

Tenderness and the Calpain System of Three Different Types of Muscles of Kejobong Does under Two Different Energy Levels

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Abstract. The purpose of research was to investigate whether there was influence of the addition of concentrates in basal feed on meat tenderness and calpastatin and calpain activities of three muscles of Kejobong does. A total of 20 heads of Kejobong does (2.0-2.5 years) with initial live weight range between 23.8-24.2 kg was studied and randomly set in two kinds of feed treatment, ie basal feed (p_0) and basal feed plus concentrates (p_1). Two kinds of feed were given to Kejobong doe for 12 weeks. The observed variables were (1) tenderness of meat, (2) calpain activity (μ -calpain activity and the activity of m-calpain), and (3) calpastatin activity. Data were processed using the General Linear Model Univariate procedure and Pearson Bivariate Correlation of the SPSS program. Results showed that feeding (basal feed and basal feed plus concentrates) significantly affected meat tenderness of Kejobong doe. The types of muscles had highly significant effect on tenderness. Feeds affected μ -calpain activity, m-calpain activity, and calpastatin activity in the muscles. Types of muscles exhibited variation in the activity of μ -calpain, the activity of m-calpain, and calpastatin activity. In conclusion, the addition of concentrates in the basal feed increased meat tenderness, and affected the activity of μ -calpain, m-calpain, and calpastatin.

Keywords: activity of μ -calpain and m-calpain, calpastatin activity, Kejobong doe

Abstrak. Tujuan penelitian adalah untuk menginvestigasi apakah ada pengaruh penambahan konsentrat ke dalam pakan basal terhadap keempukan daging, aktivitas kalpain dan kalpastatin dari tiga jenis otot kambing Kejobong betina dewasa. Jumlah kambing Kejobong yang digunakan dalam penelitian sebanyak 20 ekor (umur 2-2,5 tahun) dengan rentangan bobot hidup awal 23,8-24,2 kg, diteliti dan ditempatkan secara acak dengan dua perlakuan pakan. Perlakuannya adalah pakan basal (p_0) dan pakan basal + konsentrat (p_1). Kedua macam perlakuan tersebut diberikan selama 12 minggu. Peubah yang diukur adalah (1) keempukan daging, (2) aktivitas kalpain (aktivitas μ -kalpain dan m-kalpain), dan (3) aktivitas kalpastatin. Data dianalisis menggunakan GLM dan korelasi pearson bivariate dari program SPSS. Hasil penelitian menunjukkan bahwa pakan (pakan basal dan pakan basal + konsentrat) berpengaruh nyata terhadap keempukan daging kambing Kejobong betina dewasa. Jenis otot sangat berpengaruh terhadap keempukan daging. Pakan berpengaruh terhadap aktivitas μ -kalpain, m-kalpain, dan kalpastatin pada otot. Jenis otot menunjukkan keragaman dalam aktivitas μ -kalpain, m-kalpain, dan kalpastatin. Kesimpulan penelitian adalah penambahan konsentrat dalam pakan basal meningkatkan keempukan daging dan mempengaruhi aktivitas μ -kalpain, m-kalpain, dan kalpastatin.

Kata kunci: aktivitas μ -calpain dan m-calpain, aktivitas calpastatin, kambing Kejobong betina

Introduction

Kejobong goat is one of local goat breeds in Indonesia, mainly kept by people in district of Kejobong, Purbalingga Regency Central Java. These goats are reared as a sideline from cassava farming as the core business to earn

extra income or as savings. If the breeder needs money for families, farmers will not hesitate to sell female goats in the local markets. Kejobong area is the producer of cassava and this area had many tapioca flour mills producing a lot of industrial waste in the

form of *onggok*. In spite of its abundance, *onggok* has never been used by the goat breeders as local feed ingredients besides concentrates. In this area, goats are mainly fed on forage such as grass, gliriside leaves, and cassava leaves whose availability is limited during the dry season because the land is non-agricultural. Goats were never given concentrate feed.

