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SEMEN CHARACTERISTICS AND SPERMIOGRAM OF THE AFRICAN GREATER CANE RAT (*THRYONOMYS SWINDERIANUS,* TEMMINICK)

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ABSTRACT

Semen characteristics and spermatozoa morphological analysis were determined in twenty sexually matured domesticated African greater cane rats (*Thryonomys swinderianus*, Temminick). Semen was collected from the cane rats using the electroejaculation method followed by orchidectomy using open castration. The testis and epididymis were retrieved for sperm morphological studies. The semen of the rats was characteristically in coagulated form, opalescent in colour, gelatinously thick with no liquid fraction. The average volume of the ejaculates was 0.3 ± 0.04 ml. The total number of normal spermatozoa in the left and right testes was 308.5 x 10^9.ml and 330.1 x 10^9.ml, respectively. The percentage of abnormal spermatozoa observed in left and right testes were 9.8 % and 9.6 %, respectively. The percentages of abnormal spermatozoa observed in left and right epididymes were 10.6 % and 10.4 %, respectively. There was no significant difference (P>0.05) between the percentages of abnormal spermatozoa found in both the left and right testes and epididymes. The spermatozoa head was ovoid in shape with its surface flat, whilst its acrosome lacked any hook. This absence of the acrosomal hook, typical for the sperm head of rodents, is therefore the first of its kind in the body of literature on the spermatozoa of rodents. It is, therefore, named the African greater cane rat "hookless sperm head" (by Olukole). This study presents base-line data on the semen characteristics and sperm morphology of the African greater cane rat and is expected to be useful in artificial insemination, comparative morphology of the spermatozoa of rodents and to improve breeding of the animal.

Key words: semen; spermatozoa; testis; epididymis; rat

INTRODUCTION

The African greater cane rat also known as the grasscutter (*Thryonomys swinderianus*) is a wild herbivorous rodent erroneously regarded by some as a larger version of the rat. It is related to the African porcupine, the brush tail porcupine as well as guinea pig, the chinchilla and the capybara of the South America (NRC, 1991). It is widely distributed in the African sub-region and exploited in most areas as a source of animal protein (Asibey and Addo, 2000). Being the most preferred bush meat in West Africa, including Nigeria, Togo, Benin, Ghana and Cote' d'Voire, it contributes to both local and export earnings of most West African countries and is therefore hunted aggressively (GEPC, 1995).

Reproductive ability of a male had been reported to comprise the production of semen containing normal spermatozoa in adequate quality and quantity, in addition to the desire and ability to mate (Oyeyemi and Ubiogoro, 2005). The process of spermatogenesis is therefore a very productive and efficient mean of producing a large number of normal spermatozoa capable of fertilization. With millions of sperm produced per year, sperm morphological abnormalities do occur varying between 10 and 40 %

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(Harcourt, 1991). Spermatozoa are made up of three distinct segments: the head, mid-piece and tail. The head consists of the acrosomal cap and the post-acrosomal region. The tail is made up of the principal piece and the end or terminal piece. The sperm head contains the dense and compact DNA (Breed *et al.*, 2005).

Pioneering research reports on the African greater cane rat were concerned mainly of physiology, management, breeding and nutrition. The physiological, nutritional and pathological conditions of grasscutters are usually assessed using haematological and biochemical analysis of their blood (Awah and Nottidge, 1988; Fonweban and Njwe, 1990). Recent research reports on the anatomy of the African greater cane rat had been on the biometry of the testis and epididymis (Olukole *et al*., 2009a); histology of the kidney (Olukole *et al.*, 2009b); gross anatomy of the male reproductive organs (Olukole *et al*., 2010a) and histomorphometry of the testis and epididymis (Olukole and Obayemi, 2010). Also, the morphological characteristics of spermatozoa in bulls, boars and bucks had been described in the following reports: Oyeyemi *et al.* (2000); Oyeyemi and Ubiogoro, (2005) and Oyeyemi and Babalola (2006).

However, with the exception of the preliminary reports of Olukole *et al.* (2008) on sperm anatomy of the African greater cane rat; gonadosomatic index in relation to age (Adebayo *et al.*, 2009) and Olukole *et al.* (2010b) on sperm reserves in this rodent, reports on andrological studies of the animal are still scarce. This study, first of its kind, was therefore designed to characterize the semen and spermatozoa morphology of the domesticated adult African greater cane rat (*Thryonomys swinderianus*), thereby making available baseline data, which could be useful to improve breeding of this animal.

MATERIAL AND METHODS

Experimental Animals

Twenty domesticated adult male cane rats were used for the study. They were acquired from a commercial farm in Ogun State, Nigeria. Records on the age and feeding patterns of the animals were also obtained from the farm. The cane rats were kept at the Animal House, Faculty of Veterinary Medicine, University of Ibadan for 72 hours. They were kept on a daily ration of Guinea corn offal of about 0.5 kg per body weight supplemented with raw cassava (*Manihot species*).

Semen Collection

Semen was collected from the cane rats using the electroejaculation method, as described by Zemjanis (1977). The volume was determined using a calibrated measuring cylinder, whilst the colour was determined by visual assessment. The rats were anaesthetized using chloroform and afterward sacrificed by cervical dislocation. Orchidectomy was performed by open castration method. A midline incision was made and the testicles were milked out of the incision site. The testicles were then exposed by incising the *tunica vaginalis*. Semen samples were thereafter collected from the *cauda epididymis* as described by Oyeyemi and Ubiogoro (2005).

Sperm count and motility assay

Sperm motility was assessed by the method described by Zemjanis (1977). The spermatozoa were counted by hemocytometer using the improved Neubauer (Deep 1/10 mm, LABART,Germany) chamber, as described by Pant and Srivastava (2003). A total of 400 spermatozoa from each rat were examined for morphological changes.

Morphological abnormalities and percentage viability assay

These were determined from a total count of 400 spermatozoa in smears prepared with Wells and Awa stains (0.2 g of Eosin and 0.6 g of Fast green dissolved in distilled water and ethanol at the ratio 2:1). Live/dead ratio was determined using 1 % Eosin and 5 % Nigrosin in 3 % sodium citrate dehydrate solution according to the method described by Oyeyemi and Babalola (2006).

Statistical Analysis

All data obtained were expressed as means with the standard error of mean using the GraphPad Prism version 4.00 for Windows, GraphPad Software. Analysis of variance was performed using two-way ANOVA and significance was reported at $P<0.05$.

RESULTS

The semen characteristics and spermiogram of the cane rats used in this study are shown in Table 1. The semen was characteristically in coagulated form, opalescent in colour, gelatinously thick with no liquid fraction. The average volume of the ejaculate was 0.3 ± 0.04 ml. The sperm head was ovoid in shape with its surface flat, whilst its acrosome lacked any hook (Fig. 1). Normal spermatozoa of the adult domesticated cane rat, (making up of about 78 % of the total number of spermatozoa in one milliliter per ejaculate) are shown in Fig. 1. The mean progressive motility of spermatozoa and mean percentage of live spermatozoa for the cane rats were 73 ± 3.35 % and 95 ± 1.16 %, respectively. The mean total sperm count of the ejaculate

Table 1: Characteristics of spermatozoa of the domesticated adult African greater cane rat

Characteristics	Value/ Description
Colour	Opalescent
Volume (ml)	0.3 ± 0.04
Progressive Motility (%)	73 ± 3.35
Percentage live spermatozoa	95 ± 1.16
Total Sperm Count ($x10^9$.ml)	136.10 ± 9.15
Primary abnormality (%)	3.9 ± 1.02^a
Secondary abnormality (%)	7.9 ± 1.43^b
Tertiary abnormality (%)	11.2 ± 1.70^c

Means with different superscripts are significantly different (P< 0.05).

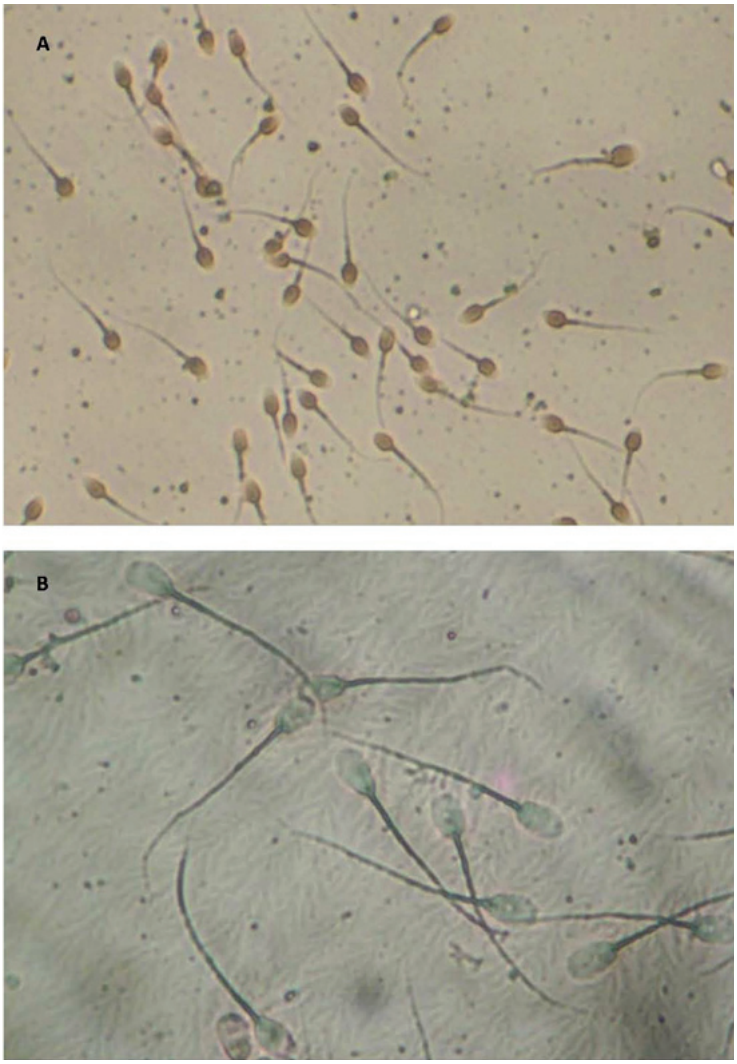

Fig. 1: Photomicrograph of the spermatozoa of the African greater cane rat (*Thryonomys swinderianus*, Temminick). A: Epididymal spermatozoa (magnification: x400); B: Testicular spermatozoa (Magnification: X1000), Eosin-Nigrosin. Note the absence of acrosomal hook on spermatozoa head

Table 2: Mean values of morphological characteristics of spermatozoa in the testis and epididymis of the domesticated adult African greater cane rat (in ml)

Morphological Characteristics	Testis	Caput	Corpus	Cauda	Epididymal mean
Tailless Head (L)	4.0	3.9	4.5	4.2	4.2
Tailless Head (R)	4.7	3.7	4.2	4.1	4.0
Headless Tail (L)	3.5	4.1	5.2	4.3	4.5
Headless Tail (R)	3.9	4.4	4.9	4.4	4.6
Rudimentary Tail (L)	1.3	1.2	1.6	1.7	1.5
Rudimentary Tail (R)	1.9	1.7	1.2	1.4	1.4
Bent Tail (L)	5.2	7.0	7.2	6.9	7.0
Bent Tail (R)	5.2	7.5	7.0	6.2	6.9
Curved Tail (L)	6.0	6.1	6.7	6.3	6.4
Curved Tail (R)	6.3	6.9	5.0	6.5	6.1
Curved Mid-piece (L)	5.8	6.7	7.2	7.4	7.1
Curved Mid-piece (R)	5.5	6.4	7.3	6.2	6.6
Bent Mid-piece (L)	6.5	7.8	8.4	8.1	8.1
Bent Mid-piece (R)	6.1	8.7	7.2	7.3	7.7
Coiled Tail (L)	0.5	0.8	1.3	1.1	1.1
Coiled Tail (R)	0.9	1.3	1.5	1.4	1.4
Looped Tail (L)	0.9	1.5	1.7	1.4	1.5
Looped Tail (R)	0.5	1.2	1.4	1.1	1.2
Total Abnormal Sperm Cells (L)	33.7	39.1	43.8	41.4	41.4
Total Abnormal Sperm Cells (R)	35.0	41.8	39.7	38.5	40.0
% Abnormal Sperm Cells (L)	9.8	10.6	11.1	10.1	10.6
% Abnormal Sperm Cells (R)	9.6	10.9	10.7	9.6	10.4
Total Normal Sperm Cells (L)	308.5	331.3*	351.0	370.1*	350.8
Total Normal Sperm Cells (R)	330.1	341.8*	330.6	363.3*	345.2
% Normal Sperm Cells (L)	90.2	89.4	88.9	89.9	89.4
% Normal Sperm Cells (R)	90.4	89.1	89.3	90.4	89.6
Total Sperm Cells (L)	342.2	370.4*	394.8*	411.5*	392.2
Total Sperm Cells (R)	365.1	383.6	370.3	401.8	385.2

L: Left; R: Right.
* Significantly different (P< 0.05).

for the cane rats was 136.10 ± 9.15 x 10^9.ml. The incidence of the spermatozoa with primary abnormalities was 3.9 % whilst those of secondary and tertiary abnormalities were 7.9 % and 11.2 %, respectively (Table 1) with significant (P<0.05) differences.

