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Clinical Signs and Blood Variables of Pregnancy Toxemia Goats during Late Gestation and Postpartum

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ABSTRACT

Pregnancy toxemia is one of the common metabolic diseases in ruminant, which has caused a huge economic impact on the dairy industry. Thus, this study aimed to describe the clinical and blood changes in pregnant goats following induction of pregnancy toxemia. Twelve pregnant goats were divided into control (n=3) and treatment (n=9) groups. The control was fed a diet with adequate energy while the treatment group was exposed to 50% reduction in the energy intake to induce pregnancy toxemia. Blood samples were collected at weekly intervals for biochemical analyses, which included glucose, beta-hydroxybutyrate (BHBA), free fatty acid (FFA), calcium, sodium, potassium, chloride, cortisol, and insulin. On days 20 (PK20) and 40 (PK40) post-induction, 3 induced and 1 control goat was slaughtered while the remaining 3 induced goats were provided with a normal balanced diet to allow recovery (PKRD). The induction resulted in acute pregnancy toxemia after 20 days with clinical signs including weakness, loss of body condition, and recumbency. At 40 days, chronic pregnancy toxemia resulted in signs such as incoordination and abortion. There was significant ($p<0.05$) decrease in the glucose, insulin, calcium, and potassium levels in the induced goats while the concentrations of BHBA, FFA, and cortisol were significantly ($p<0.05$) higher. Furthermore, the blood profiling was significantly ($p<0.05$) different between the PK20, PK40, and PKRD groups and was strongly associated with the presence of clinical signs and ketone bodies in the urine. The 50% reduction in energy intake resulted in acute pregnancy toxemia after 20 days and chronic pregnancy toxemia after 40 days. In conclusion, serum biochemical profile is a potential biomarker to assess the mild and severe pregnancy toxemia in does during the late gestation and postpartum period through changes in blood profiling.

Keywords: acute; chronic; clinical; subclinical; pregnancy toxemia

INTRODUCTION

Goat rearing plays a vital role in the economics of the farming community, where they are reared for meat, milk, and hide. However, morbidity and mortality in pregnant goats have caused an economic impact on the livelihood of, especially smallholder farmers. In Asia, goat production is predominantly operated by those groups (Morais *et al.,* 2018). Pregnancy toxemia is one of the common metabolic diseases that affect meat and milk productions of goats (Bani Ismail *et al.,* 2008). It is caused by abnormal metabolism of carbohydrates and fats as a result of negative energy balance that occurs during the late stage of gestation. It is characterized by relatively high concentrations of the ketone bodies acetoacetate and beta-hydroxybutyrate (BHBA) and a low concentration of glucose in the blood (Brozos *et al.,* 2011). A study by Moallem *et al.* (2016) revealed that approximately 60% of fetal growth took place during the last stage of pregnancy, and between 33% and 36% of the glucose circulation were channeled into the fetoplacental unit to fulfill the fetal energy requirements.

Diagnosis of clinical pregnancy toxemia is based on history, clinical signs of hepatic encephalopathy, and serum biochemical profiles (De Lima *et al.,* 2012). A previous study showed that the detection of pregnancy toxemia could be determined by observing the changes of serum biochemical profiles in blood and clinical signs of pregnant goats (Azmi *et al.,* 2016). On the other hand, Duffield *et al.* (2009) concluded that subclinical pregnancy toxemia is a common metabolic disorder that affects goats in the transition period or in early lactation.

The highest incidence of subclinical pregnancy toxemia occurs within the first 2 to 3 weeks of lactation (Vasava *et al.,* 2016; McArt *et al.,* 2011). Apart from clinical pregnancy toxemia, subclinical pregnancy toxemia also leads to changes in the serum biochemical profiles (El-Dee, 2012). Moreover, BHBA value has been regarded as the most stable ketones, and the concentration accounts for approximately 85% of the total ketones in sheep with pregnancy toxemia (Bechmann *et al.,* 2012). Indeed, this study showed that there are chances of blood profiling that correlated with the presence of clinical signs in pregnancy toxemia does. Apart from that, the study resulted in current farm practice was highly correlated with the occurrence of ketosis in tropical environments, particularly in Malaysia.

