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# Serotonin and monoamine oxidase levels on Kintamani Bali dogs (*Canis lupus familiaris*) at different origin in Bali, Indonesia

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## Abstract
Previous researchers stated that dog behavior is influenced by many factors such as place/origin, the environment, food, and training. No exception the dog's aggressiveness can be influenced by management system of the owner. This study took a sample of dogs from different places, namely from the hilly places in the Kintamani highlands and from urban Denpasar. As a result of different places and management will have an impact on different behaviors/aggressiveness. The purpose of this study was to determine levels of serotonin (SrO) and monoamine oxidase (MAO) enzyme in Balinese Kintamani dogs (*Canis lupus familiaris*) from different origins on dog aggressiveness. For this purpose, we used forty dogs, 15 male dogs and 25 female dogs were used in this study. The cross-sectional design of observational research is used in observation and sampling techniques. The results showed that the average level of serotonin in dogs from Kintamani hill were 240.2± 46.5 ng/mL (male); 265.4±38.2 ng/mL (female) and from Denpasar were 273.0±34.3 ng/mL (male); 297.5±45.9 ng/mL (female), respectively. The average level of MAO in dogs from Kintamani hill were male 3.4±0.38 ng/mL (male), female 5.07±0.39 ng/mL (female), and from Denpasar: male 4.63±1.90 ng/mL (male) and 4.75±1.28 ng/mL (female), respectively. It was concluded that the environmental conditions of the place, food, owner's style in maintaining, greatly affect the behavior of dogs, in turn affect the profile of serotonin and monoamine oxidase enzymes.

**Keywords:** Serotonin, monoamine oxidase, aggressiveness, dogs

## Introduction
Dog behavior is influenced by many factors such as place/origin, the environment, food, training and the management system (Lindsay, 2005 [20]; Blackwell *et al*., 2008 [6]; Arhant *et al*., 2010 [4]; Hughes, 2013 [19]; Bruschetta *et al*., 2014 [7]; Andrea *et al.*, 2015 [3]; Cannas *et al*., 2018 [9]). Most kintamani dogs have obedient and challenging relationships, respectful, nimble, intelligent, and loyal (Dharma *et al*., 1993 [14]; Puja, 2005 [22]) but some are aggressive. Researchers have reported perceived differences in trainability between different dog breeds and body sizes. Small dogs are generally more agile. Kintamani dogs are of medium size and have quite high energy.

Training can affect behavior including owner behavior. Arhant *et al.* (2010) [4] reported that the owner's behaviour is regarded to be a possible cause of unfavourable behaviour such as poor obedience or excitability in dogs. Abusive and repressive treatment of dogs, resulting in increased dog aggression. Tami *et al.* (2008) [27] stated that kennel-living dogs were more aggressive than house-living ones. In addition, to exercise techniques and food also influences dog behavior. Harju *et al*. (2016) [18] reported that a connection between nutrition, physiology and behaviour in dogs, as well as that canine behaviour can be regulated to some extent by altering the diet. Andrea *et al.* (2015) [3] reported that individuals fed with hyper-protein ratios presented lower serotonin values as compared to dogs receiving hypo-protein ratios. Tryptophan, the precursor of serotonin, may affect the incidence of aggression, self-mutilation and stress resistance (Bosch *et al*., 2007) [5]. Crowell-Davis *et al*. (1995) [13] reported that dogs given energy restricted diets, i.e. energy deficient diets, can show aggressive behaviour such as snaps, bites, focused barking and fighting.

Many previous studies have shown a relationship between serotonin (SrO) levels and the enzyme monoamine oxidase (MAO) (Cases *et al.*, 1995 [10]; Cakiroğlu *et al.*, 2007 [8]; Duffy *et al*., 2008 [15]; Rosado *et al*., 2010 [24]; Amat *et al.*, 2013 [2]; Alberghina *et al*., 2016 [1]), on dog

aggressiveness. Serotonin is a blood protein composed of amino acids, produced by nerve cells, and is found in the digestive system, some of which is also present in blood platelets and throughout the central nervous system. Function as a messenger and biochemical regulator, whereas in the nervous system acts as a neurotrasmitter, and as a paracrine or hormonal modulator in the process of blood diffusion. Serotonin is synthesized from the essential amino acid L-tryptophan (Rosado *et al.*, 2012) [24].

Serotonin influences behavior, such as appetite, emotional, motor, cognitive and autonomous (Richardson *et al*., 1993) [23]. Mann (2013) [21] reported that the serotonin system is involved both in diathesis (decision making) and major depression. This illustrates that serotonin is related to feelings for example in deciding a problem.