The results of morphology of spermatozoa isolated from the testis and different segments of the epididymides are presented in table 2. The total numbers of normal spermatozoa in the left and right testes were 308.5 x 10^9.ml and 330.1 x 10^9.ml, respectively. The percentage of abnormal spermatozoa observed in left and right testes were 9.8 % and 9.6 %, respectively. The percentages of abnormal

spermatozoa observed in left and right epididymes were 10.6 % and 10.4 %, respectively. There was no significant difference (P>0.05) between the percentages of abnormal spermatozoa found in both the left and right testes and epididymes. Spermatozoa with coiled tail and looped tail were the least encountered abnormal cells observed in the left and right testes and epididymes respectively (Table 2), whilst spermatozoa with bent mid-piece were the most frequently encountered abnormal cells in the left and right testes and epididymes. There were significant differences (P<0.05) in the number of normal spermatozoa found in the caput and cauda epididymis for both the right and left (Table 2). The testis, when compared with the epididymis, had lesser number of abnormal spermatozoa, whilst the cauda epididymis manifested the least number of spermatozoa in comparison with the caput and corpus epididymis (Table 2). Nevertheless, there were no significant differences (P>0.05) between these numerical observations.

DISCUSSION

The characteristic opalescent, coagulated and gelatinous ejaculated semen of the cane rats used in the study is similar to the ejaculated semen of the laboratory rat; mouse and guinea pig obtained using the electro-ejaculator method (Kishikawa *et al*., 1999). The characteristics of the ejaculate obtained in the study using the electroejaculation method agree with the findings of Oyeyemi *et al*. (2000) on the West-African Dwarf (WAD) buck. Nevertheless, the average volume of semen obtained in the study was however lower than reported in the WAD buck by Oyeyemi and Babalola (2006). The coagulation of the semen in some rodents has been reported to be due to the secretion from the coagulating glands or anterior lobe of the prostate, which contains an enzyme called vesiculase (Oyeyemi *et al*., 2000). However, Oke and Aire (1996) reported that the coagulating gland of the African giant rat is considered to be part of the seminal vesicle, rather than the prostate. Thus, the coagulating protein and enzyme appear to be derived from the structures having closer anatomical relationship than in the rat or guinea pig.

The motility and percentage of live spermatozoa obtained in the study are high enough to assure fertility. Motility of spermatozoa and percentage of live spermatozoa at the time of collection are commonly used as a measure of the fertilizing ability of sperm (Oyeyemi and Ubiogoro, (2005). The shape of sperm head of the African greater cane rat like those of most mammals is flat and oval. This is in conformity with previous reports on spermatozoa morphology in mammals (Villalpando *et al*., 2000; Breed *et al*., 2005; Oyeyemi and Babalola, 2006). The absence of an acrosome hook on the head of the spermatozoa of the African greater cane rat discriminates it from sperm cells of other rodents. The rat, Golden hamster (Blandau, 1951; Leblond *et al*., 1952), Volcano mouse (Villalpando *et al*., 2000) and approximately 11 other subfamilies (~151 species) of rodents studied by Breed *et al*. (2005) all have a sperm head that folds back onto itself producing a "hook"-like shape referred to as the apical hook. This report on the absence of acrosomal hook typical of the sperm head of rodents is, therefore, the first of its kind in the literature on the spermatozoa of rodents. It is thus named the African greater cane rat "hookless sperm head" (by Olukole).

Also, the presence of abnormal forms of spermatozoa in this study is consistent with the report of Moss *et al.* (1979), that a number of abnormal forms of spermatozoa are normally encountered in all ejaculates. Only when they are present in large numbers, they are associated with impaired fertility. Cohen (1973) has proposed that the incidence of abnormal sperm is simply an error in the process of spermatogenesis. In contrast, Barker *et al.* (1988, 1989) suggests that abnormal sperm were purposefully created and were never meant to partake in the act of fertilizing an ovum, but were constructed for the purpose of sperm competition and to hinder other male's sperm from achieving fertilization in cases when a female mates with multiple males during the oestrus.

The sperm morphological characteristics observed in the study are similar to those described by Oyeyemi and Babalola (2006) in the bull. The low level of the spermatozoa morphological abnormalities observed in the study indicates that the cane rats were fertile. The number of the spermatozoa with head abnormalities observed in the study in both the testis and epididymis put together further underscores of the potential breeding soundness of the cane rats used for this study. The higher values of secondary (bent mid-piece, curved mid-piece, bent tail and looped tail) and tertiary (tailless head, headless tail) sperm abnormalities obtained in the study, compared to primary abnormalities, are in agreement with the previous report (Oyeyemi and Ubiogogo, 2005) on the spermatozoa of mammals of sound breeding potentials. Secondary abnormalities have been reported to be due to changes that take place in the excurrent duct of the testis, whilst primary abnormalities are generated during spermatogenesis. Tertiary abnormalities are due to handling techniques like cold or heat shock, osmotic effects, toxicity of stains or changes in pH during collection and processing of semen (Moss *et al.*, 1979).

CONCLUSION

This study has shown that the sperm head of the African greater cane rat, unlike a typical rodent, lacks acrosomal hook. It also presents baseline data on the semen characteristics and sperm morphology of the animal, which hitherto had not been described yet. The findings of this work, therefore, are expected to be useful in artificial insemination, comparative morphology of the spermatozoa of rodents and to improve breeding of the animal.

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PRODUCTION TRAITS OF ROMANIAN SIMMENTAL COWS AT FIRST LACTATION

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ABSTRACT

Ninety-nine first lactation cows of the Romanian Simmental breed were used in a 305-day lactation period to study the production traits of the breed and its potential for genetic improvement. The phenotypic parameters studied were milk, fat and protein yield, as well as fat and protein percentage; variance components were calculated. The milk yield in the dairy cow population was 4053 ± 1391.8 kg, whilst the fat and protein yield were 157.6 ± 55.3 kg and 131.6 ± 46.3 kg, respectively. Phenotypic correlation between milk yield and milk fat yield was high (r = 0.9973) and between milk yield and milk protein yield was relatively low (r = 0.1403). In addition, correlations obtained between milk yield, and milk fat and protein percentages were low (r = 0.0519 and r = 0.0022, respectively), whilst the correlation between milk fat yield and milk protein yield was weak (r = 0.1414), and between milk fat percentage and milk protein percentage was moderate (r = 0.5139). The strong phenotypic correlation between the milk yield and milk fat yield indicates that the population of dairy cows can be improved by selection using the independent level of selection.

Key words: first lactation; milk yield; protein-fat correlations; Romanian Simmental cows

INTRODUCTION

Milk is a mixture of fat, protein, lactose, vitamins and minerals, either dissolved or suspended in water. This connection was already studied at phenotypic and genetic level. The usual description of milk secretion refers to the occurrence of changes in milk contents during lactation, being the decrease of milk yield accompanied by the increase in fat and protein contents (Kolb, 1987).

A dairy farmer wants to run fewer cows yielding more milk, which means that a higher profit can be obtained with less costs. Fewer cows with a great milk yield means less pollution for the environment, less forages for nourishment, less shelters for animals. All these arguments are of economic importance all over the world. Research has shown clearly that selecting for milk yield only also increases the total fat and protein yield (Linn *et al*., 1999).

Replacement heifer management is very important in dairy herds. The future producing ability of a heifer is unknown and performance at first lactation is often different from what is expected on the basis of the pedigree index alone. Thus, the selection of replacement heifers could be improved by the use of physiological markers of milk production and quality

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(Sabbioni *et al*., 2007). The value of milk is based on its composition. This is not only true from the producer side, but the consumer side as well. Consumers are looking for milk that is nutritious, has good flavour and is low in fat. Protein is a component that can contribute to flavour and nutrition without increasing the fat or calorie content of milk (Dechow *et al.*, 2007). Milk, fat and protein yields are the main economic traits for selection in dairy cows. Estimates of phenotypic parameters are required for prediction of breeding values. Phenotypic correlations show relationships between phenotype traits. These parameters are important especially in multi-trait selection and improvement programs because they are used in the calculation of selection responses (DeLorenzo and Wiggans, 1986). Selection programme is necessary to increase the total amount of milk while maintaining contents of fat and protein (Hardie *et al*., 1978).

The Romanian Simmental breed, also known as Romanian Spotted cattle breed, has been formed as the result of a long crossing between the Romanian Grey cattle native breed cows with Simmental bulls (Sas and Sas, 1996). The breed is characterized by a high variability due to the variability of the maternal breed (Romanian Grey cattle with five zonal types); the polymorphism of the Simmental breed that is due to morphological type differences from origin countries and the changing, in time, of the objects in differentiated improvement of Simmental cattle's main productive abilities.

Although Romanian Simmental is a multipurpose breed, at present it is the main supplier of beef meat in Romania, with the meat productive type surpassing in importance the milk productive type. Therefore, genetic selection is necessary in order to improve milk production type cattle of this breed. The genetic potential of the Romanian Simmental breed is estimated to be 5000 kg milk per lactation period with 3.90 % fat and 3.30 % protein, or otherwise 195 kg fat and 165 kg protein per lactation period (Sas and Sas, 1996).

Thus, the objective of this study was to determine milk yield, milk fat and protein yield, as well as milk fat and protein percentage, and to evaluate relations among the studied parameters for a first lactation dairy herd of the Romanian Simmental breed, in order to improve selection for milk yields.

MATERIAL AND METHODS

Data Collection

The study was conducted in a dairy farm located at Timiş County in the west of Romania, from February 2010 to April 2012. Ninety-nine first lactation Romanian Simmental cows were selected from 1275 dairy cows of the herd to participate in the study immediately after first calving. Cows were housed and treated in accordance with the applicable recommendations of the European Council (EEC, 1986), and the Animal Care and Use Protocol for Banat's University of Agricultural Sciences and Veterinary Medicine, Timişoara, Romania. From April to October, during the day, cows were allowed to graze on the surroundings of the farm, from 10:00 h and flocked back at 17:00 h. The rest of the year, animals were not allowed to graze. In addition, during the whole year, cows were fed a total mixed ration (TMR) (alfalfa hay 0.30, corn silage 0.40, corn grain 0.18, wheat bran 0.10, and vitamin and mineral premix 0.02, dry matter – DM basis) and water ad libitum in elongated troughs and drinkers, respectively, in the courtyard of the farm, to meet their nutrient requirements as given by NRC (2001). Cows were machine milked twice a day, for a completed lactation of at least 305 days, with a "Bradulet" type milking machine (Banat Nova Puls, Timişoara, Romania). The milking machine was equipped with a computerized management system (Banat Afimilk, Timişoara, Romania) that provides daily data report on milking efficiency, reproduction and fertility, medication and treatment procedures etc. Thus, milk yield was recorded daily at a morning and afternoon milking. During milk yield recording, milk samples were collected from each cow (after cleaning and disinfecting the teats) and kept refrigerated at 4 °C until chemical analysis within a day at the Central Laboratory of the Milk Control Association (Timişoara, Romania). Milk samples were analyzed for fat and protein with IR spectroscopy (MilkoScan^{TM}Minor4; TESCO, Denmark) according to the method 972.16 of AOAC (1990).

Statistical Analysis

Arithmetic mean (X), standard deviation (SD), range of variation and variability were computed for milk yield, milk fat and protein yield, as well as milk fat and protein percentage. An individual cow was used as the experimental unit. All dairy cows were kept in the same farm in a free nutrition system. Therefore, the quantity of food consumed was different from one cow to another.

Analyses were conducted by means of the restricted maximum likelihood (REML) procedures using the programme StatSoft (2007) for the variables milk, fat and protein yield and fat and protein percentages. The basic model (Hallowell *et al.*, 1998) fitted was as follows:

$$y = Xa + Zb + e$$

where: y, a vector of observations on first lactation

milk, fat and protein yield, fat and protein percentage; X and Z, known incidence matrices relating observations to effects; a, a vector of fixed effects consisting of month of calving, herd and times milked depending on which were significant; b, a vector of continual effects, age and calving interval with the effects of sire randomised; and e, a vector of unknown residual effects.

To evaluate relations among the studied parameters, the slope (b) of the regression coefficient was calculated using the following model (Tibshirani, 1996):

Regression Equation (y) = a + bx

Slope (b) = $(N\Sigma XY - (\Sigma X)(\Sigma Y)) / (N\Sigma X2 - (\Sigma X)^2)$

Intercept (a) = $[\Sigma Y - b(\Sigma X)] / N$

where: x and y, the variables; b, the slope of the regression line; a, the intercept point of the regression line and the y axis; N, the number of values or elements; X, the First Score, Y, the Second Score; ΣXY, the Sum of the product of First and Second Scores; ΣX, the Sum of First Scores; ΣY, the Sum of Second Scores; and ΣX^2, the Sum of square First Scores.

The coefficient of variation (CV %) was calculated as follows: CV % = $(SD/\bar{x}) \pm 100$, to describe the variation of the traits (Hendricks and Robey, 1936).

RESULTS AND DISCUSSION

Data for the milk, fat and protein yields, as well as milk fat and protein percentage, at first lactation of Romanian Simmental cows are presented in Table 1. For each parameter examined, the mean value and the variation components were estimated. The linear regression relationship and correlations among milk traits are presented in Figures 1, 2, 3, 4, 5 and 6. Results suggested that as much as the milk yield increases, the fat yield will also increase. Furthermore, when milk fat percentage increases, the milk protein percentage will increase in the same extent. The other low values of correlation between the other milk traits indicate that the relationship between the predictor and response variable is very low linearly.

