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Calpain Activity of Jawarandu Does under Four Different Energy Level in the Ration

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Abstract. The aim of the research is to investigate the effect of four different energy level in the ration into the calpain activity of Jawarandu does. The research was done during 5 months in the Experimental Farm of the Faculty of Animal Science, Universitas Jenderal Soedirman. The research material used was 16 heads of the Jawarandu doe with the aged 2.5–3 years. All the animals were randomly assigned to the ration treatment which forms four the different energy levels (82.26% TDN, 85, 87.93, dan 90.74% TDN). The replication of each treatment was four times. Variable measured was a calpain activity on the muscle of Longissimus dorsi. General linear model (GLM) of the SPSS was used to analysis variable measured. Energy content 1.63McalME/heads/day and 1.92McalME/heads/day as well as 1.73McalME/heads/day and 2.06McalME/heads/day were increased of the μ -calpain and m-calpain activities at the Longissimus dorsi muscle, respectively. However, there was decreased of the calpastatin activity at the Longissimus dorsi muscle. Different energy content of the ration increased the μ -calpain and m-calpain activities at the Longissimus dorsi muscle and of those decreased calpastatin activity.

Keywords: calpain activity, calpastatin activity, energy level, jawarandu does, *Longissimus dorsi*

1. Introduction

On Idhul Adha Holiday or Idhul Qurban Holiday, goat sate stall vendors have difficulty to get the goat as the main raw material for making satay. The scarcity of male goats lasts long, even if there are unreachable prices for the satay traders. One of the best solutions for the traders of satay stalls (in order to keep the business running) is by slaughtering the does.

Tenderness is one of the factors that influence consumers in receiving meat. High nutritious foods can increase the tenderness of lamb meat through increased intermuscular fats and decreased collagen in muscle [1]. Sufficient energy in the diet can increase the use of nitrogen food for the synthesis of body proteins. Synthesis of protein in the body encourages growth in the body of an animal in the form of weight gain. Furthermore, it is argued that the higher the live weight causing the weight of non-carcase components, carcasses, the pieces of carcass components, and the extent of the eyes of the muscle or the width of the eye muscle increases [2][3].



The lowest level of tenderness was found in *Pectoralis profundus* muscle combination, whereas *Longissimus dorsi* muscle was the most tender muscle [4]. *Longissimus dorsi* muscle has the highest level of tenderness [5]. Meat tenderness is influenced by the location of the meat in the animal body. Carcass muscle in the upper half of the body of the cattle or along the back is softer (padded) than the lower half of the body. The tenderness can vary between species, breed, animal thin n the same species, carcasses cuts, and between muscles, as well as on the same muscle [6].

Socheh et.al. [7] reported that meat tenderness in muscles *Longissimus dorsi* was respectively higher than that in muscles *Pectoralis profundus* and *Biceps femoris*. Evidence indicated that muscles *Longissimus dorsi* was tender than *Biceps femoris* and *Pectoralis profundus* muscles. This was because *Longissimus dorsi* muscles do not much move compared with the *Biceps femoris* and *Pectoralis profundus* muscles [7]. It is in accordance that the lowest tenderness level was found in the combination of *Pectoralis profundus* and *Biceps femoris*, whereas *Longissimus dorsi* muscle is the tenderest muscle [4].

Feeding (basal feed and basal feed plus concentrates) significantly affected meat tenderness of Kejobong doe. The types of muscles had a highly significant effect on tenderness. Feeds affected m-calpain activity, m-calpain activity, and calpastatin activity in the muscles. Types of muscles exhibited variation in the activity of m-calpain, the activity of m-calpain, and calpastatin activity. The addition of concentrates in the basal feed increased meat tenderness and affected the activity of m-calpain, m-calpain, and calpastatin [7].

