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*Full Length Research Paper*

# Growth performance and immunological response to Newcastle disease vaccinations of broiler chickens fed lysine supplemented diets

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The effect of supplementing varying concentration levels of dietary lysine on growth performance and immune response to Newcastle disease (ND) vaccinations of broiler chickens was assessed. A total number of 180 day-old broiler chicks of mixed sex were used for the experiment. The birds were divided into 4 treatments (Treatment A, B, C and D), in which each treatment was replicated 3 times with 15 birds per replicate. Diet A was the control diet that was not supplemented with dietary lysine and diet B contained the recommended level of lysine by National Research Council (NRC) standards of 1.12%, while diets C and D both contained 1.13 and 1.14% lysine which is 10 and 15% increment of the NRC requirements, respectively. The result of the statistical analysis showed that there was significant difference ( $P \leq 0.05$ ) among the various dietary treatments for feed intake, weight gain, final body weight and feed conversion ratio. Birds fed diet C had the highest final body weight (2.16 kg), total weight gain (2.12 kg) and feed intake (3.11 kg) while birds fed diet A had the best feed conversion ratio (1.37). The immunological response to ND vaccinations showed that birds fed diet C had the highest mean antibody titre values while those fed diet A with no lysine supplemented in their diets had the lowest antibody titre values. The haematological parameter was only significantly ( $P \leq 0.05$ ) different for erythrocyte sedimentation rate (ESR), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). It was concluded that supplementing lysine above NRC requirement at the inclusion rates used in this study had significant effect on performance characteristics and immune response in broiler chickens.

**Key words:** Lysine, immune response, growth performance, antibody titre.

## INTRODUCTION

Disease challenge is one of the many factors that will have an effect on the nutrient requirements of poultry.

Insufficient nutrient consumption will reduce the effectiveness of the bird's defense mechanisms. Therefore, poultry

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must be supplied enough dietary nutrients and energy to allow the bird to express desired growth and feed efficiency. Poultry have been produced commercially since the early 1900's, and research has been conducted for years to improve production efficiency. Poultry nutrition experts employed by the industry have access to a great amount of information allowing them to optimize the particular production parameters most important to the producers (such as breast meat yield, feed conversion, weight gain). A good example of some of this information is that supplied by the National Research Council for Poultry (NRC).

The protein and essential amino acids (EAA) requirements for broilers proposed by NRC (1994) are unable to accommodate the terms of production for modern strains of birds. In order to catch up the additional growth, levels of commercially available amino acids are generally increased (Corzo et al., 2002). Most of these amino acids, particularly lysine, are now being supplemented in free form, enabling dietary crude protein to decrease below NRC (1994) levels (Corzo et al., 2002). The development of amino acid supplementation allows meeting the EAA needs at low protein levels (Dirain and Waldroup, 2002). The use of synthetic amino acids to meet the amino acid needs of broilers leads to production of cost effective diets. Lysine is one of the limiting amino acids common in broiler diets. Lysine requirements of broilers are higher in low protein diets for maximum weight gain and feed efficiency (Labadan et al., 2001). Even at normal crude protein (CP) level, high lysine content has been reported to increase the growth rate in broilers (Holsheimer and Veerkamp, 1992). Increasing lysine over and above NRC (1994) recommendations has been reported to improve weight gain, feed efficiency and breast meat yield (Si et al., 2004) and reduce the deposition of extra fat in the carcass (Moran and Bilgili, 1990). As widely described, increasing dietary lysine generally results in improved feed intake, feed conversion, and body weight gain (Holsheimer and Veerkamp, 1992; Sterling et al., 2008).

Kidd et al. (2001) reported that dietary amino acid lysine in poultry diet in concentration recommended by the NRC (1994) support proper immune system functions in healthy chicks. Improvements in immunity, as affected by dietary lysine in animals include improved thymic weight and function, enhanced lymphocyte mitogenesis, improved immunity against tumours and enhanced wound healing (Efron and Barbul, 1998; Evoy et al., 1998).

Newcastle disease is one of the most rampant viral diseases of poultry with a prevalence rate of 28.9% (Adene, 2004). The major tools that can be used to provoke immunity in birds for both the prevention and control of the spread of the disease are vaccination, good nutrition and immunomodulation (Pangasa and Singla,

2007; Pangasa et al., 2007). Information as to the effect of lysine supplementation on growth and immunological responses to Newcastle disease in broiler chickens are rare.

This study was therefore designed to determine the effect of supplementing lysine above the minimum NRC requirement in feed of broiler chickens on their growth performance and immunological response to Newcastle disease vaccinations with a view to improving animal welfare.

## MATERIALS AND METHODS

### Experimental chickens

A total of 180 day-old broiler chicks (Arbor acre) purchased from a commercial hatchery in Ibadan, Oyo State were used for this study. The approval to conduct this study was given by the Research Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure (FUTA) Nigeria. Thus, the feeding trial was conducted at the Poultry unit of the Teaching and Research Farm of FUTA, Nigeria where adequate biosecurity measures were put in place. Brooding was done in a conventional manner with temperature ranging from 35°C at day old to 29°C at 3 weeks of age and then kept stable at approximately 25°C thereafter. Feed and water were provided *ad libitum*. They were vaccinated at stipulated times against Newcastle disease.

### Experimental layout

The completely randomized design was used. The birds were divided into 4 treatment groups A, B, C, and D during the experimental period. There were 3 replicates per treatment with 15 birds per replicate. The chicks were fed *ad-libitum* on their respective experimental diets. The live weight, weight gain and feed intake of the birds were recorded weekly.

### Experimental diets

The experimental birds were fed four different diets which were prepared at the feed mill of the teaching and research farm of the Federal University of Technology, Akure.

Diet A: was not supplemented with any dietary lysine (0%).

Diet B: contained 1.12% dietary lysine, which is the NRC requirement for lysine supplementation in broiler diets.

Diet C: contained 1.13% of lysine and this concentration was 10% increment over NRC requirement.

Diet D: contained 1.14% of lysine which was 15% increment of NRC requirement.

The gross composition of the diets is shown in Table 1.

### Vaccination

The experimental chickens were vaccinated with Newcastle disease vaccines (NDV) - NDV intra-ocular (Hithner B1 strain), NDV LaSota and NDV Komarov using a stipulated vaccination regime as shown in Table 2. The Newcastle disease vaccines used were produced by the National Veterinary Research Institute (NVRI), Vom, Jos,

**Table 1.** Gross composition of experimental diets (g/kg).

Ingredient	Treatment			
	A	B	C	D
Maize	58.63	58.50	58.49	58.48
Soya bean meal	18.50	18.50	18.50	18.50
Fishmeal	4.00	4.00	4.00	4.00
Groundnut cake	12.50	12.50	12.50	12.50
Vegetable oil	2.00	2.00	2.00	2.00
Bone meal	2.75	2.75	2.75	2.75
Oyster shell	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30
Premix	0.20	0.20	0.20	0.20
Lysine	0.02	0.10	0.11	0.12
Methionine	0.15	0.15	0.15	0.15
Total	100	100	100	100
<b>Calculated composition</b>				
Crude protein (%)	23.09	23.08	23.07	23.07
ME (MJ/Kg)	13.15	13.13	13.13	13.12
Calcium (%)	1.73	1.73	1.73	1.73
Average Phosphorus (%)	0.77	0.77	0.77	0.77
Lysine (%)	1.06	1.12	1.13	1.14
Methionine (%)	0.49	0.49	0.49	0.49

ME= metabolizable energy.

**Table 2.** Vaccination schedule for broiler chickens fed diets supplemented with varying levels of lysine.

Age of birds	Vaccination	Route of administration
3 days	NDV Hitchner B1 strain	Intra-ocular
21 days	NDV LaSota	Oral
42 days	NDV Komarov	Intra-muscular

NDV- Newcastle disease vaccine.

Plateau state, Nigeria. The vaccines were reconstituted and administered according to the manufacturer's recommendation.

#### Data and sample collection

##### Performance characteristics

**Feed intake:** This was taken by measuring daily feed consumption of birds in each replicate for the different treatment groups on a 24 h interval base.

**Weight taking:** The weight of experimental birds was taken before they were fed in the morning. The initial weight of birds was measured at day old and thereafter weight changes on a weekly basis over the trial period were measured as the difference between the initial weight and the final weight.

**Feed conversion ratio:** Feed conversion ratio (FCR) was

calculated as feed intake per unit of weight gain per replicate. All these parameters were used to evaluate the performance characteristics of experimental chickens.

##### Blood and sera collection

Samples of blood for the purpose of serum analysis were collected from 3 birds per replicate in each treatment group before the trial commenced via the heart to determine baseline maternal antibody titre levels against Newcastle disease. The birds were sedated using chloroform before the bleeding exercise. Thereafter, in each treatment 9 birds (3 per replicate) were randomly selected and blood was collected 10 days after administering each of the ND vaccines through the jugular vein for serological analysis to determine the antibody titre values. At the end of the 8 weeks experimental period blood was also collected for haematological and serum protein biochemistry analysis from 9 birds in each treatment.

**Table 3.** Performance characteristics of broiler chickens fed Lysine supplemented diets.

Parameter	Diet A	Diet B	Diet C	Diet D	±SEM
Initial weight (g/bird)	41.19	41.21	41.26	41.20	0.22
Final weight (kg/bird)	1.90 <sup>b</sup>	2.02 <sup>a</sup>	2.16 <sup>a</sup>	1.88 <sup>b</sup>	0.50
Total weight gain (kg/bird)	1.87 <sup>b</sup>	1.98 <sup>a</sup>	2.12 <sup>a</sup>	1.84 <sup>b</sup>	0.54
Weight gain(g/bird/day)	33.24 <sup>b</sup>	35.33 <sup>a</sup>	37.81 <sup>a</sup>	32.89 <sup>b</sup>	0.57
Total feed intake (kg/bird)	2.56 <sup>b</sup>	2.94 <sup>a</sup>	3.11 <sup>a</sup>	2.58 <sup>b</sup>	0.30
Feed intake (g/bird/day)	45.76 <sup>b</sup>	52.5 <sup>a</sup>	55.47 <sup>a</sup>	46.09 <sup>b</sup>	0.46
Feed conversion ratio	1.37 <sup>a</sup>	1.48 <sup>b</sup>	1.46 <sup>b</sup>	1.40 <sup>b</sup>	0.43

Means on the same column with different superscripts are statistically significant ( $p \leq 0.05$ ). Diet A- 1.06% lysine. Diet B- 1.12% lysine. Diet C- 1.13% lysine. Diet D- 1.14% lysine.

### Laboratory analysis

#### *Haemagglutination and haemagglutination inhibition test (HA/HI test)*

Serum samples were analysed using beta ( $\beta$ ) micro haemagglutination inhibition technique (Thayer and Beard, 1998) to determine the antibody titre levels as a measure of the immunological response elicited in the vaccinated experimental birds.

#### *Haemagglutination (HA) titration*

The aim of the HA titration was to determine the viability or the potency of the vaccine used. The Newcastle disease vaccine (LaSota strain) used as the antigen for the HA titration was locally produced by the National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State. Clean, dry, micro-titre plates used were labelled as required, and 0.2 ml of normal saline was dispensed into each of a pair of wells using a micro-pipette. A drop of the antigen was added into the first pair of wells and mixed thoroughly using a pair of inoculating loops and serial dilution was carried out. Finally, 0.02 ml of the guinea pig red blood cell (RBC) indicator previously diluted with normal saline was added to each well. The plates were then incubated on the laboratory bench for about 30 min at room temperature. After precisely 30 min, the end point of the titre was determined as the pair of wells where haemagglutination was clearly observed.

#### *Haemagglutination inhibition (HI) titration*

The beta haemagglutination inhibition technique was used and the stock antigen was diluted according to the HA titre obtained; thus for an antigen with a titre 1:256, the 4HA $\mu$  will be equal to 1:64 dilution of test stock. The micro-titre plates were labelled as required and 0.2 ml of the test stock antigen was then dropped into each pair of the wells on a row of the micro-titre plates. After this, a drop of the serum sample was added into the first pair of wells, thoroughly mixed and serially diluted. Lastly, a drop of the prepared guinea pig RBC indicator was added to each well. The micro-titre plates were incubated at room temperature on the bench for 30 min. The end point of the titre was determined as the pair of wells where haemagglutination inhibition is clearly observed.

#### *Haematological parameters*

The erythrocyte sedimentation rate (ESR), packed cell volume

(PCV), red blood cell count (RBC), haemoglobin concentration (HB) and white blood cell differentials were analysed. The mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and the mean corpuscular volume (MCV) were calculated as described by Lamb (1981).

#### *Serum protein biochemical analysis*

The protein content- albumin, globulin and the total protein of serum samples were estimated using diagnostic kits (Randox Laboratories Limited, UK test kits).

#### *Statistical analysis*

Data on immunological responses, haematological variables, serum protein biochemistry and performance characteristics were subjected to one-way analysis of variance (ANOVA). Where significant differences were found, the mean were separated using the statistical analysis system (SAS).

## RESULTS

### *Performance characteristics*

In Table 3, final body weight (FBW), total weight gain (TWG), total feed intake (TFI) and feed conversion ratio (FCR) of experimental birds was seen to be significantly influenced by the different diets. The FBW of birds fed diet C (2.16 kg) was significantly ( $p < 0.05$ ) higher than birds on diet D (1.88 kg), although it was not significantly ( $p > 0.05$ ) different from birds on diet B (2.02 kg). The total weight gain (TWG) also followed the same trend as the FBW. The TFI was lowest for birds fed diet A (2.56 kg) which was significantly ( $p < 0.05$ ) different from that of birds on diet C (3.11 kg) that consumed the highest amount of feed. Although birds fed diet A consumed the least amount of feed they had the best FCR (1.37) which was significantly ( $p < 0.05$ ) different from that of birds on the rest test diets.

