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# The Effect of Feeding Improvement of Local PO Cattle and It's Crossbred To Physiological Parameters andThe Expression of Extracellular Hsp70

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***Abstract:*** *Twenty calves and steers of Crossbred Ongole (CO or PO as local name) and it's crossing breed (Limousine x PO= Limpo) breed in low land (5m above sea level; environment temperature and Relative Humidity of 35ºC and 48%) of Nguling district of Pasuruan Regency, East Java Province were treated by giving commercial concentrate as much as 0.75 and 1.5 kg kg for calves and steer, respectively. There was no difference of rectal temperatures between the two's. Before the treatment, the respiration rate of Limpo's was higher than PO but the difference was not exist anymore after the treatment. The same also occured for Heat Tolerance Coefficient.Therewas no effect of breed to plasma glucose, NEFA and Hsp70, before and after the treatment. The average plasma glucose, NEFA and Hsp70 concentration was 68.9-101.2 mg/dl, 15.3-43.8 pg/ml and 2.7-3.5 pg/ml, respectively.The conclusion is that PO cattlle in low land small farm and low feeding level had better heat tolerance than Limpo. Concentrate treatment could eliminate the effect of breed to the heat tolerance of the animals.*

***Keywords:*** *extracellular Hsp70, physiological parameters, ongole crossbred*

## I. Introduction

Peranakan Ongole (PO or Ongole Cross) is one of local beef cattle of Indonesia. It is a Bos Indicus catlle, originated from the Ongole cattle, firstly imported from India in 1912and initially aimed at providing draught animal power.In Java,their crosses with the Javanese cattlegenerally named as sapi PO(Peranakan Ongole) or Ongole Cross cattle.This cattle is well adapted with hot tropical environment, low quality roughage and resistant to the thick.The cattle is distributed almost in all part of area in Indonesia (Martojo, 2012).

Many cattle breeds originated from temperate climate have been introduced into Indonesia, such Limousin and theircrosses are favored by farmers because of the bigger body size. This crosses cattle has a problem with adaptability in hot environment. The local cattle as Peranakan Ongole (PO) has better adaptability than crossing animals (Limpo/ Limousine x PO) in low land area(Nugroho, 2012).

Mostly the cattle raised traditionally by small farmers with under fed condition, especially during the dry season. Hot environment together with limited energy feed consumption make animals far from its potential productivity. In extreme hot environment, there are dry matter consumption reduction, longer time of digesta retention, small increased in digestibility, blood flow reduction and nutrient absorbed reduction (Beede and Collier, 1986). One of the strategies use for reducing the effect hot environment to the nutrition of the animals is by increasing the energy content of the feed or simply by giving commercial concentrate(Beede and Collier, 1986).

The cellular heat shock response is another component of adaptation to heat stress. Heat stress activates heat shock transcription factor-1 (HSF1) leading to increased expression of heat shock proteins (HSP) coupled with decreased expression and synthesis of other proteins, and HSP induced activation of immune system (Behl et al., 2010).

The aim of this study is to assess the physiological parameters and the expression of extracellular Hsp70 of Crossbred Ongole (CO or PO) and it's crossing breed (Limousine x PO= Limpo) at low land (5m above sea level) of East Java Province of Indonesia. Also to know if by giving commercial concentrate to the animals, the physiological and cellular heat shock responsewill be better.

## II. Materials and Methods

Ten calves and ten steers of PO and Limpo were used in the study. They were owned by the small farmers in the village of Dandang gendis, Nguling district of Pasuruan Regency, East Java Province. The animals were divided by two groups, weaned (4-7 months) and steer (8-12 months). The study was done during the dry period. Themain feed of the animals was untreated rice straw.During the study the animals stayed in farmers' own animal housing without any management intervention except for giving commercial concentrate as much as 0,75 kg for calves and 1,5 kg for steers for 21 days. The average ambient temperature and relative humidity before and after the treatment were 34.2-35ºC and 47.2-48%; 36.0-38.0ºC and 42.7-48.3%, respectively. The average initial body weights of weaned and steer were 89.4 – 133.6 kg and 142.8-162.2 kg, respectively.

