

## TECHNICAL NOTE: INFLUENCE OF FEED ON IMAGE QUALITY OF ABDOMINAL ULTRASONOGRAPHY IN NEW ZEALAND WHITE KITS

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**Abstract:** The aim of this study was to evaluate the influence of feed change on image quality of ultrasound scanning of the gallbladder, kidney and jejunum in New Zealand White kits. Thirty-nine 35-day-old rabbits were used, distributed into 4 diet groups: C, only commercial feed; CH, commercial feed and hay; C24H, only commercial feed, replaced by hay 24 h before examination; and CH24H, commercial feed and hay, with the commercial feed withdrawn 24 h before the exam. The rabbits received the diet for 42 d (from 35 to 77 d of age) and the ultrasound evaluations were performed on days 56 and 77. Ultrasonographic assessment for image quality focused on the possibility of visualisation and an image quality rating (3 scores: unacceptable, acceptable or excellent) for the gallbladder, kidneys and jejunum. The kidneys were visualised in 100% of animals of all diets and ages. There was no difference in visualisation ( $P>0.05$ ) of the gallbladder among the animals fed different diets on days 56 and 77. The C24H diet had a higher visualisation possibility ( $P<0.05$ ) of the jejunum compared to the C and CH diets on day 77. For the image quality score, there was no difference in the studied organs on day 56. However, on day 77, there was a difference for the jejunum ( $P<0.05$ ), and only the C24H and CH24H diets presented images classified as excellent. In conclusion, the image quality of the kidneys and gallbladder was unaffected by feed, and the jejunum had an improved image quality with the inclusion of hay and without the commercial feed 24 h before the test in New Zealand White rabbits at 77 d of age.

**Key Words:** feed, fibre, jejunum, ultrasonography, rabbit.

## INTRODUCTION

The primary pet rabbit production areas of Brazil are in the Southeast and Southern regions; a recent survey indicated that 41% of interview respondents considered rabbits as pets, while 39% of respondents considered them both pets and meat source (Machado, 2015). With the expected increase in demand for the rabbit as a pet, it is important to conduct research to assist the veterinarian in routine check-ups, especially in specific areas such as diagnostic ultrasound (Fischetti, 2012; Casamian-Sorrosal *et al.*, 2014; Banzato *et al.*, 2015).

A decrease in the gas content of the stomach and intestines is commonly involved in patient preparation for ultrasound examination, for these may contribute to ultrasound artifacts (D'Anjou and Penninck, 2015). One way of decreasing gas content is to maintain a good rate of bowel movement, thereby preventing the accumulation of stool and gases (Decubellis and Graham, 2013). For rabbits, the feeding of hay has been described as beneficial for health maintenance because it has a high fibre content (which maintains a good rate of bowel passage of the ingested content), contributes to the maintenance of dental health (continuous chewing of hay favours suitable dental wear) and is a dry food source. Additionally, it provides increased water intake and hence an increased micturition reflex, producing urine which is less concentrated, thereby reducing the risk of urolith formation (Ferreira *et al.*, 2006; Clauss, 2012; Varga, 2014).

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Based on these aspects, the aim of this study was to assess the influence of feed on the image quality obtained via abdominal ultrasonography in rabbit kits. The hypothesis is that the ingestion of hay, with the greater consumption of fibre, could decrease the presence of gas in the gastrointestinal tract of kits, therefore providing better quality ultrasound images. The change or adaptation of diet with hay and withdrawal of commercial feed before the ultrasound scan could improve image quality of abdominal organs in rabbits where this change could be made, for check-ups and other medical/veterinary research in which abdominal ultrasound is required.

## MATERIALS AND METHODS

The study was approved by the Animal Use Committee of Pontifícia Universidade Católica do Paraná (PUCPR) under number 0914/2014. The experiment was conducted in the rabbitry of the university's experimental farm in Fazenda Rio Grande, Paraná, Brazil, between August and November of 2015.

The study involved 39 New Zealand White rabbits (19 females and 20 males), with an initial mean weight of  $0.737 \pm 0.229$  kg. They were randomly grouped into 4 diets: C, only commercial feed (concentrate); CH, commercial feed and hay; C24H, only commercial feed, replaced by hay 24 h before the examination; and CH24H, commercial feed and hay, with the withdrawal of commercial feed 24 h before the exam. They received commercial feed and hay *ad libitum* between day of weaning (30 d of age) and the beginning of experiment (35 d of age). The rabbits received the diets for 42 d (from 35 to 77 d of age) and were housed individually in suspended wire cages with an automatic water dispenser, manual feeder and hay rack.

