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Effects of dietary supplements of zinc-methionine on milk production, udder health and zinc metabolism in dairy goats

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Twenty-two Murciano-Granadina dairy goats were used to investigate the effects of organic Zn supplementation of a diet containing a high level of inorganic Zn. Goats were kept in pens, machine milked once a day throughout lactation and fed a diet based on a dehydrated mixture of whole-plant maize and alfalfa *ad libitum*, alfalfa pellets, barley grain and a concentrate mixture. Treatments were: (1) control, and (2) supplemented with 1 g/d Zn-Methionine (Zn-Met) included in the concentrate mixture. After parturition, goats were blocked in week 3 and dietary treatments were applied until week 23. From weeks 3–20, feed intake, milk yield, milk composition, milk somatic cell count (SCC), and udder health were measured. In week 21, all goats were injected intraperitoneally with 1 g/d DL-methionine for 5 d to establish the effects of methionine under the conditions of udder stress induced by hand milking on the second day. During weeks 22 and 23, diet digestibility, and N and Zn balance were determined. Dry matter intake, milk yield, and milk contents of total solids, fat, total and true protein, and casein did not differ between treatments, but whey protein and non-protein nitrogen contents were significantly lower for the Zn-Met group. Milk SCC tended to decrease as a result of Zn-Met supplementation but differences between treatments were not significant when halves with persistent infection were excluded. Hand milking increased SCC in both groups, but udders of supplemented goats showed a lower reaction. Apparent absorption of N significantly increased and Zn retention tended to increase in Zn-Met supplemented goats. We conclude that Zn-Met supplementation can enhance resistance to udder stress in dairy goats. Effects were attributed to the organic Zn and not to the methionine component. Zn retention and protein utilization were also improved by the Zn-Met supplement.

Keywords: Zinc, methionine, somatic cell count, mastitis, dairy goat.

Use of organic trace minerals in the form of chelates for supplementation of ruminant diets has increased in recent years but whether organic forms are more effective than inorganic forms remains controversial. Greater bioavailability and more positive effects on milk quality and udder health have been reported for organic forms of Zn when compared with inorganic forms such as oxides and sulphates (Spears, 1989; Kellogg, 1990). In contrast, Whitaker et al. (1997) and Campbell et al. (1999) found no advantage to organic forms of Zn in dairy cattle.

Spain (1994) reported that supplementation with Zn-proteinates might enhance keratin synthesis in teat canal tissue and decrease occurrence of new intramammary infections (IMI). Zn is the only metal that is essential for at least one enzyme in all six enzyme classes in the body (Vallee & Auld, 1990). Moreover, Zn-Methionine (Zn-Met) has been reported to have an important role in resistance to stress in calves (Johnson et al. 1988) and lambs (Kegley & Spears, 1995).

Somatic cell count (SCC) in goat milk has received much attention since goat milk was officially included in the grade A Pasteurized Milk Ordinance in 1989 in the USA and a European regulation (92/46 EEC) to control SCC in goats milk was issued in 1992.

To our knowledge, there are no published evaluations of Zn-Met as an organic Zn supplement for dairy goats. Therefore, the objective of this study was to investigate effects in lactating goats of Zn-Met supplements to diets containing levels of Zn that exceed requirement on: (1) milk

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production, SCC and udder health, (2) response of SCC to induced udder stress, and (3) nutrient digestibility, and N and Zn metabolism.

Materials and Methods

Animals and their management

Twenty-two Murciano-Granadina dairy goats (four primiparous and 18 multiparous) from the herd of the Experimental Farm of the University Autonoma of Barcelona were used in a lactation trial from weeks 3–20 of lactation. Kids were separated from their dams within 8 h of birth and reared on milk substitutes. Goats were machine milked once daily (09.00) in a double-12 stall parallel milking parlour (Westfalia-Separator Ibérica, Granollers, Spain) with recording jars (2 l ± 5%) and low milk pipeline. Milking was at a vacuum pressure of 42 kPa, a pulsation rate of 90 pulses/min, and a pulsation ratio of 66%. Milking routine included machine milking with machine stripping before cluster removal.

Goats were assigned to two groups (n=11) in week 3 of lactation based on parity, and on milk yield and SCC recorded at weeks 2 and 3. The two groups were: an untreated control group and a supplemented group (1 g/d of Zn-Met). During the experiment, each group was kept in a separate pen.

