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Research Article **Cossian** Morphometric Traits and *Melanocortin* 1 Receptor (MC1R) Gene Polymorphism of Indonesian Muscovy Ducks of Different Plumage Color Population

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Abstract

Background and Objective: The body shape or morphological characteristics of Muscovy ducks are determined by the length of the femur, tibia, tarsometatarsus, tarsometatarsus circumference, 3rd-digit, wings and maxilla. Identification of the feather color of Muscovy ducks is important because feather color determines the physical quality of the carcass and affects the level of consumer preference. The aim of this research was to assess the genetic variation of native Indonesian Muscovy ducks based on morphometric traits and gene sequence variation of Melanocortin 1 receptor (MC1R) and its genotypic association with different feather colors. **Methodology:** Two hundred day-old Muscovy ducks consisting of the white and white-black feather color combination in the same proportion between male and female ducks were included in the study. Differences in body weight and morphometric measurements among the groups were evaluated by ANOVA with Systat version 13. Primer design used Clustal X, based on Cairina moschata MC1R gene, partial cds (KX013541.1) from the GenBank database, the primary forward sequence: 5'-GCTCTTCATGCTGCTGATGG-'3 and reverse primer: 5'-GATGAAGACGGTGCTGGAGA-'3. **Results:** The male Muscovy ducks have larger morphometric features than females. Male Muscovy ducks with the white-black feather color combination had the heaviest body weight. A general linear F-test analysis separately showed that only the neck length, length of third digit and sex significantly affected the animals' body weight. The identification of feather color of Muscovy ducks showed that there are variations within a group of white-black feather color combination of Muscovy ducks. Feather color variation was observed on the head, wings, breast, tail and plumage. The sequencing of PCR products resulted in nucleotide polymorphism. The GG genotype was observed in 293 nt in the white-black population and the CC genotype was observed in white-black and white feather colors in both male and female Muscovy ducks. Conclusion: Muscovy ducks with the white-black feather color combination had heavier body weights than those with the white feather color. The neck length can be used to predict the body weight of Muscovy ducks. Muscovy ducks had a variety of feather colors ranging from white to the white-black color combination. The MC1R gene polymorphism was observed in Muscovy ducks. Muscovy ducks with the white-black feather color grow faster and their live weight can be estimated by neck length.

Key words: Feather color, MC1R gene polymorphism, Morphometric traits, Body weight, Neck length, Muscovy ducks

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Muscovy duck is a potential waterfowl to be developed especially for meat production. Muscovy ducks are scattered almost in all parts of Indonesia because they have long been domesticated. The body shape or morphological characteristics of Muscovy ducks are determined by the length of the femur, tibia, tarsometatarsus, tarsometatarsus circumference, 3rd-digit, wings and maxillas¹. The size of the body parts can be used to estimate the live weight of a poultry. The body weight of the duck is positively correlated with its body measurements. The bones provide a basis for the external structure and shape of the body of the livestock . Characteristics or appearance of a livestock can be distinguished on both qualitative and quantitative traits. Quantitative traits are generally influenced by genetic and environmental factors and have a close relationship with the economic properties of live stocks. Some of the quantitative traits associated with poultry productivity are shank length, tarsometatarsus circumference, chest circumference, thigh length and chest circumference, whereas the increase in body measurements is determined by the size of the organs, muscles and bone growth. The difference in body weight between male and female animals is referred to as sexual dimorphism. In poultry, for instance, male Muscovy ducks at cutting age (12 weeks of age) have a 40% heavier body weight than female ducks. Sexual dimorphism is considered when determining the slaughter age in the breeding program of Muscovy ducks. Male ducks are slaughtered at a younger age because they grow faster than females².

The feathers color of Muscovy ducks found in Indonesia are white, a white-black combination and a white-gray combination. Genetic diversity of the Indonesian Muscovy ducks can be seen from the color of the wing feathers, head, back, tail and the abdomen. Most Muscovy ducks are predominantly white and the black color is only found on a minority of birds. The gene controlling feather color, W (white) gene, is dominant to the other allele (w). Gene w is recessive to the W gene causing feathers to be diverse³. The variation of feather colors in Muscovy ducks includes black, white and blue⁴.

