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Effect of dietary supplementation of sows with quillaja saponins during gestation on colostrum composition and performance of piglets suckled

S. E. Ilsley† and H. M. Miller

Centre for Animal Sciences, School of Biology, University of Leeds, Leeds LS2 9JT, UK

†Corresponding author. E-mail : sian@ilsley50.freeserve. co. uk

Abstract

The objective of this study was to assess the effect of dietary saponins, extracted from the plant Quillaja saponaria*, on the concentrations of immunoglobulins in sow colostrum and milk, and additionally to observe any effect on piglet performance. Twenty-two sows were allocated to each of two dietary treatments on day 72 of gestation, balancing for parity, fatness and past reproductive performance. Treatments were : control (C) and supplemented (Q; 2·5 g saponin per day as a top-dressing). Sows received 2·5 kg food once per day throughout gestation. Between days 72 and 93 of gestation Q sows received a saponin top-dressing as described. After day 93, all sows were given the same diet and managed in an identical manner. Colostrum samples were taken from all sows at farrowing on delivery of the first piglet then 4, 8, 24 and 72 h later and again on day 21 of lactation. A group of eight sows from each treatment were also blood sampled at farrowing, day 3 and day 21 of lactation. Concentrations of immunoglobulins IgG and IgA in the colostrum, milk and blood were not affected by sow treatment during gestation at any sampling point. Fat, lactose and cell counts were also similar across treatments in colostrum and milk. Protein tended to be higher in milk on day 3 but lower on day 21 (*P*<0·1) in Q sows. Piglet performance was not affected by sow treatment during week 1 of lactation. However pigs suckling Q sows grew more slowly between days 8 to 14 (*P*<0·05) and days 15 to 21 (*P*<0·1). Q sows also had fewer stillborn piglets in their litters at farrowing (7·67* v. *13·25%,* P*<0·05, s.e. = 1·93). It is concluded that dietary quillaja saponin given during this time period has no influence on sow lacteal immunoglobulin secretions. However an adverse effect on performance of the sucking piglet was observed, the reasons for which are unclear, but may be related to reduced milk protein concentrations. Dietary quillaja saponin during gestation does however appear to reduce the incidence of stillborn piglets.*

Keywords: *immunoglobulins, lactation,* Quillaja*, sows.*

Introduction

Previous work by Ilsley *et al*. (2003) with crude extract of quillaja, found that when offered during the final week of gestation, a reduction in the number of stillborn piglets was subsequently observed. No difference in sucking piglet performance was evident however. It is widely acknowledged that the major active component of quillaja extract is saponin. The crude nature of the product used in the previous study meant any physiological effects may well have been diluted due to a low and variable saponin content. Thus further research with a purified saponin extract was required.

Saponins extracted from the plant *Quillaja saponaria* are known to have potent effects on the immune system and are widely used as adjuvants to enhance and characterize the immune response to vaccines. They have been found to stimulate the immune system in a number of different ways, including stimulation of immunoglobulin production (Hoshi *et al*., 1999), increased cytokine secretion (Johansson and Lovgren-Bengtsson, 1999) and inducing proliferation of lymphocytes (Sjolander *et al*., 1997). These effects have been seen after several different methods of administration of the saponin including intravenous and oral. Previous work by S. E. Ilsley and H. M. Miller (unpublished data) found that when weaned piglets were given quillaja saponin for 20 days post weaning, serum IgG and acute phase protein concentrations were significantly enhanced.

The antibody content of lactating sow colostrum and milk is crucial in determining the health and survival of the newborn piglet. Work by Krakowski *et al*. (2002) showed that when sows were immuno-stimulated using either intramuscular thymostimuline or orally administered 3-hydro-3-methyl-obutyrate during the period of colostrogenesis, between weeks 6 to 3 prior to farrowing, colostral immunoglobulin concentrations were up to proportionately 0·32 higher and piglets weighed up to 0·60 more at weaning. Total protein and lysozyme content of the colostrum were also significantly increased.

This study therefore aimed to determine whether dietary supplementation of gestating sows during the period of colostrogenesis, with quillaja saponins, would enhance the immunoglobulin (Ig) profile of colostrum and the performance of piglets prior to weaning. Additionally, any influence of saponin supplementation on piglet stillbirth incidence was monitored.