Abdominal ultrasound examination was performed at 35, 56 and 77 d of age, totalling 117 tests. Day 0 of the experiment, referring to the point at which the kits began receiving the diets, was considered as day 35 of the kits' lifespan, to be used as a reference image of any rabbit. Day 35 was chosen to give 5 d for kit adaptation after weaning at 30 d of age. After the 1<sup>st</sup> exam, 21 d of rest were used between the exams (2<sup>nd</sup> at 56 d and 3<sup>rd</sup> at 77 d) to avoid excess manipulation of the rabbits and to give time for diets to change (or not) the rabbits' gastrointestinal system. Water was freely available to all animals. Chemical analyses of the concentrate and the Tifton hay used in this study was performed, evaluating the percentage of dry matter, ash, ether extract, crude protein, acid detergent fibre and neutral detergent fibre according to the recommendations of the Association of Official Agricultural Chemists (2000) (Table 1).

Prior to the ultrasound examination, all rabbits were weighed on a digital scale and a trichotomy of the abdominal region was performed. During these procedures, the animals were placed in dorsal recumbency on a padded rail, restrained by an auxiliary. The acoustic gel was applied to the abdominal area immediately preceding the beginning of the examination. All examinations were performed with a microconvex (4-8.5 MHz) transducer (Ecovet3, Chison, China).

The gallbladder, the right and left kidneys and the jejunum were evaluated. The gallbladder and a segment of the jejunum (located medial and cranial to the left kidney) were then selected for further study, owing to their direct relation to the digestive system (Varga, 2014). Jejunum was chosen for its easily standardised localisation (medial and cranial to the left kidney). The choice of one segment rather than the whole intestinal system allowed a shorter examination time. The presence of gas in the bowel could also obscure the viewing of the right and left kidneys in dorsal recumbency, so both these organs were included in the study. In the ventrodorsal view, the duodenum can hamper viewing of the right kidney by either obscuring the kidney, or distorting the image quality, while the jejunum and colon can compromise viewing of the left kidney (Dimitrov *et al.*, 2012).

A single ultrasonography technician performed all abdominal ultrasound scans and subsequently assessed all images. All structures were evaluated using both the transverse and longitudinal images. The overall gain and depth of field adjustments were made for each organ while performing the examination. The frequency base used was 8.0 MHz.

**Table 1:** Chemical analysis (%) based on dry matter of commercial feed for rabbits and hay during the experiment.

	DM	Ash	EE	CP	ADF	NDF
Hay	89.7	10.5	2.0	11.9	34.0	82.3
Comercial feed	91.7	8.4	3.8	17.3	16.3	34.2

DM: dry matter; EE: ether extract; CP: crude protein; ADF: acid detergent fibre; NDF: neutral detergent fibre.

The images were saved, de-identified, and only evaluated at a later stage (by the same examiner). To analyse image quality, 2 factors of the images were evaluated: *i*) Possibility of visualisation of each organ: assessed at 35, 56 and 77 d of age. The visualisation possibility of each organ was the number of animals that had a specific organ viewed compared with the total number of animals in the experiment; *ii*) Image quality score: performed at 56 and 77 d of age. A scoring system adapted from Sinan *et al.* (2003) was used. A numerical score on a scale of 1 to 3 was assigned: (1), 'unacceptable', whereby the image quality did not allow for adequate organ evaluation and a new examination was recommended; (2), 'acceptable', whereby the image quality was adequate for clinical purposes and there was no need to repeat the study; (3), 'excellent', whereby the image quality allowed for a clear definition of the anatomy of the organs, with observation of the anatomical structures corresponding to each organ. To compare the results among diets and organs, a proportion of each score in every diet/organ was used.

Statistical analysis was performed using the Fisher's Exact Test for the possibility of visualisation, and the proportion of scores percentages were used for image quality scores evaluation, with Kolmogorov-Smirnov test. A 5% level of significance was considered for both tests. Data were stored and analysed using Microsoft Excel 2010®.

## RESULTS AND DISCUSSION

At 35 d of age (day 0 of the experiment), in the visualisation of organs, the right and left kidneys were visualised in 100% of images for all animals, with the gallbladder being visualised in 97.4% of images, and the jejunum segment failing to visualise in any of the animals. This difficulty in visualisation can be attributed to the size of the structures necessitating the use of a higher frequency than 8.5 MHz, in order to obtain images of the organ wall.

