

Effect of Anionic Salt and Highly Fermentable Carbohydrate Supplementations on Urine pH and on Experimentally Induced Hypocalcaemia in Cows

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¹Cattle Production Medicine Research Group, Department of Large Animal Sciences, and ²Section for Experimental Animals, The Royal Veterinary and Agricultural University, Denmark, [§]College of Veterinary Medicine, Michigan State University, East Lansing, USA.

Mellau LSB, Jørgensen RJ, Bartlett PC, Enemark JMD, Hansen AK: Effect of anionic salt and highly fermentable carbohydrate supplementations on urine pH and on experimentally induced hypocalcaemia in cows. Acta vet. scand. 2004, 45, 139-147. – The objective of this experiment was to determine the effect of dietary grain on calcium homeostasis. Six rumen-fistulated dairy cows with 3 or more previous lactations and no history of parturient paresis were randomly assigned to a sequence of diets in a crossover study with 4 periods of 10 days each. Dietary treatments were: A control ration consisting of wrap grass silage alone (1), the control ration supplemented with ammonium chloride and ammonium sulphate salt solution (2), control ration following a period with supplementation (3) and control ration supplemented with increasing amounts of barley from 4 to 10 kg/cow per day, expected to produce subclinical rumen acidosis (4). Daily intake of the diets was adjusted to 14 kg DM/cow per day. On day 11, the calcium-regulating mechanisms in cows were challenged until recumbency by a standardized intravenous EDTA infusion and cows were left to recover spontaneously. Anion supplementation and the feeding of highly fermentable carbohydrate lowered urine pH below 7.0 due to subclinical acidosis. During spontaneous recovery from EDTA induced hypocalcaemia, the cows more quickly regained a whole blood free calcium concentration of 1.00 mmol/L if they had most recently been supplemented with either anionic salts or with increasing amounts of barley, as compared to the basic ration. It is concluded that so-called slug-feeding or 'steaming up' with highly fermentable carbohydrates before parturition in milk fever susceptible cows enhanced calcium homeostasis similar to the effect seen in cows on anionic diets.

Hypocalcaemia; milk fever prevention; resistance; calcium homeostasis; calcium binding; calcium mobilization; dairy cow; subclinical acidosis; EDTA infusion; anionic salt; grain feeding.

Introduction

Successful control of milk fever incidence begins with dietary management of the dry cow. The usual prepartal dietary management of a non-lactating dairy cow may involve concentrate feeding during the close-up period to adapt the microflora and forestomach mucosa to the high energy grain diets that will be fed af-

ter parturition (Gerloff 1988). Grain feeding may result in subclinical rumen acidosis (SRA) with compensated metabolic acidosis, growth of the rumen papillae and increased production and absorption of volatile fatty acids (VFA) (Dirksen *et al.* 1984). Cattle fed cereal grain diets typically are acidotic and secrete acid urine

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(Owens et al. 1998). The possible effect of such SRA derived systemic acidosis on calcium homeostasis at calving has not been reported, although previous investigations have focused on grain or sugar feeding in relation to the occurrence of milk fever (Emery et al. 1969, Erb & Gröhn 1988).

The aim of this investigation was to determine whether systemic acidosis, induced by increasing daily intake of highly fermentable carbohydrate, may have any regulating effect on calcium homeostasis as monitored by an EDTA infusion challenge to acutely bind calcium to mimic the calcium drain produced by the mammary gland at the onset of lactation. For a review of EDTA-induced hypocalcaemia as a model for spontaneous hypocalcaemia, see Jørgensen et al. (1999). For comparison, the widely used technique of inducing systemic acidosis by dietary anions was also studied. The standardized intravenous Na₂EDTA infusion test and the judging criteria were adopted from Mellau et al. (2001).

Materials and methods

Animals

The investigation was conducted with 3 Danish Holstein and 3 Red Danish dairy cattle. All cows were non-lactating, non-pregnant cows of 3rd or greater parity and with no history of milk fever. Eight weeks before the start of the experiment, 2 cows at a time were fitted with rumen cannula to facilitate anion dosing and starch supplementation. Cow weights were 660 ± 42 kg (mean ± SD). Each cow was kept in a separate pen where she could eat, drink water, turn, and lie down. Straw bedding was changed daily. All cows were healthy and their body condition remained stable during the study.

