plate. Then, 50 ul of conjugate and 50 ul of antibody were added to each well and the plate was incubated for 2 hour in room temperature at 200 rpm. After that, the plate was washed 3 times by washing buffer and evaporated in room temperature. Each well was added with 200 ul of pNPP substrate and incubated for 1 hour. Finally, 50 ul of stop solution was added to each well and the absorbance of each well were read in ELISA reader at 405 nm.

Statistical Analysis
The data are presented as mean. Analysis of variances (ANOVA) was used to calculate differences between treatment group on serum, and fecal corticosterone levels at different time point, differences in water consumption, and differences in mean body weight changes. *P*<0.05 were considered significant.

RESULTS AND DISCUSSION

Serum Corticosterone.

The blood samples were taken in 3 phases. The first phase (0-18 h post-op) was to determine the post-operative corticosterone levels of Fischer 344 rats and to see the effect of pre-emptive analgesia on post-operative corticosterone. The second phase, which was taken on 8 am day 2 until 8 am day 3, was to determine the effect of post-operative treatment with analgesic on corticosterone release and compare it with Control. The last phase, which was taken on 8 am day 2 until 8 am day 3, was to determine the normal corticosterone level in each group after all treatments were stopped. Serum corticosterone levels during 18 hours after surgery are shown in Figure 5. The serum corticosterone levels when animals in Control group regained consciousness were 250 ng/ml. After that, serum corticosterone declined 0-6 h after surgery. This profile shows the negative feedback mechanism in reducing corticosterone release from adrenal cortex. When serum corticosterone increase at 10 h post-operative show normal diurnal rhythm as the animal entering its active phase, but could also indicate lack of sufficient analgesia (Goldkuhl et al 2008).

The Nutella® group showed similar profile as the Control group. The highest level was 300 ng/ml at 0 h post-operative and the lowest was 30 ng/ml at 18 h post-op. Buprenorphine 0.4 mg/kg BW group also showed similar profile as the Control group. The highest level was 250 ng/ml at 0 h post-operative and the lowest was 100 ng/ml at 18 h post-op. Serum corticosterone in the Buprenorphine 1.0 mg/kg BW group also showed similar profile as the other groups, but there were differences on 0 h post-op. The level at 0 h was 150 ng/ml and significantly lower than other groups (ANOVA, F(3,10)=12.991 ; *p*<0.05). After that, no differences could be observed until 18 h post-op. Serum corticosterone level of this group at 18 h was 10 ng/ml and was significantly lower than Nutella® group (ANOVA, F(3,10)=4.112 ; *p*<0.05).

The serum corticosterone levels during 44-92 h after surgery are shown in Figure 6. All groups showed the same diurnal rhythm with highest level in the evening and the lowest in the morning. This may indicate that the adrenal cortex performed normally after surgery. The level in Buprenorphine 1.0 mg/kg BW group is significantly lower than Control group in the beginning of dark period in day 2 (ANOVA, F(3,8)=4.947 ; *p*<0.05).

Surgical stress can cause rapid increase in serum corticosterone levels. This is due to surgical tissue damage and anesthetic agents during surgery, including isoflurane that is used in this study, which can lead to stress and elevated corticosterone levels in the post-operative phase (Whitten et al. 1998; Martini et al. 2000). Pre-emptive analgesia is associated with reduced post-operative pain and attenuated production of pro-inflammatory cytokines. These cytokines are associated with hyperalgesia during the post-operative phase (Shavit et al. 2005).

The high serum corticosterone level at the beginning of post-operative phase (0 h post-op) on Control, Nutella® and Buprenorphine 0.4 group may indicate that these groups were suffering from post-operative pain. The levels of corticosterone, when the animals regained consciousness, were very high in these groups compared to Sprague-Dawley rat (with maximal levels at approximately 200 ng/ml) (Royo et al. 2004; Abelson et al. 2005; Goldkuhl et al. 2008). This finding confirms that F344 is more responsive to stress and have high corticosterone serum level after stressful event. The serum corticosterone declined 0-6 h after surgery. This profile shows the negative feedback mechanism in reducing corticosterone release from adrenal cortex. When serum corticosterone release increased, corticosterone will perform a binding with its receptor in adrenal cortex which will stop corticosterone synthesis. The serum corticosterone increase at 10 h post-operative show normal diurnal rhythm as the animal entering its active phase, but could also indicate lack of sufficient analgesia (Goldkuhl et al 2008).
In the present study, it was shown that oral pre-emptive analgesia with Buprenorphine 1.0 mg/kg BW suppresses the rapid increase of corticosterone during 18 h after the surgery in F344 rats. But the dose that can produce this effect in F344 is higher than what has been reported for Sprague-Dawley rats (Goldkuhl et al. 2008). Interestingly the effect of oral pre-emptive analgesia with high dose of Buprenorphine was only shown immediately after the animals regained consciousness. The level of corticosterone in Buprenorphine 1.0 mg/kg group at this time point is lower than the levels found in previous study with Sprague-Dawley rats (Royo et al. 2004; Abelson et al. 2005; Goldkuhl et al. 2008). This finding confirms that F344 is more responsive to stress and have high corticosterone serum level after stressful event.