Hence, this study is crucial because the changes of serum biochemical profiles observed in subclinical cases could be used to assess the early developmental stage of pregnancy toxemia, which further help in better diagnosis and treatment of this disease. Indeed, the use of these serum biochemical profiles as biomarkers in the developmental stage of pregnancy toxemia offers a promising opportunity to develop a rapid and accurate tests kit that could be used by dairy farmers or veterinarians to screen and diagnose herds for pregnancy toxemia, especially for the subclinical cases in the farm.

Thus, the objective of this study was to assess the establishment of clinical pregnancy toxemia through changes in the serum biochemical profiles in late gestation and postpartum periods following experimental induction of pregnancy toxemia.

MATERIALS AND METHODS

Animal and Ethical Considerations

The study was performed in accordance with the Code of Practice for The Care and Use of Animals for Scientific Purposes and was approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (Approval number: UPM/IACUC/AUP-R071/2016).

Experimental Design

A total of 12 pregnant Boer-cross does age between 2 and 3 years old and weighing between 30 and 48 kg was selected to be used in the experiment. All experimental goats were in their late stages of pregnancy (weeks 14 to 15 of gestation). They were randomly divided into two groups, the control (n=3) and the induced (n=9) groups. The control group was fed with a diet of sufficient energy of 602.5 kJ ME/body weight kg/day (NRC, 2007), which included roughly 70% of Napier grass (dry matter: 10.57%, crude protein: 14.62%, crude fiber: 30.39%, energy: 8.30 MJ/kg) and 30% palm kernel cake-based supplemented feed (moisture: 13%, crude protein: 14.5%, crude fiber: 20%, dry matter: 87%, ash: 10%, crude fat: 6.65%, and energy: 10.41 MJ/kg). The induced group was provided with 50% of the daily energy required to induce pregnancy toxemia according to the method of Cal-Pereyra *et al.* (2015). Drinking water was available *ad libitum*.

Following induction, a goat of the control group and 3 goats of the treated group were slaughtered on day 20 (PK20) and day 40 (PK40) while the remaining goats of control and treated groups were re-introduced normal diet (PKRD) from day 41 until they reached 2 weeks of postpartum period. All goats were observed daily for signs of pregnancy toxemia, which included inappetence, weakness, recumbency, teeth grinding, acetone breath, incoordination, and abortion. The presence of ketone bodies was detected in urine using keto strip tests on a daily basis throughout the study period. The body condition score was noted at five-day intervals according to the method of Koyuncu (2013) that was based on the presence of the fat layer and the visibility of spinous and transverse processes of the vertebrae.

Blood Collection and Processing

Blood samples were collected from all goats prior to and at five-day intervals throughout the experimental period in plain and EDTA tubes and were transported to the Physiology Laboratory, Universiti Putra Malaysia, in an insulated container with ice packs. Serum samples were subjected to serum biochemical profiles analysis, which included glucose, calcium, sodium, potassium, and chloride levels using a chemistry analyzer (Siemens Dimension Xpand Plus, USA). The plasma samples were subjected to measurement of BHBA, FFA, cortisol, and insulin using ELISA kits (CAYEE BIO, USA). For each parameter, two kits of ELISA were used. The optical density was measured using a computerized automated microplate ELISA reader (Infinite 200 series, TECAN). All measurements were made in duplicate.

Statistical Analysis

All data were analyzed using SPSS (Statistical Package for the Social Science) version 25.0 software. The data were analyzed using one-way ANOVA and presented as mean ± SEM and analyzed using the General Linear Model, Tukey's test. All data represent as a mean were considered significantly different when $p<0.05$.

RESULTS

Clinical Signs

Following induction, affected goats exhibited various clinical signs that change over time (Table 1) while no clinical sign was observed in control goats throughout the experimental period. Initially, no clinical sign was observed in the induced goats between days 1 and 3 of the experiment. However, they started to show several initial clinical signs of pregnancy toxemia between days 4 and 10 of induction. These acute signs included anorexia, dullness, weakness, and occasionally teeth grinding. With the time of induction, more subacute signs were observed between days 11 and 20,

which included acetone breath. Between days 21 and 40, chronic signs included incoordination, occasional nervous syndrome, and abortions. Re-introduction of the normal, energy-balanced diet on day 41, affected goats returned to normal body condition without clinical signs (subclinical pregnancy toxemia).