The data was taken before and after treatment period, i.e. at 11.30 am-13.00 pm or at the highest day temperature. The physiological parameters data obtained were rectal temperature and respiration rate. The Heat Tolerance Coefficient (HTC) for individual was calculated as HTC= Tb/38.3 + Fr/32 (Montsma, 1985), where is Tb: the average daily rectal temperature (°C), Fr: the average daily respiration rate for 1 minute, 38.3: the standar cattle rectal temperature (°C), and 23: the standar cattle respiration rate for 1 minute.Laboratory analysis was done in Faal Laboratory of Medical Faculty of Brawijaya University, Malang City, East Java to know plasma glucose, plasma NEFA and eHsp70 concentration using commercial kit test. Data obtained were tested by anova of nested design to know if there were significant difference (P<0.05). To know the effect of the treatment, the t test was used.

## III. Results and Discussion

Analysis of variance results showed that there is no effect of breed (P<0.05) to the average rectal temperature. There was a significant (P<0.05)effect of age (in breed) to animal rectal temperturer before and after treatment. The difference of animal rectal temperature in this study was caused by age (in breed). The calves had lower rectal temperature than steer, except for Limpo before treatment. As seen in Table 1, the average rectal temperature of PO calves, PO steer, Limpo calves and Limpo steer before treatment was 38.4±0.21; 38.6±0.22; 39.9±0.80 dan 38.6±0.15ºC, respectively. After treatment, the value was 38.7±0.21; 39.2±0.59; 38.8±0.22 dan 39.4±0.59 ºC, respectively. The results of t-Test analysis showed that concentrate treatment gave significant effect (P<0.05) to the rectal temperature of Limpo steer. Concentrate treatment increased the rectal temperature of Limpo steer from 38.6±0.15to 39.4±0.59°C. The addition of concentrate together with higher environmental temperature made the animals had higher heat load that permit the animals to increase their rectal temperature. The average rectal temperature in this study is still in the range of normal temperature. Nugroho (2012) in the same site of study got 38.2–38.4ºC for PO and its crossing calves (105 days of age) and 38.2–38.7ºC for steer(365 hari days of age). According to variance analysis results, it was showed that there was breed effect (P<0,05) to respiration rate of the animals before the treatment but not the age (in breed). It means that initially before treatment, the crossing cattle (Limpo) has higher respiration rate than PO, i.e 26.4±1.52 vs. 25.7±4.19 times/minuteof calves and 27.7±2.63 vs. 25.8±2.32 times/minuteof steer, but later after the treatment there was only difference caused by age (in breed) (P< 0.01). Steer had higher respiration rate than calves i.e 29.5±1,05 vs. 28.7±0.96 times/minute

Table 1. The Average Rectal Temperature (°C) and Respiration Rate (times/minute) Before and After The Concentrate Treatment.

| | Rectal Temperature (°C) | | Respiration Rate (times/minute) | |
|---|---|---|---|---|
| Animals | Before | After | Before | After |
| PO Calves | 38.4 ± 0.21 $^{a}$ | 38.7 ± 0.21 $^{a}$ | 25.7 ± 4.19$^{a}$ | 28.7 ± 0.96$^{a}$ |
| Limpo Calves | 39.9 ± 0.80 $^{a}$ | 38.8 ± 0.22 $^{a}$ | 26.4 ± 1.52$^{bc}$ | 28.4 ± 1.95$^{ad}$ |
| PO Steer | 38.6 ± 0.22 $^{b}$ | 39.2 ± 0.59 $^{b}$ | 25.8 ± 2.32$^{ac}$ | 29.5 ± 1.05$^{bd}$ |
| Limpo Steer | 38.6 ± 0.15 $^{bc}$ | 39.4 ± 0.59 $^{bd}$ | 27.7 ± 2.63$^{b}$ | 30.9 ± 1.07$^{b}$ |

$^{a,b}$different superscriptin the same column is significant difference (P<0.05)
$^{c,d}$different superscriptin the same row is significant difference (P<0.05)

of PO and 30.86±1.07 vs. 29.5±1.05 times/minuteof Limpo. The results of t-Test analysis showed that concentrate treatment gave significant effect (P<0.05) to the respiration rate of Limpo calves and PO steer.

Concentrate treatment increased the respiration rate of Limpo calves from 26.4±1.52to 28.4±1.95 times/minute and of PO steer from 25.8±2.32 to 29.5±1.05 times/minute. However, as seen in Table 1, the respiration rate of local cattle PO steer in this study still lower than of crossing breed Limpo steer (29.5±1.05 vs.