The daily ration per goat was a dehydrated mixture of whole-plant maize and alfalfa *ad libitum* (supplied at 130% of the previous week's average intake), 0·2 kg barley grain, 0·3 kg alfalfa pellets, and 0·5 kg concentrate mixture pellets. Zn in the vitamin-mineral premix was as ZnO. For the supplemented group, Zn-Met was included in the concentrate mixture at a level of 0·2% (Table 1). Goats were individually fed in the milking parlour with concentrate mixture pellets twice daily in two equal portions at 09.00 (at milking) and 16.00. Ingredients and nutrient composition of the concentrates and basal diet are shown in Tables 1 and 2, respectively. Feeds and refusals were weighed and sampled weekly for dry matter (DM) determination and calculation of amount consumed.

In week 21 of lactation, all goats were injected intraperitoneally with 1 g/d methionine (Met) for 5 d to establish any effects of methionine on the response of the udder to the stress induced by hand milking on the second day. DL-Methionine (TLC grade, >99% purity, Sigma, Paris, France) was dissolved in physiological saline and injected intraperitoneally in the lumbar depression of the right side of the animal, between the last rib and the hook bone, after the morning milking. Intraperitoneal injection is reported to be a suitable route for administration of amino acids to goats (Sahlu & Fernandez, 1992).

Nutrient digestibility and N and Zn balances were determined during lactation weeks 22 and 23. Four multiparous goats from each group, with the closest weights and milk yield to the group mean, were used. Metabolism crates with stainless steel meshes and plastic-coated trays were used to collect faeces and urine separately without Zn contamination. The trial included a 7-d preliminary period for adaptation to metabolism crates, followed by a 7-d collection period. A portable milking apparatus (Westfalia Separator Ibérica, Granollers, Spain), with the same recording jar and milking conditions as described above, was used for daily milking of goats in the crates. The same ration as that used in the lactation trial was offered each day after milking. Concentrate mixture was offered to each goat in two daily portions at 09.00 and 16.00. Daily voluntary intake was measured throughout the trial and water was available throughout the day.

Table 1. Ingredients and chemical composition of the concentrate mixtures used for dairy goats during lactation

	Control	Supplemented
Ingredient as fed, g/kg		
Barley	550	548
Soybean meal	300	300
Fish meal	70	70
Salt	30	30
Dicalcium phosphate	30	30
Premix†	20	20
Biomet Zn-10%‡	—	2
Chemical composition, on DM basis		
Organic matter, g/kg	884	879
Crude protein, g/kg	245	259
Neutral detergent fibre, g/kg	200	170
Crude fibre, g/kg	61	43
Zn, mg/kg	447	684
Cu, mg/kg	7·7	8·9

† Premix Setna ovejas y cabras (Setna, Madrid, Spain) composition per kg: vitamin A, 5 000 000 IU; vitamin D_3, 1 000 000 IU; vitamin E, 5000 mg; Fe, 15 g; Mn, 35 g; I, 1 g; Co, 0·25 g; Zn, 25 g; Se, 0·1 g; Mg, 7·5 g; BHT antioxidant, 2 g.
‡ Biomet Zn-10% (Norel S.A., Madrid, Spain): $ZnSO_4$, 30%; methionine, 25%; kaolin, 45%.

Table 2. Chemical composition of the ingredients of the basal diet used for lactating dairy goats

	Barley grain	Alfalfa pellets	Dehydrated maize and alfalfa
DM, g/kg	917	908	916
Organic matter, g/kg DM	979	874	929
Crude protein, g/kg DM	150	176	121
Neutral detergent fibre, g/kg DM	219	413	488
Crude fibre, g/kg DM	46	227	233
Zn, mg/kg DM	32·0	25·0	21·0
Cu, mg/kg DM	5·16	1·26	0·85

Sample collection

Samples of feed and refusals were collected weekly during the lactation trial and stored at room temperature for

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subsequent analysis. Milk samples were taken from each udder half for analysis of composition, SCC and bacteriology in weeks 2 and 3 of lactation and then biweekly afterwards for analysis of composition and SCC, and monthly for bacteriology until week 20. For analysis of milk composition, a sample of approximately 100 ml was collected and preserved with $K_2Cr_2O_7$ (0·3 g/l) at 4 °C. For SCC, a sample of approximately 50 ml was placed in a plastic vial, preserved with an anti-microbial tablet (Bronopol, Broad Spectrum Micro-tabs II, D&F Control Systems Inc., San Ramon, USA) and kept at 4 °C until analysed. Milk samples for the bacteriological isolates were taken aseptically from each mammary gland before milking. Each teat was washed with soap and water, dried with a disposable paper towel and the teat tip was disinfected using ethanol (700 ml/l). The initial two-to-three squirts of milk stripped from the udder half were discarded and the next 50 ml collected in a sterile plastic container and examined on the same day.

