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Morphometric analysis of small intestine of layer quails influenced by dietary phytogenic feed additives

Gagandeep Kour[1], Nazam Khan*[2], R. K. Sharma[1], Vikas Mahajan[2] and Jasvinder Singh Sasan[3]

[1]*Division of Animal Nutrition, Faculty of Veterinary Sciences and Animal Husbandry, R.S Pura, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, UT of J&K, India*
[2]*Division of Livestock Farm Complex, Faculty of Veterinary Sciences and Animal Husbandry, R.S Pura, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, UT of J&K, India*
[3]*Division of Veterinary Anatomy, Faculty of Veterinary Sciences and Animal Husbandry, R.S Pura, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, UT of J&K, India*

ABSTRACT

The effect of supplementation of different phytoadditives on the micrometrical parameters of small intestines of layer quails, 360 birds of the same hatch (6 weeks old) were randomly distributed into eight groups (n=45), having three replicas of fifteen quail layers per replica. These eight dietary groups were: Negative control (NC; maize-soya based diet with no additive), positive control (PC; herbal growth promoter- Reproforte plus™ was supplemented @ 500gm per ton feed), whereas T_1, T_2, T_3, T_4, T_5 and T_6 groups were supplemented with 1% dietary additive namely turmeric, garlic, fenugreek, cumin, aloe vera and oregano powder, respectively. These additives were fed consecutively for 22 weeks. After 22 weeks, it was observed that phytoadditive supplementation had no effect on duodenal villi base width, luminal epithelial height, crypt depth and gland epithelial height were similar among different groups (P>0.05). However, villi length (µm) showed statistically significant difference (P<0.05), being highest for PC (850.0), T_2 (867.97), T_3 (861.89) and T_4 (868.03) groups and was lowest in NC (776.65) group, whereas rest of the groups i.e. T_1 (809.97), T_5 (808.54) and T_6 (809.85) showed intermediate values. While in the jejunum, luminal epithelial height (µm) and gland epithelial height (µm) showed significant changes, whereas rest of the parameters showed no difference. The luminal epithelial height (µm) of jejunum was found significantly higher (P<0.05) in T_1 (27.75), T_2 (27.90) and T_4 (27.58) than NC (24.01), but PC (26.39), T_3 (26.11), T_5 (26.14) and T_6 (25.51) has in values. Likewise, gland epithelial height (µm) was found higher in T_4 (14.98) and lowest in NC group (12.31). But in ileum, luminal epithelial height (µm) was found to be higher in T_3 (25.09), T_4 (25.29), T_5 (24.92) and T_6 (23.77), but it was lowest in NC group (21.92). However, PC group has intermediate luminal epithelial height (µm) and had no difference with all the groups. Rest all the estimated parameters of the ileum have statistically similar values and showed no difference.

Keywords: *Quail layer, Phytogenic feed additives, Micrometry.*

Introduction

Phytogenic feed additives (PFAs) have garnered considerable attention in recent years due to their multifaceted role in layer poultry nutrition, particularly in enhancing intestinal health and improving egg quality in layer hens. Supplementation of quail's diet with phyto-additives improve the micrometry of the small intestine which helps in optimal nutrient absorption and gut health by modulating microbial populations, bolstering intestinal integrity, and fostering an environment of symbiotic harmony by promoting villi development and increasing villus height, thereby improving nutrient absorption efficiency and reducing the incidence of intestinal disorders in layers resulting in overall digestive efficiency (Akbarian *et al* 2012).

Phytogenic compounds are plant-derived bioactive compounds with favorable impacts on animal production and health and are often categorized into four different groups, i.e., botanicals, herbs, oleoresins, and essential oils (Nair *et al* 2019). These additives are derived from various plant sources and contain bioactive compounds such as essential oils, saponins, flavonoids, and tannins, among others, which exert beneficial effects on the gastrointestinal tract of poultry. The use of herbs, spices, and essential oils as alternative growth promoters (AGPs) was based on their bioactive constituents, which exhibit antimicrobial, antioxidant, anti-inflammatory, immune-modulatory, and digestion-stimulating properties (Alinian *et al* 2016; Bauer *et al* 2019; Chowdhary *et al* 2021). In the context of layer poultry nutrition, Phytogenic feed additives represent a promising avenue for optimizing intestinal health in layer quails. Continued research and application of these additives are crucial for further elucidating their mechanisms of action and maximizing their benefits in commercial layer production. Therefore, the present study was conducted to study the effect of PFA on the intestinal micrometry.