Body Condition Score

Prior to the start of the experiment, all goats were fed with sufficient energy and showed ideal body condition scores of ≥3. Following acute induction, affected goats showed scores of ≤ 2.5 by day 20, and chronic induction by day 40 resulted in scores of 2. However, the introduction of a normal diet on day 41 had improved the score back to ≥ 3 (Table 1).

Ketone Bodies Strip Test

The results of the urine test for ketone bodies are summarized in Table 1. Ketone bodies of ≥ 3+ were recorded in the urine of the induced goats (Figure 1a), while no ketone bodies were found in the urine of the control goats (Figure 1b). Furthermore, ketone bodies of ≥ 3+ were significantly ($p<0.05$) associated with the initial clinical signs observed in goats that were induced acutely for 20 days (PK20) while 4+ ketone bodies were significantly ($p<0.05$) associated with the goats that were chronically induced for 40 days (PK40) that showed severe clinical signs. Affected goats that were re-introduced to the normal diet on day 41 (PKRD) showed a ketone bodies score of 2+ with normal body score and without clinical signs.

Serum Biochemical and Hormonal Profiles

There was a significant ($p<0.05$) difference in the glucose concentration between all groups (Table 2). Glucose levels in the control group were significantly ($p<0.05$) higher (3.21±0.09 mmol/L) than the acute PK20 (2.60±0.13 mmol/L), the chronic PK40 (2.49±0.11 mmol/L). The recovered PKRD (2.98±0.09) goats were significantly ($p<0.05$) higher than the PK20 and PK40 but significantly ($p<0.05$) lower than the control. Nevertheless, the glucose level in the recovered PKRD goats was within the normal range of blood glucose of goats.

On the other hand, the BHBA concentrations in plasma of the acute PK20 (1.39±0.31 mmol/L) and the chronic PK40 (2.02±0.26 mmol/L) goats were significantly ($p<0.05$) higher than the control goats (0.44±0.02). However, no significant difference ($p>0.05$) of BHBA

Table 1. The result summary for induction of pregnant does with toxemia

Period for induction of pregnancy toxemia in does (Days)	Clinical signs	Body condition score	Ketostrip test	Group
1 to 3	No clinical sign observed	3	-	All groups
4 to 10	Anorexia, dullness, teeth grinding, and weakness	3 to 2	++/+++	PK20 and PK40
11 to 20	Anorexia, dullness, teeth grinding, weakness, acetone smell in breath, and incoordination	2	+++/++++	PK20 and PK40
21 to 40	Anorexia, dullness, teeth grinding, weakness, acetone smell in breath, incoordination, and abortion	2	++++	PK40
41 to 60	No clinical signs observed	3	+/++	PKRD

Note: All does in the control group did not show any clinical sign throughout the experiment. Clinical signs, body condition score, and ketostrip test in control group (n=3) and treatment groups (PK20= goats that were induced with toxemia for 20 days (n=3), PK40= goats that were induced with toxemia for 40 days (n=3), and PKRD= goats that were re-introduced with normal diet after 40-days-induction (n=3)); -,+ different symbols in ketostrip tests indicate the presence/absence of ketone bodies in the urine; ++ = 2+; +++ = 3+; ++++ = 4+.

(a) Positive pregnancy toxamia (b) Negative pregnancy toxamia

Figure 1. Ketostick strip result. (a) The strip showing dark purple in color indicates the presence of ≥ 3+ ketone bodies in the urine of treatment group. (b) The strip showing mild peach in color indicates the absence of ketone bodies in the urine of control group.

concentration was observed between the control (0.44±0.02 mmol/L) and the recovered (PKRD) goats (0.68±0.08 mmol/L).

The FFA in the acute PK20 (1.21±0.22 mmol/L) and chronic PK40 (1.51±0.49 mmol/L) goats were significantly ($p<0.05$) higher than the recovered PKRD (0.96±0.31 mmol/L) and control goats (0.74±0.04 mmol/L). Furthermore, the concentrations of calcium and potassium in the control goats were significantly higher ($p<0.05$) than all other groups (PK20, PK40, and PKRD). However, there was no significant ($p>0.05$) difference in sodium and chloride concentrations between all treated groups.