Calpain system controls the replacement of myofibrillar protein, the largest protein store in the body. During nutritional restriction, the protein supply is insufficient to disrupt protein synthesis and accelerate the replacement of the myofibrillar protein [8]. Calpain activity in muscle is the cause of many changes observed in the structure of proteins and this, related to tenderness [9]. Suggested that calpain activity in *post mortem* muscle tissue can cause muscle structure of protein muscle cell becomes weak. This causes the quality of meat becomes more tender [10]. Stated that calpain-calpastatin system has an important role in postmortem meat tenderness [11]. Feed affects the activity of μ -calpain, m-calpain, and calpastatin in muscle. The type of muscle indicates diversity in the μ -calpain, m-calpain, and calpastatin activities [7].

The objective of the study was to examine whether there was an influence of different energy levels in the ration on calpain activity (μ -calpain, m-calpain, and calpastatin) in *Longissimus dorsi* of Jawarandu does.

2. Methodology

This study used 16 heads of Jawarandu does age 2.5-3 years with an average initial weight of 18.03 kg. All does are placed in individual cages with size 70x70 cm.

The feed given to doe consists of forages and concentrates on the composition of feed ingredients presented in (Table 1). The ratio of forages and concentrates given to livestock is 60:40. In the morning the livestock is given a concentrate of 3% of body weight while foraging in the afternoon and afternoon.

In this study using several kinds of drugs to prevent the occurrence of disease. The drugs are as follows: Wormectin (mange drug), Terramycin (worm medicine), and Terracortril (eye medicine). The use of such drugs is given either orally or by intermuscular injection.

Table 1. Chemical composition of feed ingredients¹

Feed ingredients	DM (%)	From dry materials (%)				
		CP	RF	FF	BETN	TDN
Field grass	24.40	2.80	11.40	0.30	7.00	28.60
Rice bran	86.50	10.80	11.50	5.10	50.10	82.90
Cassava meal	88.70	1.20	11.00	0.20	74.10	72.90
Pollard	88.4	17	8.8	5.1	45	82.28

¹Result of proximate analysis of the Laboratory of animal feed, Faculty of Animal Science, Universitas Jenderal Soedirman.

Livestock maintenance

Goats are given the opportunity to adapt to treatment for 3 weeks. The length of the animal rearing period was two months (60 days). After completion of maintenance period, all livestock were cut and then made carcass. From the carcass then sampled the meat as much as 10 g of longissimus dorsi muscle. The μ -calpain activity m-calpain and calpastatin was determined on the muscle in non-freezing conditions, and pre-rigor [5] modified by [12].

The procedure for determining calpain activity

μ -Calpain activity, m-calpain, and calpastatin activity determined in the muscles in this condition are not freezing and pre-rigor according to the procedure of [5], [12], and [7]. A total of 20 g of *Longissimus dorsi*, homogenized in 2,50 volumes of extraction buffer (50mM Tris, 10mM EDTA, and 10mM b-mercaptoethanol [MCE], pH 8.30) and taken with an ice box to the laboratory to perform measurements of calpain and calpastatin activity. Two proteinase inhibitors (2 mM, phenylmethylsulfonyl fluoride; 5 Mm, trans-epoxy-succinyl-L-leucylamide-(4-guanidino)-butane) were added immediately to the extraction buffer, prior to homogenization, in order to convince proteolysis would not occur during transportation. Samples of meat then centrifuged, filtered, and dialyzed overnight (40mM Tris, 5mM EDTA, and 10mM MCE, pH 7.50). After dialysis, samples were centrifuged, filtered, and loaded on a gravimetric anion exchange column (DEA-Sephanel; Sigma Chemical, St. Louis, MO). The column was washed with elution buffer (50mM Tris, 1mM EDTA, dan 10mM MCE, pH 7.50) and eluted using a 200mM NaCl gradient in the elution buffer. Calpain and calpastatin activity was determined using casein solution ((100mM Tris, 1mM NaN₃, 5mM CaCl₂, 5mg/mL casein, and 1mL/mL MCE, pH 7.50). One unit of calpain activity is defined as the amount of enzyme required to increase 1.0 optical density unit at 278 nm for 1 h at 250⁰ C. One unit defined as calpastatin activity was the amount of inhibitor required to inhibit one unit of DEAE-purified m-calpain activity.