Materials and Methods

Experimental design: The present trial was carried out at the Division of Animal Nutrition, Faculty of Veterinary Sciences and

*Corresponding Author: ***Nazam Khan***

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Animal Husbandry, SKUAST-Jammu, India. Three hundred sixty Japanese quail (*Coturnix coturnix japonica*) layers of the same hatch (6 weeks old) were randomly distributed into eight groups (n=45), having three replicas of fifteen quail layers per replica. A maize-soya-based basal diet for layer quails was formulated as per specifications given by ICAR (2013) (Table 1). The eight dietary groups were: Negative control group contains no additive, positive control is supplemented with commercial growth promoter (Reproforte plus™ containing *Adhatoda vasica*- 20%, *Asparagus officinalis*- 15%, *Leptadenia reticulate*- 15%, *Zingiber officinalis*- 10%, *Rubia cordiolia*- 10%, *Tribulus terrestris*- 10%, *Solena amplexicaulis*- 10%, *Punica granatum*- 10% and was supplemented @ 500 gm per ton feed, supplied by Arvind Herbal Labs, Saharanpur, UP), whereas T_1, T_2, T_3, T_4, T_5 and T_6 groups were supplemented as 1% dietary additive with turmeric, garlic, fenugreek, cumin, *aloe vera* and oregano powder, respectively. All the phytoadditives used except *aloe vera* powder were purchased raw from the local market. These were dried and ground to powder form before mixing in the basal feed, whereas *Aloe vera* powder was bought from AMORVET, UK, India.

Parameters studies: At the end of the metabolism trial, 3 birds per replicate *i.e.* nine birds per group were slaughtered for assessment of micrometrical parameters. Intestinal tissue specimens/samples from the duodenum, jejunum and ileum were collected during the slaughtering of quail. Tissue samples were fixed in 10% Neutral Buffered Formalin solution for further processing. Tissue blocks were sectioned in a rotary microtome to 5 µ thickness. Slides were stained with Haematoxylin and Eosin-stain (Luna 1968). The micrometrical observations were recorded on H&E-stained sections of 5 µ thickness by using ocular micrometry, which was duly calibrated with stage micrometer. The different micrometrical observations recorded (shown in Plate 1) were: Villus length (µm), villus width (µm), crypt depth (µm), luminal epithelial height (µm), and glandular epithelial height (µm).

Statistical analysis: The data obtained from the experimental trial was subjected to one way and two-way analysis of variance (Snedecor and Cochran 1994). The significantly different means were ranked by Duncan's multiple range test as per Duncan (1995).

Results

Different micrometrical parameters *viz*. length of villi (µm), width at the base of villi (µm), luminal epithelial height (µm), crypt depth (µm) and gland epithelial height (µm) in duodenum, jejunum and ileum (small intestine different segments) were measured in layer quails and their results are presented in Table 2, 3, 4, respectively. The results revealed that in the duodenum, villi base width, luminal epithelial height, crypt depth and gland epithelial height were similar among different groups ($P>0.05$). However, villi length (µm) showed statistically significant difference ($P<0.05$), being highest for PC (850.0), T_2 (867.97), T_3 (861.89) and T_4 (868.03) groups and was lowest in NC (776.65) group, whereas rest of the groups *i.e.* T_1 (809.97), T_5 (808.54) and T_6 (809.85) showed intermediate values.

While in the jejunum, luminal epithelial height (µm) and gland epithelial height (µm) showed significant changes, whereas the rest of the parameters showed no difference. The luminal epithelial height (µm) of jejunum was found significantly higher ($P<0.05$) in T_1 (27.75), T_2 (27.90) and T_4 (27.58) than NC (24.01), but PC (26.39), T_3 (26.11), T_5 (26.14) and T_6 (25.51) has in values. Likewise, gland epithelial height (µm) was found to be higher in T_4 (14.98) and lowest in NC group (12.31).

