RELATIONSHIP OF BACKFAT THICKNESS AND RIBEYE ECHOGENICITY TO HEPATIC AND SPLENIC ECHOGENICITY IN CROSSBRED CATTLE (*Bos indicus* L.) AND WATER BUFFALO (*Bubalus bubalis* L.)

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ABSTRACT

Twelve two-year-old crossbred cattle (Brahman x Philippine Native) and crossbred water buffalo (Bulgarian Murrah x Philippine Carabao), with ideal body condition scores were used to determine the relationship between backfat thickness (BFT) and ribeye (RE) echogenicity to liver and spleen echogenicities. BFT and RE were examined between the 12th-13th rib of the left side of the animal using a 5.0 MHz linear transducer whereas the liver and spleen were scanned at the right 11th-12th intercostal space (ICS) and left 10th-12th ICS, respectively, using a 3.5 MHz convex transducer. The mean BFT was 1.867 cm for cattle and 1.683 cm for water buffaloes. A strong negative correlation was determined between echo mean values of back fat and spleen in water buffalo, while a weak positive relationship was found between RE and liver and spleen in cattle (P<0.05). No significant association was found between BFT and echo mean values of liver and spleen in cattle and water buffaloes. The results of this study can be used as reference for backfat thickness monitoring for both cattle and water buffalo in the Philippines and also serve as a guide for the features and echogenicities of backfat, ribeye, liver and spleen for both cattle and water buffalo fed with the same ration.

Keywords: backfat thickness, cattle, echo mean, liver, ribeye, spleen, water buffalo

INTRODUCTION

The beef industry aims to improve its genetic selection program by evaluation of the body composition traits for breeding stock and organization of feedlot cattle (Kim *et al.*, 1998). Two of the criteria in carcass grading are post-slaughter measurement of backfat thickness (BFT) and ribeye area (REA). Hence, other techniques for assessment of possible yield prior to slaughter were devised, an example of which is by ultrasound evaluation.

Selection of breeder animals and implementation of specific management procedures (feed formulation and amount of ration) are based on the production

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performances of the herd. To ensure that good carcass quality is achieved, pre-slaughter evaluation is performed, unlike before, wherein progeny testing was commonly done. This is accomplished through ultrasonography of the backfat (BF) and ribeye (RE), which gives thickness and size, respectively. The target is to yield less BFT and large RE size (Tuner *et al.*, 1990). However, ultrasonic measurements of BFT in water buffaloes are yet to be established.

Ultrasonography has been proven to be a reliable estimator of carcass traits in live animals with an accuracy of about 80% in grade classification (Brethour, 1990; Greiner *et al.*, 2003). This technique has been widely used in a variety of procedures for different livestock such as measurement of BFT and REA in pig, sheep and cattle (McLaren *et al.*, 1992; Leeds *et al.*, 2008; Marton *et al.*, 2009). Ultrasonography also aided in the development of body condition score (BCS) system in Murrah buffaloes (Alapati *et al*., 2010).

It is possible that evaluation of the aforementioned carcass quality in ruminants may be related to the physiological state of vital organs such as the liver and spleen, hence this study. The results of this research would aid in the selection of possible breeder stock, monitoring the status of the liver and spleen in relation to the quality of BF and RE muscling, ensuring consumer welfare by improving the quality of meat and developing the water buffalo and cattle industry in terms of improving animal health and herd management which would then translate to better meat production.

This study was conducted to determine the relationship of the BFT and echo mean values of BF and RE with the echo mean values of liver and spleen of non-pregnant crossbred female cattle and non-pregnant crossbred female water buffalo through ultrasonography.

