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RESEARCH ARTICLE

SERUM PROTEIN EVALUATION OF PIT BULL, BULLMASTIFF AND CANE CORSO BREEDS OF DOMESTIC DOG (CANIS LUPUS FAMILIARIS)

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Abstract

The diversity of proteins, their metabolism and protein functions are attributed to a wide range of responses in cells, organs and tissues of animals. The study evaluated specific serum proteins from male and female gender of 3 dog breeds (Pit bull, Bullmastiff and Cane corso). Cellulose acetate electrophoresis technique was used to ascertain the concentrations (g/dL) of the individual serum proteins and compared to the respective normal reference values for domestic dog. Results obtained indicate that the Pit bull (male and female) and the Bullmastiff (female) had serum albumin concentrations that were higher than the normal reference range. An increase above normal in serum globulin (α_1 -globulin, α_2 -globulin and β_2 -globulin) concentrations was observed in the female Cane corso breed, as other dog gender and breed fell within reference range. A comparison based on serum albumin/globulin (A/G) ratio of the dog breeds revealed a normal A/G concentration except for the female Cane corso (0.36 g/dL) which was lower than normal, and the female Pit bull (1.19 g/dL) which was found to be higher. Such abnormal decrease and or increase in these respective serum protein concentrations could be attributed to prolong dehydration due to the dogs' regular activity and low fluid (water) replacement and or incidences of mild to acute inflammatory response/disease due to consumption of a certain diet type over time.

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Introduction:-

Dogs (*Canis lupus familiaris*), a domestic mammal of the Canidae family and order Carnivora, is a subspecies of the grey wolf (*Canis lupus*), and closely related to foxes and jackals. Dogs are one of the two most ubiquitous and popular domestic animals in the world aside cats, serving various purposes to include but not limited to hunting, herding, guarding, as object of scorn or admiration, pets and as friend (DiBartola, 2012). According to Darwin, (1859), there exist a phenotypic diversity of remarkable level displayed among domestic dogs and are arguably the most morphologically variable earthly mammal on land at present, with huge physical variations (coat, colour, shape, size, leg length and width) and a host of other traits. Such developed variations and its maintenance within breeds intrigues humans (Sutter and Ostrander, 2004; Ostrander and Wayne, 2005; Wayne and Ostrander, 2007; Ostrander, 2007).

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The 'Pit Bulls' known to have originated in Europe and England in particular, represents an array of dog breed with similar but striking physical characteristics. The American Pit Bull Terrier and the American Staffordshire Terrier are common references, although Boxers and American Bulldogs are often categorized under Pit Bulls (Lockwood and Rindy, 1986; Collier, 2006). Known for its ability, athleticism and strength, Pit Bulls are used by the butcher for controlling livestock waiting for slaughter, serve as nanny dogs because of their companionship and protection ability of children, and are previously used for baiting. This breed of dog also thrives in its use for drug detection, though its ability is influenced via a combination of factors such as genetic, training, diet type and environment used for breeding. They are generally a healthy breed of dogs but with hip dysplasia, cardiovascular disease and cataract which is believed to influence their lifespan (~ 11-13 years) adversely.

The Bullmastiff believed to have originated in Britain is a large, active and powerful dog notably used for as guards to ward off poachers (Walkey, 1992). Bred by gamekeepers for agility, strength, size and speed, it was a cross of the tough, heavy and aggressive Bulldog with the large, strong and less aggressive Mastiff. It has been adopted and deemed pure by the Kennel club in 1924 to have a genetic background of about 40% Bulldog and 60% Mastiff (Walkey, 1992). Its health concerns have been reported to include hip and elbow dysplasia, bloat, lymphoma, cancer, progressive retinal atrophy and mast cell tumours (Bell et al., 2012; Edwards et al., 2003; Dobson, 2012). These health concerns resulting in its short lifespan (~ 8-10 years) is an interesting rationale for the analysis of its diversity, genome composition and function, and pathophysiology traits (Mortlock et al., 2015).

