ULTRASOUND FEATURES OF THE SPLEEN, LIVER AND KIDNEY OF BULGARIAN MURRAH BUFFALOES (*Bubalus bubalis* L.) AT DIFFERENT STAGES OF LACTATION

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ABSTRACT

Thirty-three (33) 4-8 years old lactating Bulgarian Murrah buffaloes with 2-4 parities, weighing 450-600 kg, were used to determine the ultrasound features of the spleen, liver and kidney at different stages of lactation: a) early (1-3 months, 12 animals), b) middle (4-6 months, 11 animals); and c) late (7-9 months, 10 animals). The spleen, liver and right kidney were examined using an ultrasound machine equipped with a 3.5 MHz convex-array scanner. Digital analysis of the ultrasonograms from the different organs was performed and histogram echo means were compared. Ultrasonographic features of the spleen and liver showed distinct hyperechoic capsule compared to the hypoechoic parenchyma. The echo mean values of the spleen and liver parenchyma were significantly lowest in late stage of lactation. The renal sinus, cortex and medulla can be distinguished from one another in all lactation phases. Echo mean values for gall bladder lumen were significantly lower in the late stage compared to the early stage of lactation. Thickness of the spleen, liver, kidney and gall bladder wall and gall bladder lumen diameter did not differ significantly among the different stages of lactation. The results of this study can be used to compare the ultrasound features and echogenicity of diseased spleen, liver and kidney in lactating water buffaloes at different stages of lactation.

Keywords: buffalo, gall bladder, kidney, lactation, liver, spleen, ultrasound

INTRODUCTION

Dairy water buffaloes are being used in the Philippines to supply the increasing demands for raw milk and milk products. These animals are exposed and may be susceptible to bacterial, parasitic, viral and metabolic diseases that can harm various organs of the body which can negatively affect overall productivity and longevity during the lactation period. There is a challenge to determine the effects of certain diseases at specific time in production, and ultrasonography is considered as a valuable tool in showing changes in specific organs. Determining the

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ultrasonographic features of the spleen, liver and kidney of lactating water buffaloes at different milking stages is yet to be investigated.

Diagnostic ultrasound or ultrasonography is considered as a safe, fast and non-invasive procedure for diagnosis of disorders. It is ideal for dairy production because it does not emit radiation that can be harmful to the pregnant herd and does not require anti-radiation shields, vests and building which can cause additional farm expenses. Portable ultrasound machines are also currently more affordable and readily available.

Several studies (Yamaga and Too, 1984; Braun, 1991; Braun and Hausammann, 1992; Braun and Sicher, 2005; Acorda and Alejandro, 2007; Acorda *et al*., 2009a, 2009b; Braun, 2009) have been conducted in determining the ultrasonographic features of the spleen, liver and kidney of domestic animals (sheep, goats, cattle, water buffaloes) that aim to serve as normal baseline features for comparison in diagnosing abnormal conditions affecting these animals. Information gathered in this study may aid in determining diseases or abnormalities of the spleen, liver and kidney that can affect dairy water buffaloes at specific stages of lactation.

MATERIALS AND METHODS

Animals

Thirty-three (33) apparently healthy 4-8 years old Bulgarian Murrah buffaloes, with 2-4 parities, weighing 450-600 kg, were used in this study. Twelve (12) animals were assigned to early (1-3 months), 11 were assigned to middle (4-6 months) and 10 were allocated to late (7-9 months) stage of lactation. Health status of the animals was evaluated based on the absence of lameness and abnormal discharges from the nose and eyes. Dairy buffaloes had body condition scores of 2-3. The animals were also given oral dewormer (Albendazole) and intramuscularly vaccinated against hemorrhagic septicemia twice a year.

The water buffaloes were fed with *ad libitum* grass (napier) and legume (centrosema and calopo) rations and received concentrates based on milk produced per day (0.5 kg/l produced). Milking of the herd was fully automated and was done twice a day at 4 AM and 3 PM. Fasting of animals before ultrasonographic evaluation after morning milking was not performed as it could affect daily milk production of the farm. Animals were placed in a squeeze chute to facilitate restraint without sedation and ease of handling.