The high serum corticosterone level at the beginning of post-operative phase (0 h post op) on control, nutella and buprenorphine 0.4 group may indicate that these group was suffering from post-operative pain. The levels of corticosterone when the animal regained consciousness in this groups were very high compare to Sprague-Dawley (200 ng/ml) (Royo et al. 2004; Abelson et al. 2005; Goldkuhl et al. 2008). This finding confirms that F344 is more responsive to stress and have high corticosterone serum level after stressful event.

The serum corticosterone was declined at 0-6 h after surgery. This profile show the negative feedback mechanism in reducing corticosterone release from adrenal cortex. When serum corticosterone release increased, corticosterone will perform a binding with its receptor in adrenal cortex which will stop corticosterone synthesis. The serum corticosterone increase at 10 h post op show normal diurnal rhythm as the animal entering its active phase. But the increase in nutella and buprenorphine 0.4 was not normal because it is over than normal diurnal rhythm (100 ng/ml). This may indicate post-operative pain in this group. After that the corticosterone serum was decreased untill it reach minimum at 18 h post op indicating the animals were resting in the morning.

In the present study, it was shown that oral pre-emptive analgesia with buprenorphine 1.0 mg/kg BW suppresses the rapid increase of
corticosterone during 18 h after the surgery in F344 rats, inbred strain known for its sensitivity to stressors. But the dose that can produce this effect in F344 is higher than what has been reported for Sprague Dawley (Goldkuhl et al. 2008). Interestingly the effect of oral pre-emptive analgesia with high dose of buprenorphine was only shown immediately after the animals regained consciousness. The level of corticosterone in buprenorphine 1.0 mg/kg group at this time point is lower than the levels found in previous study with Sprague Dawley rats, which is more than 200 ng/ml (Royo et al. 2004; Abelson et al. 2005; Goldkuhl et al. 2008). The corticosterone level in nutella group at 18 h after surgery was higher than buprenorphine 1.0 group suggesting that animal in nutella group was suffering from post-operative pain and animal in buprenorphine 1.0 was protected from post op pain.

Rats in all group display similar diurnal rhythmicity, with high levels during the onset of dark period and low level of corticosterone in light period as described in previous study (Atkinson et al. 2006). This return to normal level after induced stress, in this case surgery, is important since flattening in post-operative corticosterone level may indicate neuroendocrine dysfunction due to post-traumatic stress disorder (Cohen 2005). The low level of serum corticosterone in buprenorphine 1.0 mg/kg group on the beginning of dark period in day 2 show the effect of buprenorphine in suppressing corticosterone release. The level of corticosterone in this group was not significant on the beginning of dark period in day 3. This corticosterone profile shows the effect of buprenorphine in suppressing corticosterone release immediately after its administration. The levels at the onset of dark period and light period is almost similar with previous studies using AccuSampler® in Sprague Dawley rats (Royo et al. 2004; Goldkuhl et al. 2008; Siswanto et al. 2008) suggesting that there are no differences in diurnal rhythm levels between outbred Sprague Dawley and inbred F344.

Fecal Corticosterone Metabolites
The fecal corticosterone metabolites (CM) levels are shown in Figure 7. In Control group, fecal CM declined with about 50% on day 1 compared to day 0. Fecal CM on day 0 was 80 ng/kg BW*h and 40 ng/kg BW h on day 1. After that, fecal CM increased until day 2 to 60 ng/kg BW h. Nutella® and Buprenorphine 0.4 mg/kg BW groups showed the same profile as the Control. Fecal CM levels at day 0, 1, and 2 in the Nutella® group were 120 ng/kg BW h, 50 ng/kg BW h, and 120 ng/kg BW h. Meanwhile, fecal CM at day 0, 1, and 2 on Buprenorphine 0.4 group were 120 ng/kg BW h, 20 ng/kg BW h, and 70 ng/kg BW h. Buprenorphine 1.0 group showed different fecal CM profile. On day 1, fecal corticosteroid was increased about 2 times compared to day 0. After that fecal CM on day 2 declined to the same level as day 0. Fecal CM levels at day 0, 1, and 2 in this group were 60 ng/kg BW h, 110 ng/kg BW h, and 50 ng/kg BW h. Fecal CM in this group at day 1 was significantly higher than other group (ANOVA, F(3,10)=11.686 ; p<0.05).

Corticosteroid metabolites excretion can show the changes in serum corticosterone. But changes in fecal CM will be delayed for 8-12 h (Royo et al. 2004). Fecal CM on day 1 was increased due to post-operative pain and stress as shown in serum corticosterone on 0-18 h after surgery. After that the level returned to normal (day 0).