But in ileum, luminal epithelial height (µm) was found to be higher in T_3 (25.09), T_4 (25.29), T_5 (24.92) and T_6 (23.77), but it was lowest in NC group (21.92). However, PC group has an intermediate luminal epithelial height (µm) and had no difference with all the groups. All the estimated parameters of ileum have statistically similar values and showed no difference.

Discussion

Development of GIT and its health is an important factor to the productivity of all farm animals as well poultry, including quails. The digestive functions can be considered as the most important limiting factor in performances as it affects the utilization of dietary nutrients (Adibmoradi *et al* 2006).

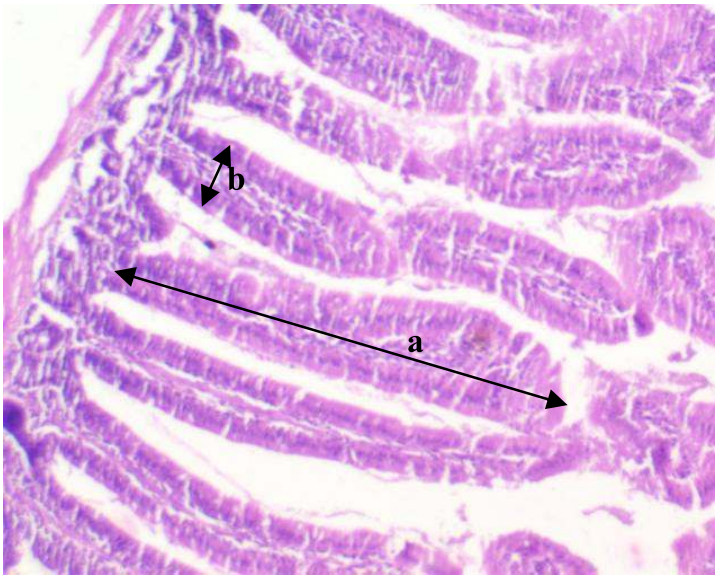
Different micrometrical parameters of all the three segments of the intestine were measured. The results revealed that in duodenum, villi length (µm) showed statistically significant difference ($P<0.05$), being highest for PC, T_2, T_3 and T_4 groups and was lowest in NC group, whereas rest of the groups *i.e.* T_1, T_5 and T_6 showed intermediate values. In line with our study, Mousa *et al* (2019) and Chowdhary *et al* (2021) reported an increase in villi height in duodenum on supplementation of garlic in poultry birds. It could be determined that the birds' morphological alterations brought about by garlic improved the layers' digestive systems. It most likely improved the activity of the enzymes and created a microenvironment that allowed the layers to better utilize nutrients (Aderemi *et al* 2013). Also, Abdel-Rahman *et al* (2014) and Qureshi *et al* (2016) observed that incorporation of fenugreek seeds at 5.33gm/ton and 1% in the diet of broilers resulted in a significant increase in the villus height in the duodenum. The beneficial effect on Fenugreek seeds on intestinal histomorphology can be because of their antimicrobial action (Qureshi *et al* 2016) which further resulted in decreased inflammatory reactions at the mucosa and increased villus height (Mahmood *et al* 2015) that further enhances the absorptive surface area for better utilization of nutrients (Adil *et al* 2010). The short or damaged villi impair the absorption of intestine, which might lead to poor performance of birds (Samanya and Yamauchi 2002). Adibmoradi *et al* (2006) in broiler chicken reported that shortening of villi decreases surface area for nutrient absorption. Increased intestinal villi height is an indicator for increased surface area for nutrient absorption (Miles *et al* 2006).

Similar to our study, Mousa *et al* (2009) and Ashayerizadeh *et al* (2023) reported no significant differences on villus height, crypt depth and villus width in duodenum and jejunum, on turmeric powder supplementation. Yason *et al* (1987) considered crypts as villus factory. A large crypt indicates fast tissue turnover, whereas a decrease in crypt depth may lead to reduction in nutrient resorption. Oregano supplementation in laying hens increased the villus height ($P>0.05$) in the duodenum compared with the control group (He *et al* 2017).