The Cane Corso is a powerful, large-boned and muscular breed of dog with a noble, confident disposition that seem intimidating to many. Its origin can be traced back to Italy as the name is Cane Corso is derived from a combination of Italian and Latin words. The Italian word "Cane" means dog and Latin term "Cohors" means "Protector" or "Guardian". Common health conditions include hip dysplasia, bloat, panosteitis, entropion, demodicosis and epilepsy, with an average lifespan of 10 to 11 years.

There are innumerable functions of proteins in the body of an animal and these include structural formation of cells, organs and tissues; metabolic role; maintenance of colloid osmotic pressure; pathogenic defence (immunoglobulins); transportation (albumin) and other multifunctional role in plasma. Since 1990s, there has been an expanded studies on individual serum proteins of domestic animals, and this realization has been influenced by monitoring acute phase proteins (APP) levels as a means of assessing inflammation and disease through an innate immune system response (Ceron et al., 2005; Murata et al., 2004; Petersen et al., 2004). A number of serum proteins have been studied and implicated in the prognosis, diagnosis and in some cases treatment of certain domestic animal diseases and some of these specific proteins have been employed as breeding trait targeted towards the production of higher resistant animals.

Methodology:-

Materials/Reagents

Standard materials, test kits and analytical grade reagents were purchased from accredited outlets and used for the purpose of this study.

Sample collection

The blood sample of various male and female dog specie (Pit bull, Bullmastiff and Cane corso) were collected from an accredited dog farm (NG Pet Universal Konzept) located at Eliozu town, Port Harcourt. The three (3) breeds of domestic dogs studied were of same age range. The blood samples were collected through venupuncture into sterile plain sample bottles, allowed to clot, dislodged, and then centrifuged immediately at 5000rpm for 10 minutes. Thereafter, the supernatant (serum) was carefully transferred into a well labelled cryovials using a pipette and kept in a specimen box before storage at -4°C for safe transportation to the research laboratory.

Assay principle and procedure

Electrophoresis fractionation method as described by Kohn (1957) was adopted for the fractionation of the different serum proteins and this involves the use of cellulose acetate as the support medium.

Cellulose acetate sheets were cut into strips, labelled and saturated with Tris Veronal buffer (pH 8.6). Excess buffer was blot-dried between two Whatman filter papers, after which 20µL of a control and test sample were respectively applied onto the plate (10-15mm from one end) with the aid of an applicator. The tank compartment was filled with barbital buffer (pH 8.6) to the point that the platinum wire was covered while the plate was curved for proper

positioning and placed in the tank, with the end containing the sample deposit at the cathode side. Voltage (130V) was passed for 20 minutes, after which the cellulose acetate strips were carefully removed and submerged in a Ponceau S stain for 10 minutes, removed and placed in 2 successive baths of destaining solution (citric acid) until the background becomes completely white. The strip was further air-dried and observed visually, while the plate was soaked in a dehydrating solution (absolute methanol) for 3 minutes before removal and scanning at 525nm. This procedure was repeated for all samples.

Results and Discussion:-

The results of electrophoretic fractionation of serum proteins of the different dog breeds indicate that all but the male Cane corso breed (3.10 g/dL) had abnormal serum albumin levels. Both the male and female of the Pit bull and the female Bullmastiff revealed a higher than normal concentrations of serum albumin (4.10 g/dL, 3.80 g/dL and 3.82 g/dL respectively), while the male Bullmastiff and female Cane corso had a lower than normal concentrations of serum albumin (2.20 g/dL and 2.30 g/dL respectively). The observed result for breeds with higher than normal serum albumin concentrations could be attributed to dehydration, while certain diseases may have likely been responsible for the drop in serum albumin concentration or synthesis as in the case of breeds with lower than normal range. The liver being the only site of albumin synthesis could develop hypoalbuminemia as a major feature of chronic liver disease. In most instances, albumin could also be lost selectively in cases of renal disease (Grauer, 2005), gastrointestinal disease (Kaneko et al., 1965; Meuten et al., 1978), and in intestinal parasitism (Dobson, 1965).

