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# Blood Haematology, Serum Thyroid Hormones and Glutathione Peroxidase Status in Kacang Goats Fed Inorganic Iodine and Selenium Supplemented Diets

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**ABSTRACT:** The effects of dietary supplementation of selenium (Se), iodine (I), and a combination of both on the blood haematology, serum free thyroxine (FT4) and free triiodothyronine (FT3) hormones and glutathione peroxidase enzyme (GSH-Px) activity were examined on twenty four (7 to 8 months old, 22±1.17 kg live weight) Kacang crossbred male goats. Animals were randomly assigned to four dietary treatments (6 animals in each group). Throughout 100 d of feeding trial, the animals of control group (CON) received a basal diet, while the other three groups were offered basal diet supplemented with 0.6 mg/kg diet DM Se (SS), or 0.6 mg/kg diet DM I (PI), or a combination of both Se and I, each at 0.6 mg/kg diet DM (SSPI). The haematological attributes which are haemoglobin (Hb), red blood cell (RBC), packed cell volume (PCV), mean cell volume (MCV), white blood cells (WBC), band neutrophils (B Neut), segmented neutrophils (S Neut), lymphocytes (Lymph), monocytes (Mono), eosinophils (Eosin) and basophils (Baso) were similar among the four treatment groups, while serum levels of Se and I increased significantly ($p<0.05$) in the supplemented groups. The combined dietary supplementation of Se and I (SSPI) significantly increased serum FT3 in the supplemented animals. Serum GSH-Px activity increased significantly in the animals of SS and SSPI groups. It is concluded that the dietary supplementation of inorganic Se and I at a level of 0.6 mg/kg DM increased serum Se and I concentration, FT3 hormone and GSH-Px activity of Kacang crossbred male goats. (**Key Words:** Kacang Goats, Selenium, Iodine, Thyroid Hormones, Glutathione Peroxidase)

## INTRODUCTION

All living organisms require minerals for proper function (McDowell, 2003). Minerals are naturally present in food or they may be added as dietary supplement to meet the requirement. The involvements of trace elements in metabolic and protective processes make them important in human and animal nutrition. It is important to supplement the animal's feed with trace elements up to requirement level. This is because there are organic and inorganic inhibitors for some of the trace elements present in natural dietary sources, as well as naturally low concentrations of trace elements in feed (Pallauf and Muller, 2006). Both I and Se are highly essential for the function of thyroid gland to synthesise thyroid hormones namely thyroxine ($T_4$) and triiodothyronine ($T_3$) (Underwood, 1977). These hormones regulate energy metabolism, thermoregulation, reproduction, growth and development (Herdt and Hoff, 2011). A study on relationships between thyroid gland activity and energy balance in goats reported that circulating thyroid hormones could reflect the nutritional and metabolic status of the animals (Todini et al., 2007). Although the majority of circulating thyroid hormones ($T_4$ and $T_3$) are bound to proteins and only a little quantity of them are free ($FT_4$ and $FT_3$) or unbound; only the free hormones correspond to the active hormone offered and able to get through into the cells and affect their function (Todini, 2007).

Selenium is important as an antioxidant and it promotes health and immune status of animals (NRC, 2005). It has been reported that Se is an integral part of the antioxidant enzyme GSH-Px (Rotruck et al., 1973). Findings of Yue et

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al. (2009) and Shi et al. (2011) demonstrated that serum GSH-Px activity in goats was significantly increased subsequent to Se supplementation.

In our study, the supplementation of I and Se (0.6 mg/kg DM) in the diet was twice higher than the recommended levels for goats (NRC, 1981) at which would be safe for the animals in term of toxicity. Thus, the haematological profile generated in the present study was to help in indicating if there were any clinical conditions associated with toxicity possibly arise due to the higher than the recommended level of supplementation of Se and I (NRC, 1981). Determination of total mineral concentration in serum could be an indicator for metabolism, immunity and antioxidant status in the farm animals (Herdt and Hoff, 2011).

Earlier studies have documented the effect of dietary Se supplementation on serum concentrations of GSHP-x (Yue et al., 2009; Shi et al., 2011) with very limited work been carried out to examine its effects on the $FT_3$ and $FT_4$ hormones in goats. Iodine is the main constituent of $T_3$ and $T_4$ hormones, while Se is important for the biosynthesis of GSHP-x involved in thyroid hormone metabolism and both elements have been associated with improved animal growth, immune system and meat quality (Schomburg and Köhrle, 2008).

