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Capsaicin supplementation does not reduce lipopolysaccharide-induced inflammation in growing beef steers[1]

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ABSTRACT: This study evaluated effects of dietary supplementation of jalapeño powder containing capsaicin on inflammation in 24 beef steers (213 ± 6.2 kg BW) exposed to lipopolysaccharide (LPS). Treatments were a 2 × 2 factorial of 2 dietary supplements that supplied 0 or 0.74 ± 0.02 mg kg-1 BW of capsaicin (**-CAP** vs. **+CAP**), and 2 infusions of sterile saline that supplied 0 or 0.5 µg kg-1 BW of LPS (**-LPS** vs. **+LPS**). Steers were limit-fed a diet for 15 d, and supplemented with dietary treatments from d 8 to 15. On d 15, LPS was infused (via i.v. catheters) 3 h after feeding. Respiration rates, rectal temperatures, and blood samples were collected at 0, 2, 4, 8, 12, and 24 h after LPS infusion. Data was analyzed using mixed models and repeated measures. A CAP × LPS interaction occurred for respiration rate ($P = 0.07$) and serum glucose ($P = 0.06$). Respiration rate was greater for +LPS than -LPS steers fed -CAP, and not different for +LPS and –LPS steers fed +CAP. Glucose was not different for +LPS and -LPS steers fed –CAP, and lower for +LPS than -LPS steers fed +CAP. An LPS × h interaction ($P < 0.01$) occurred for all variables. Respiration rates were greater for +LPS than –LPS steers at 2 h, and not different at 4, 8, 12, and 24 h. Rectal temperatures were greater for +LPS than –LPS steers at 2 and 4 h, not different at 8 and 12 h, and lower at 24 h. Cortisol and IL-6 of +LPS steers were greater at 2, 4, 8, and 12 h (IL-6 only), and not different from –LPS steers at 24 h. Serum prolactin was greater for +LPS than -LPS steers at 2 h, lower at 8 and 12 h, and not different at 24 h. Insulin and tumor necrosis factor-α were greater for +LPS than –LPS steers at 2 h, and not different at 4, 8, 12, and 24 h. Interferon-γ was not different at 0 and 2 h, greater for +LPS than –LPS at 4 h, and not different at 8, 12, and 24 h. Serum glucose was greater for +LPS than –LPS steers at 2 h, lower at 4 h, and not different at 8, 12, and 24 h. Results demonstrated that dietary supplementation of jalapeño powder containing capsaicin did not reduce LPS-induced inflammation in steers.

Key words: capsaicin, lipopolysaccharide, steer

INTRODUCTION

Morbidity in feedlot calves decreases performance and negatively impacts gross income (Waggoner et al., 2007). Calf morbidity is generally associated with exposure to infectious diseases, such as bovine respiratory disease complex, and is associated with stress from handling, commingling, and transportation to the feedlot. Exposure to infectious pathogens causes inflammation and stimulates physiological, nutritional, and immunological changes (Loerch and Fluharty, 1999). Sick cattle are commonly treated with antibiotics and anti-inflammatory drugs to reduce fever, however increased consumer pressure to minimize use of antibiotic and other drugs in the cattle feeding industry demands exploration of alternative strategies to improve animal health.

Capsaicin is a capsaicinoid that contributes to the pungent sensation in hot peppers. In addition to the pharmacological roles of capsaicin in pain, cardiovascular, and respiratory systems (O'Neill et al., 2012), previous research demonstrated that capsaicin may have anti-inflammatory properties. For example, Dogan et al. (2004) reported that intraperitoneal injection of capsaicin decreased the febrile response of rats exposed to lipopolysaccharide (**LPS**). Also, Demirbilek et al. (2004) demonstrated that capsaicin lowered pro-inflammatory cytokines (tumor necrosis factor-α, and interleukin-6) and increased anti-inflammatory cytokines (interleukin-10) in septic rats. Therefore, we hypothesized that the capsaicin in hot peppers will reduce inflammation in cattle exposed to stress and disease. The objective of this study was to evaluate effects of dietary supplementation of jalapeño powder containing capsaicin (1,280 mg/kg) on inflammation and nutrient metabolism of beef steers exposed to an endotoxin.

MATERIALS AND METHODS

Experimental Design and Treatments

The Institutional Animal Care and Use Committee at New Mexico State University approved all procedures.