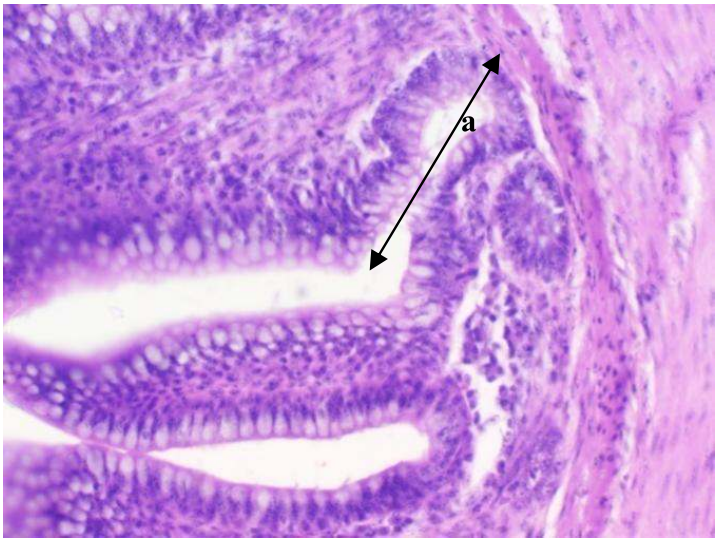
While in jejunum, luminal epithelial height (µm) and gland epithelial height (µm) showed significant changes, whereas rest of the parameters showed no difference. The luminal epithelial height (µm) of jejunum was found significantly higher ($P<0.05$) in T_1, T_2 and T_4 than NC, but PC, T_3, T_5 and T_6 has in between values. Similar to our results, Chowdhary *et al* (2021), reported increase in the luminal and glandular epithelial height in jejunum because of the supplementation of turmeric and garlic @ 1%. It might be attributed to increase in the activity of gland

on supplementation of garlic and turmeric, which results in enhanced secretion of mucin and is important for the mucinous lining of the mucosa and easy passage of the ingested feed (Schneeman 1982). Likewise, He *et al* (2017) reported no effect of oregano supplementation in laying hens micrometry *i.e.* villus length, height and crypt depth in jejunum.

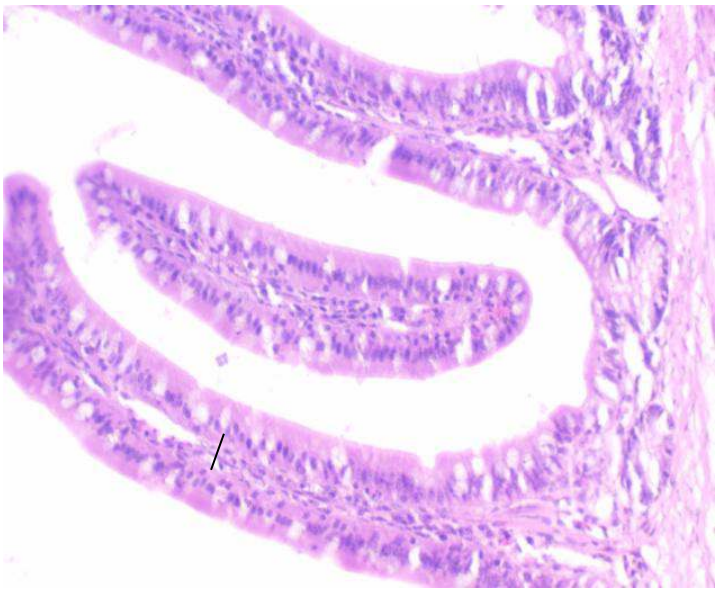
But in ileum, luminal epithelial height (µm) was found higher in T_3, T_4, T_5 and T_6, but it was lowest in NC group and PC group has intermediate values. Rest all the estimated parameters of ileum have statistically similar values and showed no difference. In line with our findings, Chowdhary *et al* (2021) and Kichloo *et al* (2023) reported increased luminal epithelial height in ileum on supplementation of 1% garlic and 0.6% *aloe vera* as dietary additives in poultry birds. But, Ashayerizadeh *et al* (2023) reported increased villus height in ileum on turmeric feeding. The increased epithelial height supplemented group describes better nutrient absorption ability and better performance, which is reflected in numerically higher digestibility coefficients in the present study. The data on effect of phytoadditive supplementation on carcass characteristics of layer quails is scanty, so the results cannot be discussed.