At 56 and 77 d, it was possible to visualise the left and right kidneys in all animals, regardless of the diet group. As for the gallbladder, at 56 d of age, the visualisation rate was 80% in the C group and 100% in others. Additionally, a visualisation rate of 100% was possible at 77 d of age, regardless of the group. At 77 d of age (Table 2), the visualisation of the jejunum was higher for the C24H group ( $P=0.047$ ) compared to those of the C and CH groups. CH24H group was similar to C24H, and it also had a higher possibility of visualisation ( $P=0.078$ ) than groups with commercial feed consumed before examination (C and CH), but it was not statistically significant. This suggests that the withdrawal of commercial feed a day before examination increases the possibility of visualisation of the jejunum in rabbits at 77 d of age. In a comparison between days 56 and 77, the CH24H diet showed a higher visualisation possibility percentage at 77 d ( $P=0.032$ ) (Table 2). Regardless of age and group, the presence of intestinal gas could be the main factor that hindered the visualisation of the jejunum, an observation, which was similarly reported by Fischetti (2012).

There was no significant difference ( $P>0.05$ ) in the image quality scores between the groups of C, CH, C24H, and CH24H for any of the aforementioned organs visualised at 56 d of age. At 77 d of age, the jejunum was the only organ that presented a significant difference between image quality scores ( $P<0.05$ ) between the groups. All animals receiving hay before the examination (CH, CH24H, and C24H) showed similar proportions ( $P>0.05$ ). However, as shown in Figure 1, C24H and CH24H diets, which received only hay 24 h before the examination, had a lower number of unacceptable images, and were the only groups to display images classified as excellent.

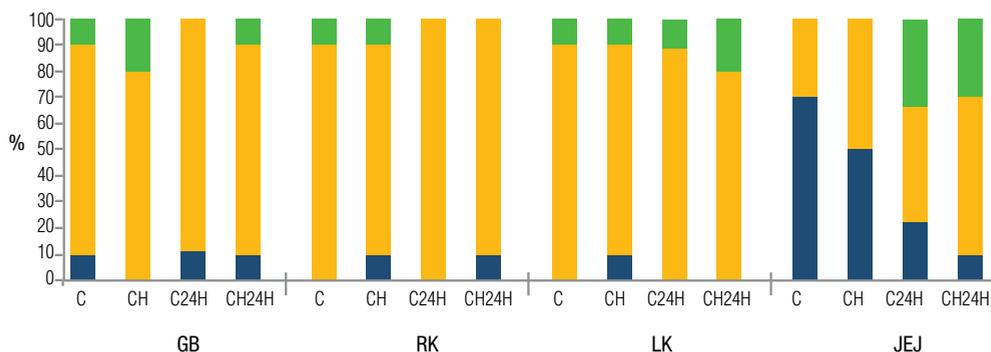
The absence of excellent images for C and CH diets suggested that the accumulation of gases may have been higher in these animals, possibly owing to commercial feed intake 24 h prior to the test. Williams *et al.* (2011) observed, via transcutaneous ultrasound, decreased intestinal motility in stabled horses, which received a higher proportion of concentrate than the grazing horses, whose feeds consisted of only pasture. The lower intestinal motility leads to a lower rate of bowel

**Table 2:** Possibility of visualisation (%) of the jejunum in New Zealand White rabbits at 56 and 77 d of age under different diets.

Diet	56 d	77 d
C (n=10)	20 <sup>aA</sup>	30 <sup>aA</sup>
CH (n=10)	20 <sup>aA</sup>	30 <sup>aA</sup>
C24H (n=9)	44 <sup>aA</sup>	78 <sup>bA</sup>
CH24H (n=10)	20 <sup>aA</sup>	70 <sup>abB</sup>

C: commercial feed; CH: commercial feed and hay; C24H: commercial feed, replaced by hay 24 h before the exam; CH24H: commercial feed and hay, with the withdrawal of commercial feed 24 h before the exam.

Percentages followed by different lower case letters in the same column and different uppercase letters on the same line are significantly different ( $P<0.05$ ).