Blood samples were taken from a jugular vein into glass vacuum tubes (Venoject, green, Leuven, Belgium) before morning feeding during weeks 3, 7, 11, 15 and 19 of lactation to determine Zn and Cu status. Serum was obtained by centrifugation at 1000 ***g*** for 15 min and preserved at –20 °C for analysis. Moreover, two samples of hair were taken from each animal in weeks 3 and 20 to study hair growth and its Zn content. A 5 × 5-cm area was identified in the flank region on the right side of the animal and hair samples were collected using a manual razor, weighed, put into plastic bags and stored at room temperature until they were analysed.

During the udder stress trial conducted in week 21, milk samples for SCC were collected daily and processed as described above. On the last day of methionine injection (day 5), blood samples were taken just before and 4 h after injection into heparinized glass vacuum tubes (Venoject, red, Leuven, Belgium). Plasma was obtained by centrifugation of whole blood at 1000 ***g*** for 15 min and was stored at –20 °C for amino acid analysis.

During the digestion trial, samples of feeds, refusals and faeces were collected daily and composited for analysis. One-tenth of the daily collection of fresh faeces was taken for analysis and frozen. Plastic containers containing 20 ml concentrated H_2SO_4 were used for the collection of urine, and a 10% aliquot taken and frozen for subsequent analysis.

Sample analysis

Feed and faecal samples were ground through a 1-mm stainless steel screen and then analysed for DM, crude protein (CP, Kjeldahl N × 6·25), crude fibre (CF), neutral detergent fibre (NDF) and ash according to AOAC (1990) procedures.

Unhomogenized milk samples were analysed using a near-infrared spectrometer (Technicon InfraAlyzer-450, Bran+Luebbe SL, Nordersted, Germany), by the method of Albanell et al. (1999), for content of total solids (TS), fat, CP (N × 6·38), true protein and casein (CN). Whey protein was calculated as the difference between CP and CN, and non-protein N (NPN) was calculated as the difference between CP and true protein. SCC was determined in the dairy herd improvement laboratory of Catalonia (Allic, Cabrils, Barcelona, Spain) using an automatic cell counter (Fossomatic 250, Foss-Electric, Hillerød, Denmark). For bacteriological isolations, a loopful (0·01 ml) of each sample was spread on blood agar plates, incubated at 37 °C and examined after 24 h. An infection was assumed to have occurred if five or more similar colony-forming units were present in the incubated sample of milk.

Plasma for amino acid determination was deproteinized by centrifugal filtration at 2000 ***g*** for 10 min using filter units of 10 000 NMWL Ultrafree-MC (Millipore Corporation, Bedford, Massachusetts, USA). The filtrate was assayed for amino acids by the AccQ-Tag 1996 method (Waters Corporation, Milford, Massachusetts, USA). Derivatization was by adding 70 µl AccQ-fluor borate buffer and 20 µl AccQ-fluor reagent to a 10-µl sample and heating at 55 °C for 10 min. Amino acid concentration was determined by injection of 10 µl of derivatized sample into a Waters HPLC system equipped with a 626 LC system heater, 717 plus auto sampler, 474 Scanning Fluorescence Detector and a Millennium Chromatography Manager. A Nova-Pak C18 Sentry Guard column was used at 37 °C, with a two-pump gradient system. Results were calculated by the external standard method using a 2·5-mM standard mixture of amino acids (Sigma, France).

Contents of Zn and Cu were determined by air-acetylene flame atomic absorption spectrophotometry (Perkin-Elmer 2100, Perkin-Elmer Corporation, Norwalk, Connecticut, USA) at a wavelength of 213·9 nm. Serum and urine were diluted with deionized water and aspirated directly into the flame. Feed and faecal samples were first burned in an oven at 550 °C and the resulting ash was dissolved in 6 M-HCl.

Statistical analysis

Data from half-udders were used in the statistical analysis for milk composition, SCC and bacteriology and were analysed by the General Linear Model procedure for repeated measurements of the Statistical Analysis Software Institute (SAS version 6.12). The model contained the effects of treatment, udder half, parity, prolificacy, the appropriate interaction terms and the residual error. Logarithmic transformations ($\log_{10}$) of SCC values were used in statistical analysis. When the probability of the interaction term was non significant ($P > 0{\cdot}20$), the interaction term was deleted from the model. Type III sums of squares was used to determine whether treatment effects were significant. Data from weeks 2 and 3 were used as a covariate to correct for differences in initial values when necessary. Udder health was analysed by the chi-square test. Data from the digestibility and metabolism trials were analysed by the General Linear Model procedure of SAS. The model included the

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Table 3. Effects of Zn-Met supplementation on dry matter (DM) intake, milk yield and milk composition in dairy goats

