# Direct Effects of Copper and Selenium Supplementation and Its Subsequent Effects on Other Plasma Minerals, Body Weight and Hematocrit Count of Grazing Philippine Goats

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**ABSTRACT** : The administration of soluble glass bolus (SGB) with cobalt (Co), selenium (Se) and copper (Cu), among 50 upgraded does in the farm of Central Luzon State University, Philippines, was effective in improving the mineral status of the animals. After one year, blood Se and plasma Cu had been significantly increased; subsequently plasma molybdenum (Mo) level had been reduced, but other macro-minerals were not affected. Soluble glass bolus supplementation did not affect plasma calcium (Ca), magnesium (Mg), and sodium (Na), although the treated does had higher plasma phosphorus (P), potassium (K), and zinc (Zn) in some parts of the year. Hematocrit count of the animals was also improved reaching 32% level after 9 months; although it was still 6% below the lower limit of a normal range of 38 to 45%. The desired improvement in Se, Cu and Mo was not able to influence live weight among the animals. After one year, body weight was not significantly different from the control group of animals. Nevertheless, the study showed the effectiveness of SGB in improving Cu and Se levels, and subsequently reducing the plasma Mo levels of grazing goats in a typical Philippine farm. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 3 : 323-328*)

**Key Words** : SGB, Philippine Goats, Copper, Selenium, Molybdenum, Hematocrit, Liveweight

## INTRODUCTION

Philippine goats in the rural areas are traditionally raised at subsistence level with negligible concentrate supplementation. Feeds provided are mostly rain-dependent grasses, weeds and other plant species such as legumes, shrubs and trees (fresh leaves and fruit litters). Rarely, however, can these forage meet the important minerals required by grazing goats.

Dominant pasture species in some parts of Luzon Island had low mineral content, adversely affecting the blood mineral profile of grazing goats (Fujihara et al., 1992). In one experimental farm in Central Luzon, almost all the animals had serum Se below critical level, and there were also indications of low plasma Ca, Zn and Cu. Although no clinical signs of Se deficiency had been reported it is feared that Se deficiency in ruminants could be associated with specific disturbances in reproduction (Hidiroglou et al., 1987; Herdt, 1995) and increased susceptibility to infectious diseases (Gerloff, 1992; Batra and Hidiroglou, 1993). Copper deficiency on the other hand, could also impose severe limitation to grazing animals. Other minerals present in excessive amount have been found to adversely affect Cu utilization (McDowell, 1985). In particular, the high level of Mo supply that could trigger Cu thiomolybdate complexes (Grace, 1991) or the increase in sulfur level renders Cu unavailable. It is in this regard that Cu and Se supplementation is necessary. Soluble glass bolus with Co, Se and Cu, plus P, Na and Mg was used because it has immediate effect of improving the level of some minerals among animals (Buckley et al., 1986; Hidoroglou et al., 1987; Givens et al., 1988; Zervas, 1988; Hidiroglou, 1989; Matsui et al., 1992).

This study investigated the effectiveness of SGB in alleviating blood Se and plasma Cu and its subsequent effects on other mineral elements, live weight and hematocrit value of grazing Philippine goats.

## MATERIALS AND METHODS

### Experimental animals and treatment

The study used 50 upgraded Philippine goats (50% Anglo Nubian × 50 Native) from the farm of Central Luzon State University, Philippines (76 m altitude at 15 °14'N, 120.54 °E). The animals were grouped into two; with SGB placed intraruminally (treated) and without SGB (untreated). The SGB (COSECURE) was a combination of Co, Se and Cu, plus P, Na and Mg; manufactured by Pilkington Controlled Release Systems at Denbigh, North Wales, UK., and obtained from the University of Leeds. The bolus was 35 gram in weight with maximum daily release of 90 mg/day and provided 12.1 mg Cu, 0.45 mg Co, 0.14 mg Se, 22.4 mg P, 16.0 mg Na, and 0.6 mg Mg per day.