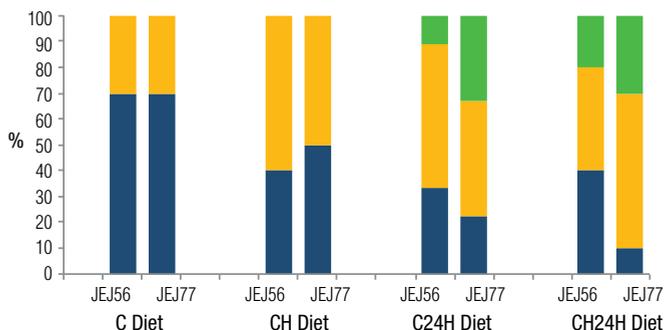


**Figure 1:** Percentage (%) of image quality scores (unacceptable, acceptable or excellent) of the gallbladder (GB), right kidney (RK), the left kidney (LK) and jejunum (JEJ) of New Zealand White rabbits, according to the diet at 77 d of age. C: commercial feed (n=10); CH: commercial feed and hay (n=10); C24H: commercial feed, replaced by hay 24 h before the exam (n=9); CH24H: commercial feed and hay, with the withdrawal of commercial feed 24 h before the exam (n=10). Columns with different lowercase letters in the same organ are significantly different ( $P<0.05$ ). ■: Unacceptable; ■: Acceptable; ■: Excellent.

movement, favouring the accumulation of gases. This observation may account for the results of the current study. With the lowest percentage of acceptable images in the jejunum of animals with the C diet, it was suggested that hay consumption in the CH, C24H and CH24H diets, in addition to the withdrawal of commercial feed in the C24H and CH24H groups had contributed to the most optimal visualisation and quality image of the jejunum after 77 d.

As the jejunum was the only organ in the study that was affected by the diets, its score ratings were compared between days 56 and 77. In the CH24H diet, there was a significant difference ( $P<0.05$ ) in the image quality scores, with more excellent and acceptable images at 77 d of age (Figure 2). This, along with the higher possibility of visualisation of the jejunum at 77 d of age (Table 2), suggested that the withdrawal of commercial feed a day before the examination had a better result for rabbits with a longer exposure to hay.

Machado *et al.* (2011) recommended ADF levels of 16.5-18.5% for breeding rabbits, 18-20% for growing rabbits and 16-18% for mixed use (both categories). Diets with ADF levels below these recommendations can prolong the total



**Figure 2:** Percentage (%) of image quality scores (unacceptable, acceptable or excellent) of the jejunum (JEJ) of New Zealand White rabbits, according to the diet at 56 and 77 d of age. C: commercial feed (n=10); CH: commercial feed and hay (n=10); C24H: commercial feed, replaced by hay 24 h before the exam (n=9); CH24H: commercial feed and hay, with the withdrawal of commercial feed 24 h before the exam (n=10). Columns with different lower case letters in the same diet, are significantly different ( $P<0.05$ ). ■: Unacceptable; ■: Acceptable; ■: Excellent.

gastrointestinal transit time, favouring the growth of undesirable bacteria and the occurrence of stasis, which may bring about health risks to the animal (Ferreira *et al.*, 2006; Decubellis and Graham, 2013). Generally, the commercial feed supplied is for mixed use, whereas the supply of hay may enable higher levels of ADF than that provided by an exclusive diet of concentrate. This could shorten the gastrointestinal transit time and decrease gas accumulation in the jejunum. Additionally, increased water consumption has been correlated with hay consumption (Tschudin *et al.*, 2011), indicating that it may be beneficial to take, as similarly observed in humans, where fluid intake before sonography allows for the differentiation of bowel loops (Nylund *et al.*, 2009).

In this experiment, there was no significant difference ( $P>0.05$ ) between the results of the C and CH diets, possibly due to the ADF levels present in the feed provided (16.3%) being close to the suitable amount required for the species (Machado *et al.*, 2011). Thus, the amount of fibre needed to meet the daily requirements of the animal (18-20%) could have been easily supplied with small amounts of hay. In the C24H and the CH24H groups, the withdrawal of commercial feed and the exclusive consumption of hay 24 h before the exam afforded the most optimal visualisation of the jejunum in rabbits, especially up to 77 d of age.

## CONCLUSION

It follows that the image quality of the kidney and gallbladder of kits were unaffected by feed, whereas the jejunum showed an improvement in its image quality with the inclusion of hay in the diet, and the withdrawal of commercial feed 24 h before examination in the New Zealand White rabbits after 77 d of age.

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