Highly nutritious feed can improve meat tenderness of the lamb through the increased intermuscular fat and decreased collagen in the muscle (Kemp et al., 1981). Whipple et al. (1990); Koohmaraie et al. (1991); Shackelford et al. (1991a); Dransfield (1994); Gill et al. (1998) stated that meat from different muscles in the same species with that from different species show different tenderness. This is allegedly due to the diversity in calpain activity. The decrease of calpain activity causes a decrease in muscle tenderness. Koohmaraie (1992b) and Pringle et al. (1997) states that, there is strong evidence that shows calpain involved in tenderness after cuts. High calpastatin activity causes a decrease in calpain activity and, therefore, decreased meat tenderness (Koohmaraie et al. (1995a). Koohmaraie et al. (1995a) suggested that high calpastatin activity led to a decrease calpain activity and, therefore meat tenderness decreased. According to Pringle et al. (1997), the activity of calpastatin had a real relation to beef tenderness from crosses of Brahman and Angus cattle.

Accordingly, it was significant to investigate the influence possibility of the addition of concentrates in basal feed on meat tenderness and calpastatin and calpain activities on Kejobong doe.

Materials and Methods

A total of 20 heads of Kejobong doe (2.0–2.5 years) was used with initial live weight range between 23.8–24.2 kg. Twenty

plots cage type stage measuring 75x75x75 cm used in this study. Feed as a treatment consisting of a mixture of feed forage (grass, leaves gliriside, and cassava leaves) served as a basal feed and basal feed plus concentrates.

Research procedures

Grouping of animals. Twenty heads of Kejobong doe were set randomly in two kinds of feed treatment, i.e., basal feed (p_0) and basal feed plus concentrates (p_1). Two kinds of feed were given to Kejobong doe for 12 weeks and each one was repeated 10 times. Composition and nutrient content of feed both treatments is shown in Table 1.

Management of feeding. Basal feed was given to goats in both p_0 and p_1 groups, 2.5 kg each. Concentrates in pellet form only served to goats group basal feed plus concentrates (p_1). The amount of concentrate given to the animal group p_1 is two percent of body weight per head per day. Comparison of basal feed and concentrate presented to group p_1 was 60:40%, given once a day for each head, respectively at 07.00 am and at 03.00 pm. Drinking water was provided to animals ad libitum.

Feeding trial. Twenty Kejobong does were kept in individual cage equipped with plastic receptacle for urine and feces. Animals were allowed to adapt well to the feed treatment and new environment for two weeks called introductory period. Concentrates feed on the Kejobong doe flock p_1 was one a day per head at 06.00 AM, while forage feed was given to all livestock at 03.00 PM. During the feeding trial period (two weeks faecal was collected and the amount of feed consumed by goats was recorded. Dried fecal matter was dried in the sun, sampled and then analyzed regarding other feed nutrients.

Operational definition. The muscle pectoralis profundus (Pp) is a muscle located on the

sternum on the front chest; (brisket) and extends posterior to the back of the chest (plate) (Soeparno, 2005). Muscles Biceps femoris (Bf) is a muscle that forms the extensor the outer hips and helps the movement of the rear leg (Frandsen, 2009). Muscle longissimus dorsi (Ld) is a very important muscle and form eyes of the flesh when cut from the rib and the loin. Ld regions in between the front quarter and rear quarter of the carcass ie between the 12th and 13th ribs (Swatland, 1984). Meat tenderness is an important factor determining the quality of meat. Perception tenderness during mastication are covered by any of the following: (1) finess, (2) resistance to tooth pressure, (3) ease of fragmentation, (4) the amount of residue after chewing. One unit of calpain activity is defined as the addition of 1.0 absorption unit at 280 nm within 60 minutes under a temperature of 25°C. One unit of calpastatin activity is defined as the number of calpastatin which inhibits the activity of one unit of m-calpain.

Livestock and muscle samples. Goat muscle obtained from the cuts on each of five Kejobong doe treated as basal feed (p_0) and basal feed plus concentrates (p_1). Carcasses were chilled at temperature of +4°C for 24 hours to improve rigor mortis. Samples of meat as much as 20 g of each muscle were taken from Pp, Bf and Ld muscles. External fat and connective tissue epimysial of each muscle were separated.