Differences in skin color/feathers in animals are caused by pigments determined by the melanocortin-1 receptor (MC1R) gene expressed on the melanocyte surface. The melanocortin-1 receptor (MC1R) gene is a 7-transmembrane receptor expressed on melanocytes. This receptor affects the induction of tyrosinase enzymes responsible for the synthesis of eumelanin and pheomelanin pigments. Typically, mutations in the MC1R encoding gene are present in mammals and birds carrying dominant alleles resulting from locus extensions in active receptors associated with black, while mutations causing loss of receptor function are associated with recessive alleles and a red-yellow color⁵. The feather colour of ducks is produced in response to melanocyte stimulating hormone (MSH) secreted by the pituitary⁶. Identification of the feather color of Muscovy ducks is important because feather color determines the physical quality of the carcass and affects the level of consumer preference. This study aimed to determine the differences in genetic diversity of Indonesian Muscovy ducks based on the body's morphological variation and to identify the presence of MC1R gene sequence variation in a population of different feather colors.

MATERIALS AND METHODS

Experimental design, birds and management: The experimental protocols were approved by the Animal Ethics Committee of Jenderal Soedirman University (4719/UN23.14/ PN.01.00/2017). Two hundred day-old Muscovy ducks white and white-black feather color combination of the same proportion between male and female ducks were included in the study. The Muscovy ducks were grouped equally into four groups: 50 ducks with white feathers (males), 50 ducks with white feathers (females), 50 ducks with white-black combination feathers (males) and 50 ducks with the whiteblack combination feathers (females). Each group of ducks was kept in 5×4 m² colony cages and fed ad libitum commercial diets: Starter diet (from 1 day to 3 weeks of age) containing 3300 kcal kg⁻¹ metabolic energy, 20.5% crude protein, 5% crude fat, 5.7% crude fiber, 0.95% Ca and 0.8% P and grower diet (from 4 weeks of age to the end of the experimental period) containing 2905.45 ME (kcal kg⁻¹), 3.91% crude fiber, 4.36% crude fat, 1.817% Ca and 1.327% P. The animals were weighed at 12 weeks of age and body measures were recorded according to literature^{7,8} which included the followings:

- Bill length: Length between the base of the beak to the tip
- Bill width: Width between two sides of the middle beak
- **Neck length:** Length between first to last cervical vertebrae
- Backbone length: Length between neck joints and backbone to coccyx

- Backbone width: Width between central thoracic vertebra
- Chest circumference: Circumference measured at the tip
 of the hind breast
- **Chest depth:** Vertically between the backbone and the beginning of the breast-bone crest
- **Chest width:** Between the anterior and the posterior border of the breast-bone crest
- Wingspan length: Length between tips of right and left wings with both stretched out in full
- **Drumstick length:** Length from the knee joint to the hock joint
- Shank length: Length between the hock joint to the spur
- Third digit length: Length from the base to the of 3rd digit

Body weight was measured in kg using a portable electronic hanging scale (1.0 g precision) and linear body measures were recorded to the nearest 1.0 mm using a plastic tailoring tape measure and caliper.

Blood Sample and DNA isolation: Three millimeter blood samples were obtained from the vena axillaries, then were put in a tube filled earlier with anticoagulant (EDTA) and were finally stored in the fridge. Deoxyribo Nucleic Acid (DNA) total genome was extracted from the blood samples and isolated with a DNA Isolation Kit (Geneaid). The resulting DNA fragments were examined using 1% agarose gel electrophoresis.

Primer design and amplification of DNA fragments with

PCR: The Clustal X program with the *Cairina moschata* melanocortin-1 receptor MC1R (MC1R) gene partial cds (KX013541.1), from the GenBank database was used for Primer design. The forward primer base sequence of the MC1R gene was 5'-GCTCTTCATGCTGCTGATGG-'3 and the reverse was 5'-GATGAAGACGGTGCTGGAGA-'3. Polymerase chain reaction (PCR) comprised several steps, namely DNA predenaturation at 95°C for 5 min, DNA denaturation at 94°C for 30 sec, annealing at 55.2°C for 45 sec and elongation at 72°C for 1 min. Final extension was performed at 72°C for 10 min. The PCR was conducted for 35 cycles. The PCR products were subjected to electrophoresis test with a 1.5% agarose gel. The PCR products were visualized under UV light.