The treated animals received two boluses; the first bolus on the first month (October) of the study and the second bolus six months later (April). All the animals received no other supplements during the

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duration of the study, thus deriving their nutrient requirement only from the pastures. The live weight of the animals was recorded every month.

**Sample collection**

Blood sampling was done monthly from November, 1992 to October, 1993. About 10 ml of blood samples were drawn from the jugular vein of each animal using heparinized vacuum tubes (VT- 100H, Veno jet, Terumo Co., Tokyo, Japan). One ml of whole blood and plasma were taken and transferred individually to an acid-washed test tube. The whole blood samples were used to determine Se level, while the plasma samples were used to determine Cu and other minerals. Whole blood and plasma samples were dried slowly over a hot plate at a temperature of about 95-100℃. Hematocrit count was determined right after collection of all blood samples except from August to October, 1993 due to malfunctioning of the centrifuge.

Samples of forage species were collected during wet and dry season by hand plucking from the pasture area. These were cleaned of possible contaminants, dried and ground to pass through a Willey mill with 2 mm screen.

Forage, blood serum and plasma samples were stored properly and shipped to Japan for mineral analysis.

**Laboratory analysis**

Blood and forage samples were subjected to wet ashing with nitric and perchloric acids (3:1) before mineral analysis. All glassware and pipette tips used were washed with acid to avoid possible contamination. Selenium concentration was measured by fluorometric detection of the 2,3-diaminonaphthalene following the procedure of Watkinson (1966). The fluorescence spectrophotometer used was Hitachi 20 (Hitachi Ltd., Tokyo, Japan) with 377 nm excitation and 520 nm emission. The other mineral elements namely, Cu, Ca, P, K, Mg, Na, Mo and Zn were determined by Inductively Coupled Plasma Emission Spectrometer (ICPS 2000, Shimadzu Co., Kyoto, Japan).

**Statistical analysis**

Data were analyzed using Students t-test (Steel and Torrie, 1980). Significant differences between means were determined at $p<0.05$ and $p<0.01$ levels.

## RESULTS AND DISCUSSION

**Mineral concentration of dominant pasture species**

Animals grazed on pastures with dominant species namely; *Pennisetum purpureum* (Schumach), *Cynodon plectostachyus* (K. Schum.) Pilger, *Calopogonium muconoides* Desv., and *Leucaena leucocephala* (Lam.) de Wit. These species showed varying levels of macro-minerals during wet and dry seasons (table 1). Levels of Ca, Mg, K, and Co were generally above the critical level recommended by McDowell (1985), while P was generally deficient. Zinc levels of *P. purpureum* during dry season and *L. leucocephala* during both dry and wet seasons were deficient. Except for *L. leucocephala*, other species had Se levels critically low. All the forage species had Fe, Mn and Mo levels more than the critical level, but *P. purpureum* and *L. leucocephala* had low Cu levels (table 1). Molybdenum level was more than twice the critical level which supports the findings of McDowell (1985) that Mo toxicity exists in some parts of the Philippines.

The average Cu-Mo ratio of dominant pasture species is about 1:2, which is the reverse of the desired ratio of 2:1 (Ward, 1978). This situation could be undesirable because it could produce physiological Cu deficiency among animals (Ward, 1978), and high Mo intake depresses Cu availability (Fleming, 1973; Ward, 1978; Mills, 1987). However, grazing goats may not show ill effects due to two plausible reasons: first; goats unlike cattle and sheep are more tolerant to high Mo doses (Haenlein, 1992); and second; forage Mo level is still much lower than the 1,042 $\mu$mol/kg DM level that could produce severe symptoms (Ward, 1978). Nevertheless, SGB supplementation is deemed necessary in order to improve the mineral contents of the animals.