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Twenty-four crossbred steer calves (213 ± 6.2 kg initial BW) were housed individually in soil-surfaced pens for the first 11 d of the study, and then were adapted to individual tie-stalls of an animal metabolism facility from d 12 to 14 before collection of samples on d 15. Catheters (J457A; Jorgenson Laboratories, Loveland, CO) were placed into the jugular vein of calves on d 14. All calves had free access to water and were individually fed a basal diet (Table 1) at 1.53 ± 0.05% of BW (DM basis) in equal portions twice daily (0700 and 1900) throughout the 15-d experiment. Steers were limit-fed to represent feed intakes typical of newly received feedlot calves (NRC, 2000).

The experiment was a randomized complete block design with calves divided into 2 blocks (12 animals per block) because of a limited number of tie-stalls in the animal metabolism facility. Within each block, calves were randomly assigned to a 2 × 2 factorial arrangement of treatments. Treatments were 2 dietary supplements that supplied 0 or 0.74 ± 0.02 mg kg-1 BW of capsaicin (**-CAP** vs. +**CAP**), and 2 intravenous infusions of sterile saline that supplied 0 or 0.5 µg kg-1 BW of LPS (**-LPS** vs. +**LPS**; *E. coli* 055:B5; Sigma Chem. Co., St. Louis, MO). Dietary supplements (Table 2) were formulated to be isonitrogenous, and jalapeño powder (contained 1,280 mg/kg capsaicin) partially replaced corn grain and soybean meal for the +CAP supplement. Dietary supplements were mixed with the basal diet at the 0700 and 1900 feedings from d 8 to 15 of the experiment. Steers were adapted to 200 g/d of dietary supplements from d 8 to 11, and received 400 g/d of the dietary supplements from d 12 to 15. For the -LPS and +LPS treatments, 50 mL of sterile saline solution (without or with dissolved LPS) was infused (Model 230 Syringe Pump, KD Scientific, Holliston, MA) at 1 mL/min via jugular catheters at 3 h after the 0700 feeding on d 15. In block 1, a steer was removed from the experiment because it was dehydrated when the jugular catheter was inserted on d 14, and in block 2 a steer was removed after the 4-h blood collection because of a severe reaction to the +LPS infusions that warranted medical treatment.

Table 1. Composition of the basal diet

Item	DM basis
Ingredient, % of DM	
Corn grain, cracked	38.2
Soybean hulls, pelleted	20.0
Alfalfa hay	20.0
Dried distiller's grains	10.0
Molasses	8.0
Soybean meal	2.5
Premix[1]	1.3
Nutrient, % of DM	
ADF	22.8
CP	16.3
Ca	0.77
P	0.30

[1]Supplied (DM basis): 0.50% urea, 0.35% limestone, 0.30% salt, 0.05% dicalcium phosphate, 20 mg/kg Fe, 18 mg/kg Zn, 3.6 mg/kg Cu, 3.4 mg/kg Mn, 0.07 mg/kg Se, 3,000 IU/kg vitamin A, 600 IU/kg vitamin D, 150 IU/kg vitamin E, and 33 mg/kg monensin.

Sample Collection and Analysis

Respiration rates and rectal temperatures were measured, and blood samples collected at 0 (immediately before), 2, 4, 8, 12, and 24 h after the infusion of LPS on d 15. Respiration rates were measured using a stethoscope and stopwatch, and rectal temperatures were measured using portable digital thermometers (ReliOn, China). Blood samples were collected via jugular catheter into 10-mL syringes, and then transferred into vacuum tubes for separation of serum (Corvac serum separator) and plasma (Monoject Sodium Heparin, Kendall, Ontario, CA). Blood samples for serum collection were allowed to coagulate for 30 min at ambient temperature, and blood samples for plasma collection were immediately placed on ice. All blood samples were centrifuged (1,500 × g; Beckman TJ-6R Centrifuge, Palo Alto, CA) for 20 min at 10°C, and aliquots of serum and plasma were transferred into multiple vials and frozen at -20°C or -80°C for later analysis.

Serum samples were analyzed for cortisol (Kiyma et al., 2004) and insulin (Camacho et al., 2012) by solid-phase RIA with commercially available antibody-coated tube technology (Siemens Diagnostics, Los Angeles, CA), and prolactin and IGF-I by double antibody RIA as described by Spoon and Hallford (1989) and Berrie et al. (1995) with modifications reported by Camacho et al. (2012), respectively. Within and between assay CV were less than 12% for all RIA. Glucose concentrations in serum were analyzed using a commercially available hexokinase reagent (Infinity TR15241, Thermo Scientific, Waltham, MA). The cytokines, IL-6, tumor necrosis factor-α (**TNF-α**), and interferon-γ **(IFN-γ**), were measured in serum using a bovine-specific multiplex sandwich ELISA kit according to manufacturer's protocol (SearchLight, Bovine Cytokine 3-Plex Assay #29-038-1-AB; Aushon Biosystems, Inc., Billerica, MA). Within and between assay CV were less than 8% for all cytokines.