Serotonin is formed when the amino acid L-tryptophan is broken down in the liver by the enzyme tryptophan hydroxylase. This breakdown process produces 5-hydroxy tryptophan (5-HTP), which is a direct precursor of serotonin. Ninety percent of serotonin in the body is produced in the gastrointestinal (GI) tract and is taken by intestinal cells and blood platelets. As a hormone in the body, serotonin is involved in various functions including digestion and appetite control, blood clotting, and sexual function. The greatest serotonin effect on the digestive tract is facilitating peristalsis, which is a rhythmic contraction of the intestine that occurs during digestion. Only 10% of serotonin in the body is made in the brain. After the breakdown of tryptophan, 5-HTP travels through the bloodstream, crosses the blood brain barrier, and enters brain tissue. Once in the brain, 5-HTP is converted to 5-hydroxytryptamine (5-HT), better known as serotonin. Rossado *et al.* (2010) [24] stated that aggressive dogs showed lower serum concentrations of serotonin (5-HT) than non-aggressive dogs (278.5 ng/ml vs 387.4 ng/ml). The lowest 5-HT concentration was found in the dog group which showed a form of defensive aggression.

Monoamine oxidase (MAO) is an enzyme containing FAD (Flavin Adenine Dinucleotide), which is iron as 2 isozymes, A and B, thus classified as flavoprotein (Richardson, 1993 [23]; Edmondson *et al*., 2003) [16]. The function of monoamine oxidase is to break down serotonin, produced by the outer membrane of the mitochondria (Youdim and Reiderer, 2011) [28]. The study found several results that monoamine oxidase levels play a role in animal behavior. Cases *et al.* (1995) [10] states that aggressive mice have higher levels of monoamine oxidase than non-aggressive mice. Chen and Shih (1997) [12] argues that mice with non-deficient MAO-A conditions exhibit decreased levels of serotonin and aggressive behavior. Lack of monoamine oxidase-A (MAO-A), an enzyme that decreases serotonin and norepinephrine, has recently been shown to be associated with aggressive behavior (Cases *et al.*, 1995) [10].

Research on serotonin and monoamine oxidase levels in kintamani dogs has never been done. Based on this, it is necessary to conduct research as preliminary data reveal levels of serotonin and monoamine oxidase, so that it can be used as a reference data base. The purpose of this study was to determine the level of serotonin and monoamine oxidase levels in the blood of Kintamani dogs (*Canis lupus familiaris*) on dry and hilly topography. The benefit of this research is to add information on basic scientific data about serotonin and monoamine oxidase levels of Kintamani dogs in Indonesia.

## Material and methods

This observational study uses a cross-sectional study design, with two independent variables being gender while the dependent variable is the levels of serotonin (SrO) and mono amin oxidase (MAO). Sampling was carried out using a purposive system, with 40 samples. Formula for calculating a minimum sample size for estimating population proportions (Snedecor and Cochron, 1994) [25]:

$$n = \frac{Z^2_{1-\alpha/2} \times p \times (1-p)}{d^2}$$

Note:

| | |
|---|---|
| $n$ | : number of samples |
| $Z^2_{1-\alpha/2}$ | : Z score at 1 - α / 2 confidence level |
| $p$ | : aggressive proportion estimation |
| $d$ | : qualify of SD |

Casey *et al.* (2013) [11] stated in their study that all types of dogs were surveyed using a questionnaire that the aggressive proportion in dogs was 15% and the standard deviation was 11%. Based on these results, the number of samples is:

$$n = \frac{Z^2_{1-\alpha/2} \times p \times (1-p)}{d^2} = \frac{1.96^2 \times 0.15 \times (1-0.85)}{0.11^2} = 40.5$$

This study used 40 dogs including 15 males and 25 females. All dogs were observed clinical symptoms and physically healthy, not on therapy. The dogs come from Sukawana village, Kintamani sub-district, Bangli district, Bali, Indonesia. Determination of serotonin and monoamine oxidase levels using the Elisa technique.

A 3-mL blood sample was collected from the saphenavein of each dog into anticoagulant (EDTA) tubes. Samples were centrifuged at 1000 x g (or 3000 rpm) at 2-8$^0$C within30 minutes. Plasma was frozen and stored at -20$^0$C until its analysis. Serotonin ELISA kit (My Bio Source Southern California, San Diego (USA) with Cat.No: MBS9368907 was used to measure serotonin levels in plasm.