Values are means with SEM for 11 animals

	Treatment			*P* value	
	Control	Supplemented	SEM	Treatment	Treatment × week
Intake, kg DM/d					
week 3 to 8	2·29	2·12	0·05	0·042	—
week 9 to 20	2·16	2·15	0·01	0·574	—
week 3 to 20	2·21	2·14	0·02	0·050	—
Milk yield, l/d					
week 3 to 8	2·22	2·24	0·06	0·929	0·496
week 9 to 20	1·91	1·97	0·04	0·674	0·001
week 3 to 20	2·00	2·05	0·05	0·769	0·018
Milk composition, g/l					
Total solids	137·5	132·9	1·42	0·729	0·660
Fat	51·2	46·7	1·04	0·063	0·470
Crude protein	36·5	35·8	0·33	0·129	0·274
True protein	33·1	32·9	0·43	0·232	0·217
Casein	26·9	27·3	0·24	0·420	0·045
Whey protein	6·2	5·6	0·31	0·046	0·190
Non-protein N	3·4	2·9	0·12	0·053	0·369

effects of treatment and experimental error. Significance was declared at $P<0·05$ unless otherwise indicated.

Results and Discussion

Ration composition and DM intake

Although concentrate mixtures were formulated to be equal in nutrient content, subsequent analysis showed the concentrate mixture of the supplemented group to be slightly higher in CP and lower in NDF and CF than that of the control (Table 1).

As shown in Table 3, animals in the supplemented group ate 7·4% less DM ($P<0·05$) than animals in the control group during the first 5 weeks of the experiment. As lactation progressed, no significant difference in DM intake was found between groups. Dietary Zn contents for control (131 mg/kg) and supplemented (204 mg/kg) were higher than the 10 and 50 mg/kg recommended for goats by NRC (1981) and Meschy (2000), respectively, but well below the toxic level for goats, 1000 mg/kg (NRC, 1981). Feed intake was not affected by Zn-Met supplementation in dairy ewes (Hatfield et al. 1995) or goats (Puchala et al. 1999). Methionine *per se* does not seem to depress feed intake since supplementation with protected methionine did not affect DM intake in dairy cows (Overton et al. 1996; Blum et al. 1999).

Milk yield and milk composition

Milk yield was slightly greater than the values reported for the Murciano-Granadina breed by Peris et al. (1997) under similar conditions. A slight (2·5%) and non-significant difference in daily milk yield was observed in favour of the supplemented group (Table 3). Similar results have been reported in dairy cows, where milk yield increased non significantly by 1–5% for diets supplemented with Zn-Met (Kellogg, 1990; O'Donoghue et al. 1995). In our experiment, Zn supply was above the minimum requirement in the control diet and therefore little or no increment in milk yield was expected. Nevertheless, supplemented goats tended to be more efficient (+5%; $P<0·08$) in conversion of feed to milk than non-supplemented goats from weeks 3–20 (data not shown) but this was a result of the lower feed intake during the first 5 weeks of the experiment.

Concentration of milk fat tended to be lower by 8·8% ($P<0·07$) for the supplemented group. This tendency was probably due to a dilution effect because supplemented goats yielded slightly more milk than control. Supplementation with Zn-Met also reduced milk fat by 2·3% in dairy cows (O'Donoghue et al. 1995) and by 9·6% in dairy ewes (Hatfield et al. 1995). This is compatible with increased molar proportions of ruminal propionate and decreased butyrogenic ratio reported when Zn was supplemented to diets for steers (Arelovich et al. 2000).

TS, CP, true protein and CN in milk were not affected by the dietary treatment (Table 3) and were similar to those reported by Peris et al. (1997) for the same breed and conditions. Nevertheless, goats in the supplemented group had lower concentrations of whey protein and NPN in their milk, which might suggest improved protein utilization in the whole body when Zn-Met was supplemented. This suggestion is supported by the results of methionine injection during the udder stress and the digestibility trials where goats in the supplemented group had higher concentrations of plasma isoleucine and leucine (Table 5) and absorbed more N than the goats in the control group (Table 7).