DNA sequencing: Sequencing of the PCR product was carried out by Genetika Science-Indonesia-Ltd. Sequencing and the result was nucleotide sequence. Electropherogram graphic

with colored peaks to differentiate nitrogen bases (nucleotide) as follows: Green for A nucleotide (adenine), black for G nucleotide (guanine), blue for C nucleotide (cytosine) and red for T nucleotide (thymine). The sequencing product in the form of an electropherogram consisting of nucleotide sequence from the MC1R gene sample of Muscovy ducks was read using Sequence Scanner v1.0. software.

SNP genotyping was determined through the BioEdit v7.2.0 program, by aligning sequence products according to the sequence in GenBank KX013541.1 database from the ClustalW menu (accessory application). The alignment result was seen in the electropherogram, to obtain SNPs in a particular position used for genotyping. The base sequence of the MC1R gene in Muscovy ducks (*Chairina moschata*) was 726 bp. The SNP was confirmed based on the electropherogram results and used for genotyping.

Morphometrics is a more objective tool for describing and evaluating the parameters of body weight and carcass rather than visual evaluation⁹. Characteristics measured in this study included body weight, beak length, beak width, neck length, backbone length, back width, chest circumference, chest depth, chest width, wingspan length, thigh length, shank length and 3rd digiti length. Data on morphometric measures are listed in Table 1.

Statistical analysis: Differences in body weight and morphometric measurements among groups were evaluated by one way ANOVA with Systat version 13. Post hoc Duncan's test procedure was used to test for differences among main effects. The general linear F-test was used to test the effect of each morphometric measure on body weight (either males or females) regardless of plumage color, because duck have the sex dimorphism characteristic. The analysis was based on the change of the residual sum of squares between the full and reduces model. Linear regression analysis and the general linear F test were performed using the R program¹⁰. The SNP genotyping of the MC1R gene was analyzed by calculating ng the gene frequency according to Pichner¹¹.

$$FAn = \frac{\sum MCIRAgene}{\sum MCIRAgene + \sum MCIRngene}$$

Keterangan: FAn = frequency of gene A at n locus

The genetic variation of MC1R was determined based on the heterozygosity formula according to Nei¹²:

$$h=l-\sum_{i\,=\,1}^m x^2$$

RESULTS

Variation of plumage colour and morphometric of muscovy

ducks: Muscovy ducks were grouped based on plumage color into white and white-black color combination groups. Color patterns are observed throughout the body. Variations in plumage color combinations are found on the head, wings, chest, tail and plumage cover as shown in Fig. 1. The current study showed that there are various patterns of white-black color combination i.e., white feathers located on 40% head, 36.7% cover, 3.3% chest, 29.30% wings and 40% tail. Black colour is located on 60% head, 63.30% cover, 0% chest, 60.7% wing and 60% tail.

The results of analysis of variance showed that body weight of male and female Muscovy ducks were highly significantly different (p<0.001). Male ducks with white-black color combination have the highest body weight. Female white ducks have heavier body weight than those of females white-black color combination. Other morphological characteristics indicated that male ducks of both white and

	Plumage variation					
Morphometric measures	White (males)	White-black (males)	White (females)	White-black (females)	SEM	p-value
Bill length (mm)	62.20ª	62.30 ^b	54.933ª	54.000ª	1.005	0.001
Bill width (mm)	33.00	31.00	31.867	30.467	0.709	0.648
Neck length (cm)	13.56 ^b	13.65 ^b	10.893ª	10.627ª	0.282	0.000
Back length (cm)	26.20 ^b	23.93ª	23.747ª	22.507ª	0.325	0.000
Back width (cm)	11.80 ^b	11.54 ^b	9.920ª	9.540ª	0.183	0.000
Chest circumference (cm)	36.86 ^b	36.96 ^b	32.360ª	32.180ª	0.425	0.000
Chest depth (cm)	10.30 ^b	10.95 ^b	7.373ª	8.200ª	0.310	0.000
Chest width (cm)	17.02 ^b	15.67 ^b	13.873ª	12.893ª	0.358	0.000
Wingspan (cm)	30.18 ^b	30.62 ^b	25.987ª	25.360ª	0.421	0.000
Drum thigh length (cm)	10.78 ^b	11.37 ^b	8.613ª	8.007ª	0.272	0.000
Shank length (cm)	8.82	8.44	9.573	9.607	0.330	0.553
3rd digiti length (cm)	6.36 ^b	7.01 ^b	4.487ª	3.707ª	0.229	0.000