Research design

Randomized Block Design was used in this study. Jawarandu does are grouped into four groups of distinct initial body weight each 15.4kg; 17.1; 18.3; and 21.3kg. All animals randomly assigned into the treatment four different energy levels in the feed (**Table 2**).

Table 2. Composition and nutrient content of the study feed

Feed ingredients	Treatments			
	E1	E2	E3	E4
	-----(% as fed)-----			
Field grass	60	60	60	60
Concentrate	40	40	40	40
Rice meal	1.82	5	1.74	1.82
Cassava meal	25.45	21.67	24.35	25.45
Pollard	10.91	11.67	12.18	10.91
Ultra mineral ¹	1.82	1.66	1.73	1.82
Nutrient content (%)				
CP ²	7.1	8.17	7.93	7.61
TDN ³	82.26	85	87.93	90.74

¹Each pack (1 kg) of Ultra Mineral production Eka Farma Semarang Central Java Indonesia contains Calcium carbonate 50.00%; Phosphor 25.00%; Manganese 0.35%; 0.20% iodine; Potassium 0.10%; Cuprum 0.15%; Sodium chlorine 23.05%; Iron 0.80%; Zincum 0.20%; Magnesium 0.15%. ²Crude protein. ³total digestible nutrient

Repetition treatment was four times. The measured variables were μ -calpain, m-calpain, and calpastatin activity in *Longissimus dorsi* of Jawarandu does. The general linear model (GLM) of SPSS is used to analyze the measured research variables.

3. Result and Discussion

Activity of calpain and calpastatin

Calpain activity and calpastatin activity were studied by sampling the meat samples of *Longissimus dorsi* muscle of Java Randu does as much as by 10g. The μ -calpain, m-calpain, and calpastatin activity were determined from the non-frozen and pre-rigorous *Longissimus dorsi* muscles by following the procedure of [5] modified by [12], [3]. Energy consumption and calpain activity of *Longissimus dorsi* muscle in Jawarandu does fed with different energy content are presented in **Table 3**.

Table 3. Energy consumption and calpain activity on *Longissimus dorsi* muscle

Variables	Treatments			
	E1	E2	E3	E4
Energy consumption				
TDN ¹ , kg/ek/hari	0.45	0.48	0.53	0.57
Calpain activity				
μ -calpain	8.13 \pm 0.07 ^a	8.22 \pm 0.08 ^a	9.03 \pm 0.23 ^b	9.00 \pm 0.30 ^b
m-calpain	6.00 \pm 0.20 ^a	5.99 \pm 0.21 ^a	7.04 \pm 0.16 ^b	7.05 \pm 0.16 ^b
calpastatin	13.32 \pm 0.59 ^a	13.29 \pm 0.57 ^a	11.05 \pm 0.18 ^b	11.03 \pm 0.20 ^b

¹Total digestible nutrient. ^{a-a}, ^{b-b}superscript of the same letter on the same line indicates no difference. ^{a-b}superscript of unequal letters on the same line indicates there is a significant difference (P <0.01). 1kg TDN=3.62MkalME, 0.45kgTDN=1.63MkalME, 0.48kgTDN=1.73MkalME, 0.53kgTDN=1.92MkalME, 0.57kgTDN=2.06MkalME.

The results of the analysis of variance showed that feeding with different energy content (1.63McalME, 1.73McalME, 1.92McalME, and 2.06McalME) had a highly significant effect (P <0.01) on μ -calpain activity *Longissimus dorsi* muscle Jawarandu does. Feeding with an energy content of 1.63McalME and 1.73McalME per head per day respectively no difference in μ -calpain activity of *Longissimus dorsi* muscle. Likewise, feeding with an energy content of 1.92McalME and 2.06McalME per head per day was no difference in μ -calpain activity of *Longissimus dorsi* muscle (**Table 3**).