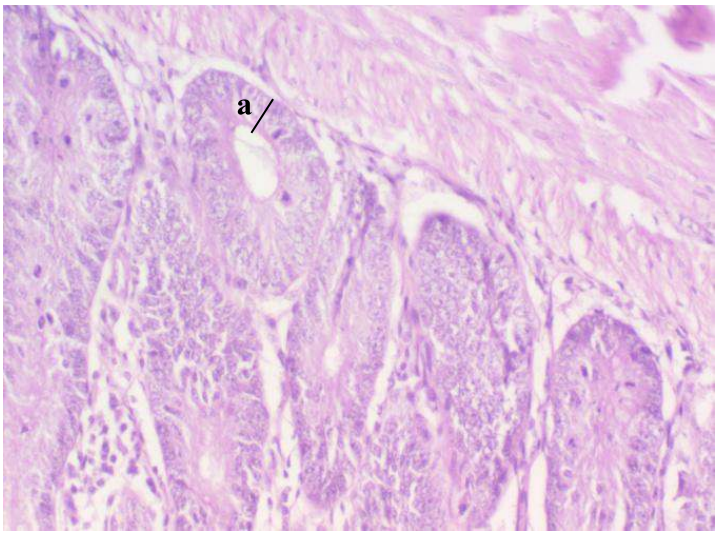
Photomicrograph showing morphometric parameters of small intestine of layer quail. a: length of villi, b: width of base of villi. x100 H&E stain



Photomicrograph showing morphometric parameters of small intestine (Ileum) of layer quail. a: crypt depth. x400 H&E stain



Photomicrograph showing the luminal epithetlial height of small intestine (Ileum) of layer quail. x400 H&E stain



Photomicrograph showing morphometric parameters of small intestine (Ileum) of layer quail. a: glandular epithelium. x400 H&E stain

Plate 1: Showing the different morphometric parameters of small intestine of layer quail which were measured

Table 1: Ingredient and chemical composition (%) of quail layer basal diet (on DMB)

Attributes	Ingredient composition (%)
Maize	62.66
Meat bone meal	4.02
Soybean meal	24.65
Salt	0.25
Sodium bicarbonate	0.01
Soybean oil	1.30
DL-Methionine	0.10
L-Lysine hydrochloride	0.12
Limestone powder	6.72
Vitamin supplement*	0.05
Trace minerals#	0.10
Chemical composition (on DMB, %)	
Organic Matter	95.31
Crude Protein	18.62
Ether Extract	5.51
Crude Fibre	4.19
Total Ash	4.69
Nitrogen free extract	66.99

Table 2: Effect of phytoadditives supplementation on micrometry of duodenum of layer quail

Attributes	Groups								Overall mean± SEM	P value
	NC	PC	T_1	T_2	T_3	T_4	T_5	T_6		
Length of villi (µm)	776.65^{a} ±21.53	850.00^{b} ±20.19	809.97^{ab} ±14.25	867.95^{b} ±21.97	861.89^{b} ±21.44	868.03^{b} ±20.31	808.54^{ab} ±20.94	809.85^{ab} ±17.60	831.61±7.55	0.007
Width at base of villi (µm)	106.88±5.23	108.64±6.72	109.24±6.32	108.49±6.70	107.61±5.63	109.58±6.89	107.08±6.78	107.32±6.41	108.11±2.17	1.000
Luminal epithelial height (µm)	25.62±0.75	26.11±0.67	26.47±1.01	26.12±0.85	26.40±0.74	26.46±0.68	26.00±1.04	26.09±0.90	26.16±0.29	0.997
Crypt depth (µm)	90.17±2.27	89.40±1.79	89.27±1.76	87.44±2.28	89.59±1.57	87.12±1.29	88.79±1.84	90.36±1.47	89.02±0.63	0.884
Gland epithelial height (µm)	15.39±0.44	15.95±0.73	16.09±0.86	15.66±0.85	16.61±0.54	15.64±0.80	14.31±0.58	15.17±0.60	15.60±0.24	0.450

[a,b,c]Mean with different superscript differs in a row significantly (P<0.05); Negative control (NC; contains no additive), positive control (PC; herbal growth promoter), whereas T_1, T_2, T_3, T_4, T_5 and T_6 groups were supplemented with 1% dietary additive namely turmeric, garlic, fenugreek, cumin, *aloe vera* and oregano powder, respectively.