### *Immunological response to ND vaccinations*

The results of the HA/HI tests in Table 4 shows the mean



**Table 4.** Average antibody titre values of chickens fed lysine supplemented diets after Newcastle disease vaccinations

Parameter	Diet A	Diet B	Diet C	Diet D
Baseline titres	Log <sub>2</sub> 5	Log <sub>2</sub> 5	Log <sub>2</sub> 5	Log <sub>2</sub> 5
Titre After NDV Hitchner B1strain	Log <sub>2</sub> 6	Log <sub>2</sub> 7	Log <sub>2</sub> 8	Log <sub>2</sub> 6
Titre After NDV Lasota	Log <sub>2</sub> 7	Log <sub>2</sub> 8	Log <sub>2</sub> 9	Log <sub>2</sub> 7
Titre After NDV Komarov	Log <sub>2</sub> 8	Log <sub>2</sub> 9	Log <sub>2</sub> 11	Log <sub>2</sub> 9

NDV-Newcastle disease vaccine. Diet A- 1.06% lysine. Diet B- 1.12% lysine. Diet C- 1.13% lysine. Diet D- 1.14% lysine.

**Table 5.** Haematological parameters of experimental birds fed lysine supplemented diets.

Parameter	A	B	C	D ±SEM
ESR (mm/h)	3.33 <sup>c</sup>	4.67 <sup>b</sup>	2.67 <sup>d</sup>	5.67 <sup>a</sup> 0.14
PCV (%)	26.00	25.00	26.67	27.00 0.53
RBC (× 10 <sup>6</sup> /mm <sup>3</sup> )	2.18	2.12	2.08	2.04 0.08
Hb (g/100ml)	8.57	8.24	8.77	8.67 0.11
MCHC (%)	32.96	32.96	32.88	32.11 0.29
MCH (pg)	39.35 <sup>b</sup>	38.82 <sup>b</sup>	42.13 <sup>a</sup>	42.51 <sup>a</sup> 2.15
MCV (μ <sup>3</sup> )	119.13 <sup>b</sup>	117.26 <sup>b</sup>	128.02 <sup>a</sup>	132.14 <sup>a</sup> 1.17
Lymphocyte (%)	59.00	60.00	60.42	59.20 0.86
Heterophils (%)	23.00	23.33	24.35	24.00 0.43
Monocytes (%)	14.00	13.95	13.67	14.00 0.64
Basophils (%)	2.67	2.33	2.67	2.10 0.05
Eosinophils (%)	0.93	1.00	0.97	0.90 0.03

ESR = Erythrocyte sedimentation rate, PCV = Packed cell volume, RBC= Red Blood cell. Hb = Haemoglobin, MCHC = Mean cell haemoglobin concentration, MCH = Mean cell Haemoglobin, MCV = Mean cell volume. Diet A- 1.06% lysine. Diet B- 1.12% lysine. Diet C- 1.13% lysine. Diet D- 1.14% lysine.

antibody titre values of experimental birds after the ND vaccinations. The table reveals that antibody titre values of experimental birds were influenced by the dietary treatments. Birds on diet C supplemented with 10% lysine above the NRC requirement had the highest mean titre values of log<sub>2</sub> 8 after NDV intraocular, log<sub>2</sub> 9 after NDV LaSota and log<sub>2</sub> 11 after NDV komarov during the study. The lowest antibody production was seen in birds fed diet A which had no supplementation with dietary lysine having antibody titre values of log<sub>2</sub> 6, log<sub>2</sub> 7 and log<sub>2</sub> 8 after each ND vaccination.

### Haematological parameters

Table 5 shows that only the ESR, MCH and MCV values were significantly ( $p < 0.05$ ) different among treatments. Birds on diet D had significantly ( $p < 0.05$ ) higher ESR (5.67 mm/h) than those fed on the other test diets, followed by those on diets B, A and C. The MCH of birds on diet D (42.51 pg) was significantly ( $p < 0.05$ ) different from those on diet B (38.82 pg) but not significantly

different ( $p > 0.05$ ) from those on diet C (42.13 pg). The MCV of birds on diet D (132.14 μ<sup>3</sup>) was not significantly different from that of birds fed diet C (128.02 μ<sup>3</sup>) but was significantly different from that of birds on diet A (119.13 μ<sup>3</sup>) and diet B (117.26 μ<sup>3</sup>).

### Serum protein biochemical analysis

Table 6 shows that the albumin and total protein content of serum of experimental birds were significantly influenced ( $p < 0.05$ ) by the dietary treatments. The albumin value of birds on diet C (1.69 g/dl) was significantly ( $p < 0.05$ ) lower than those on other test diets. The total protein value of birds on diets B (3.96 g/dl) and D (3.78 g/dl) were not significantly ( $p > 0.05$ ) different from one another but significantly ( $p < 0.05$ ) different from those fed diets A (3.12 g/dl) and C (3.14 g/dl).

### DISCUSSION

Since the possibility of disease challenge is always

**Table 6.** Serum biochemical protein values of broilers fed lysine supplemented diets (g/dl).

Treatment	Total protein	Albumin	Globulin
Diet A	3.12 <sup>b</sup>	2.08 <sup>a</sup>	1.04
Diet B	3.96 <sup>a</sup>	2.36 <sup>a</sup>	1.33
Diet C	3.14 <sup>b</sup>	1.69 <sup>b</sup>	1.45
Diet D	3.78 <sup>a</sup>	2.60 <sup>a</sup>	1.18
±SEM	0.17	0.08	0.04

Means on the same column with different superscripts are significantly different ( $p \leq 0.05$ ).

present in today's poultry operations, the bird's metabolism and immune system are constantly adjusting to the disease condition or stress of the environment and thus nutrient requirements may need to be increased at certain times. In recent times, this has led to much research attention in the area of improving the nutritional quality of poultry feed by supplementing various micro-nutrients in a bid to improve immunity against diseases. In this present study, the results is similar to findings of several researchers which stipulated that increasing dietary lysine at low levels above NRC requirement in the feed of broilers can produce highly productive and healthy poultry birds. The results showed that birds fed 1.13% of lysine (10% above NRC requirement) had the best performance characteristics as seen in their final body weight, weight gain and feed intake which is in line with Azman and Yilmaz (2005) that recorded satisfactory body weight and feed conversion ratio in chickens fed 1.5% lysine supplemented diets. Morris et al. (1987), Morris and Abebe (1990) and Surisdiarto and Farrell (1991) confirmed the positive linearity of the relationship between lysine requirement and dietary protein level which support the view that lysine requirement for maximum productivity (maximum weight gain and feed efficiency) is in the range of 1.26 to 1.33% and this is above the NRC (1994) requirement of 1.00%. According to Han and Baker (1994), the lysine requirement for broilers at 2 to 4 weeks of age was also slightly above the NRC (1994) recommendation of 1.10% for 0 to 3 week old broilers. Since the final body weight and average weight gain of birds fed 1.13% of lysine appeared to be highest in this present study, the result can therefore be said to be in line with the recommendation that lysine requirement for maximum growth is slightly above that of NRC (1994). Corzo et al. (2005) also found that lysine supplementation significantly improved the live body weight and feed conversion efficiency.

The trend of the results showed that final body weight, weight gain and feed intake was lowest in birds fed diets deficient in lysine which may be attributed to limiting

supplies of the essential amino acids. This result support the general principle by Sklan and Plavnik (2002) that chick diets should be formulated to provide sufficient amounts of all amino acids corresponding to requirement for protein synthesis. Evidence has been provided by Sakomura and Coon (2003) and by Nonis and Gous (2008) that lysine is involved in the release of the growth hormone, insulin like growth factor I (IGF-I) and modulation of bone growth by differentiation of osteoblasts and collagen synthesis and this explains why birds fed lysine supplemented diets had better growth performance. This study showed a decrease in performance and efficiency of birds at the highest lysine supplemental level of 1.14% as compared to the other test diets with lysine supplementation. This may be due to less efficient use of amino acids above the requirements for protein synthesis. In contrast, the lower growth in birds fed lysine deficient diets may be attributed to limiting supplies of the essential amino acids. Recently, the results of Nasr and Kheiri (2011) suggested that additional lysine at a level of 12% of NRC in starter and grower diets optimized body weight gain, carcass and breast percentage in Arian broiler chickens, whereas reductions in lysine level reduced growth and live weight (Kerd et al., 1998).

The birds fed diets supplemented with 1.13% of lysine which is 10% above NRC requirement elicited the highest immune response to ND vaccinations as shown in their mean HI antibody titre values. This is similar to report by Mehrdad (2012) that reported increasing lysine in diets of today's broiler in excess of NRC recommendations can improve immune system functions, FCR, abdominal fat deposition, breast meat yield and carcass efficiency. Eduardo et al. (2009) also suggested that addition of lysine to poultry diets improve immunity of the birds against different diseases. The least immune response to ND vaccinations was recorded in birds fed diets not supplemented with any dietary lysine. Similarly, Chen et al. (2003) reported that humoral immune response evaluated from antibody response to NDV vaccination was reduced in broiler chickens fed a lysine-deficient diet

and the cell-mediated immune response was also reduced due to lysine deficiency.

Also judging from this study, the lysine content of the control diet is lower than the ideal requirement while the concentration in diets containing 1.14% lysine is higher than the ideal requirement of broiler chickens currently under study. Therefore, by implication the amino acid fed to birds in the control group and those fed 1.14% lysine supplemented diets could lead to imbalance in amino acids metabolism which consequently lead to negative effects on both the weight gain and immune response as evident in this study.

In general, the requirement of lysine is higher for increased immunity than for growth. The competition for limited resources may contribute to a negative relationship between growth and immunity. However, the present study has negated this hypothesis, because the highest immune response to ND vaccinations was recorded in birds that had the best growth performance which were birds fed 1.13% of lysine. In contrast to this result, some authors (Liu et al., 1995; Parmentier et al., 1996) found that body weight and antibody titers are negatively correlated. Also, more immune competent birds have poor nutrient utilization ability. In another study, results indicated that the lysine requirement for maximum antibody response was greater than for maximum growth for broilers (Klasing, 2007).

The haematological indices aside from the ESR, MCH and MCV values recorded in the present study were not significantly affected by the different dietary treatments and this is in line with the reports of Sahir et al. (2006) and Corzo et al. (2005) which stated that lysine supplementation in feed of poultry had no significant effect on haematological parameters. Though the birds fed 1.13% lysine supplemented diets recorded the highest antibody titre levels, the percentage of lymphocytes of experimental birds in the different treatment groups was similar. This may be explained by the fact that there are many types of lymphocytes and only lymphocytes specific to ND produced antibodies and that percentage of specific lymphocytes is very small in relation to the total number of lymphocytes.

The results of serum biochemical study revealed that the varying supplementation rates of lysine had significant effect on total protein and albumin values though the birds fed the NRC requirement diet had the highest values. This can be said to be similar to the work of Sahir et al. (2006) which reported that serum total protein concentration increased with increasing dietary lysine content. The haematological and serum biochemical parameters obtained from this study suggest that dietary lysine supplementation has no deleterious effects on some physiological indices of broilers chickens since the haematological values and serum protein values fell within the normal range.

## Conclusion

The result obtained in this study shows that lysine supplementation above NRC requirement in broiler diets improved final body weight, total weight gain and feed intake and that 1.13% supplementary rate could be the most suitable inclusion level for enhanced productivity. Also, lysine supplementation at this inclusion level elicited the best immunological response to Newcastle disease vaccinations in broiler chickens. It can therefore be concluded that lysine supplementation is of benefit to high productive performance and boosting of the immune system in broiler chickens.

## Conflict of interests

The authors declare that they have no competing interests

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*Full Length Research Paper*

## Some of biosecurity measurements in different dairy farms in Khartoum State, Sudan

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Ninety dairy farms were surveyed in Khartoum State to investigate their veterinary supervision, management, husbandry, biosecurity and hygienic practices. The information was collected by using questionnaire, visits and direct interviews with farm owners. Supervision of the dairy farms revealed highly significant ( $P < 0.001$ ) variations and mostly done by the owners and herd keepers (60.8%). Cows alone as the main milk producing animals represent 60% and the predominant herds were cross breed dairy cows. Medication was usually given by the veterinarians assisted by farm owners and laborers (47%). Vaccination against some contagious diseases as a routine was done in 65.2% of the farms. The data showed that dairy cattle come in contact with other animals from different herds or farms through natural mating (56.3%), during feeding (20.7%) and drinking (23%). Dipping areas, cleaning of milkers' hands before milking, cleaning of udder, and keeping records were rare. Quarantine of newly introduced cows was practiced in 34.4% of the studied farms and 64.4% of farms owners keep their dairy cows inside the farms without grazing. Dung removal within regular intervals was practiced by 61.1% of the dairy farms and the single use of disposal syringes for one animal was practiced in 45% of the farms. Well designed pens were observed only in 13.3% of the farms, while 70% of pens were designed with local materials. The current study showed high prevalence of mastitis (90%), thileriosis (66.7%) and tick infestation (88.9%) in the surveyed dairy farms and that diseases control were not satisfactory. Hence the present study recommended the training of animal producers and laborers (formal and vocational training) to increase awareness on house designing, rearing, herd management and biosecurity and health supervision of their herds.

**Key words:** Dairy farms, biosecurity, health supervision, hygiene.

### INTRODUCTION

The term "Biosecurity" is concerned with the protection and safety of dairy cows (Cullor, 2004). Therefore,

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biosecurity is increasingly important to include in daily routines for farm management as well as veterinary practice (Anderson, 2010). Dairy farms considering expansion will have to respect sound biosecurity measures in order to maintain disease free herds and sustain maximum production. Infectious diseases can enter a herd through purchased additions or be carried onto a farm by other animal species including humans (Wallace, 2003). Therefore, strict quarantine procedures, more thorough sanitation, increased testing for pathogens and less contact between animals are important (Cullor, 2004). By identifying some of the diseases that are likely to be of greatest risk, prevention and control measures can be developed and implemented to focus on the ones that are most likely to create problems (Wallace, 2003).