**Direct effects of SGB on selenium and copper concentrations in blood**

Whole blood Se concentration was initially the same for both groups of animals at 0.20 $\mu$mol/l which is below the critical level of 0.25 $\mu$mol/l. After one month of SGB administration Se level of the treated animals increased abruptly to 0.8 $\mu$mol/l; which is significantly different from the 0.28 umol/l mean Se level of the untreated animals (table 2). The positive response of the does indicates the effectiveness of SGB in improving Se status among native does. The level of Se in the blood is related to dietary Se intake, the same conclusion made by White and Somers in 1977.

The increase in blood Se of the treated goats occurred on the 4th week after bolus administration, which was shorter than the 6-8 weeks lag time observed among cows by Hidiroglou et al. (1987). The lag time corresponds to the turnover of red blood cells in which Se is incorporated into erythrocyte GSH-Px during erythropoiesis. From the 2nd month until the end of the study, Se level of the treated animals was about 0.8 $\mu$mol/l, which indicates that the release of Se from the bolus was fairly constant. This result supports the previous observation that plasma and

**Table 1.** Mineral content of dominant pasture species in the goat farm, Central Luzon State University, Munoz, Nueva Ecija, Philippines during dry and wet season, 1992-93

| Species | Ca | P | Mg | K | Co | Cu | Fe | Mn | Mo | Zn | Se |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | | | | mg/kg | | | | | | μg/kg |
| Critical Level* | 0.3 | 0.25 | 0.2 | 0.2 | 0.1 | 10 | 30 | 30-40 | >6 | 30 | 100 |
| Grasses: | | | | | | | | | | | |
| *Pennisetum purpureum* | | | | | | | | | | | |
| *Dry* | 0.29 | 0.19 | 0.13 | 0.65 | 1.70 | 6.37 | 305.00 | 31.54 | 11.38 | 20.54 | 27.87 |
| *Wet* | 0.35 | 0.20 | 0.17 | 0.80 | 2.10 | 8.34 | 404.00 | 33.00 | 14.40 | 50.40 | 36.58 |
| *Cynodon plectostachyus* | | | | | | | | | | | |
| *Dry* | 0.45 | 0.16 | 0.25 | 1.03 | 1.50 | 12.10 | 372.47 | 59.68 | 11.68 | 40.90 | 20.20 |
| *Wet* | 0.40 | 0.29 | 0.24 | 1.02 | 1.60 | 9.60 | 402.50 | 49.38 | 13.56 | 36.60 | 25.30 |
| Legumes: | | | | | | | | | | | |
| *Calopogonium muconoides* | | | | | | | | | | | |
| *Dry* | 1.14 | 0.22 | 0.36 | 0.78 | 1.90 | 11.50 | 452.00 | 82.30 | 19.00 | 53.80 | 57.60 |
| *Wet* | 1.06 | 0.26 | 0.36 | 1.06 | 2.60 | 13.10 | 400.00 | 98.20 | 24.70 | 57.00 | 68.50 |
| *Leucaena leucocephala* | | | | | | | | | | | |
| *Dry* | 2.32 | 0.13 | 0.59 | 0.52 | 2.80 | 7.30 | 341.31 | 38.00 | 29.30 | 14.90 | >100 |
| *Wet* | 2.85 | 0.14 | 0.66 | 0.56 | 2.30 | 7.10 | 365.32 | 55.70 | 28.90 | 14.40 | >100 |

* Critical level according to McDowell (1985).