The hormonal blood profiles between control and induced subgroups of PK20, PK40, and PKRD are summarized in Figure 2. The concentration of cortisol was significantly ($p<0.05$) lower in control (21.44±2.18 mmol/L) than all induced goats, which were 76.14±8.03 mmol/L, 102.25±10.28 mmol/L, and 41.24±2.72 mmol/L for acute PK20, chronic PK40, and recovered PKRD, respectively. In contrast, the insulin concentration was significantly ($p<0.05$) higher in the control and PKRD goats compared to the goats with acute pregnancy toxemia of PK20 and chronic PK40 groups (Figure 3).

DISCUSSION

Pregnancy toxemia is caused by a negative energy balance due to inadequate energy intake (Barakat *et al.*, 2007). This study revealed that pregnancy toxemia could be acute or chronic. The first 20 days of reduced energy intake leads to acute pregnancy toxemia shown by the animal becoming recumbent due to insufficient energy to support the daily activities (Al-Qudah, 2011; Hefnawy *et al.*, 2011). By day 40, the chronic pregnancy toxemia resulted in signs such as incoordination, nervous syndrome, and abortions. However, the reintroduction of an energy-balanced diet to goats with chronic pregnancy toxemia goats resolved the clinical signs since the diet could supply sufficient energy intake required by pregnant to prevent the negative energy balance.

Pregnancy ketosis in goats happened when the animal failed to meet the energy demand for the fetal

Table 2. Serum biochemical profiles in treatments (PK20, PK40, and PKRD) and control groups

Variables	Control group Healthy pregnant does	Treatment groups of pregnancy toxemia does			Standard normal range
		PK20	PK40	PKRD	
Glucose (mmol/L)	3.21±0.09^{a}	2.60±0.13^{b}	2.49±0.11^{b}	2.98±0.09^{c}	2.70-4.20
Beta-hydroxybutyrate (mmol/L)	0.44±0.02^{a}	1.39±0.31^{b}	2.02±0.26^{c}	0.68±0.08^{a}	0.10-0.70
Free fatty acid (mmol/L)	0.74±0.04^{a}	1.21±0.22^{b}	1.51±0.49^{c}	0.96±0.31^{d}	0.00-0.79
Calcium (mmol/L)	2.26±0.02^{a}	2.02±0.04^{b}	2.05±0.03^{b}	2.05±0.03^{b}	2.20-3.20
Sodium (mmol/L)	152.04±3.41^{a}	148.68±0.93^{b}	147.77±1.26^{b}	151.49±3.46^{a}	142.00-155.00
Potassium (mmol/L)	6.19±0.42^{a}	4.79±0.08^{b}	5.71±0.34^{b}	5.66±1.44^{b}	3.50-6.70
Chloride (mmol/L)	112.01±3.21^{a}	114.40±2.64^{a}	113.64±4.16^{a}	127.79±3.63^{a}	99.00-100.00

Note: (Means ± SEM) in does with control group (n=3) and treatment groups (PK20= goats that were induced with toxemia for 20 days (n=3), PK40= goats that were induced with toxemia for 40 days (n=3), and PKRD= goats that were re-introduced with normal diet after 40-days-induction (n=3)); ^{a,b,c,d}Means in the same columns with different superscripts differ significantly ($p<0.05$).

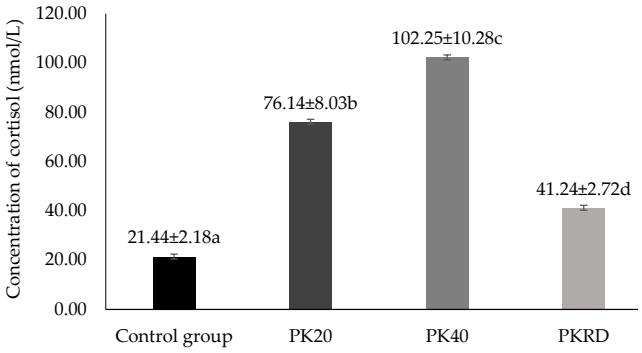

Figure 2. Concentrations of cortisol hormone in healthy pregnant and pregnancy toxemia does (PK20, PK40, and PKRD). Note: The values were expressed in means ± SEM during periparturient period in does with control group (n=3) and treatment groups (PK20= goats that were induced with toxemia for 20 days (n=3), PK40= goats that were induced with toxemia for 40 days (n=3), and PKRD= goats that were re-introduced with normal diet after 40-days-induction (n=3)); ^{a,b,c,d}Means with different superscripts differ significantly ($p<0.05$).