Milk recording provides cattle breeders with information on milk yield and milk composition for each dairy cow in the herd. The results help breeders in herd management and represent the basic source of information for the prediction of breeding value. Several variables (days in milk, milk fat and protein percentage, season) related to milk yield are collected as the parameters of the forecasting model. Fat and protein percentage are the most important components that dictate the purchase price of milk.

Milk yield for the first lactation of our Romanian Simmental cows (4053 kg) was lower than milk yield reported by Janžekovic *et al*. (2004) for first lactation of Slovenian Simmental cows (4870 kg), by Petrović *et al.* (2009) for first lactation of Serbian Simmental cows (4868 kg), and by Wolfová *et al.* (2007) and Jílek *et al.* (2008) in three herds of Czech Fleckvieh cows (5700 kg, 7651 kg and 6003 kg, respectively).

The high coefficient of variation for milk yield of 34.3 % in our Romanian Simmental cows denotes a very heterogenic population. As the data show, the herd had a very good phenotypic performance for this character, but only cows with a yield over 4000 kg/lactation were recommended to be retained for selection. Variation is a natural part of the biological process of making milk components. Age, illness,

Table 1: Mean and variability factors for milk yield and composition of first lactation Romanian Simmental cows[a]

	Mean $\bar{x}$	Standard deviation ± s	Coefficient of variation CV %	Min	Max	Standard Error ± $s\bar{x}$
Yield (kg)						
Milk	4053.0	1391.8	34.3	1055.6	7432.5	139.9
Fat	157.6	55.3	35.1	41.3	289.0	5.6
Protein	131.6	46.3	35.2	34.6	234.7	4.6
Milk percentage (%)						
Fat	3.820	0.378	9.890	2.800	4.700	0.038
Protein	3.120	0.358	11.470	2.000	4.500	0.036

[a]n = 99

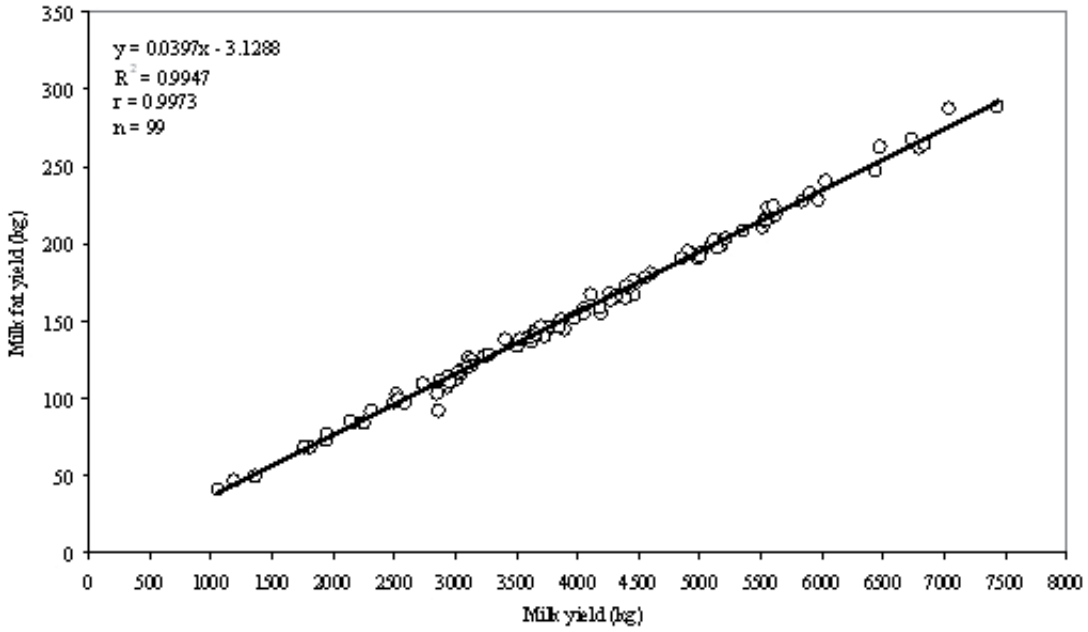

Fig. 1: The linear regression relationship between milk yield (kg) and milk fat yield (kg)

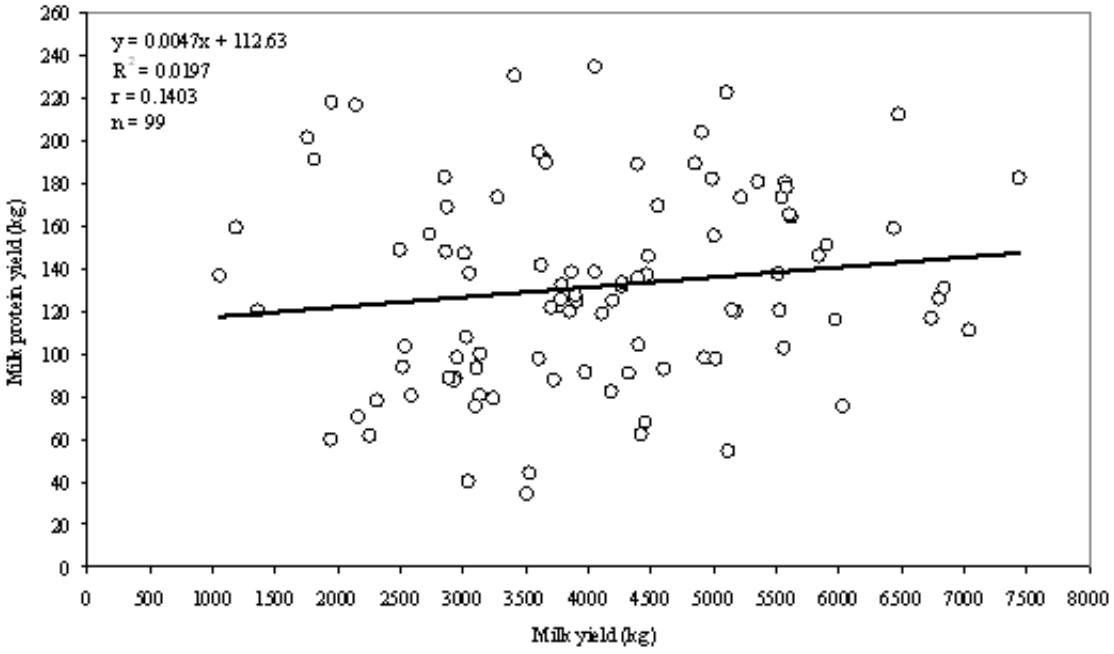

Fig. 2: The linear regression relationship between milk yield (kg) and milk protein yield (kg)

injury, feed, reproductive processes, climate, milking procedures and equipment, sampling techniques, sample shipment and lab procedures are possible sources of variability in component test results (Ježková and Dřevo, 2002). Some of these factors operate in a rather random fashion, in some cases increasing and in others decreasing component test results. Some of the factors may be systematic in nature, introducing bias into milk component test results (Gilmore and Gaunt, 1963). Furthermore, fat tests may respond quite differently from protein tests under the influence of some factors (Lee and Wardrop, 1984).

In our study, Romanian Simmental cows, in 305 days of lactation, yielded more milk fat (157.6 kg) than the Slovenian Simmental cows (133.5 kg milk fat; Logar *et al*., 2007), but less than the Czech Fleckvieh cows (275.7 kg milk fat, Bouška *et al*., 2008; and 255 kg milk fat, Čermák *et al.*, 2008). In addition, milk protein yield in this study (131.6 kg) was higher than that reported by Logar *et al.* (2007) in Slovenian Simmental cows (106.9 kg milk protein), but lower than milk protein yield found by Bouška *et al*. (2008) and Čermák *et al.* (2008) in Czech Fleckvieh (223.4 kg and 198 kg milk protein, respectively). For milk fat and protein yields, the coefficient of variation indicates a high heterogeneity in the cows' population.

In first lactation Romanian Simmental cows, milk fat and protein percentage was 3.82 % and 3.12 % respectively. Fat and protein percentage of cow milk used in this study was similar to the respective parameters in milk obtained from Simmental cows (Chládek and Kučera, 2002; Hanuš *et al.*, 2007; Krupová *et al.*, 2009). Petrović *et al.* (2009) found similar results for milk fat percentage (3.76 %) in first lactation Serbian Simmental cows. In contrast, Janžekovic *et al.* (2004) found higher milk fat (4.28 %) and milk protein percentage (3.43 %) in first lactation Slovenian Simmental cows than in our cows. Krupa *et al*. (2005) also found higher milk fat (4.10 %) and milk protein (3.35 %) percentage in Slovakian Pied cattle. Moreover, in three herds of Czech Fleckvieh cows, Wolfová *et al.* (2007) and Jílek *et al.* (2008) found milk fat percentage of 4.05 %, 4.28 % and 3.86 %, respectively, and milk protein percentage of 3.42 %, 3.45 % and 3.24 %, respectively. Low milk protein levels are frequently due to the low ration protein and/or energy level (Wolfová *et al*., 2007).

In our study, the milk fat and protein yields were highly variable with a coefficient of variation over 30 %. This suggests a very heterogenic dairy cows' population but, at the same time, there is the possibility of improving daily production by genetic means. Moreover, the milk fat and protein percentage had a coefficient of variation of 9.89 % and 11.47 %, respectively. Syrstad (1977) calculated standard deviations for cows and reported 0.364 and 0.078 percentage units for cow milk fat and protein percentage, respectively, whilst the respective standard deviations in our study were of 0.378 (for fat) and 0.358 (for protein) percentage units.

The potential fat percentage of milk from an individual cow is determined genetically, as are protein and lactose levels. Thus, selective breeding can be used to upgrade milk quality. Heredity also determines the potential milk production of the animal (Dematawewa and Berger, 1998). However, environment and various physiological factors greatly influence the amount and composition of milk that is actually produced. Fat percentage is the most variable component of milk and, besides the factors listed above, also depends on completeness of milking, sampling procedure and milking interval (Hargrove *et al.*, 1981). In our study, cows were milked at 12-hour intervals and the variation in fat percentage between milkings was negligible, but this is not practicable on most farms (Klopčič *et al*., 2003). Also, the first milk drawn from the udder is low in fat, whilst the last milk (or strippings) is always quite high in fat. Thus, it is essential to mix thoroughly all the milk removed, before taking a sample for analysis. The fat left in the udder at the end of a milking is usually picked up during subsequent milkings, so there is no net loss of fat.

Milk proteins represent one of the greatest contributions of milk to human nutrition. Protein does not vary to the same extent as fat percentage and the energy supply has the strongest impact on the protein percentage (Meinert *et al*., 1989). Longer milking intervals do not change protein percentage as much as fat percentage (Filistowicz *et al*., 1993). Milk protein has economic value because higher protein leads to higher cheese yields. Consequently, milk protein percentage of milk is emphasized (Grant, 2007). This parameter represents a decisive criterion in dairy selection. Results obtained can be also used for choosing the dams as mother sires, eliminating the individuals in which this character is situated under the standard limits.

In this study, the linear regression relationship and correlations among studied parameters were estimated, in order to have an indicator of the improvement method for these parameters. Evaluation of the relation between milk yield and milk fat yield (Figure 1) indicates a high correlation (r = 0.9973). The same high correlation (r = 0.96 to 0.99) between these milk traits was found in Holstein cows (Boujenane, 2002; Hashemi and Nayebpoor, 2008), whilst Ptak *et al.* (2004) and Al-Seaf *et al*. (2007) found a lower correlation (r = 0.73 and r = 0.70, respectively) in Holstein cows than in our study.