The amount of methionine in the Zn-Met supplement used in this study was probably too low to affect milk

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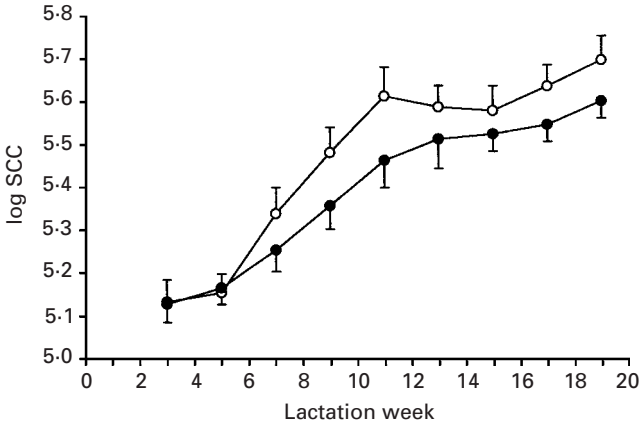

Fig. 1. Effect of Zn-Methionine supplementation on the milk somatic cell count of dairy goats throughout lactation: ○, control unsupplemented (n=11); ●, Zn-Met supplemented (n=11). Vertical bars represent SE. For details see text.

composition. Milk fat content was not affected when diets of dairy cows were supplemented with protected methionine (Overton et al. 1996; Xu et al. 1998; Blum et al. 1999; Kowalski et al. 1999). However, the effect of methionine on milk N constituents is controversial. In some experiments, supplementation with protected methionine in dairy cows did not affect the percentages of CP, CN, whey protein and NPN in milk (Overton et al. 1996; Blum et al. 1999), whereas in others, dietary inclusion of protected methionine increased milk CP content (Xu et al. 1998; Kowalski et al. 1999). Bocquier et al. (1994) showed that milk CP content can be increased in dairy ewes by the addition of 3 or 6 g/d of protected methionine at the start of the milking period.

Milk SCC and udder health

In the pre-experimental week (week 3), one udder-half from one goat from each group was found to be infected and consequently both were excluded from the SCC data. Figure 1 shows the values of log SCC during the experimental period. Overall SCC values tended to decrease by 28 and 19% (P<0·11) for the arithmetic (411×10^3 versus 570×10^3cells/ml) and geometric (269×10^3 *v.* 333×10^3 cells/ml) means in the Zn-Met supplemented and control goats, respectively. SCC decreased by 32–45% in the milk of cows supplemented with Zn-Met compared with control cows in the experiments of Kellogg (1990) and O'Donoghue et al. (1995), but had no effect on SCC, number of cases of clinical mastitis, IMI rate or recovery rate in the work of Whitaker et al. (1997).

Incidence of infection determined by the monthly bacteriological examinations is shown in Table 4. Percentage of positive samples tended to be decreased (P<0·06) by Zn-Met supplementation. Nevertheless, the number of observed IMI did not allow the detection of significant differences in infection incidence between treatments.

Table 4. Effect of Zn-Met supplementation on mammary mastitis infections in dairy goats during weeks 3 to 20

	Control	Supplemented	χ^2 (P<)
Animals†	10	10	—
Halves	20	20	—
Samples	100	100	—
Positive samples	6	1	0·062
Infected animals	3 (30)‡	1 (10)‡	0·273
Infected halves	3	1	0·301
Incidence, %	15	5	0·304

† One animal from each group was excluded at the start of the experiment because of an infection in one half-udder.
‡ Percentages.

Of the three udder-halves that showed infection in the control group, two of them persisted for more than two consecutive samplings. When halves with these persistent infections were excluded from the analysis, milk SCC in the control group were reduced to 422×10^3 and 293×10^3 cells/ml, for arithmetic and geometric means, respectively, and differences between treatment groups were not significant (P=0·29). IMI is reportedly the main cause of increased SCC (Wilson et al. 1995) suggesting that the tendency for SCC to decrease in this study when Zn-Met was supplemented, although non significant, might be due to the decrease in IMI incidence. As Zn is required for the formation of keratin, the fibrous protein that lines the teat canal, it has been hypothesized (Spain, 1994) that improving Zn status by supplementation with Zn-proteinates might enhance keratin synthesis in the teat canal tissue, thus decreasing the incidence of new infections. Moreover, antioxidants including Zn, have been implicated in promotion of efficient mammary phagocyte killing (Erskine, 1993).

Differences in milk composition between treatment groups were not affected when halves with persistent infections in the control group were excluded from the analysis.