In feeding with an energy content of 1.63McalME per head per day and 1.92McalME per head per day there was an increase in μ -calpain activity of *Longissimus dorsi* muscle as much 0.9U. Meanwhile, on feeding with an energy of 1.73McalME per head per day and 2.06McalME per head per day there was an increase in the μ -calpain activity of the *Longissimus dorsi* muscle by 0.78U (**Table 3**, **Figure 1**). Obtained references that energizing in the diet can increase the activity of μ -calpain *Longissimus dorsi* muscle. Feeding a high-energy diet will increase energy intake and protein. High-nutrient feed increases the μ -calpain activity that is useful for degrading the protein of muscle cells (myofibrils) in the muscle tissues resulting in weak muscle cell protein structure which eventually meat becomes more tender. The tenderness of the meat is closely related to the calpain system, the higher the μ -calpain activity the meat the more tender. Calpain is a proteolytic enzyme contained in two forms, namely μ -calpain and m-calpain. Calpain is useful for degrading the proteins of muscle cells (myofibrils) in muscle tissue [13]. According to [14], calpain activity in postmortem muscle tissue can cause muscle cell protein structure to become weak. This results in the quality of the meat become more tender.

m-Calpain activity

The results of the analysis of variance showed that feeding with different energy content (1.63McalME, 1.73McalME, 1.92McalME, and 2.06McalME) had a highly significant effect (P <0.01) on m-calpain activity. The results of the analysis of variance showed that feeding with different energy content (1.63McalME, 1.73McalME, 1.92McalME, and 2.06McalME) had a highly significant effect (P <0.01) on m-calpain activity *Longissimus dorsi* muscle Jawarandu does. Feeding with energy content 1.63McalME and 1.73McalME per head per day respectively no difference in m-calpain activity of *Longissimus dorsi* muscle. Likewise, feeding with energy content 1.92McalME

and 2.06McalME per head per day respectively no difference in m-calpain activity of *Longissimus dorsi* muscle (Table 3, Figure 1).