Ultrasonography

An ultrasound machine (Aloka Ultrasound Diagnostic Equipment, Aloka® SSD-500, Aloka Co. Ltd., Tokyo Japan) equipped with 3.5 MHz convex-array scanner was used in this study. Fixed machine settings (overall gain, far gain, near gain and focus) were maintained for all animals. Ultrasound gel (Trans gel®, Rothmeier Laboratories, Inc., Philippines) was applied to the transducer and the skin of the animal prior to examination. Images were recorded using a video graphic printer (Sony® UP-895 MD, Sony Corp., Tokyo, Japan).

For examination of the spleen, the left area was shaved for the visualization of the spleen and the scanner was applied on the middle to the lower left abdominal wall of the 10th intercostal space (ICS). For liver examination, a rectangular area on the right side of the animal bounded dorsally by the spinous processes of the back, ventrally at the right paramedian area, cranially by the shoulder and caudally by the flank was shaved. The scanner was applied on the 9th-12th ICS to visualize the liver, 10th-12th ICS for hepatic vessels and 11th-12th for gall bladder. Visualization of the kidney was done by removing hair on the dorsal area of the right flank from the 10th ICS to the last lumbar vertebra. Only the right kidney was evaluated due to the anatomic location of the left kidney which is situated deeper in the abdominal cavity and cannot be scanned transcutaneously using the available scanner. The scanner was applied on the area caudal to the last rib under the transverse processes of the first three lumbar vertebras. Examination was done during maximum inspiration of the animal. The ultrasound features of the different structures of the spleen, liver and right kidney were noted.

The thickness of the spleen and liver was measured using the electronic caliper of the ultrasound machine. For the measurement of the kidney, the distance between the cranial and caudal renal poles was the length (cm) while the width (cm) was taken as the distance between the lateral and medial margins of the organ. Thickness (cm) was measured as the distance between the ventral and dorsal face.

The ultrasonograms of the different organs were scanned and the echogenicity of the organ structures was evaluated through digital analysis using Adobe Photoshop CS4 Extended v.11.0.1 (Adobe Photoshop, Inc., USA. 2008). Different sample regions (one cm^2) of the organs structures were obtained and measured three times and the average of the echo mean values was computed. All echo means and measurements were analyzed using ANOVA in CRD. Data were analyzed using SAS version 9.6 (2004) and means were compared using Duncan's Multiple Range Test ($P<0.05$).

RESULTS AND DISCUSSION

The spleen of all animals in each lactation phase was visualized through ultrasonography in the left flank. The splenic capsule appeared as a distinct hyperechoic band (Figure 1). Directly underneath the capsule was the parenchyma which appeared homogenous and hyperechoic to hypoechoic. The ultrasound features of the splenic capsule and parenchyma are in agreement with the findings of Acorda *et al*. (2009a) in sheep, goat and water buffalo. Splenic vessels were not clearly seen in all stages of lactation, in contrast with the study of Acorda *et al*. (2009a), where the vessels were clearly visible.

The mean thickness of the spleen was found to be similar among lactation phases (Table 1). The thickness of the spleen was found to be lower than the gross and ultrasonographic measurements conducted by Acorda *et al*. (2009a) in non-lactating water buffaloes. The spleen normally varies in size (Nyland and Matoon, 1995); therefore, it is best to image from one site, although sometimes it is impossible because of the varying lengths of the organ. Furthermore, Frandson *et*