However, data on the influence of dietary supplementation of I and the combination of I and Se on serum concentrations of GSHP-x, $FT_3$ and $FT_4$ hormones in goats are still scarce. Therefore, the present study was conducted to examine the effects of dietary supplementation of inorganic Se, I, and a combination of both on their concentrations in the serum, the activity of GSH-Px and thyroidal hormones in the serum of Kacang crossbred male goats.

## MATERIALS AND METHODS

The experiment was conducted following the guidelines of the research policy of the Universiti Putra Malaysia on animal ethics.

### Animals and diets

A total of 24, 7 to 8 months old bucks with a mean live weight of 22±1.17 kg were randomly assigned to either one of four dietary treatment groups each consisting of six animals. The animals were housed individually in wooden slatted floor pens measuring 1.20 m×0.80 m each, 0.70 meter above ground, equipped with feeding and drinking facilities. The four dietary treatments were; control (CON): basal diet without supplementation, SS: basal diet with 0.6 mg Se/kg DM, PI: basal diet with 0.6 mg I/kg DM, SSPI: basal diet with combination of 0.6 mg Se/kg DM and 0.6 mg I/kg DM. The inorganic selenium was given in the form of sodium selenite while the inorganic iodine was in the form of potassium iodide. The basal diet offered was a concentrate mix based on palm kernel cake, corn and rice bran (Table 1). The amount of concentrate offered was based on 1% of body weight with *ad libitum* amount of fresh guinea grass for 100 consecutive days.

### Blood sampling

At d 75 of the experiment, blood samples from each individual animal were collected aseptically via jugular venipuncture into 5 mL Vacutainer K3 ethylene diamine tetraacetic acid (EDTA) tubes. The tubes were rolled gently several times to ensure enough anticoagulant mixing, and the samples were processed immediately for haemogram analysis (Brockus and Andreasen, 2003). Blood samples from all animals were collected at d 95 of the experiment, for the assessment of serum Se and I concentrations, GSH-Px activity and $FT_4$ and $FT_3$ hormones. Samples were aseptically collected by jugular venipuncture using 21-gauge needles and a 10 mL Vacutainer (BD Franklin Lakes NJ, USA) serum tube and kept slanting for 1h, followed by centrifugation at 3,000 g for 10 min. The resulted serum was frozen at -20°C until subsequent analyses.

### Analytical techniques

The haematological parameters were evaluated using haematology analyser (CELL- DYN 3700 Abbott, USA). The haematocrit [packed cell volume (PCV)] value was measured using microhaematocrit method by centrifuging (Biofuge Primo R Centrifuge, Thermo, Germany) the blood samples at 17,500 g for 5 min at 4°C. For differential cell analysis, Wright's stain technique was used. In the

**Table 1.** The ingredients (% as fed) and chemical composition (%) of the basal diet

| Ingredients | |
|---|---|
| Palm kernel cake | 43 |
| Corn | 25 |
| Rice bran | 30 |
| Limestone | 1 |
| Salt | 0.5 |
| Vitamin-mineral premix | 0.5 |
| Chemical composition [1] | |
| Metabolizable energy (Mcal/kg DM)[2] | 2.35 |
| Dry matter | 90.32 |
| Crude protein | 12.90 |
| Crude lipids | 3.28 |
| Ash | 9.43 |
| Neutral detergent fibre | 55.75 |
| Acid detergent fibre | 25.92 |
| Selenium(mg/kg DM)[3] | 0.124 |
| Iodine (mg/kg DM)[4] | 0.025 |

[1] Analysed values except metabolizable energy.
[2] Metabolizable energy: calculated (Alderman, 1985).
[3,4] Analysed by inductively coupled plasma-mass spectrometry (ICP-MS).

technique, a drop of blood was placed over a slide, air-dried, stained, and then covered with microscope cover glass. Thereafter, 200 cells were manually counted and classified. For the estimation of Se (AOAC, 1984) and I (Schone et al., 2001) concentrations in the serum, 2 mL of each sample was directly diluted in 18 mL of deionized distilled water ($ddH_2O$) and measured by inductively coupled plasma-mass spectrometry (ICP-MS) using Se standard (Perkin Elmer Pure Plus Multi-element ICP-MS Calibration Std.3, USA) and I standard (Anion Standard Iodide As-19-24 SPEX Certiprep, USA). The determination of $FT_4$ and $FT_3$ were conducted at Gribbles Pathology Laboratory, Pty. Ltd. Malaysia, using labelled antibody method by ADVIA Centaur (Siemens, USA). The ADVIA Centaur system performed the detection of $FT_4$ and $FT_3$ automatically using direct chemiluminescence technology. The activity of GSHPx enzyme in the serum was quantitatively determined using EnzyChrom Glutathione Peroxidase Assay Kit EGPX-100, (BioAssay Systems, USA). The assay measured the consumption of nicotinamide adenine dinucleotide phosphate (NADPH) in the enzyme coupled reactions by recording the decrease in absorbance at 340 nm, and was expressed as U/L, whereby, one unit (U) is the amount of GSH-Px that produces 1 μM of Glutathione disulfide (GS-SG) per min at pH 7.6 at room temperature.