30.9±1.07 times/minute) . The average respiration rate of the cattle in this study is in the range of normal respiration rate. Based on the result it was showed that PO cattle had lower respiration rate than Limpo. PO is an adapted animal to high ambient temperature. Limousine is cattle breed originated from temperate climate which are not sustain with hot environment so its crossing animal will have some of this trait. After the treatment there was a tendency that the animals had higher respiration rate. The increasing energy consumed by concentrate feeding together with higher ambient temperature during treatment period resulted in higher heat load to the animals body. The animals increasing respiration rate to dissipate the body heat through increasing respiration evaporation. The respiration rate of the animals in this study was lower than Nugroho (2012) for PO and Limpo weaning calves (105 days) of 36.8±3.90 and 41.8±4.67 times/minute and of yearling (365 days) of 41.8±3.02 times/minute.

The animals considered to have a good heat resistence if the value of HTC equal to two. The higher the value the lower the resistence (Montsma, 1985). Variance analysis results showed that there was an effect of breed to the average value of HTC before the treatment. The average HTC of Limpo was higher (P<0,01) than PO (2.2±0.06 vs. 2.1±0.18 of weaning calves and 2.2±0.13 vs. 2.1±0.18 of steer). After the treatment, there was no breed effect anymore but the age (in breed). The steer had higher HTC than the weaning calves (2.3±0.04 vs.

2.2±0.04 of PO and 2.4±0.04 vs. 2.3±0.07 of Limpo).The results of t-Test analysis showed that concentrate treatment gave significant effect (P<0.05) to the HTC of Limpo calves and PO steer. Concentrate treatment increased the HTC of Limpo calves from 2.2±0.06to 2.3±0.07 and of PO steer from 2.1±0.10 to 2.3±0.04. However, as seen in Table 2, the HTC of local cattle PO steer in this study still lower than of crossing breed Limpo steer (2.3±0.04 vs. 2.4±0.04). As stated before that after the treatment the animals added heat load from the concentrate consumed and higher ambient temperature during the treatment period. The animals did efforts to reduce heat load by increasing bodyheat loss including evaporative heat loss or increasing respiration rate. Nugroho (2012) in the same site of study obtained the HTC values of 2.6±0.15 and 2.8±0.13 for PO and Limpo of 105 days of age, respectively; and2.6±0.20 dan 2.8±0.23 for yearling animals. This difference caused by the higher respiration rate between the two studies (e.g. 36,8-41,79 vs. 25,8-28,8 times/minute of weaning calves). The average rectal temperature of the two studies more or less were same.

Table 2. The Average HTC of PO and Crossbreed Before and After The Concentrate Treatment

| Animals | Before Treatment | After Treatment |
|---|---|---|
| PO Calves | $2.1 \pm 0.18^{a}$ | $2.2 \pm 0.04^{a}$ |
| Limpo Calves | $2.2 \pm 0.06^{bc}$ | $2.3 \pm 0.07^{ad}$ |
| PO Steer | $2.1 \pm 0.10^{ac}$ | $2.3 \pm 0.04^{bd}$ |
| Limpo Steer | $2.2 \pm 0.13^{b}$ | $2.4 \pm 0.04^{b}$ |

[a,b]different superscriptin the same column is very significant difference (P<0.01)
[c,d]different superscriptin the same row is significant difference (P<0.05)

Variance analysis results on physiological paramater showed that before the treatment the average respiration rate and HTC significantly affected by the breed. After the treatment the effect was not exist anymore. It means that the effort of feeding improvement by giving commercial concentrate to the animals could reduce heat tolerance difference on crossing animals, and expected the production or body weight gain of the animals will increase.

Based on the behaviour observation of heat stressed animals (USDA, 2007) it was decided that the animals in this study were in level 1 of 6 levels, i.e respiration rate increase, spend more time standing (to increase heat loss by increasing the amount of skin exposed to air flow or wind) but not drooling. It means also that the animals were not changed the behaviour drastically.

Unlike monogastric, ruminant absorb very little glucosefrom the feed consumed, andthe peak gluconeogenesis rate is readily after feeding (Collier et al., 2008). According to Harper (1977) blood glucoselevelinruminantrange between 70-120 mg/dl. Variance analysis result of this study showed that there was no difference (P>0.05) of plasma glucose concentration caused by breed or age (in breed). The average concentration of plasma glucose was 68.9–101.2 mg/dl. The results of t-Test analysis showed that concentrate treatment gave significant effect (P<0.05) to plasma glucose concentration of Limpo calves. As seen in Table 3, concentrate treatment increased plasma glucose concentration of Limpo calves from 77.8±12.26to 96.6±10.90 mg/dl.However, this plasma glucose concentration still in the range of normal level. Rumiyani (2005) obtained66.11 mg/dl of blood glucose of PO cattle of 1 year of age feeding with kernel palm oil cake, rice bran and elephant grass. Rinayati (2005) had 71.1±11.65 mg/dl of blood glucose of PO cattle of 1.5-2 years of age feeding withremained extract of tahu (soya tofu) and cassava.