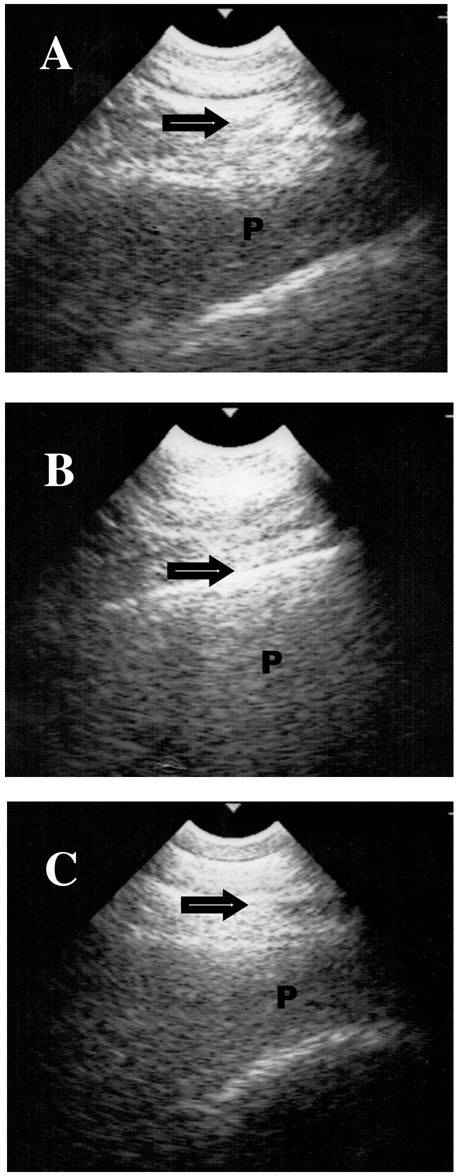

Figure 1. Ultrasonograms of the spleen in Bulgarian Murrah buffaloes during early (A) middle (B) and late (C) stages of lactation. The splenic capsule (arrow) appears as a hyperechoic band while the splenic parenchyma (P) appears homogenous and hyperechoic to hypoechoic in all stages of lactation.

al. (2003) explained that the size of the spleen also varies depending on the individual, species of animal and the amount of red blood cells (RBC) stored in the parenchyma (red pulp).

Echo mean value for the splenic capsule was significantly lowest during late lactation and was highest during early stages of lactation. The echo mean value of the splenic parenchyma was lowest during late lactation (Table 2). The spleen serves as a storage site for RBCs and produces and filters lymphocytes in animals (Reece, 1991; Frandson *et al.*, 2003). It also destroys old, damaged and abnormal RBCs and recycles and stores iron carried by the erythrocytes (Reece, 1991;

Table 1. Mean±S.D. thickness (cm) of the spleen, liver and gall bladder wall and gall bladder lumen diameter in Bulgarian Murrah buffaloes at different stages of lactation.

Organ/Structure	Early lactation	Middle lactation	Late lactation
Spleen	3.1±0.91 (n=11)	3.3±1.21 (n=10)	2.8±0.66 (n=10)
Liver	6.5±1.26 (n=12)	6.7±1.63 (n=11)	6.7±1.77 (n=10)
Gall bladder wall	0.2±0.07 (n=5)	0.2±0.10 (n=7)	0.2±0.06 (n=3)
Gall bladder lumen	3.7±0.90 (n=5)	3.0±0.93 (n=7)	4.0±1.15 (n=3)

No differences were observed among the different stages of lactation for each organ or structure ($P>0.05$).

Table 2. Echo mean values (Means±S.D.) of the splenic capsule and parenchyma of Bulgarian Murrah buffaloes at different stages of lactation.

Structure	Early lactation	Middle lactation	Late lactation
Splenic capsule	227.1±10.48[a]	230.9±9.92[ab]	220.6±8.92[b]
Splenic parenchyma	137.4±38.68[a]	120.8±33.57[a]	89.3±18.10[b]

Values in the same row with different superscripts are different ($P<0.05$).

Frandson *et al*., 2003). Abundance of these blood cells and iron in the parenchyma due to processing of larger volumes of circulating blood during the first two stages of lactation could have increased echogenicity of the structure which may explain the trend in this study. All values for splenic parenchyma echogenicity of lactating water buffaloes were lower compared with the values obtained by Acorda *et al*. (2009a) in non-lactating water buffaloes. Values obtained for the first two stages were higher while it was lower in late lactating group when compared to the histogram echo means taken from sheep, goats and water buffaloes (Acorda *et al*., 2009a).