Statistical Analysis

All data were analyzed statistically as a randomized complete block design using mixed models (SAS Inst. Inc., Cary, NC) and repeated measures with autoregressive order-one covariance structure. Data collection occurred over 2

Table 2. Dietary supplements

	Treatments[1]	
Item	-CAP	+CAP
Ingredient, % of DM		
Corn grain, cracked	70	45
Jalapeño powder[2]	-	30
Soybean meal	20	15
Molasses	10	10
Nutrient, % of DM		
CP	17.8	18.0

[1]Dietary supplements were mixed with the basal diet (Table 1) at the 0700 and 1900 feedings from d 8 to 15 of the experiment.
[2]Contained 1,280 mg/kg capsaicin.

periods (blocks), because the metabolism facility could house only 12 animals in individual tie-stalls. Individual animal was the experimental unit. The statistical model included effects of CAP, LPS, hour, and all possible interactions of CAP, LPS, and hour. Block and calf were random. Differences among treatments were considered significant when $P < 0.05$.

RESULTS

No CAP × LPS × h interactions ($P \geq 0.66$) were observed for all response variables measured. Also, no CAP × LPS interactions ($P \geq 0.26$) were observed for rectal temperature and serum concentrations of cortisol, prolactin, insulin, IL-6, TNF-α, and IFN-γ in steers. A tendency for a CAP × LPS interaction occurred for respiration rate ($P = 0.07$) and serum glucose concentrations ($P = 0.06$). Respiration rate was greater for +LPS than -LPS steers (54.1 vs 43.5 ± 7.9 breaths/min) when supplemented with -CAP, and was not different for +LPS and -LPS steers (47.8 vs 52.5 ± 7.9 breaths/min) when supplemented with +CAP. Serum glucose concentrations were not different for +LPS and -LPS steers (78.4 vs 76.9 ± 3.4 mg/dL) when supplemented with –CAP, and were lower for +LPS than -LPS steers (71.4 vs 83.4 ± 3.4 mg/dL) when supplemented with +CAP. No CAP × h interactions ($P \geq 0.15$) were observed for all response variables.

An LPS × h interaction ($P < 0.01$) occurred for respiration rate and rectal temperature, as well as for serum concentrations of cortisol, prolactin, IL-6, TNF-α, IFN-γ, insulin, and glucose. Respiration rate (Fig. 1) increased and was greater for +LPS than –LPS steers at 2 h, then decreased and was not different between LPS treatments at 4, 8, 12, and 24 h after LPS infusion. Rectal temperatures (Fig. 2) of +LPS steers increased from 0 to 2 h and were greater for +LPS than –LPS steers at 2 and 4 h (peak), then decreased and were not different at 8 and 12 h, but were lower for +LPS than –LPS steers at 24 h after LPS infusion. Serum cortisol and IL-6 concentrations of +LPS steers increased from 0 to 2 h, were greater at 2 (peak for cortisol), 4 (peak for IL-6), 8, and 12 h (for IL-6 only), and decreased and were not different from –LPS steers at 24 h after LPS infusion. Serum prolactin concentrations increased and were greater for +LPS than -LPS steers at 2 h, then decreased and were lower for +LPS than –LPS steers at 8 and 12 h, but not different at 24 h. Serum insulin and TNF-α concentrations of +LPS steers increased from 0 to 2 h, were greater than –LPS steers at 2 h, then decreased and were not different between +LPS and –LPS steers at 4, 8, 12, and 24 h after LPS infusion. Serum IFN-γ were not different between +LPS and –LPS at 0 and 2 h, increased and were greater for +LPS than –LPS steers at 4 h, then decreased and were not different between treatments at 8, 12, and 24 h after LPS infusions. Serum glucose concentrations increased and were greater for +LPS than –LPS steers at 2 h, then decreased and were lower for +LPS than –LPS steers at 4 h, and were not different between LPS treatments at 8, 12, and 24 h after LPS infusions.