Concerning the relation between milk yield and milk protein yield in our study (Figure 2), the correlation coefficient (r = 0.1403) showed a little or

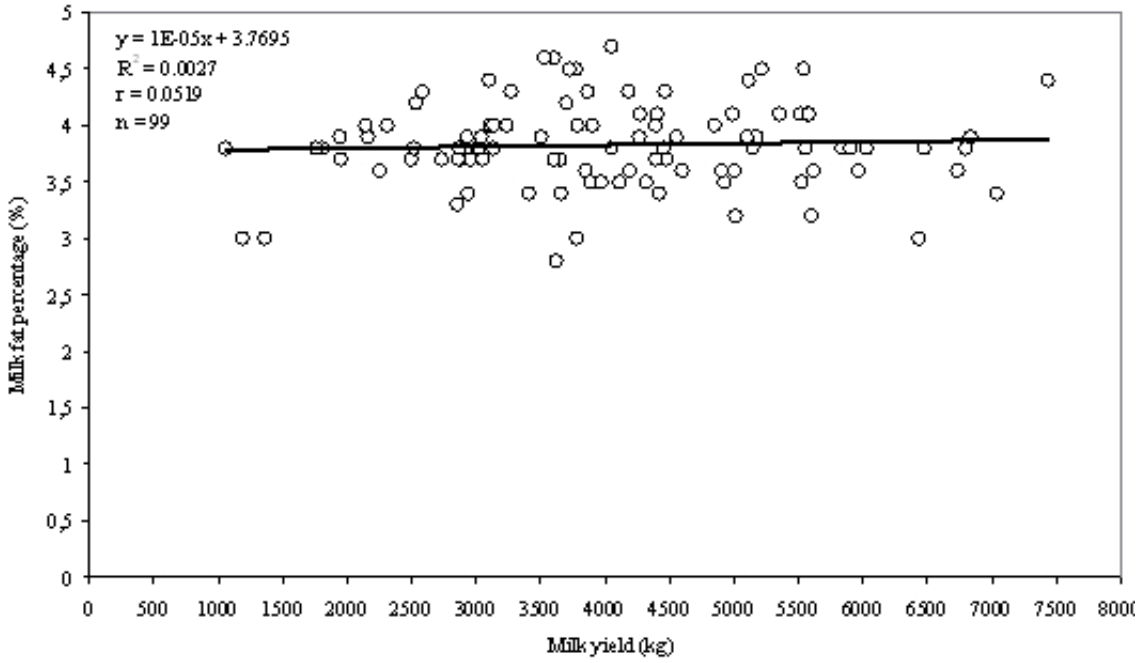

Fig. 3: The linear regression relationship between milk yield (kg) and milk fat percentage (%)

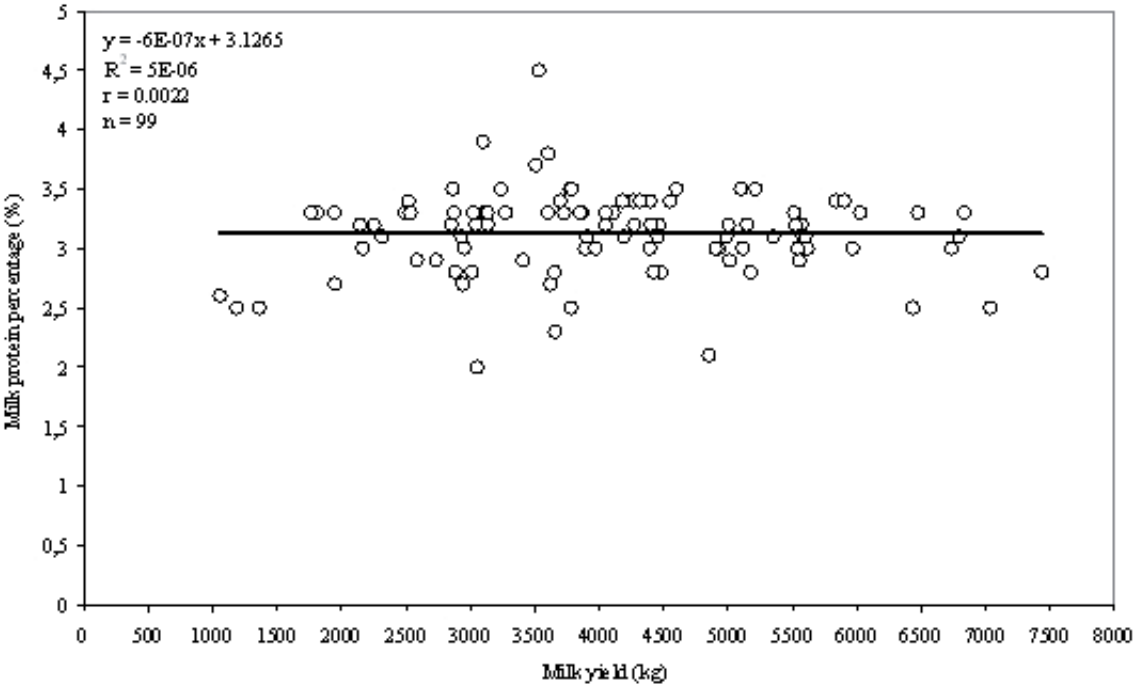

Fig. 4: The linear regression relationship between milk yield (kg) and milk protein percentage (%)

no association between analysed traits. In contrast, Ptak *et al*. (2004) and Al-Seaf *et al.* (2007) reported in Holstein cows a higher correlation (r = 0.89, and r = 0.92, respectively) between milk yield and milk protein yield than in our study.

Low correlation coefficient (r = 0.0519) was found between milk yield and milk fat percentage (r = 0.0519; Figure 3), as well as between milk yield and milk protein percentage (r = 0.0022; Figure 4), which denote no association between these two couples of milk traits. For milk yield and milk fat percentage, Gaines (1940) found r = -0.199, which suggests that this is associated with the low ratio of variability in fat percentage to variability in milk yield, whilst Boujenane (2002) and Hashemi and Nayebpoor (2008) also found in Holstein cows a negative and moderate phenotypic correlation between milk yield and milk fat percentage (r = -0.28 and r = -0.27, respectively). Moreover, Boettcher *et al.* (2004) found in Holstein cows a higher correlation coefficient between milk yield and milk fat percentage (r = 0.62) and between milk yield and milk protein percentage (r = 0.54).

Linear regression between milk fat yield and milk protein yield is shown in Figure 5, and between milk fat percentage and milk protein percentage in Figure 6. The correlation coefficient of r = 0.1414 in Figure 5 indicates that the milk protein yield did not increase similarly to milk fat yield. Al-Seaf *et al*. (2007) found in Holstein cows a higher phenotypic correlation (r = 0.74) between milk fat and protein yields. In Figure 6, the best estimate of the relationship of milk fat and protein percentages is expressed by a coefficient correlation of r = 0.5139. Musgrave and Salisbury (1952) found a lower coefficient correlation (r = 0.30) between these milk traits in Brown Swiss herd.

Excepting the phenotypic correlation value obtained between milk yield and milk fat yield, which was 0.99, all correlations values concerning other milk traits were fairly low. It is known that there is a negative correlation between milk yield and milk fat and protein percentage. As milk yield increases, milk fat percentage and milk protein percentage declines (Bilal and Khan, 2009).

CONCLUSION

The average milk yield in the studied primiparous Romanian Simmental herd was of 4053 kg, which allows the selection of individuals that produce over 4000 kg milk/lactation. Milk fat yield, in the same herd, had an average of 157.6 kg, and milk protein yield - an average of 131.6 kg. The strong phenotypic correlation between milk yield and milk fat yield indicates that the population of dairy cows can be improved by selection using the independent level of selection.

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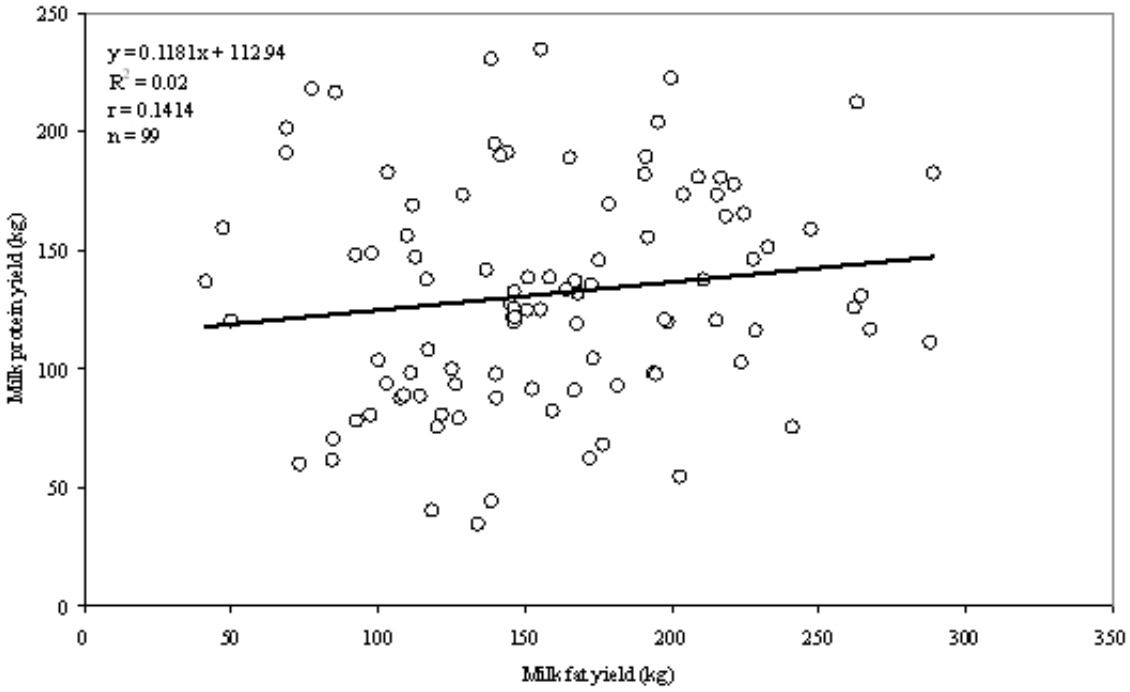

Fig. 5: The linear regression relationship between milk fat yield (kg) and milk protein yield (kg)

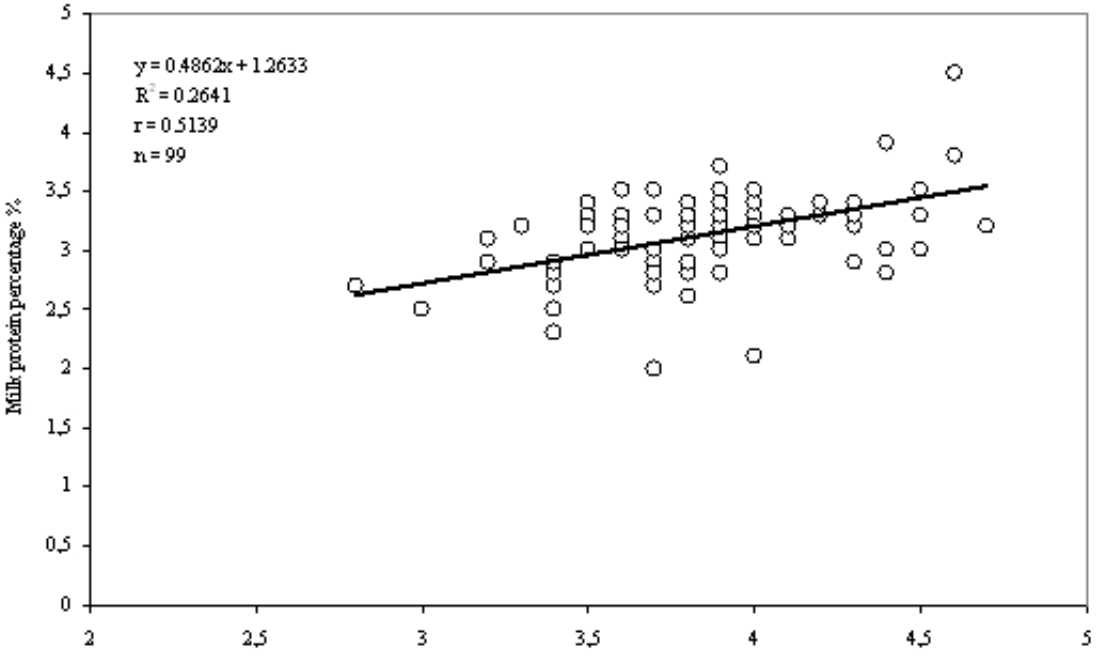

Fig. 6: The linear regression relationship between milk fat percentage (%) and milk protein percentage (%)

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MULTIVARIATE ANALYSIS FOR BODY WEIGHT AND SOME LINEAR BODY MEASUREMENTS OF NIGERIAN INDIGENOUS CHICKENS

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ABSTRACT

The use of path analysis will not only produce a regression equation for prediction of body weight but also partition correlation between two traits into direct effects of one on other and indirect effects caused by other characters which may be of importance in selection. A total number of 2641 mature cocks comprising of 1782 Yoruba ecotype and 859 Fulani ecotype were sampled from markets in Osun state, southwest Nigeria. Live weight (LW) and eight morphometric characters were measured from these birds. The biometric traits were keel length (KL), chest circumference (CC), thigh length (TL), wing length (WL), body length (BL), drum stick (DS), breast length (BrL) and shank length (SL). T-test was used to check the significance of variation in biometric traits between the two ecotypes. Correlation analysis was used to check degree of association between these traits. Regression and path analysis was also explored. There were significant differences in keel length, thigh length, wing length, body length, breast length and shank length between the two genotypes. All traits considered in Yoruba ecotype but drum stick showed significant ($p<0.05$) positive correlation with body weight whereas in Fulani ecotype all characters but chest circumference showed a significant ($p<0.05$) correlation with body weight. Body length and breast length had significant ($p<0.05$) direct effect on the body weight in Yoruba ecotype whereas significant ($p<0.05$) path coefficients were obtained for chest circumference, wing length and breast length in Fulani ecotype. Body length and breast length had the highest direct effect on body weight in Yoruba and Fulani ecotype cocks respectively. The highest indirect effect was obtained for breast length through drum stick in Fulani ecotype, while in Yoruba ecotype the highest indirect effect was obtained for body length through chest circumference. Body length and breast length can be deduced as the most important morphometric traits in determining body weight of Yoruba and Fulani ecotype cocks respectively.

Key words: Nigeria; chickens; morphometric correlation; path coefficients

INTRODUCTION

Morphometric characters have for years been used to predict body weight of animals; where different regression models are explored. These models do not only make on-farm measurement of animal weight to be less tedious but also reduce risk of hazards associated with the use of weighing scale especially in farm animals with large body size. A linear relationship between BW and shank length has been reported (Lerner, 1937). Tierce and Nordskog (1985) produced a general formula for estimation of live weight in poultry; shank length (mm) = $\alpha W (kg)^{B}$. Although accurate estimation of productivity of indigenous strains of chicken is now a difficult task due to indiscriminate cross breeding which has taken place between them and the exotic strains (Raji *et al.*, 2009; Oluyemi, 1989). Rearing of broilers is now becoming extensively popular in the southwest Nigeria. Thus, there is need for caution in sampling indigenous strains of chicken

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for characterization. Chineke *et al.* (2002) reported that the relationship existing among body characteristics provide useful information on performance, productivity and carcass characteristics of animals and these quantitative measure of size and shapes are necessary for estimating genetic parameters in animal breeding programmes. Phenotypic correlation between traits is sum of genotypic correlation and environmental correlation. Series of traits of economic importance in farm animals show pleiotropism, a situation where same portion of DNA (gene) code for more than one protein. Proper knowledge of genotypic correlation between traits is essential for selection, thus, tandem selection may be effective when positive correlations exist between the trait selected for and other characters of interest.