The liver, like the spleen, also had defined capsule in all stages of lactation which appeared as a hyperechoic line on top of the homogenous hypoechoic parenchyma (Figure 2). The hepatic vein appeared small, round or elongated while the portal vein was elongated and sometimes branched. The portal vein had a hyperechoic wall compared to the hypoechoic border of the hepatic vein. The parenchyma was homogenously hypoechoic and mottled in appearance for all lactation groups, although in late lactating animals, the deeper portion of the liver parenchyma appeared less echogenic. The sonographic features of the liver capsule, parenchyma, hepatic vein and portal vein in all stages of lactation were similar with the findings in large and small ruminants (Braun, 1990; Acorda *et al*., 2006; Acorda and Alejandro, 2007; Braun, 2009). The caudal vena cava was not imaged in all lactation phases. It was observed that the liver parenchyma was significantly less echogenic in the late stages of lactation due to decrease in echogenicity of distal areas of the liver. Severe fatty liver disease in cattle may also show anechoic distal parts of the parenchyma and regions near the abdominal wall should appear hyperechoic (Braun, 2009), although this was not observed in the study.

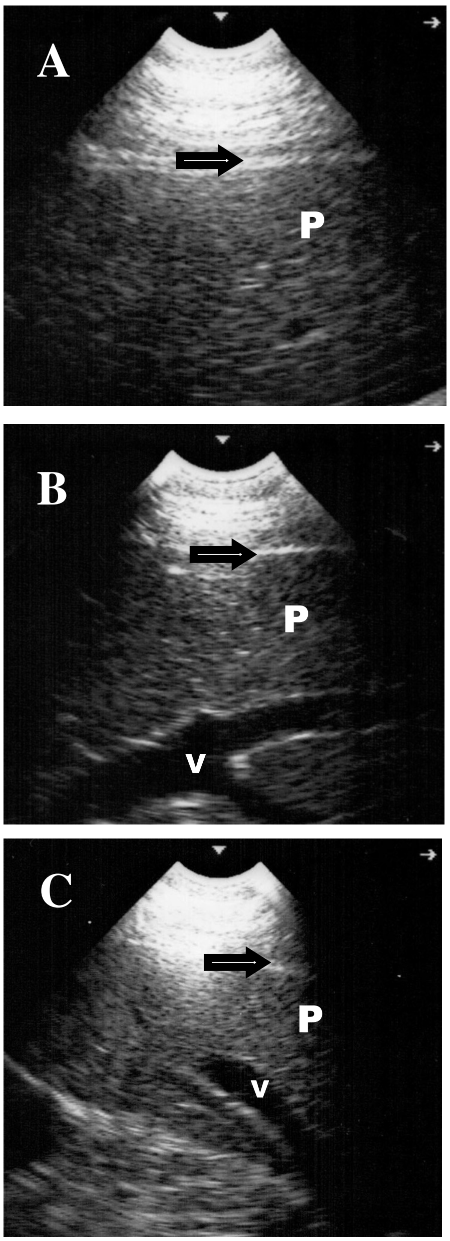

Figure 2. Ultrasonograms of the liver in Bulgarian Murrah buffaloes during early (A) middle (B) and late (C) stages of lactation. The capsule (arrow) in all stages of lactation appears as a hyperechoic line on top of the homogenous hypoechoic parenchyma (P). The portal vein (v) appears as an anechoic tubular structure with hyperechoic walls.

The thickness of the liver was maintained throughout the lactation phases (Table 1). The thickness of the liver in this study was found to be greater than the ultrasonographic and gross measurements obtained by Acorda and Alejandro (2007) for non-lactating water buffaloes. Animals used in this study were purebred Murrah buffaloes which were heavier and bigger than non-lactating cross-bred buffaloes used in other studies which may have an effect on the liver dimensions. Braun and Gerber (1994) noted that the size of the liver did not vary depending on the breed and age of the cows, although they noted that high-producing and heavier cows had thicker and larger liver.