A t test was used to assess the association between male groups and femali groups on serotonin levels (Steel and Torri, 1981) [26]. A value of $P < .05$ was considered significant for all analyses. Data were analyzed using statistical software for Windows (SPSS).

## Results

The results showed that the average serotonin level in male kintamani dogs was 245.73±53.07 ng/ml and monoamine oxidase levels 4.47±1.84 ng/ml. whereas in female, serotonin levels an average of 280.12±45.06 ng/ml and average levels of monoamine oxidase 4.91±1.37 ng/ml. Full results are presented in Table 1.

**Table 1:** Levels of Serotonin and Monoamine Oxidase in Kintamani Dogs

| No | Serotonin levels (ng/mL) | | | | Monoamine oxidase levels (ng/mL) | | | |
|---|---|---|---|---|---|---|---|---|
| | highlands | | lowlands | | highlands | | lowlands | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| 1 | 207 | 215 | 277 | 290 | 3.81 | 4.98 | 3.30 | 6.59 |
| 2 | 201 | 206 | 247 | 290 | 3.82 | 5.82 | 4.30 | 5.01 |
| 3 | 266 | 270 | 270 | 348 | 3.07 | 2.9 | 3.57 | 3.47 |
| 4 | 205 | 273 | 298 | 345 | 3,09 | 3.64 | 5.43 | 7.72 |
| 5 | 307 | 297 | 345 | 240 | 3,19 | 4.62 | 3.72 | 3.45 |
| 6 | 292 | 326 | 247 | 350 | 4,86 | 7.56 | 3.65 | 4.11 |
| 7 | 204 | 226 | 246 | 239 | 3.19 | 4.96 | 4.02 | 3.87 |
| 8 | - | 308 | 254 | 370 | - | 7.79 | 9.05 | 3.28 |
| 9 | - | 252 | - | 290 | - | 3.54 | - | 5.43 |
| 10 | - | 242 | - | 290 | - | 5.32 | - | 4.85 |
| 11 | - | 271 | - | 246 | - | 4.17 | - | 4.26 |
| 12 | - | 299 | - | 321 | - | 5.65 | - | 4.62 |
| 13 | - | - | - | 249 | - | - | - | 5.12 |
| AV | 240.2 | 265.4 | 273 | 297.5 | 3.4 | 5.07 | 4.63 | 4.75 |
| SD | 46.5 | 38.2 | 34.3 | 45.9 | 0.39 | 0.39 | 1.90 | 1.28 |

AV: average
SD: standard deviation

## Discussion

Aggressive dog behavior is normal, aggressive to the owner, other people or other dogs (Casey *et al*., 2013) [11]. Dog behavioris a complex trait influenced by genetic, epigenetic, and environmental factors (Arhant *et al*., 2010 [4]) and all these may be partly responsible for the differences found (Amat *et al*., 2013 [2]). In our study the dogs that were used as samples came from two different regions namely those from the topography of the canyon valley, dry without good management. Dogs are released wildly without being trained by the owner, so that they have a higher aggressiveness. While the other group dogs come from urban areas that get good management of feed, routine training, so they have the character of tame behavior (non-aggressive). The topography is difficult, wild and requires a lot of effort in the dog to adapt to the environment, influencing the character of the dog, especially in relation to aggressiveness. The aggressive behavior of dogs is directly related to blood profiles such as serotonin (Frazer and Hensler, 1999 [17]; Amat *et al*., 2013 [2]), the enzyme monoamine oxidase A (Harju, 2016 [18]).

Food management factors also influence serotonin levels, as in our study, dogs from the highlands that live wild get bad food, whereas dogs in the Denpasar lowlands/urban areas are generally well-managed dogs, given a good food menu (meets the element of protein/tryptophan). Amino acids such as tryptophan increase serotonin levels (Andrea *et al*., 2015 [3]), so dog serotonin levels in the lowlands (Denpasar city) are higher than dogs in the Bangli highlands. However, the results of the T-test showed no significant difference (P>.05). Amat *et al*. (2013) [2] stated that different parenting styles in certain species can cause differences in serotonin levels in off spring. The sexes of men and women also have different aggressiveness profiles, as our results show, that male dogs have a higher aggressive percentage than females. In this regard, the results showed that the average levels of serotonin and monoamine oxidase in male dogs were lower than in females.

## Conclusions

We conclude that that the environmental conditions/situation of the place, food, owner's style in maintaining, greatly affect the behavior of dogs, in turn affect the profile of serotonin and monoamine oxidase.

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