Simple correlation between traits has been commonly used in the past, its suitability as a measure of degree of association between traits is moot. Yakubu and Mohamed (2012) opined that body measurements that are used to predict body weight may affect its determination directly and indirectly. Thus partitioning of correlation coefficient between two characters into direct and indirect component (path analysis) is crucial. Path analysis partitioned the correlation coefficient into direct (path coefficient) and indirect effects (effect exerted through other variables). So it provides an effective means of partitioning correlation coefficients into unidirectional path ways and alternate pathways thus permitting a critical examination of specific factors that produce a given correlation. It is a standardized partial regression analysis that deals with a closed system of variable which are linearly related. The technique of path analysis in livestock experiment has been extensively used by several researchers (Yakubu and Mohamed, 2012; Ogah *et al*., 2011; Yakubu and Salako, 2009). This study was thus carried out with the objective of establishing a detailed relationship between body weight and linear body measurements of Yoruba and Fulani ecotype cocks using path analysis.

MATERIAL AND METHODS

Study area

Data for this study were collected from markets and traditionally managed flocks in different parts of Osun state, Southwest Nigeria.

Management of Chicken

Chicken surveyed were semi-intensively managed. They roamed freely during the day when only maize or sorghum was occasionally given to them in the morning before they were allowed to scavenge for the remaining uptake. In the evening they returned home, some of them were kept in locally made cages ‘‘ago’’ and others without cages stayed on trees till dawn.

Data collection

Data were collected from 2641 mature cocks comprising of 1782 Yoruba ecotype and 859 Fulani ecotype. Cocks were mainly selected for this study because of their market value (local farmers rarely put hen for sale) and importance of sire in breeding program. Body weights of the birds were measured using 5 kg weighing scale. Linear body measurements were performed using a measuring tape graduated in centimetres. Measurements were done with one person throughout the duration of data collection to avoid variation between individuals. The sampling of birds in market was done at random with intervals of ten (10) days between two consecutive measurements while each household was visited once to avoid repeated measurement of the same bird. Body weight and eight (8) morphometric traits were measured for each animal. The anatomical reference points were as described earlier (Monsi, 1992; Udeh *et al*., 2011). The biometric traits were keel length (KL), chest circumference (CC), thigh length (TL), wing length (WL), body length (BL), drum stick (DS), breast length (BrL) and shank length (SL).

KL: Measured as the length of the breast bone

CC: Circumference of the pectus (hind breast)

TL: Measured as the distance between knee and end of femur bone.

WL: Measured between the caput humeri to the end of the third carpal digit.

BL: Measured between the first cervical vertebra and the pygostyle.

DS: Length from the knee joint to the hock.

BrL: Measured as the distance between the right and left glenoid cavity.

SL: Distance from the hock to the extremity of the digitus pedis.

Measurements were done according to the illustrations by FAO (2012).

Statistical Analysis

Means, standard deviation (SD) and coefficient of variation (CV) of live weight and linear body measurements were done. T-test was used to check whether significant differences occurred in the morphometric characters between the two ecotypes. Pearson correlation was explored to determine the degree of association between the variables. Compound linear regression was also performed where partial regression coefficients were standardized. The standardized linear regression coefficient (path coefficient) shows the direct effect of linear

measurements (X) on live weight (Y).

$$PY.X_i = b_i \frac{SDX_i}{SDY}$$

where

$PY_{.Xi}$ = path coefficient from X_i to Y (i = KL, CC, TL, WL, BL, DS, BrL, SL)

b_i = unstandardized or partial regression coefficient

SDX_i = standard deviation of linear measurements

SDY = standard deviation of live weight

The indirect effects of X_i on Y through X_j were computed as

$$IEYX_i = (rX_iX_jP)\ (PY.X_j)$$

where

$IEYX_i$ = correlation coefficient between ith and jth linear measurements

$PY.X_j$ = path coefficient that indicates the direct effect of jth linear measurement (exogenous variable) on the live weight (endogenous variable)

The model for the multiple linear regression was

$$Y = a + b_1X_1 + b_2X_2 \ldots\ldots + b_8X_8$$

Y = body weight (dependent variable)

a = intercept

b = standardized regression coefficient

X = exogenous variable (KL, CC, TL, WL, BL, DS, BrL and SL)

The significance of each path coefficient in the model was tested by t- test procedure adapted from Yakubu and Mohammed (2012)

$$t_j = \frac{b_j - \beta_j}{\sqrt{var\ (b_j)}} \sim t\alpha\ (n\text{-}p\text{-}1)\ ;\quad j = 1, 2, \text{--------}, p$$

where

var (b_j) = the diagonal member of matrix $S^2\ (X'X)^{-1}$

S^2 = mean square of residual obtained from ANOVA

Coefficient of determination (R^2) was computed according to the method of Yakubu and Salako (2009):

$$R^2 = P^2Y.X_1 + P^2Y.X_2 + P^2Y.X_3 + P^2Y.X_4 + P^2Y.X_5 + P^2Y.X_6 + P^2Y.X_7 + P^2Y.X_8 + 2rX_1X_2PY.X_1PY.X_2 + 2rX_1X_3PY.X_1PY.X_3 + 2rX_1X_4PY.X_1PY.X_4 + 2rX_1X_5PY.X_1PY.X_5 + 2rX_1X_6PY.X_1PY.X_6 + 2rX_1X_7PY.X_1PY.X_7 + 2rX_1X_8PY.X_1PY.X_8 + 2rX_2X_3PY.X_2PY.X_3 + 2rX_2X_4PY.X_2PY.X_4 + 2rX_2X_5PY.X_2PY.X_5 + 2rX_2X_6PY.X_2PY.X_6 + 2rX_2X_7PY.X_2PY.X_7 + 2rX_2X_8PY.X_2PY.X_8 + 2rX_3X_4PY.X_3PY.X_4 + 2rX_3X_5PY.X_3PY.X_5 + 2rX_3X_6PY.X_3PY.X_6 + 2rX_3X_7PY.X_3PY.X_7 + 2rX_3X_8PY.X_3PY.X_8 + 2rX_4X_5PY.X_4PY.X_5 + 2rX_4X_6PY.X_4PY.X_6 + 2rX_4X_7PY.X_4PY.X_7 + 2rX_4X_8PY.X_4PY.X_8 + 2rX_5X_6PY.X_5PY.X_6 + 2rX_5X_7PY.X_5PY.X_7 + 2rX_5X_8PY.X_5PY.X_8 + 2rX_6X_7PY.X_6PY.X_7 + 2rX_6X_8PY.X_6PY.X_8 + 2rX_7X_8PY.X_7PY.X_8$$

where

$P^2Y.X_1$ = direct effects of predictor variables (= KL, CC, TL, WL, BL, DS, BrL, SL) in contributing to the variation of Y (body weight).

$2rX_iX_j\ (PY.X_i)(PY.X_j)$ = combined effects of explanatory predictor variable (= KL, CC, TL, WL, BL, DS, BrL, SL) in contributing to the variation of Y (body weight).

RESULTS AND DISCUSSION

Description of body weight and linear body measurements

Description of live weight and linear body measurements showing means, standard deviation (SD) and coefficient of variation (CV) for mature indigenous chicken of Nigeria is presented in Table 1. The range for average weight between Yoruba and Fulani ecotype cocks was 0.1g. Mean body weight obtained in this study for both ecotypes were similar to 1.37 ± 0.004 kg as reported for mature Nigerian cock by Yakubu and Salako (2009). Yakubu *et al*. (2009) in another related study reported lesser live weight (1.26 ± 0.004 kg) for frizzle feathered and naked neck fowl. Though sex of these birds was unspecified, the lesser weight obtained could have resulted from either sexual dimorphism or the stage of growth rather than from the major genes. Because of susceptibility of frizzle feathered and naked neck birds to cold, there are more proclivities of sampling them immature. There were significant differences in keel length, thigh length, wing length, body length, breast length and shank length between the two genotypes investigated. Fulani ecotype cocks had higher means for all significant traits. CV ranged between 13.35 % - 69.88 % in Yoruba ecotype and 8.99 % - 26.58 % in Fulani ecotype. Large variability obtained in this study indicated that these traits are still largely unselected for; therefore, they may largely respond to selection. Yakubu *et al.* (2009) reported CV of 24.26 % for live weight of adult cock. Traits that are related to bone development tend to be less variable because of large genetic influence, in spite of this; CV of 69.88 % was obtained for drum stick length of Yoruba ecotype cocks.

Correlation between traits

Matrix of correlation between body measurements of Yoruba and Fulani ecotypes cock is presented in Table 2. Correlation is a measure of degree of association between two variables; it does not produce cause and effects. All traits considered in Yoruba ecotype but drum stick showed significant ($p<0.05$) positive correlation with body weight. The low correlation of drum stick with all other parameters in Yoruba ecotype (0.11-0.18) could have resulted from

Table 1: Description of body weight and linear body measurements

Traits	Yoruba ecotype Mean ± SE	SD	CV	Fulani ecotype Mean ± SE	SD	CV
LW (kg)	1.48 ± 0.03	0.42	28.38	1.58 ± 0.05	0.42	26.58
KL (cm)	11.98 ± 2.83[b]	2.83	23.62	15.50 ± 0.49[a]	4.00	25.81
CC (cm)	25.86 ± 0.29	3.59	13.88	26.80 ± 0.42	2.41	8.99
TL (cm)	11.07 ± 0.15[b]	1.90	17.16	11.71 ± 0.24[a]	1.98	16.91
WL (cm)	14.91 ± 0.16[b]	1.99	13.35	16.66 ± 0.26[a]	2.17	13.03
BL (cm)	31.43 ± 0.39[b]	4.79	15.24	33.22 ± 0.48[a]	3.96	11.92
DS (cm)	11.82 ± 0.67	8.26	69.88	12.27 ± 0.25	2.03	16.54
BrL (cm)	10.62 ± 0.13[b]	1.62	15.25	11.42 ± 0.23[a]	1.85	16.20
SL (cm)	9.04 ± 0.12[b]	1.49	16.48	10.24 ± 0.23[a]	1.89	18.46

[ab]Means along same row with different superscripts are significantly different ($p<0.05$)
SE: Standard error for means
SD: Standard deviation
CV: Coefficient of variation

Table 2: Simple correlation analysis of body weight and linear body measurements

Traits	LW	KL	CC	TL	WL	BL	DS	BrL	SL
LW (kg)		0.42	0.13[ns]	0.45	-0.27	0.51	0.42	0.72	0.30
KL (cm)	0.22		0.23[ns]	-0.27	0.55	-0.29	0.12[ns]	-0.37	0.20
CC (cm)	0.49	0.10[ns]		0.06[ns]	0.36	-0.03ns	0.16[ns]	-0.04[ns]	0.24
TL (cm)	0.37	0.10[ns]	0.22		-0.23[ns]	0.33	0.44	0.37	0.28
WL (cm)	0.41	0.05[ns]	0.66	0.14[ns]		-0.09[ns]	0.36	0.01[ns]	0.50
BL (cm)	0.83	0.17	0.66	0.35	0.62		0.43	0.50	0.35
DS (cm)	0.15[ns]	0.11[ns]	0.14[ns]	0.17	0.18	0.15[ns]		0.52	0.80
BrL (cm)	0.34	0.15[ns]	0.06[ns]	0.24	-0.09[ns]	0.23	-0.08[ns]		0.53
SL (cm)	0.28	0.01[ns]	0.22	0.22	0.23	0.27	0.13[ns]	0.08[ns]	

Upper diagonal: Fulani ecotype
Lower diagonal: Yoruba ecotype
Correlation coefficients with superscript (ns) are not significant ($p>0.05$)

large variability (69.88 %) obtained for the trait. All characters but chest circumference showed a significant ($p<0.05$) correlation with body weight in Fulani ecotype birds. There was a significant ($p<0.05$) negative correlation of live weight with wing length (-0.27). This is critical to animal welfare because selection for larger body weight might hamper flapping ability of these birds. Correlation between body parameters obtained in this study was lower than what was reported by Yakubu and Salako (2009), though many of traits considered in the aforementioned study were not included in this finding.

Path coefficient

Path coefficients of the linear body measurements (independent variable) of Yoruba ecotype cocks are presented in Table 3. Path analysis permits the partitioning of correlation coefficient into component parts (Marjanovic-Jeromela *et al.*, 2008; Yakubu, 2010). Body length and breast length had significant ($p<0.05$) direct effect on the body weight. Body length had the highest significant ($p<0.01$) path coefficient as indicated by the t-test. Body length had both highest significant ($p<0.001$) correlation with body weight and path coefficient. This was due to the low indirect effects of

other variables on body length.

Path coefficient of the linear body measurements (independent variables) in Fulani ecotype cocks are presented in Table 4. Significant ($p<0.05$) path coefficients were obtained for chest circumference, wing length and breast length. Breast length had the highest direct effect on body weight (Path coefficient = 0.62; $p<0.01$). This infers that a unit change in standard deviation of body weight results in 0.62 units change in standard deviation of breast length.