The echo mean values for the capsule did not differ among lactation phases while echo mean value for the liver parenchyma was significantly lowest during late lactation (Table 3). Echo mean values of the liver in early and middle stages of lactation were almost half the value reported by Acorda and Alejandro (2007) in non -pregnant water buffalo while the values for late stage of lactation was six times lower than the said report. This discrepancy is probably due to the thickness of subcutaneous fat in the abdominal wall in purebred buffaloes compared to cross-bred animals used in the previous study. The liver capsule echogenicity was higher than the parenchyma values in all stages of lactation. Echo mean values of the hepatic parenchyma were found to decrease in hydropic degeneration (Acorda *et al*., 1995) and increase in fatty infiltration (Acorda *et al*., 1994) of the liver in cows. Bobe *et al*. (2008) also used digital analysis of hepatic B-mode ultrasonogram as a noninvasive method to detect fatty liver in cows and found an increase in beam attenuation, back scattering, elevated vascular blurring and fine echogenicity. A digital analysis study confirmed hepatic lipidosis using B-mode ultrasonography of the liver parenchyma (Starke *et al*., 2010). Qualitative methods of comparison between kidney and liver can also be used to determine abnormalities in the liver. Echogenicity of the right kidney cortex can be compared with the liver parenchyma which would normally show the same echo pattern (isoechoic) or appear slightly more echogenic than the former (Nyland and Matoon, 1995). This relationship was evident when comparing the echo mean values of the liver and right kidney of lactating water buffaloes in this study.

Table 3. Echo mean values (Means ± S.D.) of the liver capsule, parenchyma, gall bladder wall and lumen of Bulgarian Murrah buffaloes at different stages of lactation.

Structure	Early lactation	Middle lactation	Late lactation
Liver capsule	225.1±6.63^{a}	223.7± 0.30^{a}	222.6±10.34^{a}
Liver parenchyma	68.2±9.76^{a}	68.2±13.19^{a}	23.4±8.89^{b}
Gall bladder wall	148.9±32.86^{a}	145.5±42.50^{a}	174.4±15.62^{a}
Gall bladder lumen	9.4±2.66^{a}	5.7±2.64^{ab}	3.9±2.53^{b}

Values in the same row with different superscripts are different (P<0.05).

The gall bladder wall was closely associated with the liver parenchyma (visceral surface) in the ultrasonogram and appeared as a defined hyperechoic line (Figure 3). The lumen was also distinct and anechoic and the organ appeared elongated or oval in form. The gall bladder also showed the same features described in previous reports for small and large ruminants (Braun, 1990; Braun and Hausammann, 1992, Acorda *et al*., 2006; Acorda and Alejandro, 2007). Normal