MATERIALS AND METHODS

Six two-year old crossbred non-pregnant cattle (Brahman x Philippine Native) and six two-year old crossbred non-pregnant water buffaloes (Bulgarian Murrah x Philippine Carabao), with approximate weight of 300 kg, average daily gain of 1 kg/day and ideal body condition scores (3 for water buffalo and 7 for cattle) were used in this study. The animals were fed with high energy diet (50% forage and 50% concentrates), and were housed in a research barn (2 x 5 m/pen) of the Philippine Carabao Center at the University of the Philippines Los Baños. All animals were apparently healthy based on physical examination and absence of ocular and nasal discharges and lameness. Ultrasonographic examinations were conducted three months after fattening.

Aloka Ultrasound Diagnostic Equipment, Aloka® SSD-500 (Aloka Co. Ltd., Tokyo, Japan) with a 3.5 MHz convex-array scanner was used for ultrasound examination of the liver and spleen while a 5.0 MHz linear-array scanner was used for examination of BF and RE. An ultrasonic gel (Trans-gel®, Rothmeier Laboratories, Inc., Philippines) was applied on the transducer prior to visualization of the organ. The focus, contrast, brightness and gain settings were fixed and maintained throughout the entire procedure.

All animals were placed in a restraining chute and the left side area between the 12th and 13th rib was shaved approximately 3 in or 7.62 cm from the spinous processes of the thoracic vertebra. A 5.0 MHz linear transducer was placed transversely on the prepared site for the evaluation of BF and RE.

The area at the middle of the 11th-12th intercostal spaces (ICS) and the area behind the last rib of the right flank were shaved for ultrasonographic examination of the liver while the spleen was examined from the middle of the 10th-12th ICS at the left side. A 3.5 MHz convex-array transducer was used to examine the liver and spleen.

The images obtained from the examinations of the BF, RE, liver and spleen were printed out, the ultrasonograms were scanned and echo mean values were measured by obtaining three 1 x 1 cm samples and subjecting them to histogram analysis using Adobe Photoshop CS5 Extended v.12.0 x 64 (Adobe Photoshop, Inc., USA). The thickness of BF was measured using an electronic caliper in centimeters (cm).

Data were analyzed using Student's t-test at 95% level of confidence and Pearson's correlation analysis for the variables BFT and echogenicities of REA, liver and spleen, except that of the cattle which used Mann-Whitney U test for equality and Spearman's rho for correlation. BFT between water buffalo and cattle were compared using Student's t-test.

RESULTS AND DISCUSSION

Ultrasonograms of the BF and RE of cattle and water buffaloes are presented in Figures 1 and 2, respectively. BF and RE images of both cattle and water buffaloes were viewed transversely between the 12thand 13th thoracic vertebra approximately 3 inches on the left side of the spinous process. BF was seen as the thick hyperechoic part on the upper portion below the thin hyperechoic skin, while the RE was the oval hypoechoic region directly under the BF with light to moderate hyperechoic speckles all throughout the area. Both the BF and RE of cattle appear to be less echogenic than corresponding structures in the water buffalo. Ultrasonographic features or characteristics of the BF and RE have not been previously reported locally.

The liver of cattle and water buffalo were observed from the 11th-12th ICS on the right side, while the spleen was seen from the 10th-12th ICS on the left side. The features of the liver were similar with the location and descriptions of Acorda and Alejandro (2007) in water buffaloes and Braun (2009) in cattle of having homogenous parenchyma with weak echoes although some scans were coarse and more echogenic. The gall bladder, which consists of an anechoic lumen with hyperechoic wall adjacent to the liver parenchyma, was also observed in both cattle and water buffalo. The splenic parenchyma, on the other hand, appeared homogenously mottled gray, hypoechoic to slightly hyperechoic, while the capsule was observed to be a hyperechoic line on the distal boundary of the parenchyma as observed by Constante and Acorda (2012) in water buffaloes, Acorda *et al.* (2009a) in goats and Acorda *et al.* (2009b) in sheep.