Dietary Treatments

Cows were assigned to 4 phases of feeding regime of 10 days each in a crossover study de-

sign. Each of the feeding regimens was followed by an intravenous Na₂EDTA infusion (see below) on day 11 to measure calcium homeostasis. Feeding regimens were: 1. Control diet of wrap-grass silage (BR1), 2. Anion diet, which was BR1 supplemented with ammonium chloride and ammonium sulphate salt solution, 3. Wrap-grass silage (BR2), to avoid any possible carry-over effect of anion, 4. Control diet supplemented with rolled barley given per fistula. Daily intake of the diets was adjusted to 14 kg dry matter (DM) /cow per day, which was close to the expected amount that a cow would consume *ad libitum*, and was kept constant throughout the experiment. The DM content of BR1 and BR2 was 75%. Nutrient content of wrap grass silage was determined by Steins Laboratory (Steins Laboratorium A/S, DK 7500 Holstebro, Denmark) by standard NIR method as follows: 26.2% crude protein 23.5% crude fiber and 13.2% ash, including 0.46% calcium 0.5% phosphorus 0.19% magnesium 46 mg/kg zinc and 13 mg/kg copper (DM basis).

Anion supplementation

Ammonium chloride and ammonium sulphate was given through rumen fistula. The salts were given at the rate of 0.23 g/kg BW per day according to Wang and Beede (1992). The amount of anion salts calculated per kilogram body weight was dissolved in 1 L of tap water. At 09.00 h, 0.5 L of the solution was given after collection of morning rumen fluid samples, and the second 0.5 L was given at 15.00 h per fistula during the afternoon meal.

Starch supplementation

Cows were administered 4 kg of rolled barley per fistula in divided meals of 2 kg each on the first day. The amount of barley was increased gradually at the rate of 0.5 kg per day to provoke metabolic acidosis, defined by urine pH,

until the amount given per fistula was 10 kg/day particularly on day 10.

Blood Samples

Central venous catheters (Secalon[®] Seldy Ohmeda, Faraday Road, Swindon, London) were surgically inserted and fixed into both jugular veins. Blood samples were collected via the catheters at 09.00 h, just before offering the morning meal, and at 18.00 h, 3 hours after afternoon meal. To insert the catheters, cows were pre-medicated by intramuscular injection with a mixture of 2 ml butorphenol (10 mg/ml) (Torbugesic Vet[®], SCANVET, DK-3480) and 1 ml 1% Detomidine hydrochloride (Orion Animal Health DK-3490). Indwelling catheters were kept patent by flushing with physiological saline containing 0.2 ml of heparin/100 ml saline after collection of each blood sample. The right catheter was used for EDTA infusion and the left for collection of blood samples.

Urine Samples

Midstream urine sample were collected by gentle massaging of the perineum. Samples were collected daily at 09.00 h before the morning meal was offered and at 18.00 h, 3 hours after the afternoon meal. The pH of urine and of the rumen samples was determined cow-side using a hand-held pH meter (Horiba Twin[®] pH-meter, B-213, Spectrum Technologies Inc. 60544, Illinois, USA). The pH-meter was calibrated each test day before determinations using a 2-point calibration with pH = 7.0 and 4.0. The pH-meter was flushed with distilled water between measurements. Urine pH was determined twice on each sample and the mean figure calculated cow-side and recorded.

Intravenous Infusion with Na₂-EDTA

On day 11 of each dietary feeding regimen, cows were challenged until recumbent by intravenous infusion with an EDTA solution. Cows

were weighed on a digital electronic scale the day before intravenous EDTA challenging. The infusion was prepared by dissolving 50 g of Na₂EDTA salt (molecular weight 372.24 g/mol. Merck nr. 8418 pro analysi, E. Merck, D-6100 Darmstadt) in 1 L of sterile distilled water. The intravenous infusion flow rate was adjusted to 60 mg/kg per h equivalent to 1.2 ml/kg per h (Mellau *et al.* 2001) and infusion speed in milliliter per min was fixed using an electronic infusion pump (Masterflex[®] model No. 7523-37, Barnant Co. Barrington, IL 60010 USA).