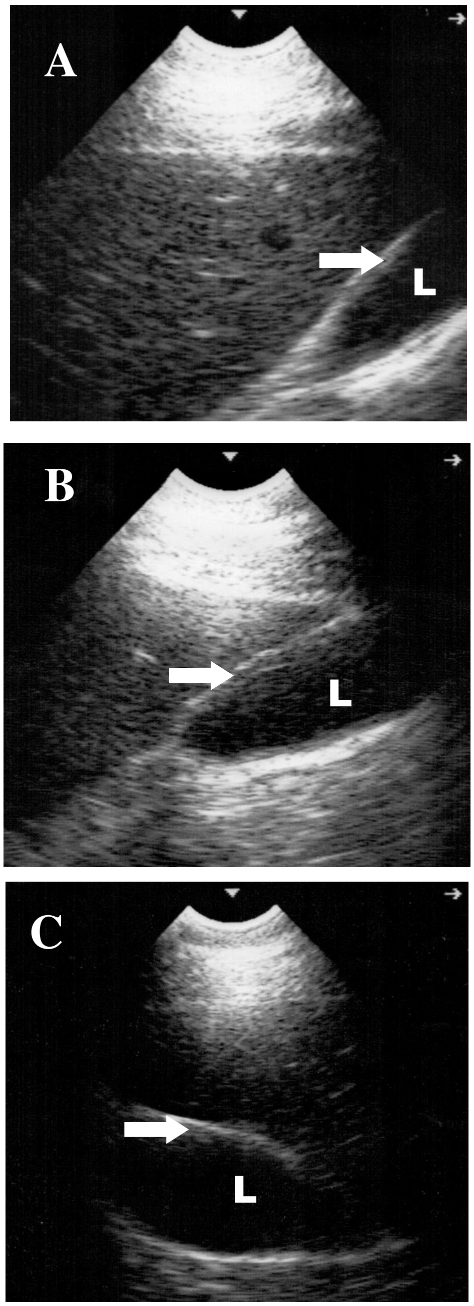

Figure 3. Ultrasonograms of the gall bladder in Bulgarian Murrah buffaloes during (A) early (B) middle and (C) late stages of lactation. The gall bladder wall (arrow) appears as a hyperechoic line and the lumen (L) was anechoic.

features of the gall bladder are important, particularly in cases of cholestasis from biliary obstruction due to fascioliasis, pus or fibrin formation and solid deposition or in rare cases, such as gall stones or tissue proliferation of the organ. Although diagnosis of these conditions entails use of several laboratory tests, ultrasonography aids in cases which require cholecystocentesis. Thickening of the wall without cholestasis could indicate edema rather than inflammation (Braun, 2009).

Gall bladder thickness and lumen diameter did not vary among lactation phases (Table 1) which may suggest normal integrity of the structure all throughout lactation. The diameter of the lumen did not vary among lactation phases, although it has been reported that the size of the organ varies greatly in the ruminant species and it is to be noted that this parameter should not be the sole basis for diagnosing abnormalities of the gall bladder (Braun, 1990; Braun and Hausammann, 1992; Acorda and Alejandro, 2007).

The echo mean value of the gall bladder lumen was significantly lower in late lactating group and highest during early lactation (Table 3). For the gall bladder wall, values were similar among all groups. Change in echogenicity of the gall bladder lumen in early stage of lactation compared to the later phase maybe due to the consistency and content of bile and difference in rate of metabolism (negative energy balance) in the early milking phase. Despite changes in echogenicity, the lumen appeared anechoic unlike in diseased gall bladder, which may appear homogenous, hyperechoic or may contain sediments (heterogenous echo pattern). Assessment of the gall bladder wall thickness, size and characteristics of lumen content when related to clinical signs would best aid in diagnosis of gall bladder diseases in ruminants (Braun, 2009).

The right kidney appeared elongated in longitudinal scan and it was impossible to view the entire length of the organ because it was beyond the scope of the scanner. For the transverse scan, the kidney was round to be ovoid and some had marked indentation on one side. The lobes of the kidney were slightly distinct and created an uneven outline for both longitudinal and transverse sections of the kidney scan (Figure 4). The ultrasonographic features of the kidney structures showed a hyperechoic capsule closely associated with the homogenously hypoechoic cortex, and the centrally located renal sinus appeared hyperechoic. The renal calyces were not obvious in the ultrasonogram. Between the areas of the renal cortex and renal sinus was the hypoechoic to anechoic medulla. Sonographic characteristics of the kidney and structures for all lactation groups were similar with the reports of Braun *et al*. (1992) in sheep and Floeck (2009) in cattle.

The thickness and width of the right kidney did not differ among stages of lactation (Table 4). Changes in dimensions are observed in cows with disease conditions such as nephrosis and pyelonephritis. The gross and ultrasonographic measurements obtained by Allauigan (2006) in non-lactating water buffalo were also comparable with the thickness and width found in this study.

The echo mean values for both renal cortex and renal sinus did not differ among the lactation phases. The echo mean value for renal medulla was highest in mid-lactation although it did not differ from the early stage (Table 5). Echogenicity of the renal sinus was greater than the renal cortex while the renal medulla was found to be the least echogenic among the renal structures for all stages of lactation.