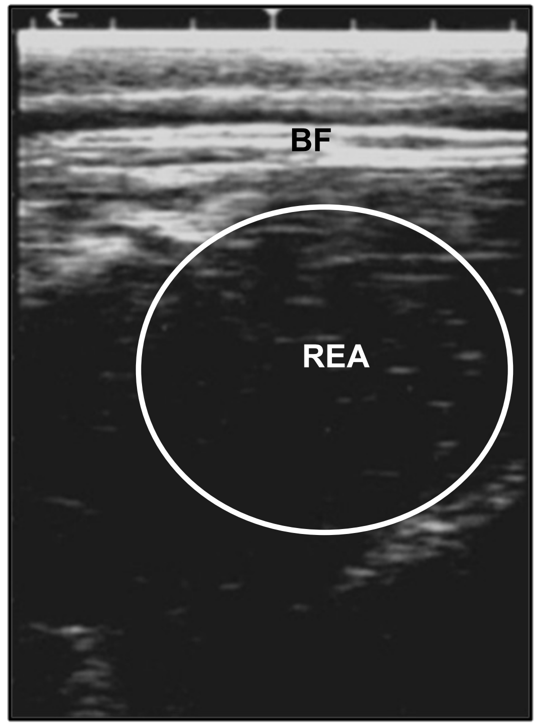

Figure 1. Transverse ultrasound image of backfat (BF) and ribeye area (REA) between the 12th to 13th rib in cattle.

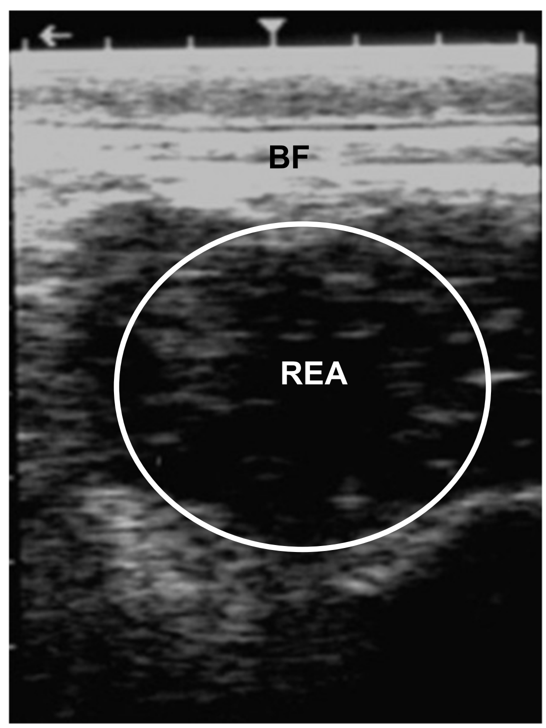

Figure 2. Transverse ultrasound image of backfat (BF) and ribeye area (REA) between the 12th to 13th rib in water buffalo.

The mean BFT of crossbred cattle and water buffaloes are presented in Table 1. The BFT between the two species were not significantly different (P>0.05) which means that in terms of fat measurement, both species are similar. The mean BFT for cattle (1.867 cm) was higher than the reported values of Perkins (1992) and Greiner (2003). This may be due to the diet ration; however, this remains to be tested because both species were given high energy ration and influence of the diet was not a variable in this study.

Table 1. Ultrasound measurement (Mean±SD) of backfat thickness (cm) of female cattle and water buffalo.

Species	Mean (cm)	SD (cm)
Cattle	1.867	0.44
Water Bufffalo	1.683	0.25

Backfat thickness is used as an indicator of fatness or over-conditioning of the animal for it reflects the amount of subcutaneous fat in the body (Starke *et al.*, 2010). One factor that may affect this is the nature (type) of diet. For instance, a growing cattle fed with high levels of concentrate is more prone to body fat accumulation (Khasrad and Ningrad, 2010). High energy ration increases subcutaneous fat, proportion of carcass fat and consequently lesser portion of meat (Arthoud, as cited by Khasrad and Ningrad, 2010). However, an increase in REA was observed as the level of concentrate increases (Khasrad and Ningrad, 2010).