The hypothesis proposed is that oral administration of the saponin to gestating sows will stimulate production of antibodies, which will enter the mammary gland and be secreted during lactation. Higher levels of immunoglobulins in the milk will offer the sucking piglet a better level of passive immune protection, which should reduce piglet morbidity and mortality during the suckling period. Additionally, piglet performance should be enhanced as a result of the improved health status.

Material and methods

Sows

Animals and housing. Forty-four hybrid sows (25% Large White, 50% Landrace, 25% Duroc) of mixed parity were selected from the commercial herd and allocated, accounting for parity, fatness and past reproductive performance to either a control or supplemented diet 6 weeks prior to their farrowing due date. Sows were housed in groups of eight in indoor straw pens equipped with individual feeding stalls during this period. Four days before they were due to farrow, sows were moved into farrowing crates in an ambient environmental temperature of 18ºC. If not already farrowed, sows were induced using a prostaglandin injection on their due date to ensure farrowings were attended.

Feeding. Throughout gestation, sows were offered a commercial reproductive sow diet once per day at the rate of 2·5 kg per sow. Sows allocated to the supplemented diet had their ration top-dressed with 2·5 g of *Quillaja saponaria* saponin (Acros Organics) between days 72 to 93 of gestation. After this point, all sows again received an identical, isonutritious ration.

Measurements. Sows were closely observed at 8-h intervals when they neared their due date. When they displayed signs that they were close to farrowing they were checked every hour. Colostrum was sampled at first appearance from three teats (anterior, medial and posterior teats on the side of the udder uppermost from the sows preferential lying position) and again at farrowing on delivery of the first piglet. Timing of the delivery of the first pig was recorded and colostrum was subsequently sampled by hand milking 4, 8, 24 and 72 h after this point. Milk was again sampled on day 21 of lactation.

From the initial group of 44 sows, eight sows from each treatment were selected to undergo more intensive measurements. These sows were matched for parity and were housed in Home Office licensed farrowing crates. Alongside the colostrum sampling, blood was sampled from the ear vein of these sows at the same time as the first colostrum sample was collected at the onset of parturition. Blood was collected into a sterile container containing no anticoagulant. Following collection it was centrifuged at 2500 **g** to separate the serum, which was stored at –20ºC for future analysis. On days 3 and 21 of lactation, blood was again sampled and milk was sampled following intra muscular injection with oxytocin.

Piglets

After farrowing, litter size, number stillborn and piglet birth weight were recorded. If necessary, piglets were cross-fostered within sow treatment groups no later than 24 h after farrowing. Piglets were weighed again at 7, 14 and 21 days of age. Any piglet mortality during the suckling period was recorded.

Analytical procedures

Colostrum, milk and serum samples were all analysed for IgG and IgA using a quantitative sandwich enzyme-linked immunosorbent assay protocol provided by Bethyl Laboratories Inc., Montgomery, Texas, USA. All antibodies used were purchased from this same company. Affinity-purified goat anti-pig antibody was diluted in coating buffer (0·05 mol/l sodium bicarbonate, pH 9·6) 1 : 100. 100 μl was added to each well of a 96-well plate and left for 60 min at room temperature. The antibody was then removed by aspiration and the wells washed thoroughly five times with wash solution (50 mmol/l Tris buffered saline, pH 8·0, 0·05% Tween 20). Postcoat solution (50 mmol/l Tris buffered saline, pH 8·0, 1% bovine serum albumin; 200 μl) was then added to each well and removed after 30 min, washing each well as before. Standards (Pig reference serum, IgG 18·2 mg/ml, IgA 0·65 mg/ml) were diluted in sample diluent (Postcoat solution containing 0·05% Tween 20; 1 to 0·0156 μg/ml dilution range). Serum samples were diluted 1 : 72800 (IgG) or 1 : 2000 (IgA) so as to fall within this range. Colostrum was diluted 1 : 250000 (IgG) and 1 : 100000 (IgA) and milk 1 : 2500 (IgG) and 1 : 20000 (IgA). Diluted standards and samples were then added to allocated wells (100 μl) and incubated for 60 min. The wash procedure was subsequently repeated and 100 μl of the detection antibody-enzyme conjugate (goat anti-pig IgG/IgA-HRP conjugate) added to each well and left for 60 min. Following a further wash sequence 100 μl of enzyme substrate (TMB (3, 3', 5, 5' tetramethyl benzidine)) was added to each well and left for 20 min. The reaction was then stopped using 2 mol/l sulphuric acid (100 μl per well) and the absorbencies read using a microtitre plate reader at 450 nm.