SEM: Standard error of the mean. Different superscripts among groups shows significantly differences (p<0.05)



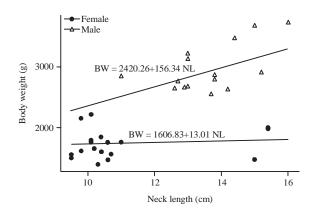
Fig. 1(a-d): Plumage patterns of Muscovy ducks, (a) White (males), (b) White-black combination (males), (c) White (females) and (d) White-black combination (females)

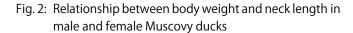
white-black combination have larger morphometrics features compared to female ducks (p<0.001) with the exception of beak width and shank length, in which both are similar. It has been shown that male ducks have larger morphometrics measures than females on all of the characteristics measures. This fact strongly supports the existence of sex dimorphism in Muscovy ducks.

The general linear F-test showed that only neck length, 3rd digit length and sex factor separately have significant effect on body weight. The interaction between sex factor and neck length has significant effect on body weight so that body weight is better predicted by a model including the interaction between sex factors and 3rd digit length. Thus, the body weight can be predicted by the following equation:

BW = 3rd digiti length+neck length+(Sex x neck length)

The linear regression analysis of the relationship between body weight and neck length neglecting





3rd digit length is shown in Fig. 2. The linear regression of body weight on neck length and sex has a determination (r^2) of 0.8381 and the prediction equation of body weight on neck length in males and females are given below:

 $y_male = 2420.26 + 156.34$ neck length

y_female = 1606.83+13.01 neck length

SNP MC1R of muscovy ducks: The PCR analysis showed that the MC1R gene is located at 203 bp (Fig. 3). After the PCR product was sequenced, it was followed by the alignment process between GenBank Acc. No. KX013541 with the MC1R gene sequence data of 203 bp. The PCR product sequencing of the MC1R 203 bp product found a sequence at 293 bp, which lies between 290 and 300 bp in GenBank Acc. No. KX01354 (Fig. 4).

The single nucleotide polymorphism (SNP) is located at 293 bp resulting in 2 genotypes of GG and CC. The result of the sequencing of the sample obtained genotype GG and CC and no heterozygous genotype was found. In the group with the white-black color combination (80 heads), 20 heads were found to have the GG genotype, with males and females having the same numbers. The rest (60 heads) were found to have the CC genotype, with males and females also having the same number. In another group, the white color Muscovy ducks, all 80 animals had CC genotype. The proportion of males and females of this group having the CC genotype is also the same (40 heads in each sex).

The gene frequency of GG is 12.5% and that of CC is 87.5% from a total of 160 heads of Muscovy ducks. The level of genetic diversity in a population can be measured by heterozygosity parameters. Heterozygosity is the value or

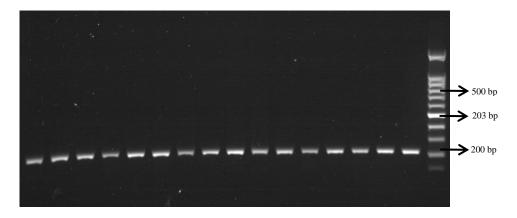


Fig. 3: Electrophoresis output of the 203-bp PCR products of the MC1R gene of Muscovy ducks

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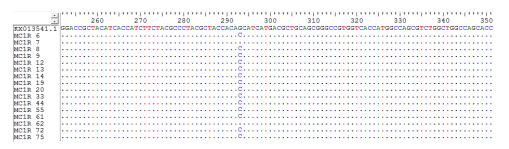


Fig. 4: Example of the alignment result of GenBank Acc. No. KX01354 and the MC1R gene sequencing of Muscovy ducks

parameter used to measure the level of genetic diversity in a population¹². The heterozygosity in this study was found to be 0.069. Criteria of heterozygosity values ranges from 0.0-1.0. If the heterozygosity value is close to 0.0, then it can be interpreted as low genetic diversity and if the heterozygosity value is close to 1.0, then it can be interpreted as high genetic diversity¹³. The heterozygosity of this study (0.069) is close to zero and thus, it can be said that the genetic diversity of the populations under study of white and the white-black combination plumage color is low.