Ahmed and El Zubeir (2013) reported that in the majority of the farms in Khartoum - Sudan, the general hygiene and sanitation measures were not satisfactory, as mastitic cows are milked directly on the floor of the pens in 83% of the farms. In another study, Mansour et al. (2014) reported that most of the farms under investigation did not quarantine the newly introduced cows and 75% of the farms did not apply proper disposal of dead calves which might be risky for dairy farms and public health. Similarly, Vasilev et al. (2007) in Bulgaria reported that the presence of high number of dirty animals has constituted the precondition for presence of high number of somatic cells in milk and increased risk of subclinical mastitis. However, Abdalla and El Hagaz (2011) found that the application of some hygienic practices prior to milking cows is an important factor in reducing the bacterial load of raw milk to produce safe milk for consumption. Hence, in order to recommend suitable, applied and economical ideal health (biosecurity) management program, the present study is designed to study the current situation of biosecurity practices for health management in dairy farms in Khartoum State, Sudan. It also aimed to test the association between the health supervision of the farms and the management practices.

## MATERIALS AND METHODS

### Study area description

Khartoum, the capital of Sudan, consists of three towns; Khartoum, Khartoum North (Bahri) and Omdurman. These three towns are situated along the riverbanks where the White and Blue Nile merge to form the River Nile. The city, with its annual average rainfall of 161 millimeters during July to September is situated in the arid and semiarid tropics. Ecological zone is between latitude 15 and 16.4° north, longitude 31 and 34.4° east (Ministry of Agricultural, Animal Wealth and Irrigation of Khartoum State, 2011). The average minimum and maximum temperatures range from 28 to 38°C during September and 16 to 31°C during January.

### Animal populations and management systems

The animal population of Khartoum State is 1, 513, 409 head (cattle 262,258; camels 6,735; sheep 552,398 and goats 692,018) as reported by Ministry of Agriculture, Animal Wealth and Irrigation of Khartoum State (2011). This was estimated by using the annual growth rate (cattle 3%, camels 0.5%, sheep 2.5% and goats 3.5%) based on the total agricultural estimation for the year 2008. The human population of Khartoum is approximately six million people. This urban area has a great demand for foods, including animal products. The management systems in the study ranged from completely closed modern to grazing-based traditional systems.

### Questionnaire and data collection

During four months between July and October, 2011, a 9 pages questionnaire was designed. Ninety commercial dairy cattle herds (producing milk for sale), representing large urban farms, were randomly chosen. The questionnaire included 3 parts: the first part (n = 8 questions) questions regarding general information about the farmers and farms. The second part (n = 8 questions) was about dairy cattle health and health problems, and the third part (n = 16 questions) covered current management and husbandry practices used on the selected dairy farms. The farms were selected according to the responders ability to participate and the 90 questionnaires were filled by direct interviewing of the responding farm' owners from Khartoum, Omdurman and Khartoum North. Observations were carried out to determine farm conditions and to identify potential problems encountered. Herds were stratified into three groups (according to the herd size). The herd size was estimated in numbers of heads in each herd, including both adult productive animals and heifers and calves for recruitment and bulls for breeding as follows: < 50 cattle; small producers, n = 41, from 51 to 100 cattle; medium producers, n = 31 and > 101 cattle; large producers, n = 18.

### Statistical analysis

The data obtained were managed in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). The analysis of the data was done using Statistical Package for Social Science (SPSS) computer program (SPSS Institute Inc., Cary, NC, USA). Descriptive analyses and Chi-Square were conducted using SPSS version 16. Correlation was also made between health supervision and some of the managerial factors.

## RESULTS

Almost all of the current studied farms were privately constructed and managed dairy farms (97.8%) compared to only two farms which were constructed by government sector capitals (Table 1). More than 55% of the farmers have more than ten years experience in dairy farming. Farmers with ten years experience were reported in 17 farms (18.9%). Newly introduced producers with only five year's experience were found to be 25.6% of total farms studied. All farmers own cross bred dairy cattle with unknown extend of foreign blood level. Some farmers had 5 to 10 years of experiences in dairy farming, owned

**Table 1.** General information about the dairy farmers and farms at Khartoum State.

Type of producer	Location of the farms (%)			Ownership of the farm (%)			Education level (%)			Farming experiences (%)		
	Khartoum	Khartoum North	Omdurman	Private	Governmental	Illiterate	Primary and intermediate	Secondary	University	> 5 years	5-10 years	>10 years
Small producers	11 (26.8)	17 (-41.50)	13 (-31.70)	41 (100)	0 (0.0)	7 (-17.10)	12 (-29.30)	13 (-1.70)	9 (22.0)	13 (31.7)	7 (-17.10)	21 (-51.20)
Medium producers	14 (-45.2)	7 (-22.60)	10 (-32.30)	30 (96.8)	1 (3.2)	6 (19.4)	10 (-32.30)	9 (-29.00)	6 (19.4)	8 (25.8)	6 (-19.40)	17 (-54.80)
Large producers	5 (-27.8)	6 (-33.30)	7 (-38.90)	17 (94.8)	1 (5.6)	3 (-16.70)	8 (-44.40)	5 (-27.80)	2 (11.1)	2 (11.1)	4 (-22.20)	12 (-66.70)
Total	30 (-33.30)	30 (-33.30)	30 (-33.30)	88 (97.8)	2 (2.2)	16 (-17.80)	30 (-33.30)	27 (-30.00)	17 (18.9)	23 (-25.60)	17 (-18.90)	50 (-55.60)

**Table 2.** Type of dairy farm in Khartoum State.

Type of producer	Specialized dairy farms (%)	Diversified dairy farms (%)					Total (%)
	Cows only	Sheep	Goats	Camels	Sheep and Goats	Poultry (Biological predators)	
Small producers	26 (-63.40)	4 (-9.80)	4 (-9.80)	1 (-2.40)	6 (-14.60)	9 (-22.00)	24 (-26.70)
Medium producers	18 (-58.10)	1 (-3.20)	11 (-35.50)	0 (0.00)	1 (-3.20)	7 (-22.60)	20 (-22.20)
Large producers	10 (-55.60)	1 (-5.60)	2 (-11.10)	1 (-5.60)	4 (-22.20)	7 (-38.90)	15 (-16.70)
Total	54 (-60)	6 (-6.70)	17 (-18.90)	2 (-2.20)	11 (-12.20)	23 (-25.60)	59 (-65.60)

local breed and cows that were crossbred with exotic breed.

The results obtained from farms visits and the questionnaire, showed that the majority of the dairy farms (60%) were specialized dairy farms (Table 2). However, the diversified dairy farms represent 40%: in which the camels, sheep and goats were found to be reared together with cows for economical values. Poultry (especially local breeds) was found in 25.6% of the dairy farms (Table 2).

Provisional specialist contributing in dairy herds' management was reported in only 5.6% farms, full-time farmers managed their animals were

6.7%, while both farmers and laborers managing the farm were found in 53.3% of the farms (Table 3). Moreover all dairy cows (100%) were milked twice a day manually; early morning (2 to 4 am) and at evening (2 to 4 pm). Farm owners milking their cows were reported in 10% of the total farms; 19.5% was in the small size producers and only 3.2% belong to the medium size farms. Herd men milking the cows were represented in the large size farms(33.3%) as shown in Table 3. The same table reflected that the small and medium producers give more care for their cows in order to reduce the risk of management and to lower the cost of production compared to large scale

producers (P > 0.05). Results of this study indicated that the majority (98.9%) of the dairy farms are under veterinary supervisions, either resident veterinarian (7.9%), through regular visits (24.7%) or on call (66.3%) as shown in Table 4. Regarding the treatment of diseased animals, the present study found that medication was usually done by the veterinarians with farm owners and laborers (43.33%) compared to the medications practiced by the veterinarian alone (18.9%). Vaccinations against contagious diseases such as anthrax, black quarter, contagious bovine pleura-pneumonia and hemorrhagic septicemia, were found to be practiced in 65.2% of the investigated

**Table 3.** Dairy herd's management and milking among dairy farms in Khartoum State.

Producer	Herds managers (%)				Herds milkers (%)			
	Owners	Herds keepers	Owners + herds keepers	Owners + herds keepers + Provisional specialist	Owner	Herds keepers	Owners + herds keepers	Herds keepers+ owner's children
Small producers	4 (5.06)	13 (16.5)	21 (26.9)	0 (0.0)	8 (10.8)	18 (24.3)	12 (16.2)	1 (1.4)
Medium producers	1 (1.3)	4 (5.06)	19 (24.05)	3 (3.8)	1 (1.4)	12 (16.2)	9 (12.2)	4 (5.4)
Large producers	1 (1.3)	3 (3.8)	8 (10.1)	2 (2.5)	0(0.0)	6 (8.1)	2 (2.7)	2 (2.7)
Total	6 (7.6)	20 (25.3)	48 (60.8)	5 (6.3)	9(12.2)	36(48.6)	23(31.1)	7(9.5)
Level of significant			0.001				Ns	

Significant difference ( $P < 0.001$ ) ns: non-significant.

**Table 4.** Veterinary supervision medications and vaccination against contagious diseases in the dairy farms at Khartoum State.

Producer	Veterinary supervision of the dairy farm (%)				Administration of medication (%)				Vaccination (%)		
	Resident	Regular visits	On call	Non	Vets	Owner	Vets +herds keepers	Vets + owners	Vets + owners + herds keepers	BQ HS CBPP and anthrax	Absent of vaccination
Small producers	1 (-1.10)	10 (-11.20)	29 (-32.60)	1 (-1.10)	7 (-8.40)	1 (-1.20)	5 (-6.02)	11 (-13.30)	16 (-19.30)	25 (-28.10)	15 (-16.90)
Medium producers	3 (-3.40)	8 (-9.00)	19 (-21.30)	0 (0.00)	4 (-4.80)	1 (-1.20)	5 (-6.02)	5 (-6.02)	15 (-18.10)	20 (-22.50)	11 (-12.40)
Large producers	3 (-3.40)	4 (-4.50)	11 (-12.40)	0 (0.00)	6 (-7.20)	0 (0.00)	2 (-2.40)	1 (-1.20)	8 (-9.60)	13 (-14.60)	5 (-5.60)
Total	7 (-7.90)	22 (-24.70)	59 (-66.30)	1 (-1.10)	17 (-20.50)	2 (-2.40)	12 (-14.50)	17 (-20.50)	39 (-47.00)	58 (-65.20)	31 (-34.80)

Vet: Veterinarian; HS: Hemorrhagic septicemia; CBPP: Contagious bovine pleura-pneumonia; BQ: Black quarter.

farms. Vaccination against Brucella was rarely used, while vaccinations against foot and mouth disease and enterotoxaemia were not reported. Some dairy cows (56.3%) come in contact with other cows during mating, and also contacted other cows, camels, sheep and goats during feeding and/or drinking water in 23% of the farms investigated (Table 5). Through investigation carried out by visiting the farms and questionnaires outcome, the farmers stated that they are sometimes faced with diseases such as foot and mouth disease (24.4%) and contagious

bovine pleuropneumonia (20%). Cases of mastitis, thielieriosis and spread of ticks were claimed to be high; mastitis occur in more than 90%.

Some of dairy keepers exchange breeding bulls for natural mating (borrowing of breeding bulls) which is practiced by small producers (26.4%) compared to others. A restriction of dairy cows to the farms only, was found to be practiced by 64.4% of total farmers. Large size farms revealed low percentages (11.1%) of restrictions (Table 5). The farm owners restricted their cows without

awareness to the restriction of laborers movements and acceptance of visitors, veterinarians and other professional without disposal or clean boots and coveralls (Table 6). The quarantine of introducing new cows to the herds was practiced in 34.4% of farms studied, the small producers adopted high rate (14.6%), compared to the medium (10%) and large producers(8.9%). Quarantine of introducing new animals has positive correlation to veterinary supervision ( $r = 0.029$ ) as shown in Table 6. In this study, 61.1% of herds' keepers remove dung



**Table 5.** Cows come in contact during feeding, sharing water, and/or natural mating, dipping areas and cup strips.

Producer	Contact during feeding (%)	Sharing water (%)	Contact during Mating (%)	Dipping areas (%)	Cup strep (%)
Small producers	7 (8.04)	8 (9.2)	23 (26.4)	0 (0.0)	0 (0.0)
Medium producers	5 (5.7)	5 (5.7)	17 (19.5)	0 (0.0)	0 (0.0)
Large producers	6 (6.9)	7 (8.04)	9 (10.3)	0 (0.0)	0 (0.0)
Total	18(20.7)	20 (23.0)	49(56.3)	0 (0.0)	0 (0.0)

within one day to three days intervals followed by 30% weekly intervals, 6.7% at 15 days intervals and only 2.2% of the farms were cleaned once per month (Table 6). The majority of farms in Khartoum State showed the absence of general hygiene and sanitation measures, most of the pens appeared heavily contaminated with dung. Regarding the using of disposed syringes, more than 51.3% of dairy farmers used one syringe for more than one cow injections (Table 6). However, dairy farmers who were adopting single use of disposal syringes were 45%. A single needle was used on multiple cattle by 35% bovine practices.

The space required by the animals was not considered and the buildings designed with local materials were observed in more than 70% of farms investigated. The walls of pens were built either from mud or corrugated irons. Five farms (8.47%) have no pens roof and fences building included red bricks in 44.07%, iron pipes (35.59%) muskeet stem (*Prosopis Juliflora*) in 5.08% and zinc in 35.5% of farms.

The veterinary extensions provided by veterinary hospitals, universities and extensions' offices were available in 61.1% of the total farms investigated (Table 8). The extension received from veterinary hospitals and universities was in 12.2% of total farms, while the veterinary extensions received from extension offices was found in 13.3% of the total farms investigated. Extensions provided by both veterinary hospitals and extension offices were found to be the majority (35.6%) in the farms. Results in Table 8 indicated that the majority of dairy cow pens (74.4%) were supplied by drinking water from general water network, while farms supplied from their own wells were 16.7% and farms that brought water to farm using donkey carts or tankers were reported as 7.8% of the total investigated farms.