Colostrum sampled at the onset of farrowing and milk sampled on days 3 and 21 were also analysed using an autoanalyser for protein, fat, lactose and somatic cell counts by Direct Laboratories (Woodthorne, Wergs Road, Wolverhampton, UK).

Statistical analyses

Piglet growth and litter data were tested using analysis of variance with the general linear model procedure (GLM) of Statistical Packages for the Social Sciences (SPSS, 2003). Sow and litter were used as the experimental unit. Data were analysed for the effects of sow treatment and parity and their interaction. Piglet birth weight and adjusted litter size were included in the model as covariates. Sow blood and milk data were analysed using the repeated measures analysis in the multivariate GLM procedure of SPSS (DATE). Effects of time (within-subjects factor) and treatment (between-subjects factor) and their interactions were measured and means compared using pair-wise comparisons and least significant difference at the 95% confidence interval. A subject was defined as dietary treatment group. Linear regression analysis was used to determine relationships between blood and milk immunoglobulin concentrations at specific time points.

Results

Piglet performance

Litter size was similar across treatments at both birth and weaning, as was piglet mortality during the suckling period. However previously supplemented sows had a lower number of stillborn piglets in their litters at farrowing ($P<0{\cdot}05$, Table 1).

Table 1 *Mean sow litter size at birth and weaning, incidence of stillbirth on a percentage of litter basis, and piglet mortality during the suckling period (all means shown are least-square means)*

	Sow diet		
	Control	Saponin	s.e.
Litter size (birth)	11·88	10·81	0·64
Stillborn† (%)	13·25	7·67*	1·93
No. died†	1·12	0·64	0·30
No. weaned†	9·18	9·36	0·33

† Litter size at birth included as a covariate in model ($P < 0{\cdot}001$).

During the 1st week of lactation, piglet growth was similar for both sow treatment groups (Table 2). However between days 8 and 14, piglets sucking previously supplemented sows gained less weight than control litters ($P<0{\cdot}05$). This effect tended to continue in the 3rd week of lactation ($P<0{\cdot}1$), resulting in overall lower average daily gain (ADG) between birth and 21 days of age.

Table 2 *Effects of sow gestation treatment on mean piglet weight (kg) and average daily gain (ADG, g per pig per day) between birth and 21 days of age (values shown are least-square means)*

	Sow diet		
	Control	Saponin	s.e.
Birth weight	1.52	1.52	0.02
Weight‡			
Day 7	2.76	2.81	0.03
Day 14	4.37	4.34	0.08
Day 21	5.69	5.40	0.14
ADG‡			
Birth to day 7	172	174	4.9
Days 8-14	229	209*	6.4
Days 15-21	201	182†	8.0
Birth to day 21	200	179*	5.5

†Approaching significance ($P < 0.1$).
‡Piglet birth weight and litter size included in model as covariate ($P < 0.001$).

Table 3 *Effect of sow gestation treatment on composition of colostrum and milk; mean proportion of fat, protein and lactose, somatic cell count (SCC; × 10^{-3}/ml) (means shown are least-square means)*

	Sow diet		
	Control	Saponin	s.e.
Colostrum			
Fat (g/kg)	45·5	51·7	8·0
Lactose (g/kg)	29·6	31·4	2·3
SCC	4426	1672	1298
Milk on day 3			
Fat (g/kg)	98·2	96·4	9·9
Lactose (g/kg)	42·8	42·0	2·8
Protein (g/kg)	52·0	67·0†	5·0
Fat: protein	1·888	1·439	2·7
SCC	5180	3865	1096
Milk on day 21			
Fat (g/kg)	76·7	80·2	3·7
Protein (g/kg)	51·6	46·6†	1·9
Lactose (g/kg)	54·0	54·8	0·9
Fat: protein	1·501	1·719†	0·07
SCC	893	641	222

† Approaching significance ($P < 0.1$).