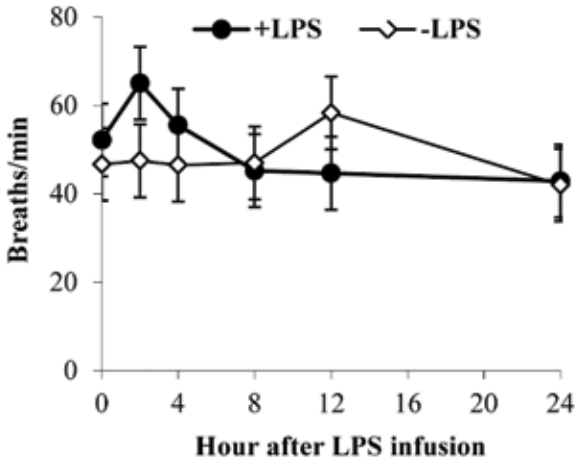

Figure 1. Respiration rate of steers in response to intravenous infusions of bacterial lipopolysaccharide (LPS) at h 0. Treatments were a 2 × 2 factorial of 2 intravenous infusions of sterile saline that supplied 0 or 0.5 μg LPS (-LPS vs +LPS; *E. coli* 055:B5; Sigma Chem. Co., St. Louis, MO) per kg of BW, and 2 dietary supplements that supplied 0 or 0.74 ± 0.02 mg capsaicin (CAP) per kg of BW. Effects were CAP × LPS × h ($P = 0.85$), CAP × LPS ($P = 0.07$), CAP × h ($P = 0.69$), LPS × h ($P < 0.05$), CAP ($P = 0.22$), and LPS ($P = 0.47$).

DISCUSSION

Lipopolysaccharide is a component of gram-negative bacterial cell walls, and has been used to induce non-infectious inflammation in cattle (Waggoner et al., 2009a, 2009b). In the current study, increases in respiration rate, rectal temperature, and serum concentrations of cortisol, IL-6, TNF-α, and IFN-γ are indicative of stress and inflammation in steers receiving LPS. Initial increases in serum concentrations of glucose in response to LPS infusion were accompanied by a brief increase in serum insulin concentrations, which in turn were likely responsible for decreased and lower serum glucose concentration at 4 h for steers exposed to LPS. These results demonstrated that LPS infusion altered energy metabolism of steers, and are consistent with those reported by Steiger et al. (1999) and Waggoner et al. (2009b). According to Spurlock (1997), uptake of glucose by peripheral tissue is inhibited by LPS resulting in transitory resistance to insulin.

Observed tendencies for interaction between CAP and LPS for respiration rates and serum glucose concentrations suggest that capsaicin may alter responses of steers to LPS infusion. However, lack of interaction between CAP and LPS for rectal temperature and pro-inflammatory cytokines indicated that capsaicin did not suppress inflammation in steers exposed to LPS. These results contrast those of Dogan et al. (2004), who reported that an intraperitoneal injection (5 mg/kg BW) of capsaicin decreased LPS-induced fever in rats during the first 4 h after LPS injection. Additionally, Demirbilek et al. (2004) demonstrated that subcutaneous injection of a single dose of capsaicin at 1 mg/kg of BW decreased the pro-inflammatory cytokines, IL-6 and TNF-α, and increased IL-10 (an anti-inflammatory cytokine) in septic rats. Dogan et al. (2004) speculated that CAP may suppress