## DISCUSSION

Data in Table 1 constituted with Mohamed (2011) who reported that most farmers constructed their farms with private capital. This may be attributed to the low education levels among the dairy cattle producers and

the absence of dairy societies which organize the governmental funds. The result regarding the farming experience goes in line with Millogo et al. (2006) who reported that the full-time farmers in Burkina Faso had more than 10 years experience in farming and their herds were essentially composed of local breeds. The dominant of cross bred dairy supported El Zubeir and Mahala (2011) who reported that the dairy herd keepers in Kuku project are older compared to those of Alrudwan project, they attributed that to the recent establishment Alrudwan project (1993), while the dairy farmers owned the cultivated lands in Kuku project since 1960.

Although the diversified dairy farms were common in Sudan, the present result showed lower value (40%) compared with the specialized one (Table 2). The farmers stated that they reared dairy cows only with no other animals or other activities because the size of land is small that make them to worry from additional costs. Poultry (especially local breeds) was found in the small producers farms mainly as biological predators to reduce the numbers of mites and ticks.

These findings were similar to that reported by El Zubeir and Mahala (2011) who reported that the dairy cow keepers in Kuku rear the cows as the main milk producing animals (60%) and few sheep (16%) and goats (4%) in addition to chicken (20%). In Alrudwan, they keep cows (60%) and chicken (40%) which were used as biological control for mites.

Provisional specialist contributing in dairy herds' management was rare; most of the farmers depend on laborers management of the farm (Table 3). This might be attributed to the involving of farmers in other works in the urban area and the limitation of understanding of farm owners regarding the advantages of consulting a veterinarian or animal specialist about managing their dairy farms. El Zubeir and Mahala (2011) and Mohamed (2011) reported similar findings. Also, the milking routine and methods were similar to those reported by Mohamed (2011) who found in his study that the hand milking was practiced twice a day in all investigated dairy farms.

The majority (98.9%) of the dairy farms are under veterinary supervisions (Table 4). This is because of the availability of graduated skilled veterinarians in Sudan

**Table 6.** Biosecurity and hygienic practices (dung removal and dealing with disposal syringes) in dairy cattle farms at Khartoum State.

Types of producers	Biosecurity			Interval dung removal (%)				Uses of syringes (%)		Keeping records (%)
	Cows restricted to the farm only (%)	Quarantine for new cows (%)	Specialist for a group of cows (%)	1 to 3 days	7 days	15 days	30 days	Single use	Use for the second time	
Small producers	29 (32.2)	14 (15.6)	0 (0.0)	22 (24.4)	16 (18.0)	2 (2.2)	1 (1.1)	18 (22.5)	17 (21.6)	0 (0.0)
Medium producers	19 (21.1)	9 (10.0)	1 (1.1)	23 (25.6)	5 (5.6)	3 (3.3)	0 (0.0)	11 (13.8)	16 (20.0)	1 (1.3)
Large producers	10 (11.1)	8 (8.9)	0 (0.0)	10 (11.1)	6 (6.7)	1 (1.1)	1 (1.1)	7 (8.8)	8 (10.0)	2 (2.6)
Total	58 (64.4)	31 (34.4)	1 (1.1)	55 (61.1)	27 (30.0)	6 (6.7)	2 (2.2)	36 (45.0)	41 (51.3)	3 (3.9)
Level of significant					0.01					

Significant difference ( $P < 0.001$ ).

and the wide spread of the private veterinary clinics in Khartoum State. Similarly, Mohamed (2011) reported that the veterinarian's roles in dairy farms were either resident 18.33%, visited the farm at regular intervals 16.67% and on call 65.0%. Similarly, Millogo et al. (2008) found that all farmers worked with veterinarians when the animals need treatment against disease. However, the medication done by the veterinarians was only 18.9%. This is because, in Sudan, the medicines are available for any producer to buy from pharmacies in the cities; without prescription (Adam, 2014). Furthermore, Said Ahmed et al. (2008) was able to detect antibiotic residues in 22.2% of the milk samples collected from the dairy farms. However the farmers seek the help of the veterinarian to examine purchased cattle before they entered the herd. This is similar to Hoe and Ruegg (2004) who reported that the proportion of herds that performed reproductive exams was highly associated with herd size and it is possible that small producers are aware of the importance of such practices, but financial constraints or simply less frequent contact with veterinarians may be limiting factors for implementation of preventive

measures.

The availability of vaccination is due to the fact that the governmental authorities have continuous programs for diseases control, this service was observed to be provided freely at Khartoum State. However some small producers did not respond to the vaccination programs as they believed that vaccination causes diseases to their cows. However vaccination against *Brucella* was rarely used, while vaccinations against foot and mouth disease and enterotoxaemia were not reported. These findings agreed with Ahmed and El Zubeir (2013) who reported that *Brucella* vaccines and foot and mouth disease vaccines were rarely used. Schaik et al. (2011) reported that vaccination did not prevent losses in milk production; it reduced the infection pressure and the clinical signs of the disease.

Some dairy cows (56.3%) come in contact with other animals (Table 5). Limitations of land size and feeding and watering facilities might be some of the reasons. Cullor (2004) reported that the three pillars of any biosecurity program are isolation, sanitation and restricted movement. Moreover large size farms revealed low

percentages (11.1%) of restrictions, which could be due to owing of cultivating lands with fodder crops, which might not be available for small and medium size producers (Table 5). Risk associated with animal movements can be reduced by producers only purchasing animals from farms with a known disease history and through isolation, disease testing and prophylactic treatment of purchased stock (Brennan and Christley, 2012). Culler (2004) reported that biosecurity program will differ from farm to farm; the overriding concerns are to keep everything as free of germs as possible and to limit contact between animals as much as possible. The farm owners restricted their cows without awareness to the restriction of laborers movements in case of infectious diseases outbreaks and acceptance of visitors, veterinarians and other professional without disposal or clean boots and coveralls (Table 6). However, Hoe and Ruegg (2004) reported that veterinarians (93%), inseminators (88%) and nutritionists (73%) who visited the farms washed their boots or wore new disposable boots every time they visited the farm. Quarantine of introducing new animals has a small positive correlation

**Table 7.** Frequencies of diseases outbreaks on the studied dairy farms at Khartoum State.

Types of producers	Frequencies of diseases occurrences (%)		
	Contagious bovine pleuropneumonia (CBPP)	Foot and mouth disease (FMD)	CBPP + FMD
Small producers	7 (7.8)	12 (13.3)	10 (11.1)
Medium producers	8 (8.9)	7 (7.8)	5 (5.6)
Large producers	3 (3.3)	3 (3.3)	3 (3.3)
Total	18 (20.0)	22 (24.4)	18 (20.0)

to veterinary supervision, which might be attributed to the understanding of herd keepers to the infectious diseases transmissions and spreads through herd contact. Also grouping of lactating cows to facilitate health observations and shared responsibilities with milkers on healthy environments maintenance was observed in one farm only (Table 6). This might be documented to the lacking of long term and expert laborers.

Mansour et al. (2014) reported non significance between hygiene, quarantine and presence of veterinary services in dairy farms at Khartoum State. The majority of farms in Khartoum State showed the absence of general hygiene and sanitation measures, most of the pens appeared heavily contaminated with dung (Table 6). This agreed with Mohamed (2011) who reported that general hygiene, cleaning programs and sanitation practices were poorly obtained. He also found that the majority of the farms had no dipping area and calving pens. Ahmed and El Zubeir (2013) also reported that the general hygiene and sanitation measures such as dung removal, disinfection, cleaning programs and maintaining minimal contamination during milking process could not be observed in the majority of dairy farms in Khartoum State. The farmers who practice one to three days and weekly dung removals, sell the animal manure to cover some of the daily farm expenditure. Similarly, Mustafa et al. (2011) reported that selling daily manure was practiced by 87.8% of the farm householders. Some of the farmers who remove the animals manure after 15 or 30 days used it as bedding of herds. However accumulations of manure with the urine might subject dairy animals to foot rot and other health problems. Cashman et al. (2008) reported that animal waste and hygiene management was applied on 56% of the farms. The single use of the disposed syringes (Table 6) could be attributed to the awareness of some farmers that clean syringe removes the risk of disease transmission, which was learned from the extension services provided by the veterinarian. Anderson (2010) reported that the principle of "one needle per cow, one cow per needle" was reported by 31% of bovine practices. It was noted that even in the farms which keep the records; the records are not well

organized and unreliable since a lot of missing data was encountered. This may be due to the ignorance of owners to the importance of records keeping. El Zubeir and Mahala (2011) reported that producers at Kuku who kept records were 64%, while only 7% kept record at Alrudwan camp. Mohamed (2011) reported that farm records were found in 36.6% of the farms at Khartoum North.

The space required by the animals was not considered in many of the investigated dairy farms. Hoe and Ruegg (2004) reported that the animal housing should be associated with herd size and providing good comfort for milking cows and other herds. The grooves in the mud walls and timber in the roofs provides good environment for ticks and other diseased insects. Also the old cars irons and woods will subject animals to injuries which increase the chances of transmitting the diseases through wounds. However, some of the pens have no access to shades; which might subject the animals to the heat stress. Similarly, Mohamed (2011) reported that metal, wood and plastic materials are used for roof in dairy farms in Khartoum North, which was also supported by Ahmed and El Zubeir (2013).

Through investigation carried out by visiting the farms and questionnaires outcome showed that diseases outbreaks such as contagious bovine pleuropneumonia (CBPP) and foot and mouth disease (FMD) occurred in more than 64% (Table 7). This attributed to poor hygienic practices during milking and absence of drying off cows programs. El Zubeir et al. (2006) reported that mastitis routine testing is very important because most of mastitis infection persist as subclinical, which will not be detected by herdsmen. Hoe and Ruegg (2004) pointed that most mastitis control practices were significantly associated with herd size, which was reflected in differences of milking systems or animal housing. Similarly, the high level of ticks might attributed to the presence of grooves in the mud walls and timber which provides good environment for ticks and other diseased insects besides lacking of dipping and sprays programs (Singh et al., 2000). The high incidence of ticks subject cows to theileriosis (Dua et al., 2012). The high incidence of

**Table 8.** Services provided to the dairy cattle farms at Khartoum State.

Types of farms	Veterinary extensions (%)			Sources of water (%)		Other sources (donkeys carts) (%)
	Veterinarians and Universities	Extension office	Veterinarians + extension office	Water network	Water wells	
Small producers	4 (4.4)	1 (1.1)	17 (18.9)	27 (30.0)	8 (8.9)	5 (5.6)
Medium producers	6 (19.4)	3 (3.3)	15 (16.7)	28 (31.1)	2 (2.2)	1 (1.1)
Large producers	1 (5.6)	8 (8.9)	0 (0.0)	12 (13.3)	5 (5.6)	1 (1.1)
Total	11(12.2)	12(13.3)	32 (35.6)	67 (74.4)	15 (16.7)	7(7.8)

of infectious diseases may have direct effects on livestock productivity and metabolism, increased mortality and decreases rates of reproduction, weight gain and milk production (Mustafa et al., 2011; Ashuma et al., 2012).

This finding concluded that veterinary extensions provided by veterinary hospitals, universities and extension offices were available in 61.1% of the total farms investigated (Table 8). However, Mustafa et al. (2011) reported that the majority (88%) of householders indicated unavailability of extension services from governmental authorities and 6.1% stated that the services were available but they did not receive it (Mustafa et al., 2011). Results in Table 8 indicated that the majority of dairy cow pens (74.4%) were supplied by drinking water from general water network. This attributed to the wide distribution of general water network.

## CONCLUSION AND RECOMMENDATIONS

The current study concluded that since biosecurity programs take time to achieve, it is advisable for Sudan dairy producers (Khartoum State) to begin thinking about proper management of their farms and to control the infectious and non-infectious

diseases to ensure successful enterprises. This could be achieved via provision of essential services such as health care, vocational education and training to the dairy farmers on good dairy farming practices. Enforcement of legislations and laws, adoption of standard methods and establishment of programs to control the diseases transmission (e.g. HACCP) are needed for clean milk production.

## Conflict of interests

The authors declare that they have no competing interests

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Full Length Research Paper

## Baseline vital, haematological and serum biochemical parameters of donkeys

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The aim of this study was to determine the baseline values of vital, haematological and serum biochemical parameters in apparently healthy experimental donkeys in North-Western Nigeria, with a view to providing additional data on normal values for donkeys. Twenty four apparently healthy donkeys comprising of 12 males and 12 females, aged 11 to 15 months were used. The animals were housed in prepared experimental pen and assigned identification neck-tags at random. Microscopic methods and mice inoculation test were used to examine and ensure that the donkeys were free from gastro-intestinal and blood parasites infections. Animals were acclimatized for 14 days, fed and salt lick blocks together with drinking water were provided *ad libitum* throughout the experiment. Vital, haemogram and some serum biochemical parameters of the animals were evaluated four consecutive times at 7 days intervals. Data was analysed using statistical package for social sciences (SPSS) Version 17. Mean of parameters were determined and tables prepared on Microsoft Excel 2010. In conclusion, these baseline data will be useful in laboratory diagnosis and further understanding of diseases of donkeys especially those within the age group of 11 to 15 months in North-Western Nigeria.

**Key words:** Donkeys, serum biochemical, haematological, parameters, vital.

### INTRODUCTION

Donkeys (*Equus africanus asinus*) are widespread in Nigeria and used as source of traction power in transport and ploughing by both pastoralists and settled farmers in

the northern region (Blench et al., 2013). Donkeys also tolerate some tropical animal diseases and parasites, survive on poor quality feeds and adverse climatic

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conditions, thereby making their management easy for their owners (Aganga et al., 2000). Despite the benefits and advantages of keeping donkeys, the research attention given to the specie is relatively small, whence; scanty biological data is available on donkeys (Starkey, 1994; Blench et al., 2013). In the present study we determined the baseline vital, haematological and serum biochemical parameters of apparently healthy donkeys acquired from Jigawa state, and conducted the experiment in Zaria, Kaduna state, both in North-Western Nigeria. It was designed with a view to providing normal values of the physiological parameters under experimental condition in order to determine alteration(s) in patients' values during laboratory diagnosis of diseases of donkeys.