**Table 2.** Monthly plasma Cu and serum Se concentration of Philippine goats treated and untreated with intrarunimal soluble glass bolus

| | Cu (μmol/l) | | Level of significance | Se (μmol/l) | | Level of significance |
|---|---|---|---|---|---|---|
| | Untreated | Treated | | Untreated | Treated | |
| 1992 | | | | | | |
| Oct | 11.85 | 12.3 | ns | 0.27 | 0.28 | ns |
| Nov | 13.2 | 12.4 | ns | 0.25 | 0.8 | ** |
| Dec | 17.7 | 20.2 | ns | 0.28 | 0.82 | ** |
| 1993 | | | | | | |
| Jan | 16.2 | 21.1 | * | 0.29 | 0.85 | ** |
| Feb | 16 | 21.3 | * | 0.29 | 0.87 | ** |
| Mar | 16.8 | 22.5 | ** | 0.31 | 0.92 | ** |
| Apr | 20.1 | 25.6 | ** | 0.3 | 0.87 | ** |
| May | 23.2 | 27.8 | ** | 0.26 | 0.83 | * |
| June | 22.1 | 25.9 | * | 0.3 | 0.86 | ** |
| July | 22.2 | 5.7 | * | 0.31 | 0.92 | * |
| Aug | 20.4 | 24.5 | * | 0.29 | 0.79 | ** |
| Sept | 21.4 | 24.7 | * | 0.28 | 0.87 | ** |
| Oct | 21.5 | 26.6 | * | 0.29 | 0.97 | ** |
| **Mean** | 19.23 | 23.11 | ** | 0.28 | 0.88 | ** |

ns=not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$.

serum Se concentration among lactating dairy cows tend to level-off after reaching peak levels in response to short term (Harrison and Conrad, 1984) or long term (Stowe et al., 1988) Se supplementation at the rate of 5-10 mg/cow/d.

The three-fold increase in serum Se concentration of the grazing does agrees with the earlier report of Zervas (1988) although the observed values in this study were relatively low. This difference in animals response towards SGB supplementation maybe due to forage types and sources, ruminal environment or genetic factors that have a profound effect on the Se level (Gerloff, 1992).

It can also be depicted in the table 2 that animals without supplementation had low Se levels which were more or less constant over one year period. This means that without supplementation there was no other source of Se among grazing goats because the Se

Table 3. Monthly plasma macro-minerals of Philippine goats treated and untreated with intraruminal soluble glass bolus

| | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | July | Aug | Sep | Oct | Mean | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1992 | | | 1993 | | | | | | | | | | | |
| Ca, mmol/l | | | | | | | | | | | | | | | |
| Untreated | 1.86 | 1.75 | 1.68 | 1.72 | 1.78 | 1.93 | 2.00 | 2.91 | 2.95 | 2.81 | 3.02 | 2.89 | 3.05 | 2.33 | 0.05 |
| Treated | 1.99 | 1.79 | 1.62 | 1.78 | 1.87 | 1.76 | 2.53 | 2.89 | 3.10 | 2.93 | 2.86 | 2.95 | 3.08 | 2.40 | 0.06 |
| Significance | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | |
| P, mmol/l | | | | | | | | | | | | | | | |
| Untreated | 2.90 | 1.69 | 2.70 | 2.71 | 2.68 | 2.84 | 2.99 | 2.41 | 2.42 | 2.12 | 3.14 | 2.43 | 2.44 | 2.58 | 0.05 |
| Treated | 2.87 | 1.68 | 2.65 | 2.47 | 2.86 | 2.71 | 2.95 | 2.73 | 2.22 | 1.88 | 2.74 | 2.80 | 2.14 | 2.52 | 0.06 |
| Significance | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | * | * | * | |
| K, mmol/l | | | | | | | | | | | | | | | |
| Untreated | 2.19 | 2.82 | 2.66 | 2.50 | 1.94 | 3.20 | 3.27 | 2.65 | 2.32 | 2.38 | 3.39 | 2.88 | 2.05 | 2.63 | 0.07 |
| Treated | 2.28 | 2.86 | 2.63 | 2.85 | 2.24 | 3.06 | 3.26 | 2.34 | 2.19 | 2.39 | 3.46 | 3.09 | 2.47 | 2.70 | 0.08 |
| Significance | ns | ns | ns | ** | ** | ns | ns | ns | ns | ns | ns | ns | ns | ns | |
| Mg, mmol/l | | | | | | | | | | | | | | | |
| Untreated | 0.84 | 0.61 | 1.01 | 1.05 | 1.03 | 1.15 | 1.16 | 0.39 | 0.30 | 0.22 | 0.47 | 0.38 | 0.51 | 0.70 | 0.02 |
| Treated | 0.83 | 0.54 | 1.01 | 1.08 | 1.12 | 1.09 | 1.30 | 0.45 | 0.30 | 0.21 | 0.38 | 0.42 | 0.48 | 0.71 | 0.01 |
| Significance | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | |
| Na, mmol/l | | | | | | | | | | | | | | | |
| Untreated | 99.35 | 79.84 | 126.00 | 122.26 | 128.71 | 140.10 | 156.09 | 202.43 | 207.62 | 187.65 | 210.25 | 202.49 | 214.59 | 159.8 | 3.50 |
| Treated | 102.97 | 76.37 | 128.06 | 126.73 | 134.32 | 134.34 | 161.01 | 190.14 | 211.21 | 200.53 | 202.42 | 200.80 | 215.52 | 160.3 | 3.22 |
| Significance | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | |
| Fe, $\mu$mol/l | | | | | | | | | | | | | | | |
| Untreated | 60.89 | 51.94 | 137.91 | 188.05 | 238.20 | 213.13 | 150.44 | 146.86 | 162.98 | 250.74 | 154.03 | 304.47 | 180.17 | 172.29 | 9.13 |
| Treated | 53.73 | 44.77 | 198.80 | 263.28 | 234.62 | 238.20 | 200.59 | 162.98 | 179.10 | 274.02 | 179.10 | 252.53 | 263.38 | 195.78 | 17.91 |
| Significance | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | |
| Mo, $\mu$mol/l | | | | | | | | | | | | | | | |
| Untreated | 6.35 | 9.38 | 8.96 | 4.78 | 3.85 | 4.79 | 6.98 | 4.06 | 8.86 | 6.25 | 5.42 | 4.06 | 6.67 | 6.15 | 0.31 |
| Treated | 6.98 | 4.79 | 4.48 | 3.75 | 2.40 | 3.13 | 4.69 | 3.85 | 5.52 | 3.75 | 2.19 | 2.60 | 2.60 | 3.90 | 0.44 |
| Significance | ns | ** | ** | ns | ns | * | ** | ns | ** | ** | * | * | ** | ** | |
| Zn, $\mu$mol/l | | | | | | | | | | | | | | | |
| Untreated | 13.62 | 13.92 | 15.15 | 18.36 | 14.08 | 11.63 | 11.47 | 9.64 | 12.24 | 9.18 | 9.49 | 14.84 | 8.41 | 12.46 | 0.46 |
| Treated | 16.83 | 14.69 | 16.83 | 16.83 | 15.30 | 14.69 | 18.36 | 16.83 | 11.17 | 10.40 | 10.71 | 12.24 | 11.17 | 14.31 | 0.61 |
| Significance | ** | ns | ns | ns | ns | * | ** | ** | ns | ns | ns | ns | ns | ns | |

ns=not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$.

content of the dominant pasture species was deficient (table 1). Long term exposure to low Se level could possibly contribute to abortion, still birth and neo-natal mortality among animals (Kott et al., 1983; Langlands et al., 1991).

Initial plasma Cu concentration was just below 15 $\mu$mol/l for both groups. No significant change was observed after one month of supplementation (table 2). Thereafter, plasma Cu level of the treated animals increased relative to untreated animals. After one year, the mean plasma level was 19.23 $\mu$mol/l for the untreated and 23.11 $\mu$mol/l for the treated animals; both are within the normal range of 9.4 23.6 $\mu$mol/l (McDowell, 1985).