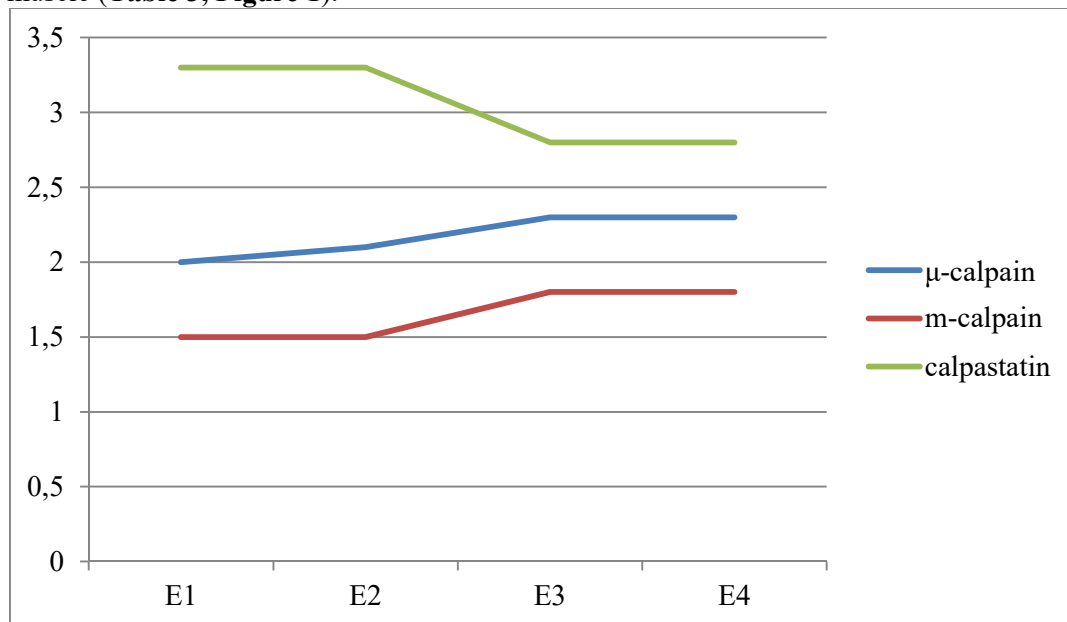


Figure 1. Calpain activity on Jawarandu does that fed different energy content

However, feeding with an energy content of 1.63McalME per head per day and 1.92McalME per head per day has an increase m-calpain activity in the muscular of *Longissimus dorsi* as much as 1.04U. On the feeding of 1.73McalME per day and 2.06McalME per head per day there was an increase m-calpain activity in the *Longissimus dorsi* muscle as much 1.06U (Table 3). Increased energy in the diet can increase the activity of m-calpain muscle *Longissimus dorsi* muscle. Meat tenderness is closely related to the calpain system, the higher the calpain activity (m-calpain), the meat the more tender [7]. Hereinafter, [7] reported that meat tenderness in muscles *Longissimus dorsi* was respectively higher than that in muscles *Pectoralis profundus* and *Biceps femoris*. Evidence indicated that muscles *Longissimus dorsi* was tender than *Biceps femoris* and *Pectoralis profundus* muscles. This was because *Longissimus dorsi* muscles does not much move compared with the *Biceps femoris* and *Pectoralis profundus* muscles. Furthermore, [3] and [12] emphasize, there is strong evidence that suggests calpain was involved in the tenderness after slaughtering.

According to [15], the lowest level of tenderness is found in the combination *Pectoralis profundus* muscle, while the *Longissimus dorsi* muscle is the most tender muscle. *Longissimus dorsi* muscle has the highest level of tenderness [5]. The tenderness of the meat is affected by the location of the meat in the livestock body part. Carcass muscle in the upper half of the body of the animal or along the back is more soft (padded) than the lower half of the body. The tenderness can vary between species, breeds, livestock in the same species, carcasses cut, and between muscles, as well as on the same muscle [6].

Calpastatin activity

The results of the analysis of variance showed that feeding with different energy content (1.63McalME, 1.73McalME, 1.92McalME, and 2.06McalME) had a highly significant effect ($P < 0.01$) on calpastatin activity *Longissimus dorsi* muscle of Jawarandu does. Feeding with energy content 1.63McalME and 1.73McalME per head per day respectively no difference in calpastatin activity *Longissimus dorsi* muscle. Likewise, feeding with energy content 1.92McalME and 2.06McalME per head per day respectively no difference in calpastatin activity *Longissimus dorsi* muscle.

However, feeding with an energy content of 1.63McalME per head per day and 1.92McalME per head per day were significantly decreased the calpastatin activity of *Longissimus dorsi* muscle by 2.14U (**Table 3, Figure 1**). On the feeding of 1.73McalME per feed per day and 2.06McalME per head per day respectively there was significantly decreased calpastatin activity of *Longissimus dorsi* muscle of 2.26U (**Table 3, Figure 1**). This means an increase in energy in the diet can decrease the calpastatin activity of *Longissimus dorsi* muscle. *Longissimus dorsi* muscle has the highest level of tenderness [5]. This means that *Longissimus dorsi* muscle has calpain activity (μ and m-calpain) which is higher than calpastatin activity. In contrast, high calpastatin activity causes a decrease in calpain activity, and therefore, tenderness of meat decreases [16].

4. Conclusion

Feeding with different energy levels can increase the μ -calpain activity and m-calpain activity of *Longissimus dorsi* muscle. Feeding with different energy levels can decrease the calpastatin activity of *Longissimus dorsi* muscle.

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