## MATERIALS AND METHODS

### Experimental animals and their management

Twenty four apparently healthy donkeys comprising 12 males and 12 females, aged 11 to 15 months estimated (using dental eruption pattern of the incisors teeth) as described by Wayne and Melvin (2000) and Joe (2012) at point of purchase in Maigatari town, Jigawa state and transported to Zaria in Kaduna state both in North-Western Nigeria were used. Prior to housing, topical fly-repellant spray (Endure<sup>®</sup>, Farnam co. inc., USA) was applied on the animals as recommended by manufacturer, to keep flies off the animals. The donkeys were housed in prepared, fly-proofed experimental animal pen in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria where the experiment was conducted. Animals were identified using neck-tags assigned at random as described by Aviva and Poul (2013). Feed was provided, equivalent to 5% of the mean body weight of the animals in form of sorghum/maize stovers and grass/legume hays (in the ratio 4:1). Concentrate feed was provided as a mixture of coarsely ground sorghum grain, bran and dried groundnut cake which was served daily in two divided rations for morning and evening. Salt lick blocks and clean drinking water were provided *ad-libitum* (Aganga et al., 2000) during the experiment. Animals were acclimatized for fourteen days during which they were standardized for the experiment.

### Standardization of animals

The animals were examined for external, gastrointestinal and blood parasites to ensure that they were free from infestations and infections. About 5 g faecal sample was scooped from the rectum of each animal using two fingers of a gloved hand, into labeled polyethylene bags and examined using floatation and sedimentation tests (Charles, 2007). Four milliliters of blood was collected from each experimental animal through jugular vein using 5 ml syringe and 19 G × 1<sup>1</sup>/<sub>2</sub> inch needle (Weiser, 2012a; Wolfensohn and Lloyd, 2013). Three milliliters of the obtained blood was anti-coagulated in blood sampling bottle containing ethylenediaminetetraacetic acid (EDTA) + K<sub>3</sub> 1 mg/ml of blood (Elaine and Margi, 2007). The blood was used to prepare 2 slides of Giemsa stained thin blood smear and 2 haematocrit centrifugation technique (HCT) capillary tubes (Wosu, 2002) which were examined microscopically for haemoparasites. The balance of 1 ml collected blood was transferred

into sodium heparin-coated bottle as anticoagulant and was instantly used to inoculate 2 representative mice per donkey (0.5 ml blood/mice) intra-peritoneally (World Organization for Animal Health (OIE), 2010) using 1 ml sterile disposable syringe with 29 G × 1/2 inch needle as recommended by Wolfensohn and Lloyd (2013). Blood of the inoculated mice were also collected from tail tip at 48 h interval and examined using 2 each, of prepared HCT capillary tubes and wet film for haemoparasites. The mice were observed for 14 days post-inoculation during which all mice were negative for haemoparasites based on which we continued with the next phase of the experiment. The animals were dewormed with Fenbendazole bolus (Fenacure<sup>®</sup>, Ashish Life Sciences PVT Ltd, India) at the dose rate of 10 mg/kg bd wt orally, once (Aliu, 2007). The experimental animals were managed as recommended in the European Union 'Directive 2010/63' contained in the report by Wolfensohn and Lloyd (2013) and approved by the Ahmadu Bello University (ABU) Research and Ethics Committee.

### Evaluation of vital parameters and blood samples collection

Respiration, pulse and rectal temperature were evaluated, and blood samples were collected 4 times consecutively at 7 days intervals between 600 and 800 h local time. Animals were observed individually while at rest for respiratory rate (breaths/minute) using costo-abdominal movement, pulse rate (beats/minute) from pulsation of external maxillary artery in the medial aspect of mandibular angle and rectal temperature in degrees Celsius (°C) using digital clinical thermometer as reported by Wosu (2002). Six milliliters of jugular vein blood was collected from each animal using 10 ml syringe with 19 G × 1<sup>1</sup>/<sub>2</sub> inch needle for determination of blood glucose in mg/dl. Three milliliters of the collected whole blood was placed in labeled blood sample bottles containing 1 mg EDTA + K<sub>3</sub>/ml of blood, and used for haemogram evaluation, while the remaining blood was saved in labeled plain vacutainer bottle and used for serum extraction. Serum was harvested by allowing whole blood to clot at room temperature for 30 min, gently decanted the sera into labeled serum vials, insufficiently sedimented blood samples were centrifuged at 5,000 g for 5 min to improve serum yield. Between 0.8 and 1.5 ml serum was obtained from each processed blood sample. Blood and sera were stored at 4 and -20°C, respectively until analysed (Charles, 2007; Weiser, 2012a).

### Laboratory protocols

#### Haemogram evaluation

Packed cell volume (PCV), total red blood cells (RBC), total leucocytes, differential leucocytes counts and haemoglobin (Hb) concentration were evaluated as reported by Weiser (2012b).

#### Serum biochemical assay

Blood glucose was evaluated with the aid of handheld, digital blood glucose monitor (Fora<sup>®</sup>G20, Fora Care Inc., U.S.A.) in mg/dl using a tiny drop of whole blood (about 0.7 µl) placed on the tip of the strip as recommended by the manufacturer. Total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine (CR) levels were determined from the stored sera with the aid of fully-automated clinical chemistry analyzer (Selectra XL<sup>®</sup>, Vital Scientific, Netherland). The machine holds 80 samples, 30 standard reagents cups, have 2 reactions

**Table 1.** Mean baseline vital parameters of experimental Donkeys (N = 96),

Parameter	Mean±SD	Unit
Respiration	19±6	Breaths/minute
Pulse	38±6	Beats/minute
Rectal temperature	37±1	°C

Standard deviation (SD)

**Table 2.** Mean baseline haematological parameters of experimental donkeys (N = 96).

Parameter	Mean ± SD unit
Packed Cell Volume, PCV	37±6%
Haemoglobin, Hb	12.2 ± 2.1 g/dL
Total Red Blood Cell, RBC	6.0±1.1 ×10 <sup>12</sup> /L
Total White Blood Cell, WBC	7.2±3.7 ×10 <sup>9</sup> /L
Neutrophils	4.9±2.3 ×10 <sup>9</sup> /L
Lymphocytes	2.1±1.5 ×10 <sup>9</sup> /L
Monocytes	0.1±0.2 ×10 <sup>9</sup> /L
Eosinophils	0.0±0.1 ×10 <sup>9</sup> /L
Basophils	0.0±0.0 ×10 <sup>9</sup> /L
Band Cells	0.1±0.1 ×10 <sup>9</sup> /L

Standard deviation (SD)

chambers that hold 24 cuvetts (cups) in a single reaction run and 2 wash stations for self cleaning after each sampling. In a run, 250 µl of each serum sample was collected using Eppendoff pipette and dispensed into sample cup. The cups with samples were loaded into the machine sample rotor as recommended by the manufacturer. The machine used between 6 to 12 µl of each sample to determine the level of a substance. Results were generated, recorded and printed automatically. After dispensing of each sample, the auto-pipettes were cleaned at the 'Wash Station' within 3 s before collection of another set of sample and reagents.

#### Serum electrolytes assay

Calcium (Ca<sup>2+</sup>), phosphate (Po<sup>4-</sup>), sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), chloride (Cl<sup>-</sup>) and bicarbonate (HCO<sub>3</sub><sup>-</sup>) ions were assayed with the aid of an automatic electrolyte analyzer (Audicom AC9900®, Audicom Medical Technology Ltd, Jena, Germany). The system holds 20 sample cups, has 2 quality control (QC) reagent loading points (QC<sub>1</sub> for electrode activation reagent and QC<sub>2</sub> for calibration reagent), one wash station containing electrode deproteinizing standard reagent and 1 emergency call. From each sample, 200 µl of the thawed sample was collected using Eppendoff pipette, dispensed into sample cup and the filled cups were loaded into serially numbered cups holders and the machine was set to run the samples as recommended by the manufacturers. Different electrodes determine the concentration levels of each element in a sample. Analysis of a sample and generation of printed result of 5 elements was completed within 120 s before collection of another sample.

#### Data analysis

The statistical package used was SPSS Version 17. Means of parameters were determined and tables prepared using Microsoft Excel 2010.

## RESULTS AND DISCUSSION

The baseline data obtained on the parameters was not analysed on the basis of gender and age differences for the fact that earlier reports showed no significant influence of variations in age, sex, lactation status and body condition of donkeys, horses and mules on values of normal haemogram and serum biochemical but variation exist between species (Gul et al., 2007). Where variation are observed between the values from this study and earlier reports on donkeys (Tables.1-3), it could be as a result of bio-variations in breeds and environmental conditions (de Aluja et al., 2001; Tesfaye et al., 2014) such as stress imposed by seasonal weather changes which can influence variations in values of normal respiratory rate, pulse rate and rectal temperature (Table 1) of donkeys (Ayo et al., 2008). Haemogram values (Table 2) and biochemical values (Table 3) are also reported as not significantly influenced by age, sex, lactation status and body condition differences in donkeys, horses and mules but variation exist between species (Gul et al., 2007). Findings by Annita et al. (2013) indicated no differences in values of creatinine kinase, albumin, urea and magnesium among age groups of donkeys. Serum biochemical values are reported to be, most of the time, within related ranges globally in donkeys, however, creatinine and aspartate amino-transferase can be low in some breeds of donkeys (Sow et al., 2014). Values of fractions of blood proteins also do not vary between sexes in donkeys (Paulo et al., 2012). Therefore, knowledge of the deviations of laboratory clinical examination findings from the published normal physiological values is an essential tool for accurate diagnoses of diseases of donkeys (Mori et al., 2003, 2004). The results were; respiratory rate = 19 ± 6 breaths/minute; pulse rate = 38 ± 6 beats/minute and rectal temperature = 37 ± 1°C. PCV was 37 ± 6%, Hb = 12.2 ± 2.1 g/dl, red blood cell (RBC) = 6.0 ± 1 × 10<sup>12</sup>/L, white blood cell (WBC) = 7.2 ± 3.7 × 10<sup>9</sup>/L, neutrophils =



**Table 3.** Mean baseline serum biochemical parameters of experimental donkeys (N=96).

Serum/blood parameter	Means±SD	Unit
Total protein, TP	6.9±3.6	g/dl
Albumin, ALB	3.9±3.9	g/dl
Aspartate-aminotransferase, AST	38.5±15.0	IU/L
Alanine- aminotransferase, ALT	50.0±14.6	IU/L
Alkaline-phosphatase, ALP	72.2±8.4	IU/L
Creatinine, CR	71.6±7.5	mmol/L
Blood Urea Nitrogen, BUN	4.3±0.8	mmol/L
Calcium, Ca	2.4±0.1	mmol/L
Phosphate, PO <sub>4</sub>	1.1±0.1	mmol/L
Sodium, Na	139.6±2.9	mmol/L
Potassium, K	4.0±0.5	mmol/L
Chloride, Cl	98.8±3.5	mmol/L
Bicarbonate, HCO <sub>3</sub>	24.0±2.3	mmol/L
Blood glucose	76.0±6.9	mg/dl

Standard deviation (SD).

4.9 ± 2.3 × 10<sup>9</sup>/L, lymphocytes = 2.1 ± 1.5 × 10<sup>9</sup>/L, monocytes = 0.1 ± 0.2 × 10<sup>9</sup>/L, eosinophils = 0.0 ± 0.1 × 10<sup>9</sup>/L, basophils = 0.0 ± 0.0 × 10<sup>9</sup>/L and band cells = 0.1 ± 0.1 × 10<sup>9</sup>/L. Blood glucose was 76.1 ± 6.9 mg/dl, total protein = 6.9 ± 3.5 g/dl, albumin = 3.9 ± 3.9 g/dl, aspartate aminotransferase = 38.5 ± 15.0 IU/L, alanine aminotransferase = 50.0 ± 14.6 IU/L, alkaline phosphatase = 72.2 ± 8.4 IU/L, creatinine = 71.6 ± 7.5 mmol/L, blood urea nitrogen = 4.3 ± 0.8 mmol/L. Electrolyte values were: calcium = 2.4 ± 0.1 mmol/L, phosphate ion = 1.1 ± 0.1 mmol/L, sodium = 139.6 ± 2.9 mmol/L, potassium = 4.0 ± 0.5 mmol/L, chloride = 98.8 ± 3.5 mmol/L, and bicarbonate = 24.0 ± 2.3 mmol/L.

## Conclusion

The values generated from this work will serve as additional baseline data for accurate laboratory diagnosis of diseases of donkeys especially for the young animals aged 11 to 15 months in North-Western part of Nigeria.

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## Conflict of interest

The authors have no conflict of interest.

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Review

## Elastography: Principles and considerations for clinical research in veterinary medicine cibe

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Since the beginning of medicine, palpation has been the primary semiologic method used to detect abnormalities during clinical examination. The mechanical properties of soft tissues are usually related to changes in stiffness. However, changes in stiffness and echogenicity of soft tissues are not always correlated. Elastography has emerged in the last decade as a new method that, when used in association with ultrasonography, can provide information about the acoustic properties (echogenicity and texture) and mechanical attributes (stiffness) of a region of interest in a specific tissue. The objective of this article is to review the literature on the physical principles of elastography and to describe several features of the elastographic imaging process to provide a better understanding of this new technique and its potential utilization in the veterinary field.

**Key words:** Ultrasonography, elastography, shear wave, stiffness, tumor.

### INTRODUCTION

Since the beginning of medical practice, palpation has been used to detect the presence of abnormalities in tissue consistency that could indicate the presence of pathology. The success of this method rests on the fact that the mechanical properties of diseased tissue are typically different from those of the adjacent normal tissue. However, palpation is of limited usefulness in detecting masses located deeply in relation to the skin surface. The assessment of other properties associated with diseased tissue, including water content, tissue density and acoustic interaction capacity, has prompted the emergence of the diagnostic imaging field, which currently makes possible imaging that offers resolution far beyond the limits of palpation (Konofagou, 2004).