Variable measured. The observed variables were (1) tenderness of meat, (2) calpain activity (μ -calpain activity and the activity of m-calpain), and (3) calpastatin activity.

Meat tenderness. Meat tenderness measurement procedure was done by use of a penetrometer PNR-6.

Testing calpain and calpastatin activity. Activity of μ -calpain, m-calpain, and

calpastatin determined in the muscles in this condition are not freezing, and prerigor according to the procedures of Koochmaraie (1991) modified by Pringle et al. (1997). A total of 20 grams of each muscle i.e. the muscle of Pp, Bf and Ld, homogenized in 2.5 volumes of extraction buffer (50 mM Tris, 10 mM EDTA, and 10 mM b-mercap to ethanol [MCE], pH 8.3) and taken with an ice box to the laboratory to perform measurements of calpain and calpastatin activity. Two proteinase inhibitors (2 mM, phenylmethyl sulfonyl 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 (40 mM Tris, 5 mM EDTA, and 10 mM MCE, pH 7.5). After dialysis, samples were centrifuged, filtered, and loaded on a gravimetric anion-exchange column (DEAE-Sephacel; Sigma Chemical, St. Louis, MO). The column was washed with elution buffer (50 mM Tris, 1 mM EDTA, and 10 mM MCE, pH 7.5) and eluted using a 200 mM NaCl gradient in elution buffer. Calpain and calpastatin activity was determined using casein solution (100 mM Tris, 1 mM NaN₃, 5 mM CaCl₂, 5 mg/mL casein, and 1 mL/mL MCE, pH 7.5). 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 250°C. One unit defined as calpastatin activity was the amount of inhibitor required to inhibit one unit of DEAE-purified m-calpain activity.

Data Analysis

Variables of meat tenderness, calpain and calpastatin activity analyzed using 2x3 factorial experimental design pattern (2 treatments feed, the as basal feed (p_0) and basal feed plus concentrates (p_1) x 3 types of muscle). Replications were 5 times. If the F test results

showed significant differences, the analysis continued with the least significant difference test (Gaspersz, 1991). Data processed by the General Linear Model Univariate procedure of the SPSS Program.

Results and Discussion

Comparison between the consumption of does and their nutritional needs

Based on observations during the feeding trial period, all animals in both groups of p_0 and p_1 consumed all the feed offered. Group p_0 spent fresh forage as much as 2.5 kg of basal feed, while p_1 groups in addition to livestock feed to spent as much as 500 g of concentrate basal. It showed that all goats had adapted to the feed treatment.

Results showed dry matter consumption of doe in group p_1 was equal to the demand but higher than that of p_0 . TDN energy consumption in the group p_1 was higher than p_0 groups and needs. Total protein consumption of doe in group p_1 was higher than p_0 and needs (Table 2). When compared with the nutrient needs of doe weighing 25 kg and daily gain expectations of 50 g (PHILSAN, 1996), then the energy consumption of TDN and protein in group p_1 was higher. It indicated that high energy feed increased palatability of feed, thus encouraging livestock to improve feed ingestion that more feed to consume made the movement of feed consumed in the digestive tract becomes faster, resulting in the quicker digestive tract empty. In addition, the provision of concentrate to the doe during the twelve-week maintenance could increase the dry matter consumed. Therefore, the number of doe consuming dry ingredients as well as other nutrients such as energy and protein increased.

This was consistent with the statement of Owen and Goetsch (1988) and Aritonang (2009), feed with a high energy level will

increase the delicious taste and therefore will encourage the livestock to consume the feed more. This fits well with reports of Salim et al. (2002) concerning the addition of increasing concentrations of dry matter eaten.

Tenderness of meat

Results of analysis of variance showed that, the interaction between feeding and the type of muscle did not significantly affect meat tenderness of Kejobong does.

Results of analysis of variance showed that feeding (basal feed and basal feed plus concentrates) significantly affected ($P < 0.05$) meat tenderness of Kejobong doe. Doe fed on basal plus concentrates produced a more tender meat than goats fed only on basal (Table 3). It indicated an increase in level of protein concentrates in the form of a mixture of rice bran and *onggok* could improve the tenderness of Kejobong doe meat. Proteins are substance in food required for growth. Increased levels of protein concentrates can stimulate growth, and rapid growth can increase the formation of fatty meat. According to Soeparno (1989b), the accumulated fat will dissolve and lower content of collagen, which in turn expected to improve meat tenderness.