Serum concentrations of Zn and Cu

Dietary addition of Zn-Met did not affect concentrations of Zn in serum (0·64 *v.* 0·65 mg/l, respectively, for control and supplemented; P=0·67), but Cu concentration tended to be lower (1·62 *v.* 1·53 mg/l, respectively, for control and supplemented; P<0·11). King (1990) reported that plasma concentration of Zn does not reflect true Zn status in the body because metabolic conditions unrelated to Zn status cause it to change. Goats in the present study consumed approximately 3·2 and 3·6 mg Cu/kg DM feed in control and supplemented groups, respectively. Meschy (2000) recommends a level of 8 mg Cu/kg DM, and that includes a safety margin. However, no signs of Cu deficiency in any of the goats were noticed in this experiment despite levels of Zn exceeding requirement. Serum Cu in both groups was within the normal range reported for Murciano-Granadina goats (Gómez et al. 1996) making it unlikely that goats

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supplemented with Zn-Met suffered from Cu deficiency. Week of lactation significantly affected ($P<0·01$) serum Zn concentration; concentration in week 19 was higher than in weeks 3, 7, 11 and 15 (data not shown). Serum Cu levels did not differ during the experimental period. No interactions between treatment and week of lactation for serum Zn and Cu concentrations were detected.

Growth of hair and its Zn content

Weight of greasy hair in the area of 25 cm^2 in week 3 was 0·359 and 0·362 g for the control and supplemented groups, respectively. Weights in the same area in week 20 were 0·217 and 0·268 g for the control and supplemented, respectively, but these differences were not significant. White et al. (1994) suggested that a plasma Zn concentration of 0·5 mg/l supports normal wool growth in Merino sheep, whereas the mean plasma concentration of Zn in control animals of the present study (0·64 mg/l) was above the value recommended by the authors. Moreover, mohair production did not differ between goats with plasma Zn concentrations between 0·72 and 0·97 mg/l (Puchala et al. 1999). There is insufficient information on the influence of Zn on hair growth in goats; therefore, it was assumed that according to data concerning wool growth, which is far more important, Zn may not be limiting for hair growth.

There were no significant differences in hair Zn contents between groups at week 3 (0·21 mg Zn/kg for both groups), but at week 20, hair of goats in the control group contained more Zn than that of goats in the supplemented group (0·22 *v.* 0·15 mg Zn/kg, respectively; $P<0·02$). This may be explained by the pattern of storage observed for bioavailable Zn (Henry et al. 1997). It is moderately stored in structural and keratinized tissues (bone, muscles, skin, hair, hooves and cornea) and intensively stored in many body organs (liver, kidneys and pancreas) when supplemented in relatively high levels, as in our study.

Effect of methionine injection and hand milking on milk SCC

SCC during the udder stress trial are shown in Fig. 2. Injection of methionine did not affect ($P>0·05$) SCC levels in either treatment when compared with values reported on days without methionine injection, which showed similar values to those observed at week 19 of lactation (Fig. 1). Similarly, supplementation with protected methionine did not affect SCC in milk of dairy cows (Polan et al. 1991; Blum et al. 1999).

When compared with values before hand milking (days 0, 1 and 2), SCC increased by 121% on day 3 ($P<0·01$) and by 45% on day 4 ($P<0·05$) for goats in the control group; and, by 53% on day 3 ($P<0·01$) and not significantly on day 4, for goats in the Zn-Met group. This shows that hand milking induced a high level of stress in the udders, indicating that under our conditions, goats were well adapted to machine milking and that an occasional hand

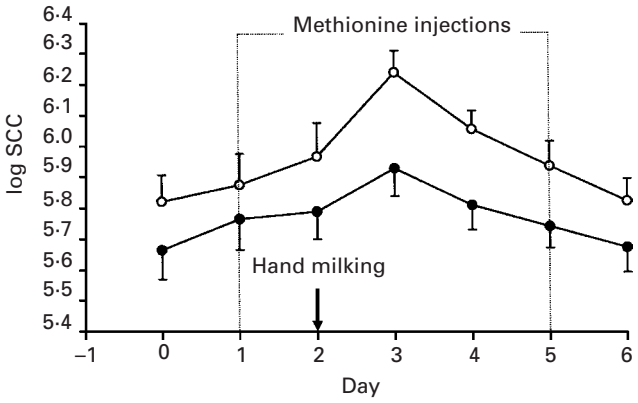

Fig. 2. Effect of Methionine injection (day 1 to day 5) and hand milking for 1 d (day 2) during week 21 of lactation on the milk somatic cell count of dairy goats: ○, control unsupplemented ($n=11$); ●, Zn-Met supplemented ($n=11$). Vertical bars represent SE. For details see text.

milking was a stressor. Murciano-Granadina goats used in our study have good machine milkability as reported by Peris et al. (1996). Zeng & Escobar (1996) observed the highest level for SCC for hand milking compared with pipeline machine and bucket methods (10·2 *v.* 8·7 and $9·3 \times 10^5$ cells/ml). Nevertheless, these results do not agree with the findings of Sheldrake et al. (1981) and Kosev et al. (1996) who reported lower SCC levels for hand milking than for machine milking, although the machine milking conditions are not reported in their studies.