Whole Blood Free Calcium Monitoring

Ten milliliters of blood was collected into sodium-heparinated test tubes (Vacutainer[®] System) at time zero (before the start of infusion) and every 20 min during infusion until involuntary recumbency. Before each sampling, 10 ml of blood was drawn from the catheter and discarded. Na₂EDTA infusion was stopped when paresis or other clinical signs of hypocalcaemia ensued and the cow became involuntarily recumbent. During spontaneous recovery from hypocalcaemia, hourly blood samples were collected and analyzed for free calcium until the concentration of 1.00 mmol/L or above was regained. This level of 1.00 mmol/L was chosen based on previous studies showing this to be the lower limit for normal smooth muscle contraction (Daniel 1983, Desmecht *et al.* 1995, Jørgensen *et al.* 1998). The period of time in min from involuntary recumbency until the cow had regained blood free calcium concentration of 1.00 mmol/L was defined as Calcium Regaining Time (CRT). Whole blood free calcium was determined cow-side using a transportable acid-base analyzer (IRMA[®] Blood Analysis System, Diagnostics Medical Inc., St Paul, MN, USA). (Since these studies were performed the analyzer has been validated for use in cattle by Hansen *et al.* ((2000)).

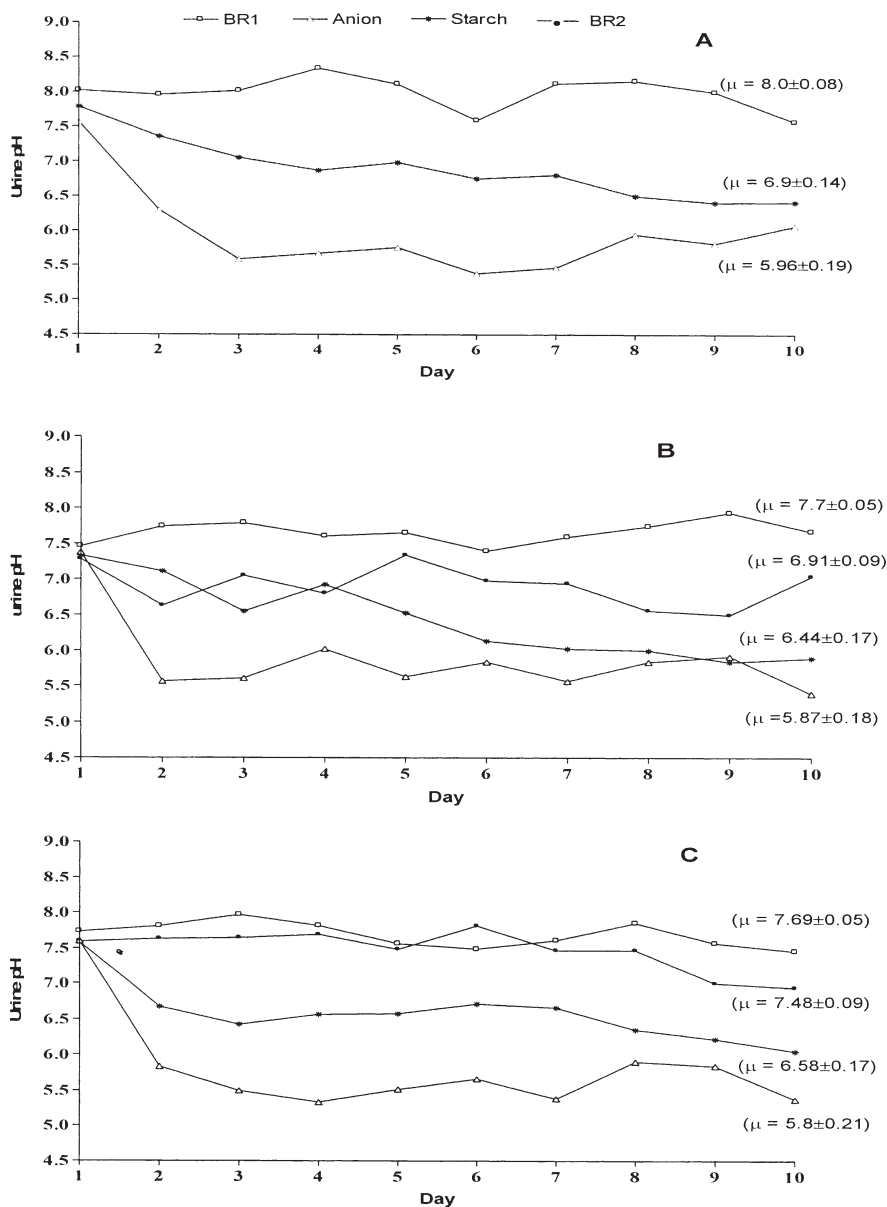


Fig. 1. Average pH of urine for the 1st pair (A), 2nd pair (B) and 3rd pair (C) of cows during 4 periods of 10 days each. BR1 and BR2: Periods in which basic rations were fed with no supplements. ANIONS and STARCH: Periods in which basic ration feeding was supplemented per fistulam with anion salts and increasing amounts of barley, respectively. Figs. in brackets are mean values of all ten pH determinations of that particular graph. Initially (1st pair) urine pH determinations were not done during the BR2 period.

Table 1. Time (min) spent by cows before involuntary recumbency due to EDTA-induced hypocalcaemia. Cows were administered one of 4 experimental diets for 10 days each prior to EDTA challenge to their calcium homeostatic mechanism. The time until involuntary recumbence was recorded.

Cow number	BR1	Anion	BR2	Starch
1	200	220	160	135
2	180	140	140	120
3	195	140	130	130
4	100	120	100	80
5	90	100	90	90
6	155	115	115	130

Statistical Analysis