Coefficient of determination

The coefficient of determination where direct and combined effects of linear measurements on body weight were determined is presented in Table 5. For Yoruba ecotype, the highest direct contribution to variation in body weight was made by body length ($R^2 = 0.824$). Very low combined effects were obtained between all variable pairs. Direct coefficient of determinant of 0.00, 0.01, 0.00, 0.02, 0.00, 0.01 and 0.00 were obtained for keel length, chest circumference, thigh length, wing length, drum stick, breast length and shank length respectively. The low R^2 for all these traits indicate the importance of body length as a predictor variable for body weight of Yoruba ecotype cock. The preliminary regression equation where all traits were considered in Yoruba ecotype was:

BW = -1.09 + 0.06KL - 0.07CC + 0.03TL - 0.12WL + 0.91BL + 0.03DS + 0.10BrL + 0.06SL

Table 3: Direct and indirect effect of linear body measurements on live weight of Yoruba ecotype cock

Traits	Correlation of linear measurements with live weight	Direct effect	Indirect effect								Total
			KL	CC	TL	WL	BL	DS	BrL	SL	
KL (cm)	0.221	0.06	-	-0.01	0.00	-0.01	0.15	0.00	0.02	0.00	0.16
CC (cm)	0.485	-0.07	0.01	-	0.01	-0.08	0.60	0.00	0.01	0.01	0.56
TL (cm)	0.365	-0.03	0.01	-0.02	-	-0.02	0.32	0.01	0.02	0.01	0.34
WL (cm)	0.408	-0.12*	0.00	-0.05	0.00	-	0.56	0.01	-0.01	0.01	0.53
BL (cm)	0.825	0.91**	0.01	-0.05	0.01	-0.07	-	0.00	0.02	0.02	-0.06
DS (cm)	0.145ns	0.03	0.01	-0.01	0.01	-0.02	0.13	-	-0.01	0.01	0.12
BrL (cm)	0.336	0.10*	0.01	0.00	0.01	0.01	0.21	-0.00	-	0.01	0.24
SL (cm)	0.283	0.06	0.00	-0.02	0.01	-0.03	0.25	0.00	0.01	-	0.23

Table 4: Direct and indirect effect of linear body measurements on live weight of Fulani ecotype cock

Traits	Correlation of linear measurements with live weight	Direct effect	Indirect effect								Total
			KL	CC	TL	WL	BL	DS	BrL	SL	
KL (cm)	-0.416	-0.02	-	0.07	-0.001	-0.20	-0.03	0.03	-0.23	-0.03	-0.40
CC (cm)	0.134	0.29**	0.00	-	0.00	-0.13	0.00	0.04	-0.02	-0.04	-0.15
TL (cm)	0.447	0.01	0.00	0.02	-	0.09	0.04	0.11	0.23	-0.04	0.44
WL (cm)	-0.268	-0.37**	-0.01	0.10	-0.001	-	-0.01	0.09	0.00	-0.07	0.10
BL (cm)	0.507	0.11	0.00	-0.01	0.00	0.03	-	0.10	0.31	-0.05	0.39
DS (cm)	0.418	0.24	0.00	0.05	0.00	-0.13	0.05	-	0.33	-0.12	0.17
BrL (cm)	0.712	0.62**	0.01	-0.011	0.00	0.00	0.06	0.13	-	-0.08	0.10
SL (cm)	0.303	-0.14	0.00	0.07	0.00	-0.19	0.04	0.19	0.33	-	0.45

Table 5: Direct and combined effects of the biometric traits

Traits	Coefficient of Determinant R^2	
	Yoruba Ecotype	Fulani Ecotype
Direct effects		
P^2LW. KL	0.0006	0.0004
P^2LW. CC	0.0125	0.0824
P^2LW. TL	0.0004	0.0001
P^2LW. WL	0.0184	0.1441
P^2LW. BL	0.8217	0.0117
P^2LW. DS	0.0004	0.0581
P^2LW. BrL	0.0106	0.3883
P^2LW. SL	0.0008	0.0188
Combined effects		
KL and CC	0.0007	0.0004
KL and TL	0.0005	0.0002
KL and WL	0.0009	0.0093
KL and BL	0.0212	0.0003
KL and DS	0.0004	0.0006
KL and BrL	0.0006	0.0141
KL and SL	0.0007	0.0004
CC and TL	0.0003	0.0003
CC and WL	0.0091	-0.0825
CC and BL	-0.0826	-0.0003
CC and DS	0.0004	0.0213
CC and BrL	0.0002	-0.0115
CC and SL	0.0005	-0.0184
TL and WL	0.0003	0.0003
TL and BL	0.0241	0.0003
TL and DS	0.0006	0.0007
TL and BrL	0.0004	0.0002
TL and SL	0.0002	0.0014
WL and BL	-0.1413	0.0131
WL and DS	0.0007	0.0582
WL and BrL	0.0004	-0.0026
WL and SL	0.0008	0.0483
BL and DS	0.0084	0.0214
BL and BrL	0.0427	0.0664
BL and SL	0.0342	-0.0132
DS and BrL	0.0003	0.1622
DS and SL	0.0006	-0.0641
BrL and SL	0.0001	-0.0914
Sum total	0.7908	0.8393

In Fulani ecotype cock nevertheless, the highest lone contribution to body weight was by breast length, closely followed by wing length, chest circumference and drum stick (R^2 = 0.39, 0.14, 0.08 and 0.06 respectively). Combined effects of drum stick and breast length (R^2 = 0.16) was highest among the variable pairs. The pilot regression equation where all traits were considered in Fulani ecotype was:

BW= -0.48 - 0.02KL + 0.29CC + 0.01TL - 0.37WL + 0.12BL + 0.24DS + 0.62BrL + 0.14SL

Deletion of less significant predictor variable in the estimation of body weight

In Yoruba ecotype, the path coefficient of keel length, chest circumference, thigh length, drum stick and shank length were statistically insignificant ($p>0.05$). The path coefficient of wing length though significant ($p<0.05$) negatively influenced the body weight. This informed the inclusion of only body length and breast length as the predictor variables for body weight. After the deletion of six less important independent variables (KL, CC, TL, WL, DS and SL), the path coefficients for body length and breast length were 0.82 and 0.15 respectively. The new regression equation was:

BW= -1.21 + 0.82BL + 0.15BrL

In Fulani ecotype, the path coefficients of chest circumference, breast length and wing length were statistically significant ($p<0.05$) as indicated by t-test. Wing length was primarily expunged from the equation because of its negative influence on body weight. The decision to include it in the analysis was prompted by its relatively large direct effect (R^2 = 0.14). After the deletion of less significant predictor variables, the regression equation was:

BW= -0.09 + 0.30CC + 0.74BrL - 0.38WG

CONCLUSION

Apart from body length, other linear measurements considered in this study had low correlation, though significant, with body weight in Yoruba ecotype birds. In Fulani ecotype, breast length had the highest correlation with body weight. Path analysis indicated that body length and breast length have the highest direct effect on body weight in Yoruba and Fulani ecotype cocks, respectively. The direct effects of body length and breast length on body weight in Yoruba ecotype were positive and significant whereas negative significant direct effect was obtained for wing length. In Fulani ecotype, path coefficients of chest circumference, wing length and breast length were significant but wing length

had negative influence on body weight. The highest indirect effect was obtained for breast length through drum stick in Fulani ecotype while in Yoruba ecotype, highest indirect effect was obtained for body length through chest circumference. It was concluded that body length and breast length were the most important morphometric traits in determining body weight of Yoruba and Fulani ecotype cocks.

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WATER BALANCE AND SOME BLOOD PARAMETERS IN WATER-RESTRICTED GOATS DURING HOT-DRY SEASON

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ABSTRACT

Six West African Dwarf (WAD) and 6 Red Sokoto (RS) dry female goats were subjected to volumetric water restriction (WR). The experiment was arranged in a modified cross-over design with 3 treatment periods (TPs) of 1 week each and 2-week wash-out (WO) period. The goats were subjected to 3 graded levels of WR daily: *ad libitum* (0 %), 33 % and 67 %. Blood samples on day 1 and day 7 of each TP were collected. Daily urine and faeces were collected for 3 days each TP. Combining data for the two breeds, WR had no significant ($P>0.05$) effect on initial value, final value or differences in the values of the packed cell volume (PCV), red blood cells (RBC), haemoglobin (Hb) concentration, plasma urea, plasma osmolality, blood glucose, total serum protein, albumin and globulin. No significant ($P>0.05$) effect of water restriction on urine volume, fresh faeces, faecal DM and volume of water in faeces was recorded. However, based on metabolic weight, water loss in faeces was significantly ($P<0.05$) higher in 0 % WR grade level than in 33 % and 67 % WR grade level groups. Similarly, water intake-urine ratio was higher in 0 % and 33 % than in 67 % group. Taking each breed separately, there was significant ($P<0.05$) effect of WR on faecal output and faecal water content (FWC). RS goats voided more faeces (DM) than WAD goats at 0 % but not at 33 % and 67 % restriction levels. FWC per metabolic weight was higher in RS than in WAD goats at all WR levels. In RS goats, there was a gradual decrease in FWC with increase in water restriction level, whilst there was no significant difference in WAD goats. WR did not affect the blood parameters, however WAD goats proved superior to RS in regulating amount of water losses in faeces, thereby showing a higher capacity to cope with water shortage.

Key words: water restriction; goat; water balance; haematology

INTRODUCTION

Water is one of the most important nutrients, consumed in larger quantity than other nutrients by livestock (Mustafa *et al.*, 2010) and being the most abundant molecule in all living cells (NRC, 2007). It is involved in virtually all physiological functions of the animals (Wilson and Brigstocke, 1981). The significance of water in ruminant livestock production was reviewed by Aganga *et al.* (1986) and more recently by Araújo *et al*. (2010). Water is widely distributed in the body of animals covering both intra and extracellular spaces. Positive balance of water in tissues is an essential pre-requisite for the normal maintenance of life (Aganga *et al*., 1989). Sources of water to goats include drinking water, dietary water in feed ingested and metabolic water from catabolism of nutrients (Araújo *et al.*, 2010). Water is taken by goats intermittently, however its loss from body system is continuous in sweat, in transpiration, in urination and in defaecation. Water is lost in milk production and neonates during parturition. Therefore, goat farmers

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need to pay attention to the supply of adequate and clean water for the animals. Sometimes however, goats are exposed to varying degrees of water deprivation especially during dry season. The first physical response of an animal to the restriction of water intake is restriction of voluntary feed intake, which has profound negative effect on productivity of the animals (Abioja *et al.*, 2010).

In Nigeria, it is generally known that animals in the Guinean and Sahelian savannah zones experience water deprivation almost throughout the year. Consequently, the animals that will survive there, must have ability to cope with little water. The assumption is that ruminant animals reared in the humid forest zone of the southern Nigeria do not experience such situation. This is not totally correct, especially during the hot-dry season (February-April) when the available pastures are dried and fibrous in south-western Nigeria. At such a time, the ambient temperature is usually above 30 °C. Goats, therefore, experience varying degrees of water deprivation. The effects which these conditions may have on body water balance and on the haematological picture have not been fully studied in goats reared in the region.

West African Dwarf (WAD) goats are of importance in the humid tropics of South-Western Nigeria because of their relative trypanotolerance (Akusu, 1994). On the other hand, Red Sokoto (RS) goats are more adapted to the drier condition of the Northern Nigeria. RS goats are being introduced into the south and can serve as a good breed for comparison with WAD goats that are common in the south on the effects of water deprivation. It has been reported that when WAD and RS goats were subjected to water restriction up to two-third of normal intake during hot-dry season, they exhibited higher respiratory and pulse rates and reduced voluntary feed intake and loss of weight (Abioja *et al*., 2010) compared to the unrestricted group. Changes in drinking behaviour and water intake, as affected by water restriction in German black-head mutton sheep and Boer goats, had also been reported by Al-Ramamneh *et al.* (2012). Alamer (2009) has earlier reported that water restriction had effect on lactation performance of Aardi goats under heat stress conditions. Togashi and Tanaka (1979) reported that water restriction has effect on haematocrit, haemoglobin and serum protein in fattening beef cattle. Adogla-Bessa and Aganga (2000) however reported that Tswana goats can survive water deprivation without severe dehydration even when watered once in 72 hours. This tolerance in Tswana goats to water deprivation was attributed to the animals' ability to limit urine and faecal water excretion. This has not been confirmed in WAD and RS goats. Therefore, the present study is aimed at determining the effect of water restriction on haematological and water balance responses of WAD and RS goats.