This effect was not shown in Control, Nutella, and Buprenorphine 0.4 mg/kg groups. It could be due to that the animal in these three groups defecated less as a consequence of reduced food intake during 18 hour post-surgery, due to insufficient analgesic effect. This may cause retention of corticosteroid metabolites excreted through out the faeces. Another explanation is that these animals may suffer from paralytic ileuses. Anesthetics agents like isoflurane can cause post-operative ileus (Livingston & Passaro 1990) and reduced food intake after surgery can worsen the post-operative ileus. However, post-operative ileus in these rats was not studied since no analyses of gastric motility could be performed during present study.

Figure 7 Fecal corticosterone in each group.
Post-operative Body Weight Changes and Water Consumption

Body weight changes were compared during the blood sampling period and shown in Figure 8. The mean body weight of the rats that were used in this study is 246 gram. In the Control group, body weight was reduced with 10 gram (4%) on day 1 after surgery. On day 2 and 3, the reduction in body weight was 20 gram (8%) compared to pre-operative values (day 0). On day 4, there was slight increase in body weight, and the body weight loss was 15 gram (6%) on day 4 compared to day 0. In the Nutella® group, body weight was reduced up to 16 gram (6.5%) compared to day 0. After that, there were no additional changes in body weight. In the Buprenorphine 0.4 mg/kg BW group, body weight was reduced on day 1 (12 gram or 4.8% compared to day 0) and day 2 (20 gram or 8%). On day 3, the body weight was increased and the mean body weight change was 15 gram (6%) compared to day 0. But on day 4, the body weight loss was further decreased to 18 gram (7.3%) compared to day 0. The lowest body weight reduction was shown in Buprenorphine 1.0 mg/kg group, in which no significant changes could be observed, compared to day 0. When the treatment was stopped on day 3, the body weight declined and at day 4 the body weight loss was 6 gram (2.4%).

Water consumption before and after surgery is shown in Figure 9. In the Control group, water consumption day 0 to 1 was reduced to 10 gram. After that, the water consumption showed an increase day 1 to 2 and day 2 to 3, and day 3 to 4 the water consumption was almost back to before surgery. The Nutella® and Buprenorphine 0.4 mg/kg BW groups showed the same profile as Control. But, in the Buprenorphine 0.4 mg/kg BW group, water consumption day 3 to 4 was decreased to almost the same level as day 0 to 1. On Buprenorphine 1.0 mg/kg BW group, water consumption was increase day 0 to 1. Water consumption day 0 to 1 was 28 gram. After that, water consumption declined back to normal day 1 to 2, day 2 to 3, and day 3 to 4. Water consumption day 0 to 1 and day 1 to 2 in Control and Buprenorphine 0.4 mg/kg BW groups were significantly lower than before surgery (t-test, p<0.05).

Reduced body weight and water consumption as shown in the Control, Nutella, and Buprenorphine 0.4 mg/kg BW group is very common in rodents after surgery and may be an indication of post-operative stress and lack of sufficient analgesia (Goldkuhl 2008). It is known that high level of CRF due to stress can suppress the appetite of animal since this peptide is a potent anorexic agent (Sapolsky et al. 2000). The high levels of plasma corticosterone may contribute to reduced body weight and water consumption since this effect was suppressed in Buprenorphine 1.0 mg/kg group, which had significantly low levels of corticosterone at this time point compared to the other three groups. Another explanation is that F344 is very responsive to an environmental stressor (single housing) in the present study. It has been reported before that F344 response very poorly in novelty environment (Baumann et al. 2000) and this strain show no habituation or adaptation of the corticosterone stress levels during repeated stress (Dhabhar et al. 1993) and this could also explain the loss of body weight during the first day when the animal arrived in the lab (Figure 10). This is not the case with Sprague-Dawley (Goldkuhl et al. 2008).

High levels of corticosterone during stress can also suppress immune function. It has been reported before that rapid increase and high level of corticosterone serum in F344 during stressful event or even surgery can protect this animal from autoimmune diseases since corticosterone will suppress the immune system. However, this robust increase of corticosterone makes F344 more vulnerable to diseases like cancer or infection by bacteria or virus since immune system is suppressed due to high level of corticosterone (Dhabhar 1993). This could be the case in Control, Nutella®, and Buprenorphine 0.4 mg/kg groups. The animal in these groups might have suffered from subclinical bacterial infections in the earlier post-operative phase which could impair the animal’s ability to maintain the body weight. Buprenorphine 1.0 mg/kg may suppress this effect since this drug suppresses...
the corticosterone in the early post-operative phase and therefore did not suppress the immune system during this critical phase.

Figure 9 Post-operative mean water intake in each group.

Figure 10 Body weight during adaptation before surgery (7 days)

**CONCLUSION**

In the present study it was shown that high dose of orally administered Buprenorphine can reduce the effect of surgical stress on body weight changes and water consumption and suppress rapid increase of corticosterone during 18 hour after surgery. Rat strain F344 need higher dose of Buprenorphine to suppress post-operative corticosterone release and have higher post-operative corticosterone level compare to Sprague-Dawley strain. For future studies, the animals may benefit from housing with another male partner after surgery and during the blood sampling period to minimize environmental stressor that may occur during study. However it should be taken into consideration that the animal should be recovered from anesthesia before introducing the partner.

**REFERENCES**