The results of daily urine pH measurements were analyzed by ANOVA. Time until involuntary recumbence and the CRT were analyzed using the general linear model (SAS, 1997). Tukey's multiple comparison test was used for specific dietary contrasts for urine pH, and for CRT when the difference between diets was found to be statistically significant. The statistical model for time to involuntary recumbency and CRT was as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij};$$

Where:

Y_{ij} = Time in min until involuntary recumbence or the CRT.

μ = overall mean time in min

α_i = fixed effect for the i_{th} diet

β_j = random effect for the j_{th} cow

ε_{ij} = random error variation other than that due to the effect of the diet or cow

Results

Daily Urine pH

The trend of urine pH fluctuation during each dietary feeding regimen and the 10 day mean \pm SEM for each pair of cows are shown in Fig. 1. The daily urine pH differed significantly among cow pairs ($p < 0.0001$). Urine pH was significantly lower during anion supplementation

Table 2. Calcium Regaining Time (CRT) in min in cows administered one of 4 experimental diets. Each diet was given for 10 days, and the calcium homeostatic mechanism for each cow was challenged by a standardized intravenous 5% Na_2EDTA infusion on day 11. Intravenous infusion was continued steadily until the cow went involuntarily recumbent. Whole blood free calcium was monitored cow side during spontaneous recovery from the induced hypocalcaemia. The first blood sample was collected at recumbence. Subsequent blood samples were collected hourly until a whole blood free calcium concentration above 1.00 mmol/l was regained. CRT at 1.00 mmol/l was then extrapolated graphically from plots.

Cow number	BR1	Anion	BR2	Starch
1	360	180	360	240
2	300	120	300	180
3	540	120	310	360
4	380	130	240	240
5	240	240	240	120
6	420	300	240	240

compared with periods of BR1 ($p < 0.001$), BR2 ($p < 0.001$) or starch ($p < 0.05$). During starch supplementation, cows had significantly lower urine pH compared to BR1 ($p < 0.001$), but urine pH did not differ significantly between periods of starch supplementation and BR2.

Time to Involuntary Recumbence

Time (min) to involuntary recumbence due to EDTA-induced hypocalcaemia is shown in table 1. Significant variation ($p < 0.05$) was observed among diets. Cows on BR1 went recumbent faster ($p < 0.01$) than cows supplemented with anion salts and similarly faster than starch supplemented cows ($p < 0.05$). Time to involuntary recumbence was not statistically significant between BR1 and BR2 cows. Comparison of time to involuntary recumbence among individual cows was not significant.