MATERIAL AND METHODS

Experimental animals and management

Twelve non-pregnant (6 WAD and 6 RS) does used for this study were managed intensively in an open-sided slatted-floor individual pens at the Small Ruminant Unit of the University Teaching and Research Farms, Federal University of Agriculture, Abeokuta, Nigeria. They were allotted randomly to treatments in a modified cross-over design with 3 treatment periods. The three treatments were: *ad libitum* water supply (A), 33 % reduction (B) and 67 % reduction (C) from average water intake. A preliminary period of 7 days was

Table 1: Average weather conditions during the experimental period

Climatic factor	Mean	Diurnal variation		Treatment period			
	± sem	08.00 h	14.00 h	1	2	3	sem
Minimum temperature (°C)	26.5 ± 0.45			27.1	25.9	26.4	0.78
Maximum temperature (°C)	36.3 ± 0.58			37.9	34.9	36.0	0.92
Mean temperature (°C)	31.4 ± 0.45			32.5	30.4	31.2	0.74
Relative humidity (%)	81.1 ± 1.58	92.1 ± 1.23	70.1 ± 2.40	76.4	83.2	83.7	2.55
Temperature-humidity index	98.0 ± 1.81	88.6 ± 1.81	107.4 ± 2.13	100.3	94.6	99.2	3.14
Dry-bulb temperature (°C)	29.2 ± 0.44	27.1 ± 0.39	32.7 ± 0.55	30.8	29.0	30.0	0.75
Wet-bulb temperature (°C)	26.7 ± 0.25	26.0 ± 0.29	27.5 ± 0.30	26.7	26.2	27.3	0.43

sem- standard error of mean

allowed for determination of the average water intake of the experimental animals. This was followed by three treatment periods (TPs) of one week each. Between each TP there was a two-week period (wash-out period, WP) during which the effects of earlier treatment were expected to wear off before the next treatment.

The daily minimum and maximum temperatures, relative humidity, wet- and dry-bulb temperatures at 0800 h and 1400 h were monitored using suitable thermometers. The temperature-humidity index (THI) was calculated as described by Palmer (2000). The composition of the feed given to the animals was wheat offal (54 %), brewer's dried grain (20 %), rice bran (24 %), common salt (1 %) and bone meal (1 %). They were fed on 4 % body weight basis.

Haematological and blood biochemical studies

On the first and the last day of each of the three TPs, blood samples were obtained by jugular vein puncture at 07.00 h before feeding. Plasma from EDTA-supplemented bottles and serum from clotted blood, collected into hypodermic syringes, were harvested. The packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (Hb), plasma urea (URE), plasma osmolality (OSM), blood glucose level (GLU), total serum protein (TSP), serum albumin (ALB) and serum globulin (GLO) were determined using standard analytical methods. The blood samples in the EDTA-supplemented bottles were centrifuged and the plasma was stored at -20 °C until analyses. Wintrobes microhaematocrit and colorimetric methods (Lamb, 1991) were used to determine packed cell volume (PCV), haemoglobin concentration (Hb) and red blood cell count (RBC). Blood samples collected into labelled EDTA-supplemented bottles were placed into the microhaematocrit centrifuge and spun for 5 minutes at a speed of 11000 rpm. The PCV values were subsequently determined by measuring the height of the red blood cell column and expressing this as a ratio of the height to the total blood column using microhaematocrit reader. RBC count was done by diluting the blood sample with 0.9 % NaCl and shaking well. The diluted blood was placed on a haemocytometer and the number of erythrocytes was counted under a microscope. Serum biochemical indices, plasma urea (URE), plasma osmolality (OSM), total serum protein (TSP), serum albumin (ALB) and serum globulin (GLO) were determined using spectrophotometer, as described by Werner *et al.* (1976). Blood glucose level (GLU) was measured by enzymatic colorimetric test (GOD-POD). The values of blood parameters on the first day were subtracted from those of the day 7 to reveal changes in blood picture as a result of water restriction.

Faecal and urine output

Six (3 WAD and 3 RS) does were transferred into metabolism cages where the daily (24 h) faeces and urine samples were collected for three days for each TP. Faeces collected were weighed before being transferred into an oven for faecal dry matter and water content determination. The samples were dried to constant weight. Faecal water content was taken as the difference

Table 2: Effect of water restriction on the change in blood constituents and chemistry (final values minus initial values) in goats

	Water restriction			
Parameter	0 %	33 %	67 %	sem
Water intake (ml)	1489[a]	1436[a]	841[b]	128.80
PCV (%)	2.7	3.2	2.7	0.85
Hb concentration (g.dL)	0.9	1.0	0.9	0.29
RBC (x10^6.mm^3)	0.27	0.29	0.33	0.101
Urea (mg.dL)	-0.1	1.2	0.1	1.67
Glucose (mg.dL)	5.8	7.1	5.2	1.94
Osmolality (mOsmo.kg)	17.9	24.0	21.0	7.15
Serum total protein (g.dL)	3.3	7.6	5.3	2.10
Serum albumin (g.dL)	2.9	5.3	3.2	1.29
Serum globulin (g.dL)	0.3	2.3	2.1	1.09

[a,b]Row means with different superscripts differ significantly ($P<0.05$)

between weights of fresh faeces and the dry matter. The volume of the daily urine output for individual goat was also measured.

Statistical analyses

The data obtained were subjected to analysis of variance using a modified cross-over design. The daily mean temperature and relative humidity were included as covariates. All analyses were done with SYSTAT analytical computer package version 5.0 (Systat Inc., 1992). Means with probability value less than or equal to 0.05 were considered to be significantly different and were separated using Duncan multiple range test.

RESULTS

Meteorological conditions

The summary of climatic data during the experimental period is shown in Table 1. The minimum, maximum and mean temperatures averaged 26.5 ± 0.45, 36.3 ± 0.58 and 31.4 ± 0.45 °C, respectively. The relative humidity averaged 81.1 % during the experimental period while the temperature-humidity index was 98.

Haematology and biochemistry

The result of haematological responses and serum biochemical indices in the goats is shown in Table 2. Water restriction had no significant (P>0.05) effect on all the haematological and blood biochemical parameters examined. As well, there was

Table 3: Effect of water restriction on water losses in urine and faeces in goats

	Water restriction			
Parameter	0 %	33 %	67 %	sem
Live weight (kg)	17.3	17.5	17.1	0.80
Metabolic weight(kgW$^{0.75}$)	8.4	8.5	8.4	0.29
Urine output (ml)	621.3	616.3	503.8	48.73
Urine output (ml.kgW$^{0.75}$)	74.2	72.4	60.1	5.18
Fresh faecal output (g)	606.6	513.5	537.7	52.80
Faecal output (g.DM)	182.3	211.9	243.8	20.94
Water in faeces (ml)	424.2	301.6	294.0	36.28

Table 4: Effects of water restriction on water losses in urine and faeces of WAD and RS goats

	Water restriction						
Parameter	0 %		33 %		67 %		sem
Breed	WAD	RS	WAD	RS	WAD	RS	
Live weight (kg)	17.7	16.9	18.4	16.5	17.3	16.9	1.13
Metabolic weight(kgW$^{0.75}$)	8.6	8.3	8.8	8.2	8.4	8.3	0.42
Urine output (ml)	629.1	613.4	654.3	578.3	532.1	475.6	68.91
Urine output (ml.kgW$^{0.75}$)	73.3	75.1	74.1	70.6	63.0	57.3	7.33
Fresh faecal output (g)	439.1^{ab}	774.0^{a}	373.8^{b}	653.2^{a}	422.6^{b}	652.9^{a}	74.67
Faecal output (g.DM)	144.3^{b}	220.3^{a}	179.3^{ab}	244.5^{a}	193.1^{ab}	294.5^{a}	29.61
Water in faeces (ml)	294.8^{b}	553.7^{a}	194.5^{b}	408.7^{a}	229.5^{b}	358.4^{ab}	51.30
Water intake : urine output	2.3	2.4	2.2	2.8	1.7	1.8	0.29

^{a,b}Row means with different superscripts differ significantly (P<0.05)

no significant (P>0.05) change in these parameters before and after 7 days of water restriction. There was no significant (P>0.05) interaction between water restriction and breed in the blood parameters (data not shown).

Water metabolism

The effect of water restriction on water losses in faeces and urine in goats (data combined) is shown in Table 3 and in Figures 1 and 2. Daily urine volume and amount of faeces voided by goats (fresh or dried) were not significantly (P>0.05) affected by water restriction. Water content of faeces was however significantly (P<0.01) lowered by water restriction. 0 % goats had the higher water loss in faeces than 33 % and 67 % goats; moreover the latter groups were not different from each other (Figure 1). Ratio of water

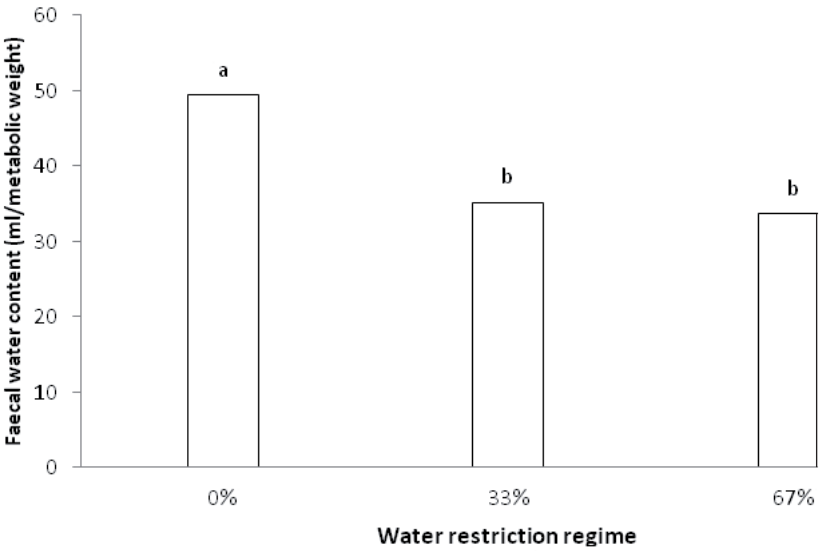

Fig. 1: Effect of water restriction level on faecal water content in goats

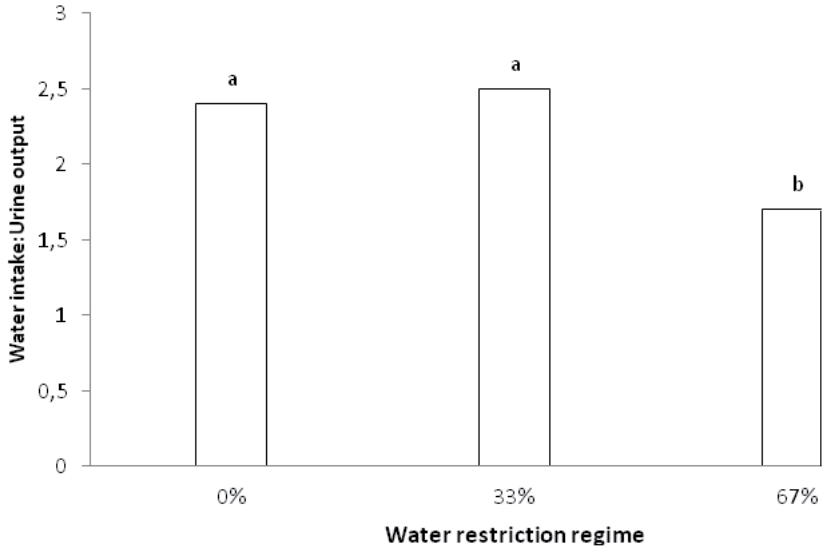

Fig. 2: Effect of water restriction level on water intake : urine output ratio in goats

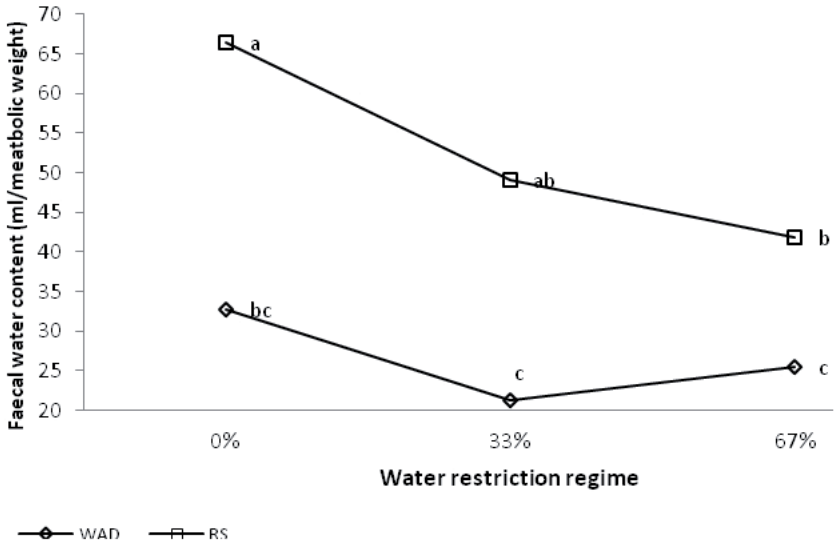

Fig. 3: Effect of water restriction on faecal water content in WAD and RS goats

intake to daily urine voided was significantly ($P<0.05$) affected by water restriction (Figure 2). There was no difference between 0 % (2.4 ± 0.17) and 33 % (2.5 ± 0.17) restriction level in water intake-urine ratio, but these values were higher than those of the 67 % group (1.7 ± 0.17).

The result of water losses by WAD and RS goats, as affected by water restriction, is presented in Table 4. In both WAD and RS goats, the daily volume of urine (in millilitre and millilitre per metabolic weight) was not different ($P>0.05$) among the three water restriction groups. However, both fresh ($P<0.05$) and dry matter ($P<0.01$) faecal material voided daily were significantly affected by water restriction. Considering fresh faecal weight, RS goats had higher weight than WAD goats at 33 % and 67 % water restriction level. However, the dried faecal weight in RS goats was only higher than that of WAD goats at 0 % restriction level. Faecal water content (FWC) per metabolic weight was higher for RS than WAD goats at all water restriction levels (Figure 3). In RS goats, there was a gradual decrease in FWC with increase in water restriction level, whilst there was no significant difference in FWC of WAD goats.