DISCUSSION

Variation of plumage colour and morphometric of muscovy

ducks: Local Muscovy ducks have color variations of black and white feathers. Plumage color variation is controlled by the W gene which is dominant to the other gene (w) of the same locus. This dominant-recessive gene action causes the feather color to vary³. The black plumage color theoretically arises because of the gene factor E, whereas its allele pair i.e., e^b causes a brown color. E is dominant against e^b. The color source is caused by the C gene factor, whereas the allele pair, c, causes a white colour (recessive). White color plumage is formed due to the absence of the O gene factor (supporting gene factor for gene C so that color can be formed through oxidation process). Without the O gene, it turns out that the C gene is unable to produce colour, so the resulting color is white¹⁴. The plumage color variation in chicken is determined by a SNP in MC1R as indicated by the correlation between MC1R polymorphism and the difference of the allele at locus E⁵.

Morphometric research of Muscovy ducks has been conducted according to Yakubu *et al.*¹⁵ in adult African Muscovy ducks including variables of body weight, body length, body circumference, thigh or tibia, beak length, neck length, leg length and wing length. The results showed that sex is very influential on body weight. Male Muscovy ducks have heavier body weight compared to female ducks. The linear regression of body weight on neck length and sex has determination (r^2) of 0.838. These results are consistent with the research of Ismoyowati *et al.*², who reported that at the same age, male ducks produced heavier weight gain and greater relative growth compared to female Muscovy ducks. The greatest body weight gain of Muscovy ducks was reached at 3 weeks of age and then the growth rate began to decrease at 4 weeks.

Body conformation is affected by the size and shape of the animal. The livestock breed can be distinguished primarily from the size and shape of the body¹⁶. Physical characteristics such as size, shape and plumage color can be used to assess individual livestock¹⁷. Literatures has shown that male ducks have bigger body measures than females, except in the beak width and shank length (tarsus). The length and diameter of the femur are not affected by the body weight. Femoral bone growth and mineralization are slower than the tibia. Selection for weight gain is directly related to muscle enlargement in the chest without significant change in the skeleton¹⁸. Other studies, though have reported that medullary and cortical dimensions are associated with weight. The difference in cortical dimensions between femur of different poultry groups is largely determined by weight¹⁹. Other factors, such as differences in weight distribution, activity and material properties within the cortex, can also affect the femoral dimension. Wyets et al.20 found that the tibial (tibio-tarsal) cortex was significantly thicker in heavy weight turkey than light weight turkey strains, from 3-12 weeks of age, even when both strains were compared at equivalent weight. Heavy weight poultry has larger leg bone structure than light weight poultry.

Based on a principle component analysis of the Peking duck shape is influenced by the length of the femur, tibia and wings. The local Muscovy duck shape is heavily influenced by the length of the tibia, the circumference of the tarsometatarsus and the wings length, whereas the shape of imported Muscovy ducks is influenced by the length of the femur, the circumference of tarsometatarsus, the length of the 3rd digiti and the wings length¹. The results of the current study showed that the length of the femur length (drum thigh length) of male Muscovy ducks is longer than the females but the shank (tarsus) is relatively similar. The increase in poultry body weight is mainly due to muscle accumulation on the chest rather than on the legs, which causes the foot to have a heavy burden in supporting the body¹⁸.

In most avian species, poultry including Muscovy ducks, have a long neck. The long and flexible neck acts as shock absorber, protecting the weak brain tissue from a considerable vibration at the time the birds land. The long, stiff neck allows Muscovy ducks to pick up food on the ground more easily and allows the center of gravity of the poultry to adjust when the poultry changes from the upright position or perching to a more horizontal position when flying²¹. Male Muscovy ducks have longer neck so that in a flock they can get more feed than females. More feed consumption allows the males to grow faster so that the body weight is heavier.