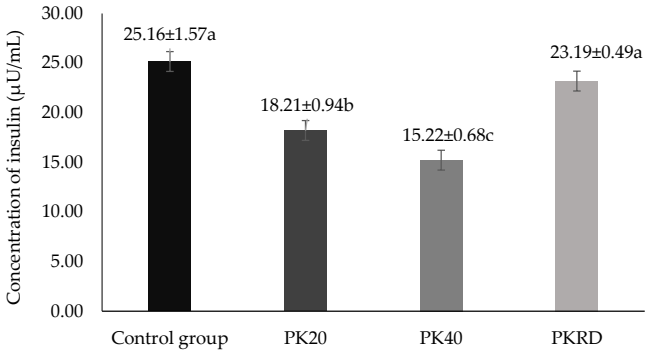

Figure 3. Concentrations of insulin hormone in healthy pregnant and pregnancy toxemia does (PK20, PK40, and PKRD). Note: The value express in means ± SEM during periparturient period in does with control group (n=3) and treatment groups (PK20= goats that were induced with toxemia for 20 days (n=3), PK40= goats that were induced with toxemia for 40 days (n=3), and PKRD= goats that were re-introduced with normal diet after 40-days-induction (n=3)); ^{a,b,c,d}Means with different superscripts differ significantly ($p<0.05$).

unit and may cause hypoglycemia as well as ketonemia (Menzies, 2011). Glucose is the main source of energy in the body, and the blood glucose level indicates the animal energy status and inhibits the production of ketone bodies (Sordillo & Raphael, 2013). During the last 4 to 6 weeks of gestation, glucose is very crucial to animals as approximately 60% of fetal growth takes place, which is about 26% to 33% of the glucose circulation is focused into the fetoplacental unit to fulfill its energetic requirements (Roberts *et al.,* 2012). Hypoglycemia is caused by a dietary deficiency of energy intake along with the increased demand for energy in the latter part of pregnancy due to twin or triplet, which resulted in ketonemia following lipid lysis (Ondieki & Renita, 2012). According to Balikci *et al.* (2009), the goats having blood glucose concentrations lower than 2.70 mmol/L are suffering hypoglycemia. This study revealed that the induced goats of PK20 and PK40 had significantly lower glucose levels than the control and standard normal range of healthy goats (2.70-4.20 mmol/L). The low glucose level leads to a negative energy balance and production of ketone bodies (BHBA). During the elevation of blood BHBA, hepatic gluconeogenesis is inhibited and further enhanced the maternal hypoglycemia (Schlumbohm *et al.,* 2007). This condition is in line with the findings by Motshakeri *et al.* (2014), in which a significant drop of glucose turnover will occur due to increment of BHBA concentration. However, it was the elevation of blood BHBA that leads to the associated clinical signs.

In this study, the concentrations of BHBA in PK20 and PK40 goats were significantly higher ($p<0.05$), thus, it could be used as a biomarker for diagnosis of pregnancy toxemia in goats as being used in ewes and cattle (Bechmann *et al.,* 2012). This is because the production of ketones is related to the levels of BHBA and FFA (Rahman, 2015). As time progress, higher levels of BHBA are accompanied by lower levels of glucose, producing more ketone bodies that lead to more severe clinical signs. Roberts *et al.* (2012) concluded that pregnant ewes with more than 3.0 mmol/L of BHBA concentration could suffer from severe pregnancy toxemia, while this study revealed that the lower level of BHBA at 2.02±0.26 mmol/L could produce severe signs of pregnancy toxemia in goats.