Table 4 *Effect of sow gestation treatment on mean immunoglobulin levels (IgA, IgG; mg/ml) in colostrum and milk (values shown are least-square means)*

	Sow diet		
	Control	Saponin	s.e.
IgA			
Pre-farrowing	34·10	35·27	3·66
Farrowing	56·62	70·74	6·21
+4 h	29·53	29·08	4·18
+8 h	21·20	27·04	2·76
+24 h	15·78	13·14	3·93
+72 h	4·61	2·85	1·56
+21 days	8·24	8·08	1·33
IgG			
Pre-farrowing	77·25	80·89	4·91
Farrowing	85·59	72·45	7·18
+4 h	64·44	61·09	8·51
+8 h	55·38	55·33	9·81
+24 h	30·81	21·70	4·71
+72 h	0·90	1·20	0·45
+21 days	0·61	0·53	0·10

Table 5 *Effect of sow gestation treatment on concentrations of immunoglobulins (IgA, IgG; mg/ ml) in sow blood (values shown are least-square means)*

	Sow diet		
	Control	Saponin	s.e.
IgA			
Farrowing	5·076	5·250	0·722
Day 3	5·424	4·706	0·654
Day 21	4·384	4·816	0·463
IgG			
Farrowing	5·375	9·530	1·881
Day 3	10·326	10·828	1·592
Day 21	11·071	13·296	1·255

Milk composition

Fat and lactose did not differ in colostrum or milk between treatment groups (Table 3). Protein concentrations however varied and tended to be higher in milk from supplemented sows on day 3, yet lower in the same animals on day 21 ($P<0{\cdot}1$). Unfortunately, protein values are not available for colostrum samples due to a technical problem. Somatic cell counts (SCC) were similar across treatments at all sampling points. SCCs did however markedly decrease between days 3 and 21 of lactation.

No difference in colostral or milk immunoglobulin concentrations was observed between treatments at individual sampling points (Table 4). There was however a temporal effect; concentrations of both IgA and IgG decreased as lactation progressed, although IgA increased again between the 72-h and 21-day sampling point.

Similarly, no effect of gestation treatment on serum immunoglobulin concentrations was observed, although there was again, a temporal effect. IgG concentrations were greater on days 3 and 21 than at farrowing. IgA also declined between farrowing and day 3 but had increased again by day 21 of lactation. (Table 5).

There was no relationship between serum and milk Ig levels at any sampling point, with the exception of IgA on day 3

Table 6 *Regression analyses: sow blood (x) v. sow milk (y) immunoglobulins (IgG, IgA)*

	R^2	Equation	Significance
IgG			
Farrowing	0·091	$y = 66·94 + 1·89x$	
Day 3	0·029	$y = 0·54 + 0·048x$	
Day 21	0·034	$y = 0·36 – 0·005x$	
IgA			
Farrowing	0·008	$y = 62·08 + 1·38x$	
Day 3	0·259	$y = -2·42 + 1·21x$	*
Day 21	0·038	$y = 5·89 + 0·99x$	

Table 7 *Effect of sow gestation treatment on sow blood metabolites; non-esterified fatty acids (NEFA, meq/l) and urea (mmol/l) (values shown are least-square means)*

	Sow diet		
	Control	Saponin	s.e.
NEFA			
Day 0†	0·79	0·81	0·18
Day 3	0·53	0·51	0·12
Day 21	0·58	0·73	0·21
Urea			
Day 0	3·54	5·33‡	0·57
Day 3	4·65	4·21	0·36
Day 21	5·38	5·71	0·46

† Days are in relation to farrowing.
‡ Approaching significance ($P<0·1$).

where serum IgA levels proved a significant predictor of milk concentrations (R^2 0·259, $P<0·05$, Table 6).

Non-esterified fatty acid concentrations in sow blood were not affected by diet (Table 7). They were at their highest levels in both treatment groups at farrowing. Urea levels in the blood of sows supplemented with quillaja saponin during gestation tended to be higher than control animals at farrowing ($P<0·1$). However levels were similar between treatments after this point.