The concentration of Non Esterified Fatty Acids (NEFA) is an indicator of lipid mobilisation. During fasting the plasma NEFA is increasing because the fatty acids are released from adipose tissues as metabolism fuel. Adewuyi (2005) in his review stated that the increased level of serum NEFA level is an indicator of Negative Energy Balance (NEB) in post partum cow. According to variance analysis results there was no effect of breed but the age (in breed) (P<0.05) to the average concentration of plasma NEFA. Numerically, the average concentration of plasma NEFA in Limpo was almost twice than PO's. Based on the result, it could be stated that Limpo has higher lipid metabolism than PO. High concentration of plasma NEFA indicate that lipid catabolism activity originated from lipid body reserve is also high. Heat stress reduce dry matter consumption (Beede and Collier, 1986). Heat stress also increase energy maintenance requirement needed to cope with the stress. The requirement was overcome by increasing fat body reserve catabolism resulted the increase of plasma NEFA concentration. The average concentration of plasma NEFA of PO steer was higher (P<0.05) than calves (before treatment: 22.5±9.43 vs. 18.4±5.22 pg/ml and after treatment: 18.0±8.04 vs. 15.3±3.84pg/ml). But in

Limpo, the average concentration of plasma NEFA of calves was higher than steer (before treatment:41.2±4.25 vs. 37.7±6.83pg/ml and after treatment: 43.8±3.39 vs. 41.3±3.17pg/ml). The results of t-Test analysis

Table 3. The Average Concentration of Plasma Glucose (mg/dl) and Plasma NEFA (pg/ml) Before and After The Concentrate Treatment.

| Animals | Plasma Glucose (mg/dl) | | Plasma NEFA (pg/ml) | |
|---|---|---|---|---|
| | Before | After | Before | After |
| PO Calves | 92.0 ± 14.9 | 83.0 ± 14.07 | 18.4 ± 5.22 [a] | 15.3 ± 3.84 [a] |
| Limpo Calves | 77.8 ± 12.26[c] | 96.6 ± 10.90[d] | 41.2 ± 4.25 [a] | 43.8 ± 3.39 [a] |
| PO Steer | 68.9 ± 34.47 | 101.2 ± 23.02 | 22.5 ± 9.43 [bc] | 18.0 ± 8.04 [bd] |
| Limpo Steer | 74.5 ± 11.33 | 79.5 ± 15.76 | 37.7 ± 6.83 [b] | 41.3 ± 3.17 [b] |

[a,b]different superscriptin the samecolumnis significant difference (P<0.05)

[c,d]different superscriptin the samerowis significant difference (P<0.05)

showedthat concentrate treatment gave significant effect (P<0.05) to plasma NEFA concentration of PO steer. As seen in Table 3, concentrate treatment decreased plasma NEFA concentration of PO steer from 22.5±9.43to 18.0±8.04pg/ml. This result indicate that local PO cattle had better adaptability than crossing animals.

The expression of extracelluler Hsp70 was detected during the observation ranged between 2.7-3.5 pg/ml. This range was same as reported by Kristensen et al. (2004) for Friesian-Holstein cow (0.24–26.47 ng/ml Hsp72) but higher than of Kristensenand

Table 4. The AverageConcentration of Plasma Hsp70 (pg/ml)

| Animals | Before Treatment | AfterTreatment |
|---|---|---|
| PO Calves | 3.1 ± 0.42 | 3.5 ± 0.31 |
| Limpo Calves | 2.9 ± 0.14[c] | 3.3 ± 0.38[d] |
| PO Steer | 2.7 ± 0.29[c] | 3.3 ± 0.37[d] |
| Limpo Steer | 3.0 ± 0.13[c] | 3.5 ± 0.34[d] |

[c,d]different superscriptin the samerowis significant difference (P < 0.05)