**Statistical analysis**

The experiment was of a completely randomized design (CRD). The experimental unit was the animal for all the variables measured during the conduct of the entire study. The data were statistically analyzed using the general linear model (GLM) procedure of Statistical Analysis System package (SAS) Version 9.2 software (SAS, 2007) and statistical significance was set at $p<0.05$. Duncan multiple range test was used to test the significance of variance between the means of the studied parameters.

## RESULTS AND DISCUSSION

**Blood haematological parameters**

The data of complete blood count (CBC) test of the animals are as presented in Table 2. The erythrocyte indices (Hb, PCV, and MCV) of the animals of all treatments were in the normal physiological reference range. Results of these parameters did not show any significant differences between the supplemented groups and control. The values of the RBC counts were apparently similar across the supplemented groups ($p>0.05$) and were within the normal reference range of healthy goats. Similarly, no significant differences were found in the differential leukocyte parameters (B Neut, S Neut, Lymph, Mono, Eosin, and Baso) between the supplemented goats and those of control. Additionally, means of WBC count for the supplemented animals was comparable with that of control, and was also within the normal reference range of healthy goats.

In the current study, animals were all healthy, and that probably is why the hematological parameters were not different between the supplemented and control groups. Increased immune function by Se supplementation may only be apparent when animals are challenged by mitogen or pathogens *in vivo* or *in vitro* (McClure, 2008).

Studies related to the effect of dietary supplementation of I and Se on goat haematology are rather limited. However, Juniper et al. (2008) and Alhidary et al. (2012) showed that dietary supplementation of Se did not affect ($p>0.05$) the haematological parameters of lambs and sheep, respectively. In line with our findings, the concentrations of Hb and PCV were also unaffected by the iodine supplementation in male goats (Rajendran et al., 2001). Contradicting with the present results on the effect of

**Table 2.** Haematological parameters of goats fed different dietary treatments

| | Dietary treatments[1] | | | | | |
|---|---|---|---|---|---|---|
| | CON | SS | PI | SSPI | SEM | Ref |
| RBC $\times10^{12}$/L* | 12.75 | 12.97 | 13.05 | 12.68 | 0.27 | 5-17 |
| Hb g/L | 100 | 101 | 106 | 96 | 3.92 | 66-146 |
| PCV L/L | 0.19 | 0.20 | 0.21 | 0.20 | 0.01 | 0.22-0.38 |
| MCV f/L | 15.16 | 15.50 | 15.67 | 15.50 | 0.33 | 16-25 |
| WBC $\times10^9$/L | 12.32 | 12.97 | 12.38 | 12.52 | 0.72 | 4-13 |
| B Neut $\times10^9$/L | 0 | 0 | 0 | 0 | 0 | 0 |
| S Nuet $\times10^9$/L | 5.96 | 6.35 | 6.15 | 6.26 | 0.49 | 1.2-7.2 |
| Lymph $\times10^9$/L | 5.74 | 5.26 | 6.15 | 5.39 | 0.48 | 2-9 |
| Mono $\times10^9$/L | 0.52 | 0.50 | 0.47 | 0.49 | 0.05 | <0.55 |
| Eosin $\times10^9$/L | 0.23 | 0.22 | 0.25 | 0.27 | 0.07 | 0.05-0.65 |
| Baso $\times10^9$/L | 0.06 | 0.07 | 0.08 | 0.07 | 0.01 | <0.2 |

[1] CON = Control, basal diet without supplementation; SS = Basal diet+0.6 mg Se/kg DM; PI = Basal diet+0.6 mg I/kg DM; SSPI = Basal diet+ (0.6 mg Se/kg DM+0.6 mg I/kg DM); SEM = Standard error of means; Ref = Reference range of healthy goats (Rasedee, 1981).