Calcium Regaining Time (CRT)

The CRT (Table 2) differed significantly between diets ($p < 0.01$) as well as among cows

($p < 0.001$). The mean \pm SEM CRT of 373 ± 42 min was observed in BR1 cows, 282 ± 20 min in BR2 cows, 230 ± 33 min in starch supplemented cows and 182 ± 30 min in anion supplemented cows. There was a significant difference in CRT between BR1 and anion supplemented cows ($p < 0.01$), and between BR1 and starch supplemented cows ($p < 0.05$).

Discussion

In this study, calcium regulation was challenged by a standardized intravenous EDTA infusion until involuntary recumbence (Mellau et al. 2001). The resistance to hypocalcaemia was measured by the dose (minutes of EDTA administration) needed to induce recumbency, and by CRT. By the first criteria, the ability of the cows to resist hypocalcaemia varied significantly among diets. Starch supplemented cows resisted induced hypocalcaemia better than either BR1 or BR2 fed cows ($p < 0.01$) although anion supplemented cows resisted significantly longer than others. Variation among cows in the same context was not significant, indicating that we were successful in maintaining repeatable experimental conditions during each pair of cows. Lack of variation among cows in time to involuntary recumbency entails an equal chance for each individual cow to develop hypocalcaemia manifested by paresis and recumbence as long as the EDTA infusion volume and flow rate was maintained to chelate calcium.

Specific comparisons for the effect of diets on time to involuntary recumbency indicated that cows on BR1 became recumbent faster than cows supplemented with either anions or starch. The reduced CRT in cows supplemented with either anions or starch, compared to cows offered BR1 or BR2, indicated enhanced calcium regulation mechanisms following EDTA-induced hypocalcaemia. Urine pH in these cows supplemented with either anions or starch

was also observed to be less than 7.0, indicating a mild systemic acidosis.

Acidosis has been shown to improve calcium homeostasis in cows (Jorgensen 1974, Block 1984). The positive aspect of acidosis is not fully understood, but at least part of it lies in its effect on the conversion of vitamin D to its active hormonal metabolite $1,25(\text{OH})_2\text{D}_3$, a key component stimulating intestinal absorption of dietary calcium, bone resorption and renal calcium reabsorption (Lunn & McGuirock 1990). The resulting metabolic acidosis improves calcium homeostatic mechanisms by enhancing the effect of PTH and $1,25(\text{OH})_2\text{D}_3$ on bone, intestinal absorption and renal regulation of calcium (Goff & Horst 1997). These responses follow a negative calcium balance caused by increased urinary excretion of calcium during metabolic acidification (Wang & Beede 1992). In accordance to this, plasma hydroxyproline and other indicators of bone resorption have also been reported to increase in cows fed acidogenic diets (Block 1984). In our study, the possible carry-over effects resulted from previous acidogenic salt supplementation. Perhaps the invasive effect of EDTA infusion itself could describe the relative similarity of the degree of acidosis, as reflected by low urine pH, between cattle on starch or BR2. The response seen to anionic salt supplementation concurred with a report involving supplementation of cows with NH_4Cl and $(\text{NH}_4)_2\text{SO}_4$ (Wang & Beede 1992). The ability for improved CRT of starch-supplemented cows most likely resulted from increased production of VFA and lactic acid in the rumen as a result of starch fermentation. Wadhwa & Care (1999) suggested that short chain fatty acids (SCFA) stimulate calcium absorption across the reticulo-rumen in sheep by providing hydrogen ions to stimulate $\text{Ca}^{2+}/2\text{H}^+$ exchange. Although the dietary cation-anion difference of the experimental diets was not determined during the study, going

from 14 kg grass silage to 4 kg grass silage plus 10 kg rolled barley is very likely to change the dietary cation-anion difference (DCAD) independent of organic acid load. Noteworthy here is that adding grain in the daily ration dilutes the effect of potassium, thereby changing the DCAD of the ration. Rumen acidosis may lead to systemic acidosis if the amount of organic acids absorbed into blood exceeds the removal of these acids from the circulation by the liver and the kidney.