Swatland as cited by Khasrad and Ningrad (2010) reported that deposition of fat during fattening has three phases: marbling in internal organs like kidneys and mesentery, subcutaneous and intermuscular areas, and lastly, intramuscular fat or marbling. The same was reported by Ribeiro *et al.* (2008).

The nutritional status, which is generally reflected by the BCS, is a reflection of the overall health status of an animal and directly affects the animals' reproductive performance specifically on its fertility and estrous cycle. Nutrition is important to aim for the target mating weight of heifers and target BCS at calving. BCS is monitored starting at weaning stage for gradual diet adjustment (Penny, 2013). Adequate dietary fat enhances ovarian follicular growth, enables early return to estrus after calving, improves fertility due to increased progesterone concentration and inhibits regression of corpus luteum, which are important in both dairy and beef cattle (Santos, 2001; Staples and Thatcher, 2005). However, it was found that the risk of hepatic lipidosis, infertility ketosis and milk fever is high at overconditioned periparturient cows (Sinclair, 2010). Hence, BFT of the animal must be established for monitoring to ensure that overconditioning of the animal is prevented.

The BFT of cattle and water buffalo, as shown in Table 2, was found to have no significant linear relationship with that of the echogenicities of RE (36.24), spleen (106.82) and liver (96.12) (P>0.05), which may indicate that fat thickness has no influence on the degree of echogenicity of the RE, liver and spleen for both species.

Table 2.Correlation between backfat thickness (BFT) to echo mean values of liver and spleen in female cattle and water buffalo.

Organ	BFT of Cattle	BFT Water Buffalo
Liver[1]	0.694	0.422
Spleen[2]	0.173	0.531

[1]Pearson's correlation.
[2]Spearman's rho correlation.

The mean ultrasound echogenicities of BF, RE, liver and spleen of cattle and water buffaloes are shown in Table 3.

Comparing the two species of large ruminants, the echo mean values of BF and RE for water buffalo (167.27 and 63.36, respectively) were observed to be higher than those of cattle (131.99 and 36.24, respectively). This affirms the more echogenic appearance of BF and RE of water buffalo compared to those of cattle. The opposite trend was observed when comparing the echo mean values for the liver of water buffalo (60.91) and cattle (96.12). Water buffalo echo mean value for the spleen was lower (75.52) compared to cattle (106.82) ($P<0.05$). This could be explained by the observations of Lapitan *et al.* (2008) wherein crossbred water buffalo had higher body fat proportion and lower lean meat yield than crossbred cattle. Comparisons of liver and spleen echogenicities to BF and RE between the two species are the first known reports, locally.

The echo mean values for the liver and spleen of water buffaloes were lower than the values obtained from the study of Acorda and Alejandro (2007) and

Table 3. Echo mean values (Mean±SD) of backfat, ribeye, liver and spleen of cattle and water buffaloes.

Parameter	Cattle (Mean±SD)	Water Buffalo (Mean±SD)
Backfat	131.99±44.38[a]	167.27±42.11[b]
Ribeye area	36.24±21.21[a]	63.36±34.46[b]
Liver	96.12±14.63[a]	60.91±26.31[b]
Spleen	106.82±45.17[a]	75.52±22.47[b]

Means with different superscripts between species are different ($P<0.05$).

Constante and Acorda (2012), while, the mean value of the liver in cattle obtained in this study was slightly higher than the values reported by Acorda *et al.* (1994). No studies have been previously reported for the echogenicities (echo mean values) of cattle BF, RE and spleen and water buffalo BF and RE.

A correlation was found between the echogenicities of BF and spleen of water bufflalo (Table 4) with strong negative linear relationship, $\rho = -0.616$ ($P<0.05$) which indicates that the echogenicity of the BF of water buffalo is indirectly proportional to the spleen echogenicity. Aside from this, the echogenicity of RE of cattle (Table 3) had weak positive linear relationship with the echogenicities of the liver and spleen, $\rho = 0.543$ and 0.581, respectively ($P<0.05$).