The mechanical attributes of soft tissues depend on

their molecular constitution (the presence of fat, collagen fibers, elastin and water, among others) and on the micro- and macroscopic structural organization of the tissues. These attributes include the elasticity, stiffness and mobility shown by a tissue in response to forces applied to it (Konofagou, 2004). Pathological changes are usually correlated with changes in tissue stiffness in many cases, the small size of a lesion and/or its deep location prevents its detection or evaluation by palpation, despite the fact that it differs from normal tissue in stiffness and mobility. In addition, lesions may or may not exhibit acoustic properties that render them detectable by ultrasonography. Because the mechanical attributes and echogenicity of tissues are not always correlated, combining data on these properties is expected to reflect

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tissue structure and pathology and to provide greater diagnostic accuracy. For example, although harder than adjacent tissue, prostate tumors may be sufficiently small to be not observable by normal ultrasonography. This is also true in the case of diffuse diseases; liver cirrhosis, for example, significantly increases the stiffness of liver tissue, although in early stages of the disease sonographic images may be within normal limits on conventional ultrasonography examination (Konofagou, 2004).

Elastography, which has emerged in the last decade, offers a very promising imaging method when combined with ultrasonography. Used together, these methods provide data on the acoustic properties and mechanical attributes of an area of interest in relation to adjacent tissues (O'Brian and Holmes, 2007). The proper interpretation of images generated using this technological resource requires an understanding of the mechanical attributes of the tissue in question, that is, the relationship between stiffness and elasticity, as well as knowledge of the various processing methods that can be used with elastographic images (O'Brian and Holmes, 2007). This study aims to perform a review of the literature published on the physical principles of elastography with the goal of helping practitioners to understand the potential applications of this new diagnostic tool.

## TISSUE MECHANICAL PROPERTIES

The responses of tissue to applied mechanical forces (that is, compression, traction, tension and elasticity) are considered their mechanical properties. Depending on the material (live tissue) and temperature, the tension (stress) applied to a medium is approximately proportional to the deformation (strain) on the tissue. The ratio of proportionality between the applied stress and the resulting strain is called the elasticity modulus or Young's modulus (Lai, 2009). Young's modulus ( $E$ ) is a measure of a material's resistance to compressive deformation. Tissue deformations occur in response to stress ( $\sigma$ ) applied to the tissues, and this stress is related to the force applied. The deformation that occurs is known as the strain ( $\epsilon$ ). Tissues with higher Young's moduli, such as fibrous tissues, are more resistant to deformation than other more compliant tissues such as fat.

To understand the application of these physical principles to the elastographic method and because soft tissues are typically composed of heterogeneous and complex material, it is also important to understand the following:

1. The force applied to the tissue is considered static; that is, the data acquisition time is very short compared to the time during which the force acts to cause changes. Thus, the applied force may be considered constant during data acquisition, reducing the complexity of the resulting dynamic

dynamic viscoelasticity equation.

2. The tissue axial displacement is very small, on the order of less than 1%; therefore, the resulting equation is considered to describe a linear force (that is, the amount of strain resulting from an applied stress is not a function of the absolute stress applied).

3. The material is elastic (that is, the tissue returns to its non-deformed state when the applied stress is removed, and the deformation state is not dependent on the rate of the applied stress), isotropic (that is, the tissue's material properties are not orientation-dependent) and incompressible (that is, the volume of the tissue remains the same when strained due to its high water content) (Sarvazyan, 1993).

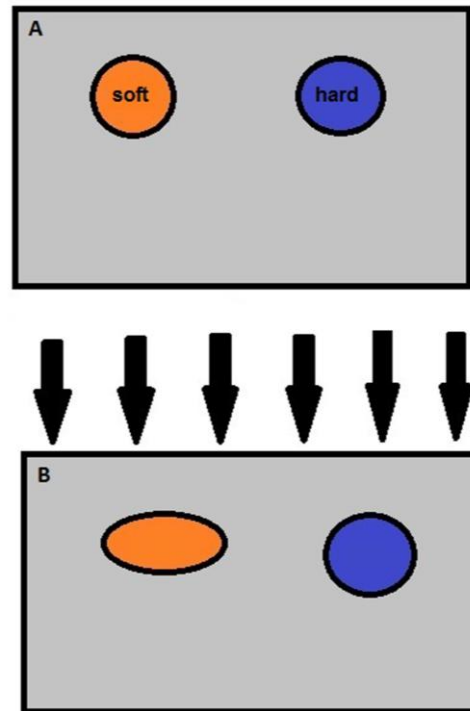
To simplify the analysis and interpretation of elastography, stress and strain can be related to each other by Young's modulus (that is, stress is a function of strain):  $\sigma = E\epsilon$ .

When an elastic medium undergoes compression due to the application of a constant uniaxial force, all points in the medium whose main components are arranged along the axis of compression and motion experience some level of longitudinal displacement. The resulting level of displacement of a given element will be greater or smaller if one or more tissue elements have stiffness parameters that differ from those of other elements (Ophir et al., 1991; Sarvazyan, 1993). A harder tissue element will undergo less deformation when subjected to compression than other elements of lower stiffness (Figure 1).

The longitudinal (axial or lateral) displacement of the elements of a tissue can be estimated through the analysis of ultrasound signals detected using a diagnostic ultrasonography device. If the amount of tension necessary to obtain a certain degree of deformation of the medium is known, the elasticity of the medium being studied can be calculated. The higher the modulus of the medium is, the greater the tension required for deformation of the medium; thus, the stiffer the material. The linear relationship between these quantities is known as Hooke's law (Sarvazyan, 1993). These data are usually gathered in the following order:

1. A sample of radiofrequency echoes obtained in the region of interest in the tissue under study is collected.
2. A small compression of the tissue is performed using a transducer along the same direction of the sound beam.
3. A second linear echo sample is acquired after compression of the same region of interest (Ophir et al., 1991; Palmieri and Nightingale, 2011).

The data are compared using cross-correlation techniques in which similar linear echoes are subdivided into small time windows and paired, thereby permitting mathematical calculation of the change in the region of interest after compression at several points within the area (Ophir et al., 1991). Tissues may show greater or



**Figure 1.** Effect of a compression force on a tissue with focal lesions of various origins. The harder lesion is less deformed than the normal tissue.

smaller viscoelastic behavior depending on the presence and quantity of certain components, including fluid flow at the site and the ability of fibers within the tissue to undergo stress relaxation. Thus, most soft tissues appear isotropic when a mechanical stress force is applied (Parker et al., 1990; Sarvazyan, 1993; Palmieri and Nightingale, 2011), although there is evidence that some soft tissues, for example, muscles, possess anisotropic mechanical and ultrasonographic properties (Levinson, 1987).

The introduction of viscosity to the tissue description allows tissue stiffness to be analyzed as a function of the excitation frequency (that is,  $E(f)$ ). Higher frequency excitations yield stiffer tissue responses than lower frequency excitations (Sarvazyan, 1993). The use of a device that permits results to be obtained in real time, as is the case in ultrasound imaging, is optimal for mathematically describing the mechanical behavior of the analyzed tissues and determining all the factors involved. Elasticity imaging modalities generate images of tissue stiffness (Ophir et al., 1991; Palmieri and Nightingale, 2011). They accomplish this by applying stress to the tissues using an external excitation source, an internal, physiological motion source or an acoustic radiation force and measuring the resulting deformation (displacement) that occurs in response to the applied stress. Based on a known stress/strain relationship, the deformation that occurs in response to the applied stress can be related to the

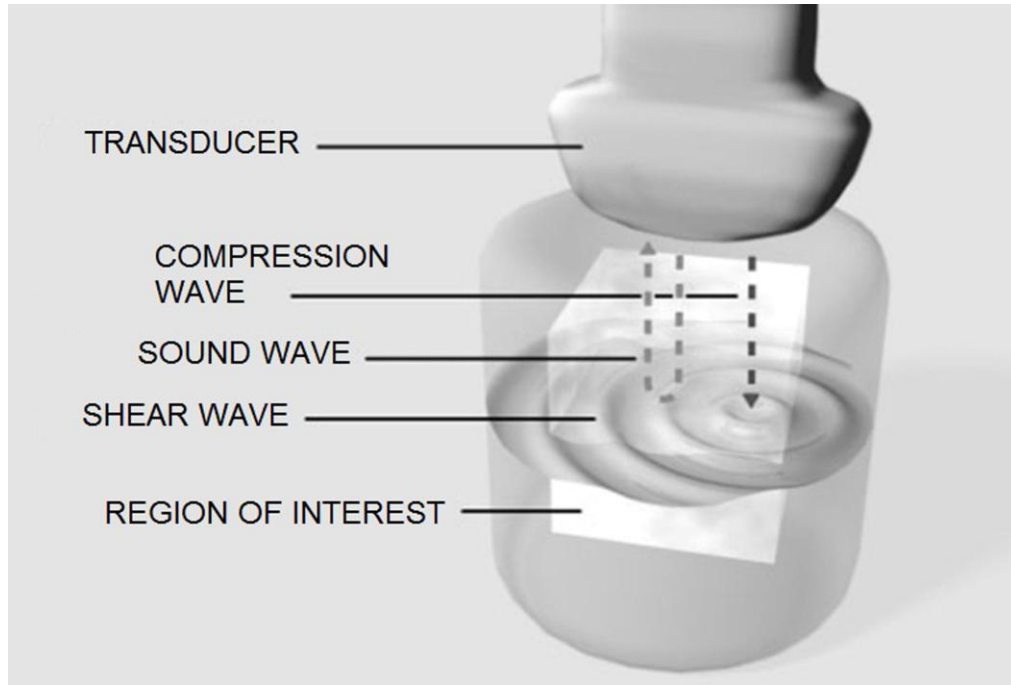
tissue stiffness.

## FORCES AND STRESS SOURCES

Elasticity imaging requires a source of stress to deform tissue so that relative or absolute responses to that stress can be measured to generate elasticity images. These excitation sources can be external to the body and include mechanical punches, vibrating rods and compression plates. We may consider two types of waves resulting from a specific stress source traveling through a medium: compression and shear waves (Figure 2).

### Compression waves

With compression waves, the particles of a medium oscillate in the direction of motion. The tissues of the body have substantially different mechanical properties because their mechanical functions are different. Furthermore, the estimation and imaging of tissue displacement is, by definition, a three-dimensional problem. When a tissue is deformed, the apparent proximal non-compressibility of most soft tissues means that stress components are generated in all directions simultaneously (Levinson, 1987; Han et al., 2003). In ultrasound



**Figure 2.** Schematic drawing showing compression and shear waves traveling through a medium. The compression waves move particles in a medium along the direction of motion, while, when shear waves are applied, the particles oscillate perpendicular to the direction of motion.

elasticity imaging, the ultrasound transducer can be used to apply compression to the tissues of interest through the skin surface. The advantage of using this excitation source is that known stresses or strains can be applied to large volumes of tissue, which can be useful when screening for lesions. A potential drawback to external excitation, however, is the limited penetration of these applied stresses into deep organs of interest (example, the liver in an obese patient) due to attenuation of the stress from the skin surface or a physical barrier such as the presence of abdominal ascites.

### Shear waves

With shear waves particles oscillate perpendicular to the direction of motion. Shear waves are important for elastography because calculation of stiffness is performed using a mathematical formula (Young's modulus) that requires wave velocity data (Hoskins, 2012). Local displacement of the tissue occurs as shear waves pass through it. These local displacements cause changes in the returning echo pattern over time that can be monitored using methods of axial image correlation. However, this requires that the time between subsequent ultrasound pulses be less than 0.5 ms, which is equivalent to a minimum pulse repetition frequency (PRF) of 2,000 Hz. This parameter can be easily adjusted in devices that produce one-dimensional images (for

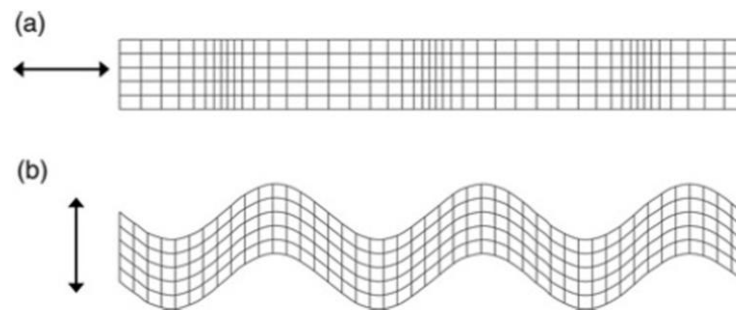
example, in M-mode). However, a PRF is required to produce two-dimensional multiaxial images; such images cannot be obtained using conventional technology devices (Levinson, 1987; O'Brian and Holmes, 2007; Hoskins, 2012).

Another stress source that can be used in ultrasound elasticity imaging to overcome the challenge of introducing stress into internal organs of interest is the acoustic radiation force, the force that results from transfer of momentum from the propagating ultrasonic wave to the sound-absorbing soft tissue. This transfer of momentum applies a force in the direction of the wave propagation; thus, through the use of longer and/or stronger acoustic pulses which are typically used in diagnostic ultrasound, transient tissue deformation on the order of microns can be generated. The acoustic radiation force ( $F$ ) is a force applied over a volume of material, and it is related to the acoustic attenuation of the tissue ( $\alpha$ ), the acoustic intensity ( $I$ ) and the speed of sound ( $c$ ) by the formula  $F=2\alpha I/c$ .