Discussion

Immune profile

Saponin supplementation did not enhance the antibody profile of sow colostrum or milk, nor did it alter the levels of antibodies found in sow serum during lactation. Previous studies using oral doses of quillaja saponin in chickens showed a large increase in serum IgG levels 4 weeks after administration with an antigen (Hoshi *et al*, 1999). Previous work by our own research group, also found that when weaner pigs were given dietary quillaja saponin for 20 days post weaning, IgG levels in the serum were significantly enhanced on day 20 of supplementation (Ilsley *et al*., 2004). Thus the lack of effect in this situation is surprising. In the 6th week prior to parturition, colostrogenesis has begun. Antibodies found in the initial lacteal secretions partly (IgA) or solely (IgG) originate from the sows circulation (Watson, 1980) and a boost to serum antibodies during this period would therefore be thought to lead to an improvement in colostrum levels. Certainly work by Krakowski *et al*. (2002) showed that when sows were administered with one of three proven immunostimulants between weeks 6 and 3 prior to farrowing, colostral IgG levels were subsequently enhanced. This led to a massive increase in piglet weaning weight although the viability of the study was impaired by the small sample size of only five sows per treatment used. It is possible that due to the lack of a controlled antigen, the response to the saponin in the current trial was unpredictable. Although the sows used are considered to be of high health status, variations in pathogen load and presence of infection will occur which is likely to affect any immune response. The timing of supplementation or dose rate may also have been inappropriate. It would perhaps have been beneficial to extend the period of supplementation up to farrowing to enhance the chances of an effect as serum antibodies continue to enter the mammary gland up to this point (Bourne and Curtis, 1973b). The dose rate used was calculated based on results of previous research showing induction of an immune response following oral saponin intake, scaling to the body weight of the sow. However it may have been advantageous to conduct a dose response study as previous research has been in small animals under laboratory conditions. The current study used large, commercially farmed animals which are subjected to unregulated factors including disease, which is likely to affect any physiological response.

As the piglet is born devoid of passive immunity, passage of antibodies, particularly IgG, from sow colostrum through the intestinal mucosa into the piglet's circulation is essential in determining the piglet's chances of survival and health. Colostrum-deprived piglets are much more susceptible to disease and death. Klobasa *et al.* (1981) showed that piglets that die before weaning have between proportionately 0·1 to 0·5 lower serum immunoglobulin concentrations than surviving littermates. Tuchscherer *et al.* (2000) found that piglets surviving the first few weeks of life were born earlier in the birth order and so reached the udder and sucked more rapidly, thus ensuring maximum intake of immunoglobulins. IgG has a half life in piglet serum of between 7 and 22 days (Bourne and Curtis, 1973a), thus initial ingestion of maternal IgG in colostrum provides the piglet with systemic immune protection for a significant period of time.

Piglet growth and milk composition

Contrary to our hypothesis, piglet pre-weaning growth was not enhanced by sow treatment but was actually impaired in litters sucking previously supplemented sows. This effect materialized in the 2nd week of lactation and continued through to weaning on day 21. Piglet birth weight and growth during the first 7 days were not different between treatments. Colostrum composition was similar for both treatment groups for all factors measured including immunological content, which correlates with the similar initial growth rate. However as lactation progressed, milk protein tended to be higher on day 3, yet lower on day 21 in sows supplemented during gestation. On day 21, fat to protein ratios in the milk tended to be higher in previously supplemented sows, which may have affected nutrient partitioning in the piglet. Pluske and Dong (1999) stated that when milk protein to energy ratio is decreased (i.e. fat to protein ratio is increased), lower milk to tissue conversion efficiency can occur in the piglet. Because no milk samples were taken between days 3 and 21, it is impossible to determine when the protein content shifted and it may be that the change occurred in time to influence piglet performance during the 2nd week. During the 1st week of lactation, milk composition is in a transitional period whilst developing into mature milk (Klobasa *et al.,* 1987),

thus samples taken on day 3 may only be representative of this specific time point and subject to substantial change.

A previous study (Ilsley *et al*., 2003) found that when a crude quillaja extract was given to sows during the last week of gestation and throughout lactation, piglet performance was not affected. This may be due to the difference in timing of supplementation. If the saponin is affecting milk composition in the present study it would indicate that it is long acting in the sow. However, the previous trial results most likely differ due to the greatly reduced saponin levels found in the crude extract used.