Producing goats feeding on live weight and carcass weight is higher, in addition it is also more muscular and leaner carcass (Oman et al., 1999). Highly nutritious feed can improve meat tenderness of the lamb through increasing inter muscular fat and decreasing collagen in the muscle (Kemp et al., 1981). This shows ST muscle is tenderer than SM muscle even though the difference is not significant. This is because the SM muscle has larger file fibers than ST muscle (Lawrie, 1979), therefore it has less tenderness.

Results of analysis of variance showed that type of muscle had a highly significant effect ($P < 0.01$) on meat tenderness of Kejobong doe.

Table 1. Composition and nutrient content of feeds

Feed material	p ₀ (%)	p ₁ (%)
<i>Nutrient composition</i>		
Grass field	50	50
Cassava leaves	30	30
Glyriside	20	20
Onggok	-	65
Rice bran	-	35
Ultra of Mineral ¹ (kg)	-	2
<i>Nutrient content</i>		
DM	87.79	89.60
CP	13.12	9.88
TDN ³	45.17	49.27

p₀ = basal feed; p₁ = feed (basal + concentrate); ¹Each pack (1 kg) of Ultra mineral produced by Eka Farma Semarang, Central Java, Indonesia, contains Calcium carbonate 50.00%; Phosphorous 25.00%; Manganese 0.35%; Jodium 0.20%; Potassium 0.10%; Cupprum 0.15%; Sodium Chlorine 23.05%; Iron 0.80%; Zincum 0.20%; Magnesium 0.15%; ²Group III feed (Hartadi et al., 1980); ³%TDN = $-54,820+1,951(CF)+0,601(EE)+1,602(NFE)+1,324(Pr) -0,027(CF)^2+0,032(EE)^2 -0,021(CF)(NFE)+0,018(EE)(NFE)+0,035(EE)(Pr)-0,0008(EE)^2(Pr)$.

Table 2. Comparisons between treatments with nutritional needs for doe (60% forage + 40% concentrate) (dry matter basis)

Description	Requirements ¹	Treatment	
		p ₀	p ₁
BHA ² , kg	25	24.80	27.70
DMI ³ , kg	0.71	0.43	0.71
E-TDN ⁴ , kg	0.41	0.23	0.58
Total Protein, g	63	47	75.7

¹ PHILSAN (1996); ²Initial live weight; ³Dry matter intake; ⁴(1 kg TDN = 3.62 Mkal ME; 0.23 kg TDN = 0.83 Mkal ME; 0.58 kg TDN = 2.10 Mkal ME); p₀ = basal feed; p₁ = basal feed + concentrate.

Table 3. The influence of feed and type of muscle into the tenderness, calpain and calpastatin activities

Variables	Feed		SEM	P-value	Type of muscle			SEM	P-value
	p ₀	p ₁			Pp	Bf	Ld		
Tenderness, mm/gr/dt	0.255 ^a	0.267 ^b	0.004	0.017	0.242 ^p	0.262 ^q	0.280 ^r	0.005	0.005
μ-calpain, U/20 g	5.32 ^a	6.10 ^b	0.057	0.020	4.67 ^p	4.78 ^p	7.68 ^q	0.070	0.003
m-calpain, U/20 g	5.42 ^a	6.00 ^b	0.093	0.050	4.98 ^p	5.41 ^q	6.60 ^r	0.113	0.036
Calpastatin, U/20g	27.09 ^a	29.61 ^b	0.205	0.036	30.41 ^p	29.44 ^q	25.20 ^r	0.251	0.023

Values bearing different superscript at the same row (between the feed and the type of muscle) differ significantly p₀ = basal feeds; p₁ = feed (basal + concentrate); Pp = Pectoralis profundus muscle; Bf = Biceps femoris muscle; Ld = Longissimus dorsi muscle.