The result of serum globulin concentrations (particularly α_1 -globulin, α_2 -globulin and β_2 -globulin) were increased above normal in the female Cane corso breed, while others (dog breed and gender) fell within reference range. Such abrupt increase in α -globulins could be attributed to early symptoms of inflammatory disease. From scientific reports, elevation in α -globulins (α_1 -antitrypsin and haptoglobin) has been described in dogs with chronic hepatic disease (Sevelius and Andersson, 1995). However, an increase in β -globulins could be associated with acute hepatic disease, suppurative dermatopathies and in cases of nephritic syndrome.

The serum albumin/globulin (A/G) ratio of the dog breeds revealed a normal A/G concentration in all but the male Bullmastiff (0.55 g/dL) which is slightly below normal, the female Cane corso (0.36 g/dL) which is significantly ($p < 0.01$) lower than normal, and the female Pit bull (1.19 g/dL) found to be significantly ($p < 0.01$) higher than the normal range.

Table 1:- Fractionation of different serum proteins of dog breeds.

Breed	Male	Female	Reference range
Serum albumin estimation (g/dL) of different dog breeds			
Pit Bull	4.10	3.80	2.60 – 3.30
Bullmastiff	2.20	3.82	2.60 – 3.30
Cane corso	3.10	2.30	2.60 – 3.30
Serum alpha-1 globulin estimation (g/dL) of different dog breeds			
Pit Bull	0.40	0.30	0.20 – 0.50
Bullmastiff	0.45	0.61	0.20 – 0.50
Cane corso	0.30	0.70	0.20 – 0.50
Serum alpha-2 globulin estimation (g/dL) of different dog breeds			
Pit Bull	0.85	0.70	0.30 – 1.10
Bullmastiff	0.90	0.66	0.30 – 1.10
Cane corso	0.90	1.48	0.30 – 1.10
Serum beta-1 globulin estimation (g/dL) of different dog breeds			
Pit Bull	0.66	0.40	0.70 – 1.30
Bullmastiff	0.50	0.42	0.70 – 1.30
Cane corso	0.60	0.90	0.70 – 1.30
Serum beta-2 globulin estimation (g/dL) of different dog breeds			
Pit Bull	0.60	0.30	0.60 – 1.40
Bullmastiff	0.70	0.54	0.60 – 1.40
Cane corso	1.48	1.44	0.60 – 1.40
Serum gamma globulin estimation (g/dL) of different dog breeds			

Pit Bull	1.39	1.50	0.70 – 1.90
Bullmastiff	1.40	1.75	0.70 – 1.90
Cane corso	0.90	1.80	0.70 – 1.90
Total serum protein estimation (g/dL) of different dog breeds			
Pit Bull	8.00	7.00	5.40 – 7.10
Bullmastiff	6.15	7.80	5.40 – 7.10
Cane corso	7.28	8.62	5.40 – 7.10
Serum albumin/globulin (A/G) ratio of different dog breeds			
Pit Bull	1.04	1.19	0.59 – 1.11
Bullmastiff	0.55	0.95	0.59 – 1.11
Cane corso	0.74	0.36	0.59 – 1.11

Comparison was between gender and breed. Values are considered significantly different at $P < 0.01$ with post hoc LSD test * $P < 0.01$.

Conclusion:-

The elevated levels of albumin in the serum of the Pit bulls (both gender) and that of the female Bullmastiff breed suggests a case of prolong dehydration occasioned by insufficient water consumption due to effect of dry/sunny weather or animal's activity (barking or playing). The low levels of albumin in the serum of the male Bullmastiff and female Cane corso breeds could suggest an onset or mild disease condition likened to renal or gastrointestinal disease, or acute hepatic disease occasioned by loss of albumin, inadequate or impaired albumin synthesis. The increased levels of the globulin subunits (α - and β -) could be attributed to signs of mild to acute inflammatory disease, liver cirrhosis, suppurative dermatopathies or nephritic syndrome in dog which is greatly influence by their prolonged diet type or formulation.

Conflict of Interest

Authors express no conflict of interest in this research work.

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