* RBC = Red blood cell; Hb = Haemoglobin; PCV = Packed cell volume; MCV = Mean cell volume; WBC = White blood cells; B Neut = Band neutrophils; S Neut = Segmented neutrophils; Lymph = Lymphocytes; Mono = Monocytes; Eosin = Eosinophils; Baso = Basophils.

dietary I supplementation on Hb concentration, Pattanaik et al. (2011) reported higher ($p<0.05$) Hb concentration in goats subjected to dietary supplementation of iodine. The authors attributed this high concentration of Hb in iodine-supplemented goats to their superior protein status, which could be resulted by not only better nitrogen utilisation, but also enhanced consumption of digestible crude protein.

**Serum selenium and iodine concentrations**

In the present study, animals subjected to the dietary supplementation of Se (SS) presented higher ($p<0.05$) serum concentration of Se than the control group (Table 3). The present results further support a previous report by Shi et al. (2011) which documented greater blood Se values in the supplemented than the control growing male goats. Likewise, the dietary supplementation of I significantly increased I concentration in the serum of supplemented animals (PI) compared with the control group (Table 3). In line with our results, Meyer et al. (2008) reported significant elevation of serum I concentrations in I supplemented bulls. In the present study, goats supplemented with both Se and I (SEPI) demonstrated positive results in terms of the concentration of Se and I in serum, and this suggests possible synergistic effects of combined Se and I. It has been reported that Se concentration in the blood could be an indicator for Se content in goat meat (Shi et al., 2011: Aghwan et al., 2012). Furthermore, Aghwan et al. (2012) concluded that increasing iodine levels in the serum resulted in increased I content in the muscles and organs of supplemented goats.

**Serum free thyroxine and free triiodothyronine**

Dietary supplementation of iodine, selenium, and combination of both had no effect on $FT_4$ levels ($p>0.05$) (Table 3). However, the $FT_3$ level of animals supplemented with combination of I and Se (SSPI) was significantly increased with respect of the animals supplemented with I (PI) (Table 3). Additionally, no significant differences in $FT_3$:$FT_4$ were observed between the supplemented animals and those of control group (Table 3).

In our study, the concentrations of $FT_4$ and $FT_3$ were not influenced by Se supplementation (SS). This could be explained by the Se level in the basal diet (0.124 ppm) which was enough for the regulation and control of thyroid gland metabolism and hormone biosynthesis. In agreement with our findings, dietary Se supplementation (0.11 Se in the basal diet) did not show any significant effects on the serum concentration of thyroid hormones ($T_3$ and $T_4$) in sheep (Chadio et al., 2006).

The absence of effects of I supplementation (PI) on the thyroid hormones in our study could be explained by the fact that the diets offered to the animals in different treatments were isonitrogenic and isocaloric. Todini, (2007) reported that the concentrations of total plasma $T_3$ significantly correlated with nitrogen and energy balance. The concentrations of $T_3$ and $FT_3$ in adult sheep were decreased in case of energy deprivation, whereas subsequent realimentation of energy elevated their concentrations. It has been reported that the dietary supplementation of I significantly increased serum $T_4$ concentration as well as $T_4$:$T_3$ in goats (Pattanaik et al., 2011). The authors documented that the higher serum $T_4$ noted in their study was correlated with higher ME intake.

In this study, the concentration of $FT_3$ but not that of $FT_4$ of the animals supplemented with both Se and I (SSPI) was significantly higher than those of I supplemented animals (PI). Arthur et al. (1990) showed that type 1 5'-deiodinase (D1) is the selenoprotein mainly established in the liver, kidney, and thyroid, and it regulates $T_4$ conversion into the biologically active form, $T_3$. Furthermore, selenium deficiency in rats fed a purified amino acid diet depressed the activity of D1 (Beckett et al., 1993). In rats, supplemental selenium was found to be able to mitigate the toxic effects of excessive iodine and increased the activities

**Table 3.** Serum iodine, selenium, thyroid hormones concentrations and glutathione peroxidase (GSH-Px) enzyme activity of goats fed different dietary treatments

| | Dietary treatments[1] | | | | |
|---|---|---|---|---|---|
| | CON | SS | PI | SSPI | SEM |
| Serum iodine (μg/L) | 56.17[b] | 61.90[b] | 172.64[a] | 158.84[a] | 5.18 |
| Serum selenium (μg/L) | 53.11[b] | 154.02[a] | 54.79[b] | 137.00[a] | 10.56 |
| Serum GSHPx (U/L)[2] | 54.35[b] | 138.63[a] | 41.40[b] | 109.76[a] | 12.48 |
| $FT_4$ (pmol/L)[3] | 12.66 | 13.40 | 13.90 | 14.30 | 0.85 |
| $FT_3$ (pmol/L)[4] | 3.63[ab] | 3.68[ab] | 3.33[b] | 4.03[a] | 0.18 |
| $FT_3$: $FT_4$ | 0.30 | 0.28 | 0.26 | 0.30 | 0.03 |

[1] CON = Control, basal diet without supplementation; SS = Basal diet+0.6 mg Se/kg DM; PI = Basal diet+0.6 mg I/kg DM; SSPI = Basal diet+ (0.6 mg Se/kg DM+0.6 mg I/kg DM).