Similarly, following hypoglycemia, fat would be mobilized from fat stores causing high blood FFA levels (Hefnawy *et al.,* 2011) and the formation of fatty liver. Both cortisol and insulin levels are associated with the breakdown of body fats. A high level of cortisol in goats with pregnancy toxemia promotes gluconeogenesis by stimulating the breakdown of the substrate, for instance, promoting lipolysis (Tadesse, 2012). The low insulin level reduces the use of glucose in the body but accelerates lipolysis and increases hepatic ketogenesis (Brockmanr & Laarveld, 2010). On the other hand, the re-introduction of an energy-balanced diet significantly improved the blood glucose and reduced the BHBA and FFA levels, indicating a recovery.

Moreover, the level of calcium was found lower with slightly locomotion disturbance in pregnancy toxemia does; PK20, PK40, and PKRD as compared to healthy pregnant does, which was in line with the findings of Anoushepour *et al.* (2014). Decreased calcium level in pregnant does due to the high need of calcium for fetal skeleton development during the late stage of pregnancy (Hefnawy *et al.,* 2011). A study by Abd-Elghany *et al.* (2011) reported that pregnant does in the last trimester tend to get hypocalcemia and pregnancy toxemia compared to those in the early trimester.

Based on the result, the cortisol concentration has a strong positive correlation with BHBA and FFA and also was negatively correlated with the concentration of glucose and insulin. Basically, cortisol is one of the hormones that act as an indicator of pain and stress during the toxemia state in ruminant (Forslund *et al.,* 2010). In addition, gluconeogenesis is stimulated by the cortisol in the liver by stimulating the breakdown of the substrate, for instance, promoting lipolysis (Tadesse, 2012). The insulin-mediated glucose uptake by muscle, adipose tissue, and other tissue that uses glucose is inhibited, which eventually reduces the use of glucose in the body. However, the concentration of cortisol in plasma of PK40 does is significantly higher ($p<0.05$) than PK20 and PKRD does. It is possible that an early stage of toxemia may reflect the mild severity of pregnancy toxemia, while in advanced stages of the disease, a significant change might be observed due to the reduced excretion by the liver or increased adrenal input (Ford *et al.,* 2010).

The deficiency of insulin in plasma of all pregnancy toxemia could lead to accelerated lipolysis development, reduced ketone body utilization, and increased hepatic ketogenesis (Brockmanr & Laarveld, 2010). Secretion of insulin plays an important role in regulating the utilization of ketone bodies and uptake of BHBA as well as acetate. The plasma concentration of insulin decreased more distinctly in PK40 as compared to other groups. According to a study by Koyuncu (2013), the values of plasma insulin concentration are even lower in the does that showed severe clinical signs of pregnancy toxemia and low glucose value. However, the concentration of insulin was found higher in the control group as well as recovered PKRD along with the increased glucose value in blood circulation. This could be related to some evidence that during pregnancy and lactation, the responses to the effects of insulin are increased, resulting in an increment of lipolysis development and elevation of glucose consumption in insulin-sensitive tissues during late pregnancy (Schlumbohm *et al.,* 2007).

Based on the previous study by Bechmann *et al.* (2012), the concentration of electrolytes was decreased in the plasma of pregnancy toxemia ewes. It is in line with the current finding, which showed a significant decrease of sodium and potassium concentrations in PK40 and PK20 does as compared to the healthy does. It is suggested that there are disturbances in the electrolytes which may attribute to stress of starvation, dehydration, and kidney involvement of the kidney in the pathogenesis of pregnancy toxemia or also due to enhanced lipolysis that could induce hypocalcemia and hypomagnesemia (Forslund *et al.,* 2010). In addition, hypokalemia and hypocalcemia that occur during the toxemia state may be associated with anorexia and metabolic acidosis

as well as inadequate food intake and incomplete reno tubular absorption of potassium (Tadesse, 2012).

CONCLUSION

In conclusion, a 50% reduction in energy intake by pregnant goats leads to acute pregnancy toxemia after 20 days (PK20) and chronic pregnancy toxemia after 40 days (PK40), which is observed through blood profiling changes and clinical signs. In particular, the PK40 group showed significant changes in serum biochemical profiles and clinical signs compared to the PK20 group. However, re-introducing an energy-balanced diet to the goats suffering from pregnancy toxemia might allow recovery. Indeed, the changes in blood profiling could be used as a potential biomarker to detect stages of pregnancy toxemia, either mild or severe, by using serum biochemical profiles.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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