for digestion (Yason *et al.*, 1987; Paulus *et al.*, 1992; Yasar & Forbes 1996; Buclaw *et al.*, 2016). This contradicts with the view of Kalmendal *et al.* (2011). In this study, while GITL and GITW increased ( $p > 0.05$ ), the muscular layer, the VCR, and the M value increased linearly in the treatment groups compared to the control group

( $p < 0.01$ ) (Shahverdi *et al.*, 2013). These findings are in agreement with the observation of Cardoso *et al.* (2012) who showed that M value was expanded with dietary piperine supplementation. In addition, a decrease in the muscular layer in the 100 and 400mg/kg HPWP supplementation groups can be a sign of increasing gut health like VCR, because if there were any stress factors or deterioration in the gut, the muscular layer would increase (Figure 1). Silva *et al.* (2009) demonstrated that oral inoculation with *Eimeria tenella* increased Lamina muscularis mucosae 2 or 3 times more than antibiotics and anticoccidials. On the other hand, the antimicrobial effect of HPWP could have also been effective on the beneficial and harmful microorganism population in the structure of the small intestine (Orndorff *et al.*, 2005). At the end of the experiment, the FI increased. All these results did not reveal any negative effects as a result of using HPWP in quail rations. Katayama *et al.* (1986) found similar results in rats. They reported that dietary hot pepper supplementation increased the stomach contents in the gut and produced better growth performance.

**Figure 1** – Hot pepper waste powder effects of Japanese quail villus properties.



A: Serosa, B: Muscular layer, C: Submucosa layer, D: Crypt, E: Goblet cell (PAS Staining, 40x)



The results of this study are the first record of dietary HPWP supplementation in quail feed. The main component of HPWP is capsaicin-like. Sim & Sil (2008) reported that HPWP has 80% antioxidant activity like hot pepper. Because capsaicin is an antioxidant, the basic effect of capsaicin may be on meat quality and the shelf life of the meat, but this was not determined by this study. Different studies are needed to determine how HPWP supplementation affects the quality of poultry meat and its shelf life. It was concluded that dietary HPWP supplementation in quail feed can be used to increase gut health and improve digestion. Further studies should be conducted to determine dietary HPWP supplementation with different stress factors, such as the challenge of pathogenic bacteria, low protein, high fiber, etc. in the diet in different animal species.

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## CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest associated with the publication.

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