Løvendahl (2006) forheat stresed Jersey's calves age of 76.7±4.5 days i.e. 0-1.3 ng/ml of Hsp72.Statistically, there is no effect of breed or animal's age (in breed) to the concentration of Hsp70 extracellular in this experiment. The results of t-Test analysis showed that concentrate treatment gave significant effect (P<0.05) to plasma Hsp70 concentration of Limpo calves, PO steer and Limpo steer. As seen in Table 4, concentrate treatment decreased plasma NEFA concentration of Limpo calves, PO steer and Limpo steer from 22.5±9.43to 18.0±8.04,2.7±0.29 to3.3±0.37 and 3.0±0.13 to 3.5±0.34pg/ml, respectively. It means also that concentrate treatment increased the expression of Hsp70 extracellular in this study.It is still in doubt if this increased purely caused by the concentrate treatment or not. Haque et al. (2012) stated that Hsp70productionstarted atenvironment temperature of 2 or 3ºC higher than animal body temperatureor 40ºC.

There was significant increase (p<0.05) of all physiological parameter (rectal temperature, respiration rate, pulse dan skin temperature of some body parts) on young and mature buffaloes aftersubjected to 40, 42 and 45°C for 4 hourscompare to 38°C. The average consentration of Hsp70 plasma significantly higher (P<0.05) in 40, 42 dan 45ºC thanof 38ºC of young and adult buffaloes.It should be that the environment temperature of present experiment site (34-38ºC) was not higher enough to increasethe expression of extracellular Hsp70. Many factors affect the environment temperature i.e. the wind, the roof and the shading trees. Most animals in the experiment were housing in roofed barn.

Based on regression analysis between Heat Tolerance Coefficient (HTC) and plasma metabolites and Hsp70 concentration it was konwn that the only significant effect (P<0.05) was in relationship between HTC and plasma Hsp70 concentration of PO and Limpo steer.

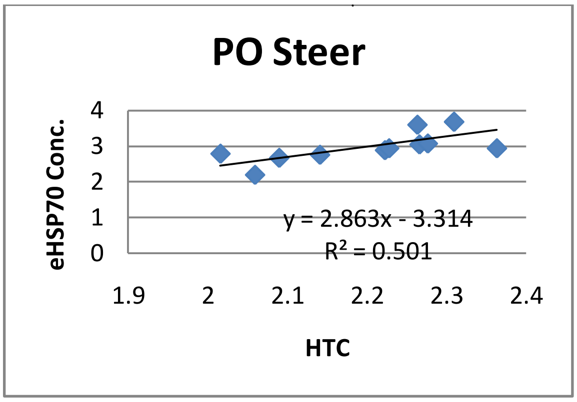


**Figure 1**. Relationship between eHsp70 concentration and HTC on PO steer.

On the PO steer,the relatioship was stated as Y=2.863 X–3.314 with $R^2$=0.501. If in PO steer there is an increasing value of HTC from 2.0 to 2.5 there is high possibility an increasing concentration of eHsp70 as much as 1.43 ng/ml. Figure1show the relatioship between plasmaHsp70 concentration (Y) and HTC (X) on PO steer. On the Limpo steerthe relatioship was stated as Y=2.000 X- 1.437 with $R^2$ = 0.380.Figure 2 show the relatioship between plasmaHsp70 concentration (Y) and HTC (X) on Limposteer.

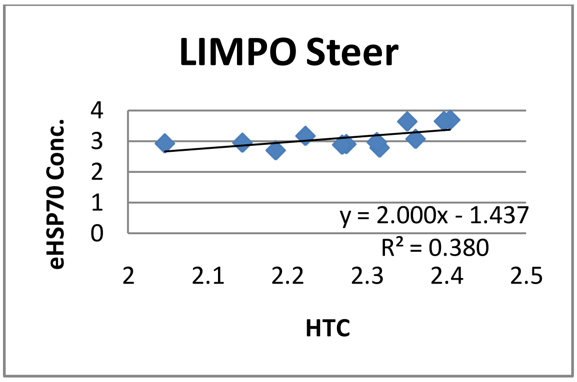


**Figure 2.**The relatioship between eHsp70 concentration dengan HTCon Limpo steer.

Based on these relationship, it also means that in PO and Limpo steer there is a possibility to use eHsp70 expression as an indicator of heat stress.

## IV. Conclusion

The conclusion of this study is that PO in low land small farm yard and low feeding level had better heat tolerance than Limpo. Concentrate treatment could eliminate the effect of breed to the heat tolerance of the animals. There was a positive correlation between HTC and eHsp70 expression. There was no effect of the treatment to plasma metabolites concentration except for NEFA.

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