Meat tenderness in muscles Ld was respectively higher ($P<0.01$) than that in muscles Pp and Bf (Table 3). Evidence indicated that muscle Ld was tenderer than Bf and Pp muscles. This was because Ld muscles does not much move compared with the Bf and Pp muscle. It is in accordance with the

Hafid and Sham (2007) that the lowest tenderness level was found in combination of Pp muscles, whereas Ld muscle is the tenderest muscle. According to Soeparno (2005), the tenderness of meat influenced by the location of the meat is the concern of the livestock body. Carcass muscle in the upper

half of the animal body or along the back is softer (tender) than the bottom half. Tenderness can vary among species, breed, livestock in the same species, pieces of carcass, among muscles as well as the same muscle. Sunarlim and Setiyanto (2005) stated that non soft-textured muscle has coarser texture than smooth muscle. Rough texture indicates that the muscle in question consists of a large muscle fibers or thick connective tissue.

Calpain dan calpastatin activity

Results showed that the interaction between feeding (basal feed and basal feed plus con-centrate) and the type of muscles (Pp, Bf and Ld muscles) to the activity of calpain (μ -calpain and m-calpain) and activity calpastatin showed no significant effect. It means the addition of energy feed and muscle type did not affect to the calpain and calpastatin activity.

Calpain-calpastatin system have an important role in the tenderness of meat postmortem (Koohmaraie et al., 1988; Dransfield, 1992; Koohmaraie, 1992; Perez et al., 1998; Morton et al., 1999; Lametsch et al., 2004). Calpain activity and calpastatin activity can be studied in this research by taking a sample of meat as much as 20 g of each muscle Pp, Bf and Ld muscles. μ -calpain activity, m-calpain, and calpastatin activity determined in the muscle in not frozen conditi-on, and prerigor according to the procedures of Koohmaraie (1990) modified by Pringle et al. (1997).

Results showed that, feed giving was a significantly different ($P<0.05$) against μ -calpain activity. μ -calpain activity in the goat group p_1 (basal feed + concentrate) and goat p_0 group (basal feed) respectively amounting to 6.10 U and 5.32 U (Table 3). This means that the activity of μ -calpain in the goat group p_1 (basal feed + concentrate) is higher than the group p_0 (basal feed). Obtained evidence

that, the energy consumption on goats group p_1 can increase the μ -calpain activity 6.10 U, whereas activity μ -calpain in the goat group p_0 just as much as 5.32 U.

Results showed that, feed giving was significantly different ($P<0.05$) to the activity of m-calpain. m-calpain activity of goats on the p_1 and p_0 groups were 6.00U and 5.42U respectively (Table 3). This means that the activity of m-calpain goat in the group p_1 was higher than p_0 . Obtained evidence that the energy consumption of 0.58 kg TDN (Table 2) in goats p_1 group can increase the activity of m-kalpain of 6.00 U, whereas the activity of m-kalpain on goat p_0 group just as much as 5.42 U with the energy consumption of 0.23 kg TDN (Table 2).

Results showed that, feed giving was a significantly different ($P<0.05$) against calpastatin activity. Calpastatin activity in goat groups p_1 and p_0 groups 29.61 U and 27.09 U, respectively (Table 3). This means that the activity of calpastatin in the goat group p_1 higher than goats p_0 group. Obtained evidence showed that the energy consumption of 0.58 kg TDN (Table 2) in the goat group p_1 can increase the activity of calpastatin 29.61 U, whereas calpastatin activity in the goat group p_0 only amounted to 27.09 U with the energy consumption of 0.23 kg TDN.