**Subsequent effects of SGB**

1) Concentration of other plasma minerals

The monthly plasma mineral concentrations of the two groups of does are shown in table 3. There were no treatment differences for plasma Ca, Mg, Na, and Fe concentrations, but there were significant differences ($p<0.05$ or $p<0.01$) in some months on plasma P, K, Mo and Zn concentrations. The insignificant effect of Se supplementation on plasma Mg supports the conclusion of Batra and Hidiroglou (1993) that intraruminal administration of 30 g Se pellets did not influence plasma level of Mg in dairy cattle.

Molybdenum was affected more by supplementation than plasma P, K and Zn. Mo level in goats plasma were reduced significantly by mineral supplementation during the study period; counteracting the possible contribution of high Mo content of the available pastures.

2) Body weight and hematocrit value

Changes in monthly body weight of does treated and untreated with intraruminal SGB are shown in table 4. After one year, mean body weight was not affected by the treatment. Soluble glass bolus increased

**Table 4.** Monthly body weight and hematocrit value of Philippine goats treated and untreated with intraruminal soluble glass bolus

| | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | July | Aug | Sep | Oct | Mean | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1992 | | | 1993 | | | | | | | | | | | |
| Body wt, kg | | | | | | | | | | | | | | | |
| Untreated | 23.1 | 23.4 | 25.9 | 28.0 | 29.1 | 29.3 | 28.5 | 29.0 | 29.2 | 29.6 | 30.5 | 30.0 | 27.1 | 27.9 | 1.54 |
| Treated | 25.2 | 25.5 | 27.0 | 29.1 | 29.2 | 28.9 | 28.3 | 28.5 | 29.3 | 29.6 | 29.6 | 29.3 | 28.8 | 28.3 | 1.27 |
| Significance | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | |
| Hematocrit, % | | | | | | | | | | | | | | | |
| Untreated | 27.7 | 29.1 | 27.5 | 30.0 | 28.4 | 26.7 | 27.5 | 27.8 | 28.2 | 27.5 | md | md | md | 28.0 | 0.69 |
| Treated | 28.4 | 62.0 | 30.6 | 32.9 | 32.0 | 32.7 | 32.7 | 32.0 | 32.4 | 32.8 | md | md | md | 32.2 | 0.54 |
| Significance | ns | * | * | * | ** | ** | ** | ** | ** | ** | | | | ** | |

ns=not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; md=missing data.

the Cu and Se status of the animals but this did not affect their body weight. This conforms with the previous results of Hidiroglou et al., (1985 and 1987) that Se supplementation failed to effect gain in weight of cattle grazing on Se-deficient pastures. However, under marginal Se deficiency or sub-clinical insufficiency, Se supplementation with or without Vitamin E could improve live weight of cattle (Spears et al., 1986) and sheep (Langlands et al., 1991).

Prior to SGB administration, hematocrit values of the two groups of animals were the same at about 28%. Hematocrit count of the treated animals increased abruptly to 32.0 one month after bolus administration, and remained more or less at that level for the next eight months. Mean hematocrit count was significantly different, 28% for the untreated and 32% for the treated animals, after nine months. However, even the hematocrit value of the treated animals is still below the normal range of 38 to 45% (40% at resting stage) as suggested by Swenson (1993). One plausible reason is that the high forage Mo level reduced the synthesis of hemoglobin which eventually affected hematocrit count (Ward, 1978).

## CONCLUSION

Blood Se and plasma Cu of upgraded Philippine does significantly increased after SGB supplementation. Without supplementation, this could not been achieved because of low mineral contents of dominant pasture species. The increase in Cu intake reduced Mo level, which minimized the possible adverse effects of excessive forage Mo concentration. No other macro-minerals were subsequently affected by SGB, likewise live weight was not affected by SGB. Hematocrit count of the animals was improved by 4% after treatment; although it was still 6% below the 38% critical limit. Results of the study form part of the growing literature that shows the effectiveness of SGB in improving the mineral status of goats in the tropics.

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