However, hand milking produced a differential increment in SCC according to the dietary treatment (Fig. 2). SCC was lower for the Zn-Met group than for control goats on day 3 ($P<0·01$) and day 4 ($P<0·05$). This suggests that goats supplemented with Zn-Met suffered less traumatic damage to the udder when hand milked, in agreement with the reported beneficial effect of Zn-Met in resistance to stress. Cunningham et al. (1995) found that heifers stressed by supplementation with excess Fe had less udder oedema when supplemented with Zn (0·8 g/d as Zn-Met and $ZnSO_4$) suggesting a positive, site-specific effect of Zn. Also, when challenged with BHV-1 virus, cattle given supplemental Zn-Met at 220 mg/kg of diet had significantly lower rectal temperatures than unsupplemented controls (Reddy & Frey, 1990). Moreover, Kegley & Spears (1995) found that 3 d after transporting over 350 km, unstimulated lymphocytes from lambs supplemented with Zn-Met had a greater *in vitro* blastogenic response than those from lambs supplemented with ZnO.

Hence the favourable effect of Zn-Met on SCC during the udder stress trial were attributed to organic Zn and not to the methionine.

Effect of methionine injection on plasma amino acids

Plasma amino acid levels before methionine injection were similar between treatments (Table 5). Concentrations of

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Table 5. Effects of supplementation with Zn-Met and time after methionine injection on amino acid concentrations (μmol/l) in plasma of dairy goats

Values are means with SEM for 11 animals

	Treatment					*P* value			
	Control		Supplemented			Treatment		Time	
Amino acids	0 h	4 h	0 h	4 h	SEM	0 h	4 h	Control	Supplemented
Alanine	633	644	667	720	44·1	0·714	0·499	0·976	0·800
Arginine	66	63	74	125	2·0	0·535	0·033	0·975	0·111
Cysteine	141	230	160	261	19·0	0·499	0·552	0·016	0·114
Glutamic acid	294	332	321	414	25·8	0·663	0·090	0·941	0·292
Glycine	1121	1209	1077	1199	70·6	0·668	0·312	0·584	0·228
Histidine	555	755	610	987	49·8	0·366	0·025	0·049	0·001
Isoleucine	497	635	582	901	48·6	0·449	0·011	0·054	0·025
Leucine	238	360	265	485	25·2	0·545	0·047	0·012	0·001
Lysine	166	247	199	309	17·0	0·217	0·190	0·049	0·002
Methionine	66	142	68	186	13·6	0·887	0·008	0·006	0·001
Phenylalanine	345	736	358	880	54·6	0·858	0·327	0·006	0·002
Proline	250	327	298	447	22·1	0·289	0·026	0·028	0·003
Serine	31	42	34	50	3·4	0·746	0·367	0·350	0·340
Threonine	242	253	275	312	18·2	0·562	0·062	0·804	0·426
Valine	596	789	634	966	47·5	0·699	0·125	0·042	0·001

histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline and valine increased ($P<0·05$) in both groups 4 h after feeding and methionine injection. At this time, concentrations of methionine ($P<0·01$), arginine, histidine, isoleucine, leucine and proline ($P<0·05$) were greater in the Zn-Met supplemented goats (Table 5). Blum et al. (1999) found that, in dairy cows, concentrations of amino acids in blood remained stable from 2–10 h postprandially. Hence, allowing 4 h after feeding and methionine injection should be appropriate for collecting representative blood samples. Moreover, the increase in the branched-chain amino acids, isoleucine and leucine, indicators of a greater protein absorption from the intestine (Lobley, 1992), suggests a positive effect of Zn-Met on N absorption. This is also supported by the improvement in the apparent absorption of N in Zn-Met supplemented goats (Table 7).

Nutrient digestibilities, and Zn and N metabolism

Apparent digestibilities of DM ($P<0·05$) and CP ($P<0·01$) were greater in the Zn-Met group (Table 6). There was no difference between treatments in CF and NDF digestibilities, but OM digestibility tended to increase ($P<0·07$) when Zn-Met was supplemented. Kegley & Spears (1994) also reported that supplemental Zn tended to increase DM digestibility in lambs. Froetschel et al. (1990) also found that high intakes of Zn increased the escape of dietary amino acids in steers, suggesting an interaction of Zn with dietary CP.