This means that the addition of energy on basal feed could increase the activity of μ -calpain, m-calpain, and calpastatin activity on goat meat. The addition of concentrate on basal feed increased energy and protein feed. The high energy and protein content of feed given to doe produced tenderer meat when compared with female animals which were only fed on forage. Meat tenderness is closely related to system-calpastatin calpain. The high activity of m-calpain and μ -calpain made the meat tenderer. Conversely, the decreased activity of calpain made the meat less tender. Decreased calpain activity further was

followed by increasing activity of calpastatin a subsequent drop in meat tenderness. The fact was in line with Koohmaraie et al. (1988); Dransfield (1992); Koohmaraie (1992b); Perez et al. (1998); Morton et al. (1999); and Lametsch et al. (2004) that system-calpastatin calpain has an important role in meat tenderness postmortem. A decreased calpain activity causing decrease in muscle tenderness was concerned. Koohmaraie (1992b) and Pringle et al. (1997), states that there is strong evidence that shows calpain involved in tenderness after cutting. It is argued that high calpastatin activity led to a decrease calpain activity and, therefore, decreased meat tenderness (Koohmaraie et al., 1995a). Calpastatin activity at 24 hours post mortem in beef steers causes a decrease in tenderness Morgan et al. (1993).

The results showed that types of muscles which include Pp, Bf and Ld muscles provide a highly significant difference ($P<0.01$) against the activity of μ -calpain. μ -calpain activity in Pp, Bf and Ld muscles up to 4.67 U; 4.78, and 7.68 U (Table 3). This means μ -calpain activity was higher in Ld muscle ($P<0.01$) than in Pp and Bf. Pp and Bf muscles showed the same μ -calpain activity.

Based on the results of the study of m-calpain activity, Pp, Bf and Ld muscle types had a highly significant effect ($P<0.01$) on the activity of m-calpain. m-calpain activity in muscles Pp, Bf and Ld muscles was 4.98 U; 5.41, and 6.60 U (Table 3). Evidence obtained indicated that the activity of m-calpain at Ld muscle was higher ($P<0.01$) than Pp and Bf muscles. However, the activity of m-calpain in Pp and Bf muscles was not different.

The results showed that different types of muscles i.e. s Pp, Bf and Ld muscles provided a significant influence ($P<0.01$) on calpastatin activity. Calpastatin activity in Pp, Bf and Ld muscles was 30.41 U; 29.44, and 25.20 U respectively (Table 3). This means calpastatin

activity at Ld muscle was lower ($P<0.01$) than that on the other two types of muscle, namely Pp and Bf muscles.

High μ -calpain and m-calpain activity in Ld muscle was probably due to the nature of the Ld muscle that never moves. Evidence obtained showed that in different types of muscle (Pp, Bf and Ld muscles) with different treatment feed groups (basal feed and basal feed plus concentrates) there were diverse calpain activities which led to the different tenderness level of each muscle (Table 3). This fact is in accordance with the statement of Whipple et al. (1990); Koohmaraie et al. (1991); Shackelford et al. (1991a); Dransfield (1994); Gill et al. (1998), the meat from muscle of the same species is different and meat from different species shows different real tenderness. This is allegedly due to the diversity in calpain activity. This situation is also in accordance with the findings of Ibrahim et al. (2008) that calpain activity in Longissimus muscle (LM) cow is high, in that LM of Waguli cow is higher than LM of Brahman cows. In contrast, calpastatin activity in LM of Brahman cattle is higher than that in LM of Waguli cow.

However the findings above were not the case in calpastatin activity. Ld muscle (25.20 U) showed lower activity calpastatin ($P<0.01$) compared to Pp (30.41 U) and Bf (29.44 U) muscles. Calpastatin activity in Bf muscle (29.44 U) was lower than Pp (30.41 U) (Table 3). Based on the high and low calpastatin activity obtained that, Ld muscle is more tender when compared with muscle Pp and Bf muscle. This is due to the high the activity calpastatin inhibiting calpain activity so calpain activity decreased. It was concerned that the decreased calpain activity caused a decrease in muscle tenderness. Koohmaraie (1992b) and Pringle et al. (1997) state that there is strong evidence that shows calpain involved in tenderness after cutting. It is

argued that high calpastatin activity causes a decrease in calpain activity and therefore, decrease meat tenderness (Koohmaraie et al., 1995a).

Conclusions

The addition of concentrates in the basal feed increased meat tenderness. The addition of concentrate in the basal diet increased the activity of μ -calpain, m-calpain, and calpastatin activity. Longissimus dorsi muscle had a μ -calpain activity, a higher m-calpain activity but lower calpastatin activity than *Pectoralis profundus* and *Biceps femoris* muscles.

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