The ultrasound systems that are currently commercially available can be classified according to their capacity for wave emission and detection (Ophir et al., 1991; Nightingale et al., 2002; Palmieri and Nightingale, 2011; Jaffer et al., 2012). The elasticity of tissue can be inferred in three ways: by generation of shear waves using an external mechanical agent; by sonoelastography that involves automatic generation of shear waves and by generation of shear waves using acoustic radiation force

**Table 1.** Comparison of the main features of currently available elastographic techniques.

Method	Stress source	Equipment	Vibration source	Advantages	Limitations
Static elastography	Mechanical compression	Hitachi Toshiba Esaote	Manual	Commercially available	Operator- dependent
Transient elastography	Automaticcompression	Fibro scan	Mechanical transient force	Validated in humans	Body conditions
Acoustic radiation	Shear wave	Siemens phillips	Transient radiation force	Potentially useful in all organs	Expensive; few studies
Supersonic image	Shear wave	Aixplorer	Acoustic radiation force impulse	Faster	Expensive; no standardization

**Figure 3.** Schematic drawing showing the motion of particles in the medium according to the type of acoustic wave. (a) compression waves; (b) shear waves.

or shear force associated with a B-mode image (Table 1). The types of stress sources described above can be applied (quasi) statically (that is, under conditions in which a stress state is applied and held), dynamically (the stress is applied impulsively, that is, there is a transient excitation typically lasting tens to hundreds of microseconds) or harmonically (that is, the stress is applied as a sinusoid of one or more frequencies).

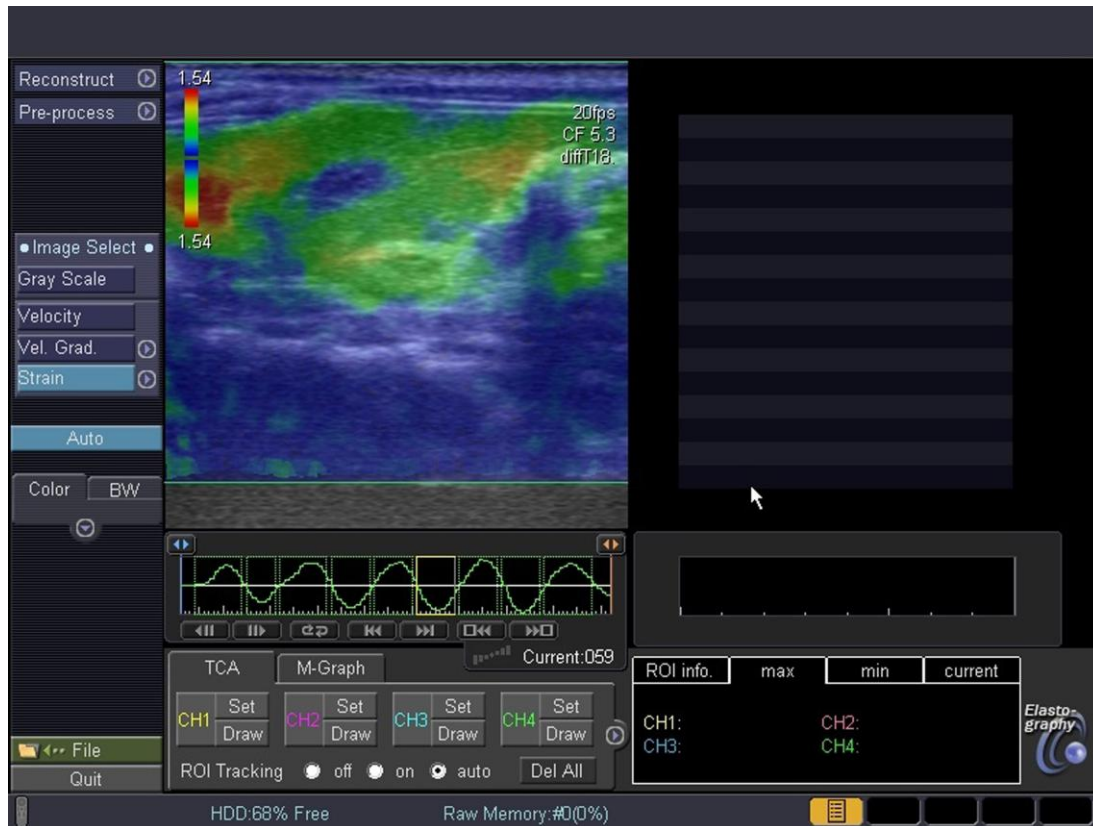
### ELASTOGRAPHY IMAGE PROCESSING

Quantitative evaluation of the mechanical parameters of tissues has yielded a broad range of values (Parker et al., 1990). Most studies of the mechanical parameters of tissues have been performed on tissues (muscles, arteries, lungs, tendons, bones, skin, ureter) that were subjected to a tensile force. Conversely, limited data on the compression features of tissues within organs have been gathered. Even fewer studies have measured the elastic resultants of these tissues *in vitro* (Parker et al., 1990). Some *in vivo* studies were performed; the results of these studies indicate that the mechanical properties of tissues are significantly different under normal conditions and in the presence of tumorous lesions (Han et al., 2003).

The existence of significant differences in the responses

of normal and abnormal tissues to a mechanical stimulus has enabled the development of imaging techniques that reveal the mechanical attributes of a tissue or a region of interest within a tissue (Nightingale et al., 2002; Jaffer et al., 2012). Currently, a variety of techniques can be used to obtain measurements of tissue mechanical properties and process the measurements into an image format that can be used as a diagnostic tool. The commercially available methods can be subdivided into two main types: strain elastography or elastography by mechanical compression waves and shear wave elastography performed by shear wave emission (Figure 3) (Hoskins, 2012).

In shear wave elastography, a force is exerted on the tissue, and the imaging system measures the response of the tissue to that force. There are two basic techniques that can be used; both are based on tissue compression. In the first method, the transducer is moved along an axis; this technique is termed strain elastography (A-line correlation) or static elastography. The other technique, which is called sonoelasticity imaging, transient elastography or Fibroscan, involves the application of low-frequency vibration energy to the tissue followed by simultaneous detection of Doppler ultrasound waves showing the hemodynamic disturbance caused by vibrations (Nightingale et al., 2002). In the latter case, data obtained using the Doppler tool generate a result that shows the elastic modulus calculated from the wavelength



**Figure 4.** Elastographic image of a liver with a hard nodule (in blue color) centrally located in the left medial lobe. The colored bar at the side of the image illustrates the degrees of relative stiffness (harder tissues are shown in blue, and softer tissues range from yellow to red in color). A plot of sinusoids that shows the mechanical compression exerted in the study area is presented below the color image.

at the vibration site; that is, they show only the displacement amplitude related to the elasticity of this modulus also along an axial path (Hoskins, 2012).

The technique termed strain elastography measures the local response of a tissue subjected to a force and transforms the calculations of longitudinal (axial) displacement of the relevant components at different points of a single tissue into an image (Ophir et al, 1991). The resulting image is known as an elastogram (Figure 4). This technique can be used to detect tissue movement, tissue compression and extension along the axis of displacement and are especially used in real-time in clinical practice (Hoskins, 2012). They may be performed in real-time or not, using ultrasound devices equipped with specific software that is capable of evaluating the physical properties of a tissue (Figure 5) and assessing its degree of stiffness in relation to the adjacent tissue through compression of the evaluated site (Jaffer et al., 2012).

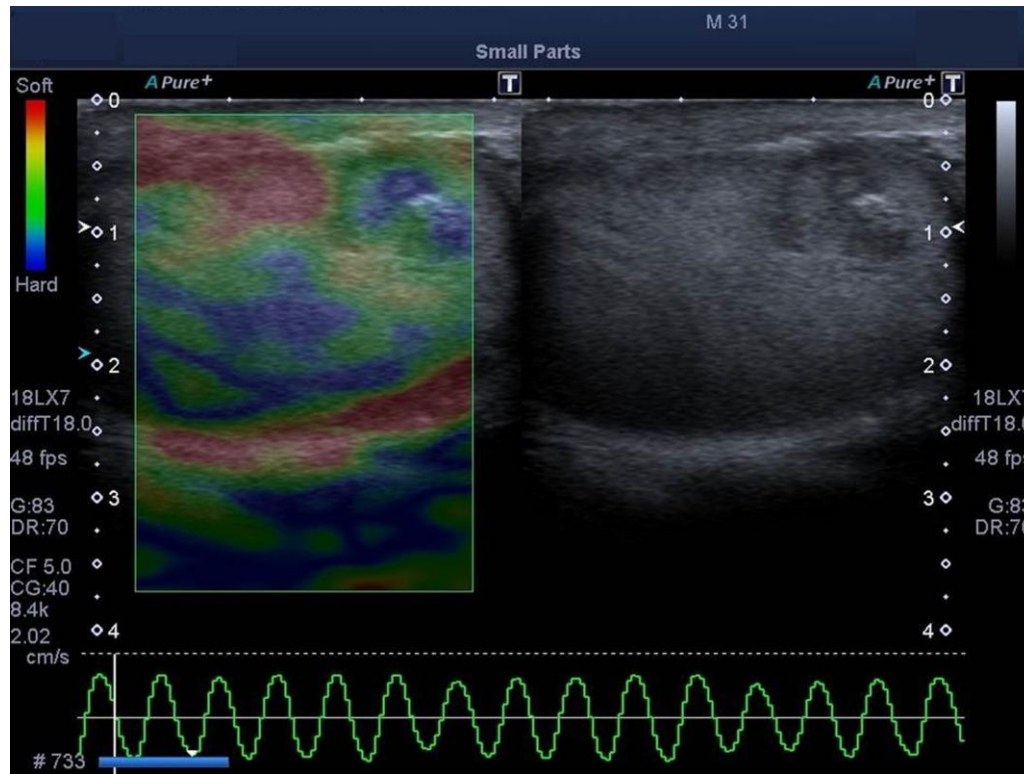
In static elastography, a sound beam is emitted by the transducer while a slight manual mechanical compression is simultaneously performed. An elastographic image is generated from the tissue deformation, with

the software performing a comparative analysis of the moment of compression and the resultant after compression. Harder tissues deform less upon compression, while tissues more susceptible to compression show greater deformation (Saftoiu et al., 2007). The deformity experienced by the tissue is represented on a color scale, according to the elastic variation: red corresponds to softer tissues, green to tissues showing intermediate deformity, and blue to tissues with less deformation, that is, of greater stiffness (Saftoiu et al., 2007).

It is important to note that strain elastography does not provide an exact mathematical image of the elastic modulus itself. In strain elastography, a stiffness index can be estimated by comparing the stiffness of the lesion with that of an adjacent reference region. Thus, the operator must remember that the measurement is sensitive to the positioning of the reference region; to obtain a reliable index, the reference region should be at the same depth as the lesion under study (Figure 6). This is important because the stress caused by compression (that is, the force per unit area) often changes with depth.

In a uniform tissue such as the liver or at a phantom, stress and elasticity decrease with increasing depth





**Figure 5.** Strain elastography of a testicle with a nodular focal lesion of greater stiffness than the adjacent tissue and suggestive of malignancy.

(Hoskins, 2012). Thus, depth becomes a limitation because lesions at depths greater than 5 cm cannot be compressed sufficiently to be evaluated by this imaging technique. This primarily occurs in organs located at greater depths or in obese patients (Hoskins, 2012). Variations of elastography techniques, including real-time elastography and transient elastography, have been described in the literature. These variations are the result of technological development aimed at finding optimal methods for different clinical indications (Hoskins, 2012).

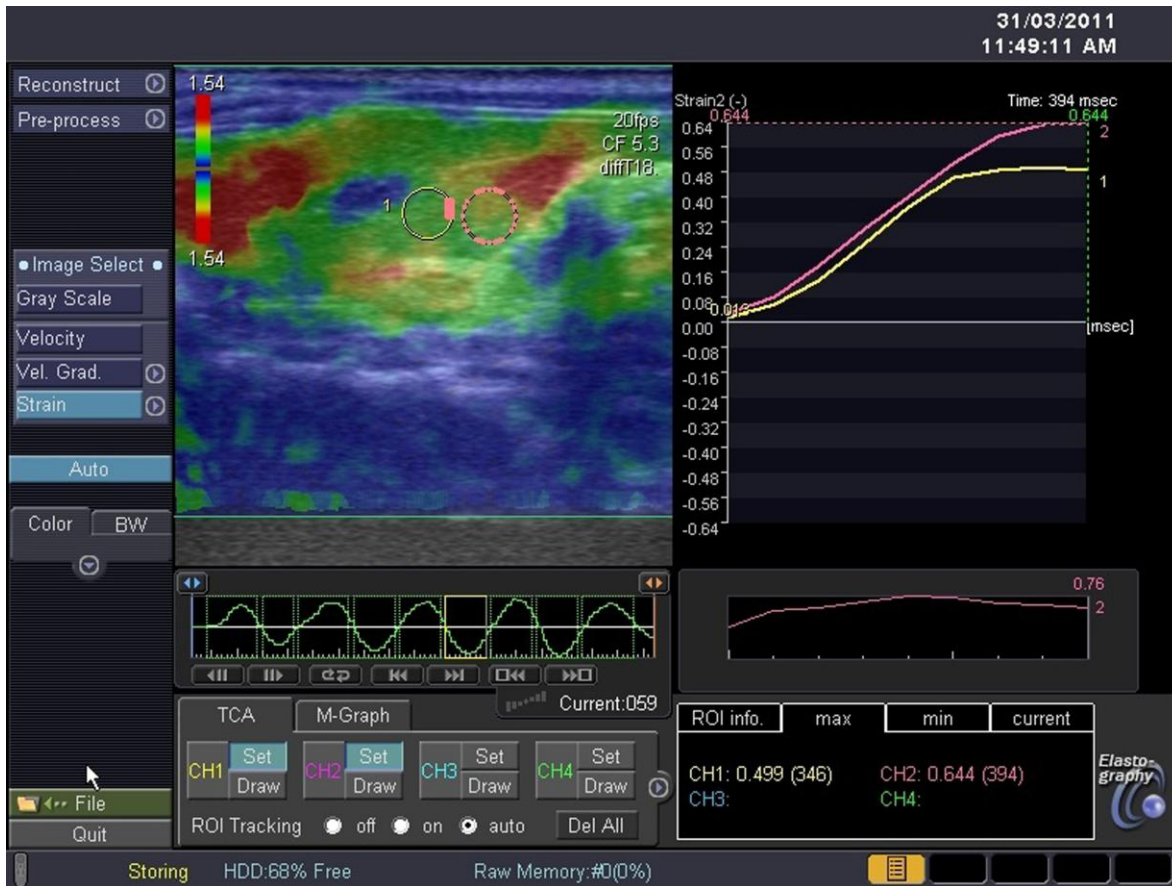
Another technique that has been described in the literature is based on the propagation of an acoustic radiation force impulse (ARFI) or a shear waveform. This force is capable of causing tissue displacement without the need for manual compression. This technique can provide both strain and sheare wave data (Figure 7). The displacement caused by the shear wave is minimal, although the focus of a high-mechanical index pulse may produce displacements of up to 20  $\mu\text{m}$ , with the tissue returning to its initial position in 5 ms (Nightingale et al., 2002). The displacement can be detected using the strain elastography technique and can be used at greater depths and they are permitted by simple strain elastography because the force originates directly from the focal region of the transducer. Moreover, the subjectivity of the measurements resulting from the variation in compression force applied by the operator

ceases to exist with this method. Thus, the method is more accurate and involves less inter-observer variation and greater reproducibility (Jaffer et al., 2012).