Previous experiences with a possible link between grain feeding and calcium availability were inconclusive. *Hibbs & Conrad* (1966) analyzed large data sets involving lactating cows and found that the availability and absorption rate of calcium and phosphorous were markedly improved by the addition of 1.4-2.3 kg of grain concentrates per day. They were unable to explain this effect, but in their later study *Conrad & Hibbs* (1973) observed a 55% increase in absorbability of calcium in cows receiving 9 kg of concentrates/day compared to 38% in cows fed alfalfa hay. In contrast, *Jones & Luthman* (1978) supplemented concentrates containing 82% starch and 18% crude protein with silage to sheep but gastrointestinal absorption of labeled calcium remained the same. According to *Braithwaite* (1976) the effect of sugars and of grain in promoting calcium absorption might be due to a decreased intestinal pH resulting from the products of their digestion. *Favus* (1992) observed that plasma ionized calcium concentration increased during compensated metabolic acidosis due to competition of Ca^{++} with H^{+} to the negatively charged binding sites on the protein molecules.

Kendall et al. (1966) increased the rate of concentrate mixture to 1% of body weight daily for a period of approximately 3 wk prior to calving in 11 cows. All cows had had milk fever during the previous lactation. Interestingly, they observed that none of the treated cows developed

milk fever and the lowest blood calcium was 1.83 mmol/L, as compared to 1.37 mmol/L during the previous calving.

In contrast to this, in an ecological analysis of risk factors for postpartum disorders, *Correa et al.* (1990) recorded an increased likelihood of milk fever and left displaced abomasum on farms in which the stated policy was 'to lead feed'. They suggested that acidosis from grain feeding may also cause hypocalcaemia, although this conflicts with the findings of *Bushinsky et al.* (1985) who found that acidosis increased bone calcium availability by releasing calcium from amorphous CaPO_4 and CaCO_3 in the bone matrix.

In the present study, anion and rolled barley supplementation lowered urine pH to below 7.0. According to *Emmanuele & Staples* (1994), supplementation of readily fermentable carbohydrates may increase stomach absorption of calcium and magnesium because of more acidic conditions resulting in greater movement of these minerals to the lower digestive tract. They suggested a greater release of minerals from foodstuffs or a reduced absorption through the rumen wall under acidic rumen conditions to be responsible for their observations. It was apparent from our study that grain supplementation provoked systemic acidification although at a lower level compared to anions. Therefore, an on-farm trial on dry-cow grain supplementation to monitor the ability to resist parturient hypocalcaemia should be conducted.

Concluding from our findings, anions supplementation or an increasing daily dose of highly fermentable carbohydrate had a measurable and significant effect on calcium mobilization.

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Sammendrag

Effekt af supplerende indgivelse af anionsalte og letfordøjelige kulhydrater på urinens pH og på eksperimentelt induceret hypocalcæmi på køer.

Formålet med dette forsøg var at undersøge effekten af hurtig optrapning med korntilskud på calciumhomeostasen. Seks vomfistulerede malkekøer, der havde gennemgået mindst 3 laktationer og ikke tidligere havde haft mælkefeber, blev tilfældigt fordelt i behandlingsgrupper i et såkaldt crossover-studium bestående af 4 perioder, hver af 10 dages varighed. Behandlingerne bestod i følgende: En basisration bestående udelukkende af wrapensileret græs (1), basisration suppleret med ammoniumklorid og ammoniumsulfat (2), en anden basalration (3), og endelig en basalration tilsat byg i stigende mængder (fra 4 til 10 kg/d) med henblik på at provokere en subklinisk

acidose (4). Anionsalte og byg blev givet via vomfistlerne. Dagligt indtag var justeret til 14 kg tørstof per dag. Vom- og urin-pH blev bestemt daglig således: Før morgenfodringen (kl. 9) og 3 timer efter eftermiddagsfodringen (kl. 18). På dag 11 testedes de calciumregulerende mekanismer ved en standardiseret intravenøs infusion med calciumbinderen EDTA, der blev givet, indtil koen lagde sig ufrivilligt. Anionsupplering såvel som hurtig stivelsesoptrapning reducerede urin-pH til under 7, formentlig som udtryk for en subklinisk acidose. Ved infusionstestning efter perioder med aniontilsætning såvel som efter perioder med hurtig stivelsesoptrapning sås, at køerne restituerede sig hurtigere målt ved den tid, det tog koen at hæve niveauet af fri calcium i blodet til 1.00 mmol/l, end efter perioder med basisration uden tilsætninger.

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