[2] U = Amount of GSH-Px that produces 1 μM of Glutathione disulfide (GS-SG) per minute at pH 7.6 in room temperature.

[3] $FT_4$ = Free thyroxine hormone. [4] $FT_3$ = Free triiodothyronine hormone.

[ab] Means with different superscripts within the same row differ significantly at $p<0.05$.

SEM = Standard error of means.

of D1 (Xu et al., 2011). In our study, the four dietary treatments were analysed and found to be similar in their protein and energy levels. The probable elucidation for our results is that Se supplementation in the animals supplemented with both I and Se (SSPI) could have enhanced the activity of D1, which then increased the conversion from $T_4$ to $T_3$.

**Serum glutathione peroxidase enzyme activity**

In comparison with animals in the Con and PI groups, glutathione peroxidase concentration was significantly higher in the SS and SSPI groups (Table 3). Glutathione peroxidase plays vital defence function in the body by reducing lipid and hydrogen peroxides to less hazardous hydroxides through oxidation (Arteel and Sies, 2001). The measurement of GSH-Px activity in the blood can also be used as indicator for the assessment of Se status (Herdt and Hoff, 2011).

In this study, the results of serum GSH-Px activity were found to be parallel with higher concentration of serum Se noted in the animals of SS and SSPI groups. This may be due to larger proportion of Se in the blood which has been incorporated into functional GSHPx (Juniper et al., 2009). It has been reported that Se must be converted to selenocysteine (Se-Cys) before it can be incorporated to GSHP-x (Forstrom et al., 1978). Furthermore, Wang et al. (2011) reported that Se in the form of Na Selenite was metabolised efficiently into Se-Cys and rapidly incorporated into GSH-Px.

In line with our results, Yue et al. (2009) and Shi et al. (2011) reported significantly higher serum Se and GSH-Px activity in Taihang Black goats supplemented with Se compared to the non-supplemented animals. However, Chung et al. (2007) reported no differences in serum GSH-Px activity between goats supplemented with 0.25 ppm of inorganic or organic Se and the non-supplemented ones. The authors suggested that it could be attributed by a plateau possibly attained by Se even in the basal diet. The concentration of GSHP-x did not differ between the iodine supplemented (PI) and the CON groups. The level of I supplemented in this study may be not high enough to cause changes in the concentration of GSHP-x. Reports by Xu et al. (2011) documented that the activities of GSH-Px and Type 1 5'-deiodinase were significantly decreased in rats supplemented with excessive I. Additionally, Qin et al. (2011) reported that the activity of serum GSH-Px in the goats supplemented with 2 mg I/kg DM was not affected, while the activity of serum GSH-Px was decreased significantly in the animals supplemented with excessive I (4 mg/kg DM) compared to the non-supplemented group. The authors elucidated that excessive I could have resulted in the production of extreme free radicals during thyroid hormones metabolism which in turn, could increase oxidative damage in thyroid gland and consequently result in extra consumption of GSH-Px for the protection of thyroid gland.

In the present study, the dietary supplementation of both Se and I (SSPI) significantly increased serum GSH-Px activity in the animals. This could also display similar trend of possible synergistic effects of combined supplementation of Se and I at the level of 0.6 mg through increased serum GSH-Px activity in the animals from the SSPI group.

In conclusion, the present study demonstrated that the dietary supplementation of combined inorganic I and Se at the level of 0.6 mg/kg DM has resulted in higher concentration of both elements in the serum, and these were not different compared to the supplementation of I and Se alone. The concentration of $FT_3$ has markedly increased following supplementation of combined I and Se treatment. Serum GSH-Px activity increased significantly in the animals of SS and SSPI groups. These suggest possible synergistic effects of combined Se and I in improving metabolism, immunity and antioxidant status of the animals. The data generated from this study also provide a basis for future work on growth performance, carcass characteristics, and meat quality traits in relation with the supplementation of Se and I.

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