Shear wave elastography involves a step forward in the reconstruction of the elastic modulus resulting from axial displacement in real-time, as it measures the waves able to cause elastic deformation in the direction of motion, thereby enabling the user to calculate the tissue stiffness from the data gathered in the region of interest (Kallel et al., 1999). Several studies that show the accuracy of estimates of the displacement of waves in various tissues have been published (Konofagou et al., 1999). Shear wave elastography or shear wave elastography is currently performed using devices that rely on focused ultrasound wave propagation to generate marginal and orthogonal (or shear) waves within the organ. This process may be performed using either ARFI imaging devices or supersonic shear wave imaging devices (Konofagou et al., 1999).

## PRIMARY APPLICATIONS OF ELASTOGRAPHY

Among the primary applications of the method, it is important to emphasize the use of so-called transient elastography. Transient elastography was the first elastography method to be commercially used. It is performed



**Figure 6.** Elastographic image of two adjacent regions of interest and the resulting plots of comparative stiffness.

using a device termed Fibroscan® that was specifically developed to quantify liver fibrosis without, however, forming an image. This device has the ability to send elastic waves together with the sound beam. These waves go through the tissue at speeds directly proportional to tissue stiffness. The technique offers advantages over conventional biopsy for the quantification of liver fibrosis: it is risk-free for patients, it is painless, and it evaluates liver tissue within a 1-cm-thick and 2-cm-deep cylindrical fragment that is 100 times larger than the fragment removed by biopsy (Serejo et al., 2007; Rivero-Juárez et al., 2012). However, studies show that some conditions associated with chronic liver disease, including liver inflammation and congestion, biliary obstruction and portal hypertension, may increase the elasticity of the liver, making the method inconclusive in 20% of cases (Friedrich-Rust et al., 2008; Castera and Pinzani, 2010). Furthermore, some technical difficulties, including the high cost of the device, the long user learning curve and the impossibility of performing the technique in the presence of ascites and in obese patients, limit the use of the method (Hoskins, 2012).

Mechanical or real-time elastography involves the use of ultrasound devices with specific software and offers

the advantages of lower cost and ease of operation. Most ultrasound device manufacturers produce machines that use this method. Notwithstanding its advantages, mechanical elastography is considered operator-dependent because it requires manual application of the force; for this reason, it has low reproducibility. The literature reports that mechanical elastography has been used as an auxiliary method in conventional ultrasound imaging for differentiating malignant from benign focal lesions (O'Brian and Holmes, 2007). Preliminary studies of its use in liver tissue have aimed to standardize the technique and evaluate its diagnostic value in detecting focal and diffuse lesions, differentiating malignant and benign processes (Carvalho et al., 2012), and detecting degenerative and inflammatory processes (Orlacchio et al., 2012) in addition to quantifying fibrosis in human (Boozari et al., 2010) and canine (Rivero-Juárez et al., 2012) patients. For these reasons, many expectations have been generated regarding this new technique (Jaffer et al., 2012).

Lastly, methods based on shear wave propagation (ARFI and supersonic shear waves) offer several advantages compared to other methods. They enable the procedure to be combined with routine real-time ultrasound



**Figure 7.** Shear wave elastography of a focal liver lesion showing a speed ( $V_s$ ) of 2.03 m/s at a depth of 5.4 cm, suggesting a greater stiffness in the region than in the normal adjacent tissue.

ultrasound even in overweight patients or in patients with ascites because they generate greater penetration of the mechanical wave. Furthermore, the technique provides better accuracy in the calculation of tissue elasticity and better reproducibility because the result is independent of the skill of the operator (Friedrich-Rust et al., 2009; Muller et al., 2009; Boursier et al., 2010). The primary disadvantages of the shear wave propagation techniques are related to the cost of the device, which often precludes its use outside of reference centers for human and veterinary medicine. We may also add that another disadvantage of the shear wave propagation method is the size of the sample measured, which is small and not always significant in relation to the whole organ in the case of diseases without diffuse parenchymal infiltration (Friedrich-Rust et al., 2008). Studies using this technique are still very recent, and there are no established benchmarks in the veterinary literature.

Elastography has the potential to be more challenging in animals than humans. Obtaining measurements from deeper regions within the liver was sometimes problematic as these regions had a tendency to show increased movement during the respiratory cycle, compared with the more subtle movement of the superficial tissues. Breath holding is commonly used in human elastography but, in veterinary medicine, is not possible to request conscious patients to breath hold or remain

still; therefore, the values obtained are likely to be more variable. Some patients that were uncooperative and moving and patients that were panting heavily were noted to produce invalid readings (XXX readout). To minimize the impact of respiratory motion on the accuracy and reliability of results, efforts can be made to time measurements such that acquisition to occur during the normal end-expiratory pause. Another possible solution, in some instances, would be to sedate or anesthetize animals for the procedure to prevent voluntary movement, permit control over respiration and allow breath-hold techniques to be employed (Holdsworth et al., 2014). Besides the application of the Elastography on the evaluation of the hepatic parenchyma, recently, in several case reports this technique is also being used on the evaluation of different organs.

This technique has proved to be immensely useful in assessing lymph nodes in the neck and maxillofacial region (Tan et al., 2010). Information on lymph node stiffness would likely be clinically useful for guidance of percutaneous biopsy and/or nodal dissection. Such information might also improve patient follow-up by enabling early detection of cancer recurrence (Lyshchik et al., 2007). Elastography has been used to identify metastatic lymph nodes, measure masseter stiffness (Ariji et al., 2009) for the purpose of massage and to evaluate focal lesions in major salivary glands (Bhatia et

al., 2010; Das et al., 2011).

Pancreatic applications of ultrasound elastography are relatively recent because of the difficulty in pressing the pancreas, which is located deep in the body. The lesion detection rate and diagnostic rate could be improved to over 90% by the combined use of B-mode and elastographic images, compared to using b-mode images alone (Uchida et al., 2009; Park et al., 2014). This technique has been reported to be useful in characterizing and differentiating normal pancreas, chronic pancreatitis and acute pancreatitis. In a study using ARFI elastography to evaluate the pancreas tissue, the authors conclude that mean ARFI values less than 2,2m/s for pancreatic parenchyma may be exclude an acute pancreatic inflammatory disease. If mean ARFI value is higher than 2,2m/s, an acute pancreatitis attack may be diagnosed. Higher ARFI values in acute pancreatitis are due to increased fluid content in the inflamed organ making the tissue hard (Mateen et al., 2012). Another study (Janssen et al., 2007) concluded that chronic pancreatitis and hard tumors cannot be distinguished by elastography, probably because of their similar fibrous structure and calcification, leading to high ARFI values (Janssen et al., 2007; Park et al., 2014).

Spleen stiffness as measured by noninvasive imaging modalities, as elastography, has more recently been reported to be a marker for prediction of portal hypertension (Grgurevic et al., 2011). Spleen stiffness as measured by the mean shear wave velocity has a close relationship with portal vein pressure and can be used to predict the presence of cirrhosis and varices in patients with liver fibrosis and portal hypertension. When portal vein pressure is higher, the spleen is stiffer, and the mean shear wave velocity is increased, whereas when portal vein pressure is lower, the spleen becomes softer, and the mean shear wave velocity is decreased (Gao et al., 2012). The explanation for these results would be that portal hypertension would initially and directly induce hypersplenism with associate histologic changes in the spleen (Ran et al., 2012).

Some recent studies showed that acoustic radiation force impulse (ARFI) elastography was useful for the differentiation between benign and malignant thyroid nodules. The mean shear wave velocity value of malignant thyroid nodules was significantly higher than that of normal tissue or benign nodules (Zhang et al., 2014). In a study about clinical application of thyroid elastography including 92 consecutive patients with a single nodule, the researchers calculated the sensitivity to be 97% and the specificity to be 100% from predicting thyroid malignancy (Rago et al., 2007). In practice, elastography is usually performed as an extension of conventional US and not as an independent test (Kwak and Kim, 2014). Vorländer et al. (2010), confirmed the possibility of measuring the increased stiffness in Hashimoto disease by using magnetic resonance elastography and in other study was reported the possible

possible usefulness of ARFI to confirm the presence of Graves disease and chronic autoimmune thyroiditis (Zaleska-Dorobisz et al., 2014).

Another common application of ultrasound elastography is in breast examinations. The combination of conventional ultrasound, elastography and virtual touch tissue quantification technology are proven to have high diagnosis performance on detecting breast lesions and diagnostic of breast cancer. The stiffness of breast lesions is closely associated with the degree of malignancy, and is the basis for the evaluation of benign or malignant tumors by elastography. However, due to the overlap in the hardness of benign and malignant tumors, actual clinical application would benefit from combining these methods with conventional ultrasound to optimize the accuracy of breast tumor diagnoses. Furthermore, elastography would increase the sensitivity of B-mode sonography in distinguishing benign and malignant lesions, in consequence, could potentially reduce unnecessary biopsies. This seems to be an important aim because nearly 80% of biopsied breast lesions turn out to be benign, according to the American Cancer Society (Jiang et al., 2014; Zaleska-Dorobisz et al., 2014). In a study to evaluate the applicability of acoustic radiation force impulse elastography as a complementary method in diagnosing mammary neoplasia in 50 female dogs, the authors conclude that elastography helped to differentiate between malignant and benign mammary neoplasm. Future studies with larger number of samples should be performed in order to determine the sensitivity and specificity of this imaging technique in the evaluation of mammary tumors in dogs (Feliciano et al., 2014).

Elastography is constantly being improved and it also allows us to show the lesions that are in the prostate gland. This method targeted approach detects high-risk prostate cancer more reliably than biopsy, requires a reduced number of cores for prostate cancer detection, and enhances the overall sensitivity in the combined biopsy setting (Junker et al., 2014). In consequence, it helps in making a decision on whether to carry out a biopsy and thanks to higher precision additional contuses is not necessary (Zaleska-Dorobisz et al., 2014). Trans rectal elastography revealed carcinoma in 93% cases in a study with 97 patients. In these study, the detectability of carcinoma was higher than in trans rectal ultrasound – 59% in 16 patients, but the authors (Junker et al., 2014) noticed that the effectiveness of sonoelastography of the prostate gland depends on malignant tumor size. In the case of foci of 0 to 5 mm the effectiveness of this method is 6/62, 6 to 10 mm is 10/37, 11 to 20 mm is 24/34 and the highest detectability of 14/14 is in the case of tumors bigger than 20 mm (Zaleska-Dorobisz et al., 2014).

Application of these new ultrasound techniques to the kidney has been shown to be possible and the first results are encouraging. However, the kidney is a much more complex organ than the liver, with two compartments and a high vascularity. Increased cellularity and

increased intratubular and interstitial hydrostatic pressure would change and bias the elasticity values obtained within the renal parenchyma. But the correlation between renal elasticity quantification and intrarenal pathological changes is quite controversial in the literature. One possible explanation for such discrepancies is the non-specificity of stiffness changes related to interstitial fibrosis. The results of recent studies suggest that the degree of renal cortical stiffness does not reflect any specific intrarenal change, such as fibrosis, but rather the association of several renal microlesions, especially chronic lesions. Therefore, more experience is needed in preclinical models and in patients' cohorts with pathological correlation to better understand which are the physical factors of variation and the histopathological causes of elasticity changes (Grenier et al., 2013).

## Conclusion

The primary challenges that complicate the widespread and routine use of elastography in human (Palmieri and Nightingale, 2011) and veterinary medicines are:

1. The difficulty of access in obese patients and to some organs, including the liver, given the thoracic skeleton; this is true especially in the case of veterinary medicine because some breeds of dogs have keel-shaped deep chests, complicating liver examination.
2. The need for devices that support the new technology and are affordable.

However, elastographic imaging methods have a promising future given their safety and the good image resolution of current conventional diagnostic ultrasound devices (Table 1) (Palmieri and Nightingale, 2011). The main areas in which we may expect significant progress and clinical research for the application of this technique are:

1. Characterization of the stiffness of tissues with respect to the presence of structural lesions in organs (Holdsworth et al., 2014; White et al., 2014).
2. Monitoring of processes of tissue fibrosis in various organs.
3. Tumor characterization based on the image pattern associated with the tumor's mechanical properties (including stiffness and mobility);
4. Early detection of malignant lesions using three-dimensional characterization of the affected area.
5. Lastly, applications at the cellular level through the use of high-frequency transducers for small organs (Konofagou, 2004).

These are some examples of the numerous possibilities various elastography methods may provide for clinical applications in veterinary medicine in the coming years.

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## Conflicts of interest

The authors declare that they have no conflicts of interest.

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