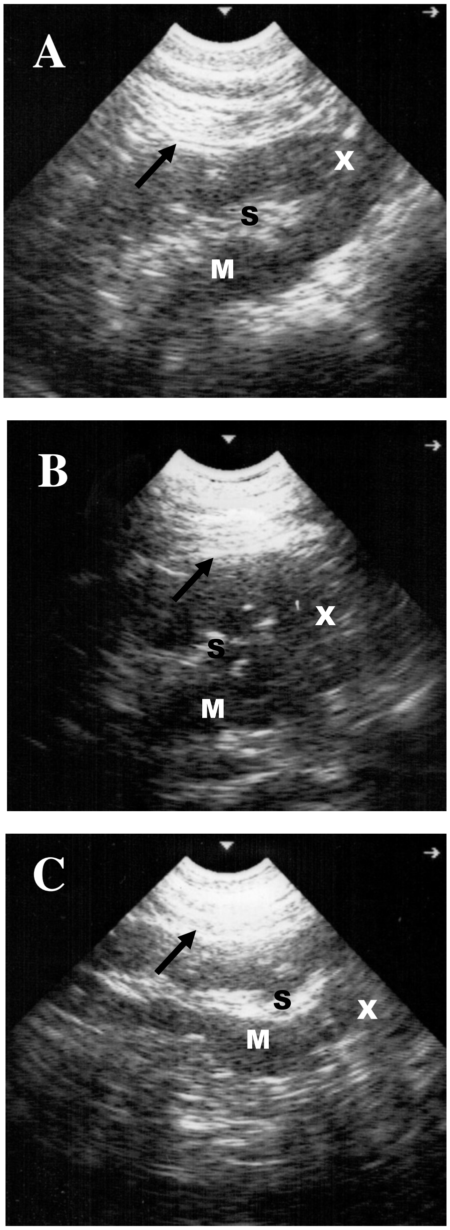

Figure 4. Longitudinal scans of the right kidney of Bulgarian Murrah buffaloes during early (A) middle (B) and late (C) stages of lactation showing a hyperechoic capsule (arrow), homogenously hypoechoic cortex (X) and hypoechoic to anechoic medulla (M) with a centrally located hyperechoic renal sinus (S).

Difference in intensity of echoes of the different renal structures was also reported by Allauigan (2006) in non-lactating water buffalo, although values obtained from this study were lower. Comparing the echogenicity of the spleen parenchyma and kidney cortex would normally show great difference between echoes produced (Nyland and Matoon, 1995), and this feature of variation of echoes between these organs in water buffalo at different stages of lactation were also evident. There are diseases of the kidney which may affect the ultrasound features and echogenicity of the organ. Changes in echo patterns of the kidney parenchyma occur in acute renal failure and causes increase in size or dimension. The organ may also appear hypoechoic to hyperechoic depending on the severity of edema and amount of cellular infiltration. The cortex may become hyperechoic in cases of glomerulonephritis while inflammation of the renal sinus would show hyperechoic debris within the enlarged calices. Presence of renal cysts in the parenchyma also produces distinct anechoic round to oval structures in this region (Floeck, 2009).

The above results show that the dimensions of the spleen, liver, gall bladder and right kidney were not significantly affected by the stage of lactation. The echo mean values of the different organs obtained through digital analysis could be used to evaluate the organs for possible diseases or disorders at different stages of lactation.

Table 4. Mean±SD thickness (cm) and width (cm) of the right kidney of Bulgarian Murrah buffaloes at different stages of lactation.

Dimension	Early lactation	Middle lactation	Late lactation
Thickness	5.4±0.92	5.4±0.76	5.1±0.46
Width	8.4±1.05	8.4±1.19	8.1±1.64

No differences were observed among the different stages of lactation for each dimension (P>0.05).

Table 5. Echo mean values (Mean±SD) of the right kidney cortex, medulla and sinus of Bulgarian Murrah buffaloes at different stages of lactation.

Structure	Early lactation	Middle lactation	Late lactation
Kidney cortex	81.1 ± 10.04[a]	81.9 ± 13.07[a]	83.3 ± 22.53[a]
Kidney medulla	11.8 ± 9.07[ab]	14.0 ± 10.23[a]	5.0 ± 3.06[b]
Kidney sinus	194.9 ± 27.56[a]	186.2 ± 33.05[a]	171.6 ± 40.72[a]

Values in the same row with different superscripts are different (P<0.05).

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