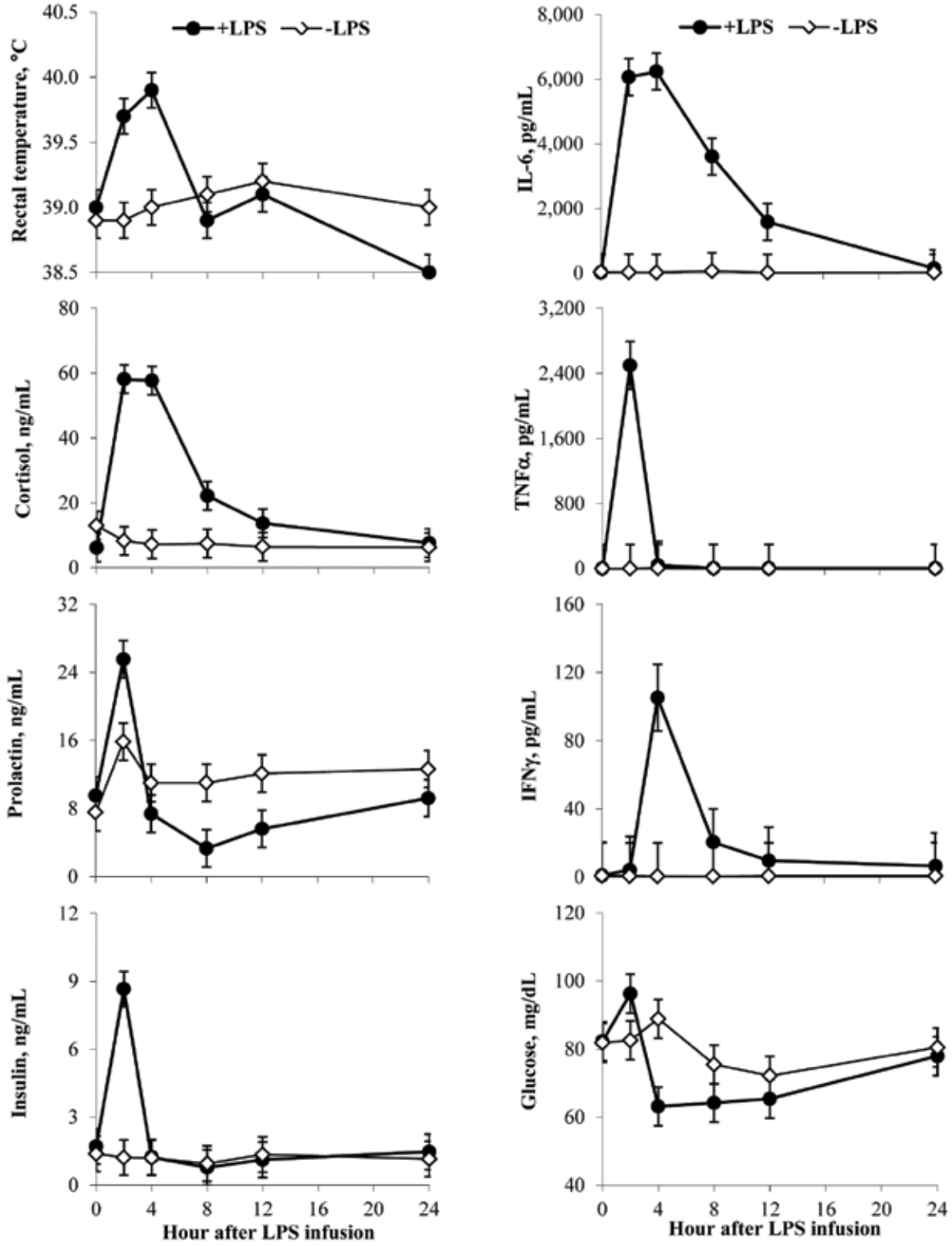

Figure 2. Rectal temperature, serum cortisol, prolactin, insulin, IL-6, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and glucose concentrations of steers in response to intravenous infusions of bacterial lipopolysaccharide (LPS) at h 0. Treatments were a 2 × 2 factorial arrangement of 2 intravenous infusions of sterile saline that supplied 0 or 0.5 μg LPS (-LPS vs +LPS; *E. coli* 055:B5; Sigma Chem. Co., St. Louis, MO) per kg of BW, and 2 dietary supplements that supplied 0 or 0.74 ± 0.02 mg capsaicin (CAP) per kg of BW. Effects for rectal temperature, cortisol, prolactin, insulin, IL-6, TNF-α, IFN-γ, and glucose were CAP × LPS × h ($P \geq 0.66$), CAP × LPS ($P \geq 0.06$), CAP × h ($P \geq 0.15$), LPS × h ($P < 0.05$), and CAP ($P \geq 0.22$); main effects of LPS ($P < 0.05$) for cortisol, insulin, IL-6, and TNF-α, and no effects of LPS ($P \geq 0.11$) for rectal temperature, prolactin, IFN-γ, and glucose.

production of PGE2 by macrophages in organs that process LPS. The lack of inflammatory response to capsaicin in the current study compared with positive inflammatory response observed in previous studies (Demirbilek et al., 2004; Dogan et al., 2004) may be explained by differences in the amounts and methods of capsaicin administration. In the current study, jalapeño powder was supplemented to the diet and supplied 0.74 ± 0.02 mg capsaicin per kg of BW daily for 7 d before steers were infused with LPS. Although fed for a longer period of time, the amount of capsaicin fed in the present study was lower than the amount infused into rats. Furthermore, preliminary in vitro research in our laboratory revealed that capsaicin may be partially degraded in the rumen, and Alford et al. (2014) demonstrated that supplementation of capsaicin in jalapeño powder altered rumen microbial fermentation. Therefore, it is likely that the dietary supplementation of jalapeño powder to steers resulted in a lower post-absorptive supply of capsaicin than initially anticipated. However, greater respiration rates for -LPS steers in response to capsaicin supplementation (CAP × LPS interaction) may be an indication that capsaicin affected airway smooth muscle contraction and (or) pulmonary inflammation as reported in rats (Mandal et al., 1994).

In conclusion, results of this study demonstrated that supplying capsaicin via dietary supplementation of jalapeño powder did not reduce LPS-induced inflammation in growing steers. Alternative administration routes may be required to observe the post-absorptive effects of capsaicin on inflammation in cattle.

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