Table 4. Correlation coefficients of echogenicities in cattle and water buffalo.

Parameter	Cattle		Buffalo	
	Liver[1]	Spleen[2]	Liver[1]	Spleen[1]
Backfat	0.350	0.307	-0.379	-0.616[+]
Ribeye area	0.543*	0.581*	0.334	-0.075

[1]Pearson's correlation.
[2]Spearman's rho correlation.
[*]Weak positive linear relationship (0.33 to 0.66) ($P<0.05$).
[+]Strong negative linear relationship (-1 to -0.66) ($P<0.05$).

The relationship found between the echogenicity of RE and liver of cattle can be attributed to the role of the liver in skeletal muscle development in general. Ribeye area, also known as the longissimus muscle, is divided into four parts: capitis, cervicis, thoracis and lumborum. It is the largest muscle of the epaxial muscles as well as the other muscles (transversospinalis and iliocostalis) on both sides of the spinous processes. These muscles are accountable for the extension, and unilateral flexion of the vertebral column (Frandson *et al*., 2009).

Muscle development, such as in RE, is known to be influenced by the Insulin -like Growth Factor (IGF) which is secreted by the liver through the stimulation of growth hormone (GH) (Gahr *et al*., 2007). Fiber number at birth determines the extent of muscle mass and, consequently, limits the compensatory hypertrophy through growth and exercise. Furthermore, nutrition affects the GH-IGF cascade (Hammon and Blum, as cited by Georgieva *et al.*, 2003). IGF is recognized to be important for growth later in life. Overexpression of IGFs elicits muscle hypertrophy or increase in muscle mass (Barton-Davis as cited by Stewart and Pell, 2010). Studies on the relationship of muscle mass and IGF expression have been well-known (Georgieva, 2003; Klöting *et al.*, 2008; Stewart and Pell, 2010). A study by

Hannon *et al.* (1991) proved that IGF-1 is primarily responsible for the increased muscle development in bulls than in heifers or steers.

Aside from muscle cell growth, Klöting *et al.* (2008) reported an *in vitro* study of Scavo in humans, wherein lipogenesis has been proven to be regulated also by IGF-1. This indicates that the liver affects muscle development and lipogenesis, which may explain the relationship between ribeye muscling and liver. Because of the relationship of RE to liver and spleen of cattle, it is possible that BFT of cattle is also associated with the liver and spleen echogenicities based on the study of Turner *et al.* (1990) wherein a moderate genetic correlation between RE and BFT has been established. Furthermore, Starke *et al.* (2010) found a weak positive correlation between BFT (ultrasonically measured) and liver triacylglycerol that confirmed the predisposition of cows with good body condition to fat accumulation.

The splenic echogenicity of cattle, on the other hand, can be related to its RE echogenicity through its physiological role. During muscle development, an increase in volume of circulating blood is needed to facilitate transportation of nutrients and oxygen. When there is an increase demand for oxygen, blood stored in the spleen is mobilized into circulation through the sympathetic nervous system by stimulating the post-ganglionic sympathetic neurons in the smooth muscles of the splenic capsule with catecholamines. Upon stimulation, the smooth muscles contract and the stored erythrocytes are released. However, unlike in other species, ruminants, swine and humans have little smooth muscles (Swenson and Reece, 1993)

The relationships found may indicate that the leaner the animal, the more functional the liver and the spleen are as shown by their correlated echogenicities.

The results of this study can be used as reference for backfat thickness monitoring for both cattle and water buffalo in the Philippines, and also serve as a guide for the features and echogenicities of backfat, ribeye, liver and spleen for both cattle and water buffalo fed with the same ration. Deviation from the normal ultrasonographic features and echogenicities obtained from this study may indicate errors in the overall production management and abnormal health status of these large ruminants.

Further studies can be done to determine the echogenicities of BF, RE, liver and spleen in cattle and water buffalo with different diet (control vs. high energy diet) as well as BFT monitoring in different stages of feedlot cattle.

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