SNP of MC1R gene of muscovy ducks: Melanocortin 1 receptor (MC1R) is in the protein-coupled receptor (GPCR) subfamily. Melanocortin receptor (MCR) varies from 1 to 5. MCR is activated by a group of peptide hormones related to melanocortin biosynthesis (MC) that contributes to the regulation of important physiological processes such as the production of glucocorticoid hormones in the adrenal gland (MC2R), food and homeostatic energy (especially MC4R), sebaceous glands activity (MC5R) and others²². Melanocortin is a bioactive peptide produced on the cleft of prohormone protein pro-opiomelanocortin (POMC) precursor in several locations by two endoproteases²³.

Color pigment is theoretically produced in response to melanocyte stimulating hormone (MSH) secreted by the pituitary⁶ gland. The MC1R activation occurs when MSH is bound. As a result, the enzyme activity of tyrosinase increases and limits the synthesis of melanin. Eumelanin will be produced when the enzyme tyrosinase reaches the highest level in melanocytes, resulting in black and -/or brown. Furthermore, if there is no stimulation of MSH from MC1R, it causes the level of enzyme tyrosinase to be low, so that pheomelanin is produced expressing red and dull yellow. If cAMP concentration increases in cells, the C nucleotide will be activated. Increased cAMP concentration also increases the synthesis of tyrosinase resulting in increased eumelanin synthesis and the reduction of pheomelanin synthesis. This results in the formation of a black or brown color²³.

One of the controlling factors of several programs related to neurogenesis and neural crest is microphthalmia-associated transcription factor (MITF). After birth, MITF controls 3 enzymes that potentially control the production of melanin. One of the enzymes, tyrosinase, produces *eumelanin* and *pheomelanin*. Mutations in MITF will inhibit melanin production and consequently *eumelanin* and *pheomelanin* are not produced, so the expressed color is white²⁴.

The single nucleotide polymorphism found in the MC1R 293 bp gene shows polymorphism in Muscovy ducks. Sequence variation of the MC1R gene in SNPs causes amino acid changes at 98-nt, which include a change of serine (Ser/S) to threonine (Thr/T) where the amino acid AGC is converted into ACC. Polymorphisms in the MC1R genes have previously been reported in several mammals such as bulls²⁵ and goats²⁶ and in poultry such as Maguk Ducks²⁷, chicken and guails²⁸ and geese²⁹. The polymorphism occurs as a result of mutations that produce black/dark plumage color, while loss of function mutations causes red/yellow or white plumage color²⁶. Based on the observed electropherogram (Fig. 4) it is believed that there is base alteration from Guanin to Cytosine. A base alteration at SNP c.293G >C indicates a transition mutation. Transition mutation occurs because of the substitution of a purine base (adenine and guanine) and other purine bases or between a pyrimidine base (thymine and cytosine) and other pyrimidine bases³⁰. Ran et al.³¹ have conducted a study of the relationship between mutation of the MC1R gene and the color variation of plumage in pigeons. The SNP1 (G199A) and SNP3 (A466G) lead to a change in amino acids (Asp67Asn and Thr156Ala). In genetics, a missense mutation, a type of nonsynonymous substitution, could result in truncation of the resulting protein and a non functional protein.

Genetic variation of Muscovy ducks can be used in the selection by duck breeders and breeding systems can be applied to produce white feathered Muscovy duck, to improve the physical quality of the carcass.

CONCLUSION

It can be concluded that male Muscovy duck with the white-black color combination plumage have greater body weight than the others. The length of the neck can be used to predict the body weight of Muscovy ducks. There is DNA polymorphism of the MC1R gene in the local Indonesian Muscovy ducks (*Cairina moschata*) distinguished by the plumage color (white and white-black color combination). The genetic diversity of Indonesian Muscovy ducks of white and the white-black color combination is low.

SIGNIFICANCE STATEMENT

This study discovers how the morphometric as quantitative characteristics and feather color as qualitative characteristics could be potentially used as the basis for selecting Muscovy duck regarding its growth. Selection based on morphometric characteristics and feather color has advantages to obtain Muscovy duck with high body weight and good carcass physical quality. This study found that neck length could be used to predict body weight and discover the existence of polymorphism of the MC1R gene, which responsible for feather color coding which have not been discovered in the previous research. Therefore, a new theory on the existence of a positive correlation between neck length and body weight is introduced.

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