Table 3: Effect of phytoadditives supplementation on micrometry of jejunum of layer quail

Attributes	Groups								Overall mean± SEM	P value
	NC	PC	T_1	T_2	T_3	T_4	T_5	T_6		
Length of villi (µm)	672.85±13.63	680.66±11.10	713.51±11.53	707.41±16.16	699.44±12.08	710.35±18.41	687.61±15.88	691.53±15.63	695.42±5.16	0.426
Width at base of villi (µm)	85.58±3.99	89.25±3.40	87.27±4.94	86.89±5.69	88.09±4.38	88.29±5.40	86.81±5.10	85.76±5.30	87.24±1.65	0.953
Luminal epithelial height (µm)	24.01^{a} ±0.60	26.39^{ab} ±0.87	27.75^{b} ±0.73	27.90^{b} ±0.99	26.11^{ab} ±0.73	27.58^{b} ±0.87	26.14^{ab} ±0.94	25.51^{ab} ±0.78	26.42±0.31	0.019
Crypt depth (µm)	73.56±2.68	74.69±3.29	72.28±3.14	73.49±3.93	72.65±1.81	74.22±2.75	73.72±4.00	74.12±3.00	73.59±1.07	0.999
Gland epithelial height (µm)	12.31^{a} ±0.60	12.69^{ab} ±0.47	13.20^{abc} ±0.58	14.73^{cd} ±0.76	14.32^{bcd} ±0.65	14.98^{d} ±0.49	12.96^{ab} ±0.46	12.65^{ab} ±0.52	13.48ab±0.22	0.004

[a,b,c]Mean with different superscript differs in a row significantly (P<0.05); Negative control (NC; contains no additive), positive control (PC; herbal growth promoter), whereas T_1, T_2, T_3, T_4, T_5 and T_6 groups were supplemented with 1% dietary additive namely turmeric, garlic, fenugreek, cumin, *aloe vera* and oregano powder, respectively.

Table 4: Effect of phytoadditives supplementation on micrometry of ileum of layer quail

Attributes	Groups								Overall mean± SEM	P value
	NC	PC	T_1	T_2	T_3	T_4	T_5	T_6		
Length of villi (µm)	324.52±15.71	340.42±14.77	365.12±10.37	368.46±6.57	335.92±15.30	367.78±10.00	353.05±10.49	346.63±13.67	350.24±4.53	0.122
Width at base of villi (µm)	65.86±2.47	69.85±3.98	70.25±4.16	74.14±4.26	70.84±4.69	73.68±3.59	71.24±3.94	69.85±3.94	70.71±1.36	0.883
Luminal epithelial height (µm)	21.92^{a} ±0.81	23.09^{ab} ±0.67	25.65^{b} ±0.77	25.85^{b} ±0.76	25.09^{bc} ±0.69	25.29^{bc} ±0.75	24.92^{bc} ±0.83	23.77^{abc} ±0.77	24.45±0.29	0.003
Crypt depth (µm)	70.49±2.94	73.06±3.12	75.93±2.46	74.66±1.70	73.23±2.82	75.19±2.24	76.50±3.64	72.41±4.58	73.93±1.06	0.880
Gland epithelial height (µm)	13.19±0.42	14.22±0.41	13.38±0.64	14.26±0.53	14.21±0.63	13.92±0.66	13.14±0.49	13.79±0.68	13.76±0.20	0.230

[a,b,c]Mean with different superscript differs in a row significantly (P<0.05); Negative control (NC; contains no additive), positive control (PC; herbal growth promoter), whereas T_1, T_2, T_3, T_4, T_5 and T_6 groups were supplemented with 1% dietary additive namely turmeric, garlic, fenugreek, cumin, *aloe vera* and oregano powder, respectively.

Conclusion

Intestinal micrometry parameters *viz*., length of villi in the duodenum, luminal epithelial height in jejunum, were found higher (P<0.05), indicating a positive effect of PFA supplementation on micrometry of small intestines in layer.

Conflicts of interest: The authors declare that there is no conflict of interest.

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