The mechanisms by which the saponin may be affecting milk protein are unknown. Milk protein is notoriously difficult to manipulate by dietary means as the sow has a high capacity to buffer milk production with her own body reserves. It is possible that the saponin is having an effect on protein metabolism in the sow, which in turn is affecting milk composition. The greater serum urea levels seen at farrowing in the saponin group would indicate an increase in protein catabolism although this effect does not continue into lactation. Boyd and Kensinger (1999) stated that a single amino acid deficiency can limit milk yield and milk protein in lactating sows.

Another saponin rich plant, *Yucca schidigera*, has been found to influence nitrogen metabolism in a number of species (Hussain *et al.,* 1996). One mechanism by which this might be occurring is through modification of the gastro-intestinal microflora. Work in ruminants indicates saponins have pronounced anti-protozoal activity and can modify bacterial populations (Wallace *et al*., 1994) however effects in the monogastric are unclear. Scheline (1978) considered the mechanisms by which saponins might exert their biological activity and concluded that either effects in the gut lumen via interaction with other dietary components, enzymes, microflora etc. or effects on the cell membrane affecting absorption are the most likely. Glycoside compounds are among the most likely to react with the gut microflora, involving a range of reactions. The sugar moiety of saponins serves to decrease the likelihood of absorption in the small intestine thus rendering the compound available in the large intestine. The fewer sugar molecules associated with the aglycone component then the more likely the saponin is to be absorbed (Hostettmann and Marston, 1995). These authors also suggested that saponins may be partially cleaved into the glycone and aglycone components and subsequently absorbed, thus having potential systemic effects or more likely, entering the enterohepatic circulation.

Stillbirth incidence

A very interesting observation of this study is the reduction in stillborn piglets seen in the litters of previously supplemented sows. This effect was also observed in a previous trial (Ilsley *et al*., 2003) when sows received crude quillaja extract for 1 week prior to farrowing. This firstly suggests that it was the saponin component of quillaja extract that elicited the effect, and secondly indicates that the saponin has long acting activity due to the difference in the timing of supplementation. It is highly unlikely that the saponin is having a direct effect on the piglet as saponins and their metabolites are large in structure and this factor, coupled with the epitheliochorial placentation of the pig, means large molecular weight compounds do not pass the placental barrier to reach the foetus. Thus an effect on maternal oxygen supply to the foetus appears to be the most likely explanation. The predominant cause of stillbirth is anoxia caused by prolonged farrowing and commonly seen in piglets late in the birth order. Farrowing duration did not differ between sow treatment groups in the current study. Previous work by Cline *et al.* (1996) found that when sows were given yucca extract (also high in saponin) during the last week of gestation, piglet blood oxygen levels were elevated at birth. Saponins are known to have the ability to increase cell membrane permeability due to their surfactant properties. Thus it is possible that oxygen diffusion across the placenta is being enhanced in supplemented sows, so increasing foetal supply and thus piglet viability at parturition. However due to the large molecule size, few saponin molecules will be absorbed whole from the gut which makes this mechanism unlikely. Alternatively, the oxygen carrying capacity of the sows blood may have been increased by some unknown mechanism, possibly resulting in an increase in erythrocyte numbers or haemoglobin content. Erythrocytes have a lifespan of 65 days in pig blood (Ruckebusch *et al*., 1991), thus such an effect would result in long lasting results. On average 9% of piglets in UK litters are lost to stillbirth (Meat and Livestock Commission, 2003), thus reducing this figure would markedly improve sow productivity. However unfortunately the current study offers no firm insight into the mechanisms behind this effect.

Conclusions and future research

Supplementation of sows with quillaja saponin using the timing and concentrations of this study did not modify the immunoglobulin concentrations of sow colostrum and milk. However, performance of sucking piglets was reduced after sow supplementation possibly due to a lower milk protein concentration. One beneficial effect of dietary saponin seen was its ability to reduce piglet stillbirth incidence, supporting previous findings (Ilsley *et al*., 2003). A potential alternative means of application of saponin to lactating sows is using intramammary immunization. IgA secreted in milk is vital to the sucking pig in providing enteric immune protection throughout lactation. The majority of milk IgA is locally produced in the mammary gland, thus direct intramammary injection of the saponin, coupled with the piglet's natural inoculation of the sow whilst sucking, may result in an increase in milk IgA. Salmon (1999) found that injecting live virus into the mammary gland of gestating sows resulted in an increase in milk IgA. This would be a route for further investigation.

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