Although N intake was similar for the two treatment groups (Table 7), N retention tended to be higher as a result of the significant decrease in faecal N excretion when Zn-Met was supplemented. Apparent absorption of Zn ($P<0·12$) and Zn retention ($P<0·08$) tended to be higher for the supplemented group (Table 7). Stake et al. (1973) found that Zn retention was positively correlated to N retention in calves. A similar effect was observed in our results in the control group ($R=0·55$; $P<0·05$). Moreover, Spears (1989) observed that heifers fed Zn-Met had lower plasma urea N than those receiving ZnO, suggesting a greater utilization of

Table 6. Effect of Zn-Met supplementation on daily dry matter intake, milk yield and nutrient digestibility in dairy goats in the digestibility trial (weeks 22 and 23)

Values are means with SEM for four animals

	Treatment			
	Control	Supplemented	SEM	*P* value
Intake, g DM				
Concentrates†	921	921	—	—
Forages	886	747	81	0·301
Total	1807	1668	81	0·301
Intake, g DM/kg $BW^{0·75}$				
Concentrates†	57	58	3	0·852
Forages	54	47	4	0·253
Total	110	104	5	0·391
Yield				
Milk, ml/d	1469	1443	121	0·904
Fat, g/d	66	68	5	0·832
Protein, g/d	51	50	4	0·922
Digestibility, %				
Dry matter	67·3	70·4	0·8	0·048
Organic matter	69·6	72·7	0·9	0·067
Crude protein	71·3	75·6	1·0	0·009
Crude fibre	40·9	42·5	2·3	0·652
Neutral detergent fibre	50·5	53·4	1·3	0·323

† Sum of concentrate mixture pellets, alfalfa pellets and barley grain.

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Table 7. Effect of Zn-Met supplementation on nitrogen and zinc metabolism in dairy goats during the digestibility trial on weeks 22 and 23 of lactation

Values are means with SEM for four animals

	Treatment			
	Control	Supplemented	SEM	*P* value
Nitrogen				
Intake, g/d	47·4	45·6	1·2	0·462
Faecal excretion, g/d	13·6	10·9	0·7	0·031
Urinary excretion, g/d	20·8	17·6	1·5	0·324
Apparent absorption, %	71·3	76·0	1·0	0·008
Retained, g/d	13·0	17·0	1·4	0·182
Zinc				
Intake, mg/d	237·0	340·0	19·6	0·007
Faecal excretion, mg/d	208·0	275·0	13·9	0·008
Urinary excretion, mg/d	7·4	12·5	2·0	0·223
Apparent absorption, %	12·1	19·1	2·2	0·122
Retained, mg/d	21·4	52·5	9·1	0·079
Milk excretion, mg/l	3·7	3·4	0·7	0·894

amino acids for protein synthesis in animals fed Zn-Met. In our study, improvement in N utilization associated with supplementation may have been reflected in lower whey protein and NPN contents in milk. Again, a positive effect of Zn-Met on N absorption is supported by greater plasma concentrations of isoleucine and leucine in supplemented goats (Table 5).

We cannot overlook the possibility that at least part of the improved N absorption and retention observed in animals receiving Zn-Met was due to methionine rather than Zn. However, this seems unlikely because the amount of methionine provided by Zn-Met in our experiment was small (0·25 g/d per goat or 0·01 g/d per kg of $BW^{0·75}$, for an average body weight of 45 kg). Protected methionine is usually supplemented at a level of 0·10–0·19 g/d per kg of $BW^{0·75}$ to dairy cows (Overton et al. 1996; Xu et al. 1998; Kowalski et al. 1999) and 0·14–0·28 g/d per kg of $BW^{0·75}$ to dairy ewes (Bocquier et al. 1994).

Dietary Zn stimulates production of metallothionein in some tissues (Blalock et al. 1988). Rojas et al. (1995) found that metallothionein concentration in liver, kidneys and pancreas of lambs supplemented with Zn-lysine or Zn-Met was higher than in unsupplemented lambs or in lambs supplemented with $ZnSO_4$ or ZnO. Hence the higher retention of Zn in the supplemented group might result from greater accumulation in the internal organs.

Despite the differences in Zn intake ($P<0·01$) and the tendency for greater Zn retention ($P<0·08$), Zn content in milk did not differ between treatments (Table 7). Jelínek et al. (1996) found a low but significant correlation ($r=0·30$; $P<0·01$) between Zn concentration in blood and Zn concentration in milk of dairy ewes. Since serum Zn concentrations in our study were similar in both groups, differences in milk Zn concentration were not expected.

Although it is difficult to detect differences in bioavailability between different Zn sources when the level of Zn in diets is relatively high, Zn retention was improved when Zn-Met was supplemented in the conditions of this experiment. Changes in plasma amino acids and reduction in NPN and whey protein in milk of supplemented goats also suggest a positive role of Zn-Met in body protein synthesis. In conclusion, Zn-Met supplementation seems to enhance udder resistance to stress and improve milk quality as expressed in SCC.

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