DISCUSSION

The non-significant effect on water restriction observed in the present study on haematological and biochemical results of analyses of goats is opposite to the reports of various previous studies in ruminant animals (Togashi and Tanaka, 1979; Abdelatif and Ahmed, 1994; Adogla-Bessa and Aganga, 2000). Water deprivation causes a shift in the dynamic water balance, which results in certain responses from the body systems. The initial response to a negative water balance is the withdrawal of fluid from tissues to maintain the normal blood volume (Radostits *et al.*, 2000). During dehydration, water is reabsorbed to accompany sodium absorption in the colon, which results in the return of water to the blood. It means the animals in this study were able to maintain the blood components by drawing water from other tissues into the blood system. The absence of a significant effect observed here might also be due to goat´s superior adaptability to water shortage (Devendra and McLeroy (1982) and the shortness of the treatment period. However, in agreement with this study, Qinisa (2010) reported that though blood urea concentration increased as the level of water restriction increased, the values were not significantly different for the animals on *ad libitum*, half *ad libitum* or quarter *ad libitum* water supply.

In this present study, water restriction had no effect on daily urine volume. In contrast, Adogla-Bessa and Aganga (2000) reported a decrease in urine volume in Tswana goats as the period of water deprivation lengthened. The reason for the differences in results may be adduced to higher capacity to withstand water shortage in WAD and RS goats, used in the present study, than in Tswana goats used in the previous study. Moreover, the present study dwelt on volumetric water restriction, as against 0, 24, 48, 72 and 98 hour water restriction regimes, applied by Adogla-Bessa and

Aganga (2000). Ahmed and El Kheir (2004) reported that whether Sudanese desert goats were offered good or bad quality feed, water restriction decreased faecal water losses and increased water losses in urine. In the same vein, Dahlborn and Karlberg (1986) reported that a decrease in water intake even for a single day gradually reduced secretion of urine in goats. However, result similar to the present one was observed by Little *et al*. (1976) in cattle, where 40 % reduction in WI resulted in no significant reduction in urine output, though there was evidence for the reduction in total body water. The weights of fresh faecal and faecal dry matter from goats, subjected to different regimes, were not different but RS goats voided more faeces than WAD goats. In the other study, water deprivation decreased faecal output in Tswana goats (Adogla-Bessa and Aganga, 2000). The result of the present study might be due to a decrease in the number of rumen bacteria and protozoa following water deprivation, as suggested by Fluharty *et al*. (1996). Water loss in faeces was lower in goats subjected to 67 % water restriction, showing that the animals were able to reduce water loss during the short period of water deprivation. WAD goats have greater ability to conserve water and reduce water loss in faeces than RS goats, as they voided drier faeces. This is in contrary to the expectation from RS goats, as they are adapted to drier conditions of the Northern Nigeria.

CONCLUSION

WAD goats proved superior to RS in regulating amount of water losses in faeces, thereby showing a higher capacity to cope with water shortage under conditions prevalent in the southern Nigeria during hot-dry season.

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EFFECT OF FORAGE LEGUME SUPPLEMENTATION OF MAIZE COBS ON THE PERFORMANCE OF WEST AFRICAN DWARF SHEEP

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ABSTRACT

The supplementation of maize cobs with forage legumes was used to evaluate the performance of sixteen West African Dwarf sheep aged 10-15 months and average weight of 18.01 ± 0.30 kg in a complete randomized design. The basal diet of maize cob plus 100 g of palm kernel cake was offered alone (control) or supplemented with 200 g on dry matter basis of forages of *Enterolobium cyclocarpum*, *Leucaena leucocephala* and *Gliricidia sepium*, respectively. Data were taken on feed intake, body weight changes, feed conversion ratio, digestibility and haematological parameters. The result of the study showed that maize cobs supplemented with forage legume improved sheep performance with best ($p<0.05$) feed intake and weight gain observed in sheep supplemented with Leucaena (745 g.day^{-1} and 33.57 g.day^{-1}, respectively) and Gliricidia forage (720 g.day^{-1} and 30.89 g.day^{-1}, respectively) with best dry matter, crude protein and fibre digestibilities. Though, there were significant differences ($P<0.05$) observed in values of blood parameters with the exception of white blood cells, these values were within the normal range reported for healthy sheep production. It was therefore concluded that maize cobs supplementation with forage legumes can play an important role in improving the performance of sheep with the supplementation of Leucaena and Gliricidia forage producing the best performance.

Key words: maize cob; forage legume; sheep; intake; digestibility; haematology

INTRODUCTION

The prohibitive cost of concentrate diets for sheep production has necessitated continuous search for less expensive and high nutritive feedstuffs that could represent cost-effective supplements for sheep on poor quality crop residues.

Maize cob is a common and readily available feedstuff in Nigeria which is an underutilized by product from the processing of harvested maize. The amount of maize cob generated annually in the country increases as more people venture into the cultivation of maize, but they have low feeding value because of its poor protein content, energy, minerals and vitamins (Akinfemi *et al*., 2009). However, supplementation is perhaps a cheaper and simpler way of improving the feeding value of crop residues in situ, involving practical methods that are realistic of small farm situations. Foliage from tree legumes and shrubs which are readily available and persist during the dry season when pasture is either scarce or of poor quality has been found to be beneficial to ruminants as they offer a cheaper alternative to supplementation of poor quality roughages (Odeyinka, 2001; Phimphachanhvongsod and Ledin, 2002), contributing protein-rich forage, digestible energy and minerals when used either as supplements or as sole feed (Abdulrazak *et al.*, 1997). There exists extensive and diverse literature on the effects of leguminous tree supplementation on the productivity of ruminants. Forage tree leaves, particularly of Leucaena and Gliricidia, have been used as supplements to a wide range of forages and agricultural by products in

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ruminant nutrition (Norton, 1994).

This study therefore evaluates the effect of forage legume supplementation on the intake, digestibility, growth and haematological profile of West African Dwarf sheep fed maize cob.

MATERIAL AND METHODS

Experimental Animals and Management

Sixteen (n = 16) sheep of the West African Dwarf breed selected from the farm flock in the Small Ruminant Experimental unit of Teaching and Research Farms, Federal University of Agriculture, Abeokuta, Ogun State were used in a 56 day study. The animals aged 10-15 months with an average body weight of 18.01 ± 0.30 kg were allotted to four treatments in individual feeding pens with wooden slatted floors, balanced for weight in a complete randomized design. The animals were assigned to a basal diet of maize cob plus 100 g of palm kernel cake offered alone (control) or supplemented with 200 g on dry matter basis of forages of *Enterolobium cyclocarpum*, *Leucaena leucocephala* and *Gliricidia sepium* respectively, at 4 % of their body weight with fresh and clean water provided *ad libitum*.

Experimental Diets

Dried maize cobs were procured from maize sellers in a local market and the leaves of the forage legumes namely *Leucaena leucocephala*, *Enterolobium cyclocarpum* and *Gliricidia sepium* were harvested from established plots within the university campus. Maize cobs were sun-dried and grounded while the forage legumes were harvested, chopped, sun dried for 5 days.

Data Collection

The body weights of the animals were taken using a spring balance at the beginning of the experiment and on weekly basis thereafter. The feed offered and feed refusal were also taken to get the feed intake. During the last 7 days of the experiment, the animals were transferred into metabolic cages where faecal samples were collected from each sheep. Also, the faeces were weighed to get the faecal voided. The faecal sample was dried in oven to constant weight and bulked for the determination of its proximate composition.

Blood samples were also collected from each animal in ethylenediaminetetraacetic acid bottles at the end of the experiment via jugular vein puncture with a 5 ml guage syringe for the determination of haematological parameters namely packed cell volume, white blood cells, red blood cells, haemoglobin and total protein (AOAC, 1995).

Chemical Analysis

The proximate composition of the feeds and faeces was determined (AOAC, 1995). The dry matter was determined by oven drying at 65 °C for 24 hours, crude protein (CP) by Kjeldahl method and fat by Soxhlet fat extraction method. The concentration of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) both in feed and faecal samples were also determined by the method of Van Soest and Robertson (1985).

Statistical Analysis

Data collected were subjected to one way analysis of variance in a completely randomized design (SAS, 1999). Significant means were separated using Duncan multiple range test (Duncan, 1955).

Table 1: Chemical composition (% DM) of experimental diets fed to sheep

Parameters	MC	EC	LL	GS	PKC
Dry matter	90.37	90.81	91.14	91.07	92.20
Crude protein	2.43	20.27	27.02	20.80	18.67
Ether Extract	0.26	6.91	3.11	2.91	6.88
Ash	1.40	5.92	9.89	9.73	5.08
Neutral detergent fibre	81.30	67.43	49.22	56.31	68.43
Acid detergent fibre	47.20	36.54	20.39	24.65	45.22
Acid detergent lignin	8.89	12.02	6.01	8.56	12.78

MC – Maize cob, EC – *Enterolobium cyclocarpum*, LL – *Leucaena leucocephala*, GS – *Gliricidia sepium*, PKC – Palm Kernel cake

RESULTS AND DISCUSSION

The chemical composition of maize cobs and forage legumes is shown in Table 1. Maize cob had a low content of crude protein (2.43 %), ether extract (0.26 %) and ash (1.40 %) with a high content of fiber fractions. The high NDF content in maize cob was consistent with previous reports where it is characterized by high NDF contents implying that the material is high in lignocelluloses component and low in nitrogen (Adebowale, 1988).

The CP contents of *Enterolobium cyclocarpum* (EC), *Leucaena leucocephala* (LL) and *Gliricidia sepium* (GS) were consistent with values reported in the literature (Odeyinka 2001, Babayemi 2006, Fasae *et al*., 2011) with Leucaena having the highest crude protein (27.02 %). The CP content of these forage legumes were above 10-12 % as recommended by Gatenby (2002) for moderate level of ruminant production suggesting the potential of these forages to provide adequate nitrogen required by rumen micro organism to maximally digest the dietary fiber components which will lead to the production o f volatile fatty acids.

The fiber contents of the plant species were similar with the report of another group of workers (Larbi *et al*. 1996). Although Meissner *et al.* (1991) reported that browse species with NDF above 60 % will reduce the intake of such fodder by ruminants, thereby reflecting the benefits of low NDF content of forage legumes used in this study in improving the DM intake of these plant species. In all, Leucaena species exhibited the lowest values of the fiber fractions.

The data on feed intake, weight changes and feed conversion ratio of the experimental animals are shown in Table 2. The high DM intake of maize cobs supplemented with forage legume by the experimental sheep indicates the potential of these forages as a good supplement to low quality roughages such as maize cob. The DM intake differed significantly ($P<0.05$) among the treatments and increased with supplementation. This response could be attributed to the stimulating effect of these forages on the intake (Tolkamp, 1988) of the basal diet. In addition, the CP content of the forage legumes being able to provide the rumen microbial requirements of nitrogen (Abdulrazak *et al*., 1997) could have had an effect in increasing the microbial population thereby improving the breakdown of digesta. When the rate of breakdown of digesta is increased, there is a corresponding increase in feed intake (Ngwe and Kona, 1996). This however, reflects the ability of these forages to provide a favourable environment for microbes to grow and multiply, hence colonizing more of the cobs leading to an increase in intake.

Moreover, sheep supplemented with LL had significantly ($P<0.05$) higher feed intake of 745.0 g.day^{-1} which ranked the same ($P>0.05$) with animals supplemented with GS (720.0 g.day^{-1}). Animals supplemented with EC had a lower ($P<0.05$) feed intake of 677.5 g.day^{-1} relative to sheep on other forage legumes but higher ($P<0.05$) than the control (567.5 g.day^{-1}). The higher fiber fraction content of EC might explain the lower level of intake observed in the sheep compared to the other forages. Also, the low feed intake of the control treatment could be accentuated by high fiber fractions of diet to be digested. This increases the retention time in the recticulo-rumen (Orskov and Ryle, 1990) thereby depressing the intake of more feed. From this, one would expect both voluntary intake and digestibility of maize cobs to be low and an addition of supplemental

Table 2: Performance indices of West African Dwarf sheep fed maize cobs supplemented with forage legumes

Parameters	MC + EC	MC + LL	MC + GS	MC	SEM
Average feed intake (g.day^{-1})	677.5^{b}	745.0^{a}	720.0^{a}	567.5^{c}	13.05
Initial weight (kg)	17.75	17.60	18.48	19.20	0.31
Final weight (kg)	19.60	19.48	20.20	20.13	0.30
Weight gain (kg)	1.55^{b}	1.88^{a}	1.73^{a}	0.93^{c}	0.12
Weight gain (g.day^{-1})	27.67^{b}	33.57^{a}	30.89^{a}	16.61^{c}	2.03
Metabolic weight gain (kgW$^{0.75}$)	12.06 ^{b}	13.95^{a}	13.01^{a}	8.23^{c}	1.01
Feed conversion ratio	24.49^{b}	22.19^{c}	23.31^{bc}	34.17^{a}	3.73

a,b,c means with same superscripts within the same rows are not significantly different ($p>0.05$)
MC – Maize cob, EC – *Enterolobium cyclocarpum*, LL – *Leucaena leucocephala*, GS – *Gliricidia sepium*

forages would be required to support reasonable levels of production. However, the utilization of crop residues have been found to be limited because they contain a large proportion of lignocellulosic compounds and little nitrogen (Butterworth and Mosi, 1985).

Among the forage supplements, Gliricidia was the least consumed initially but was all consumed subsequently. This could not be unconnected with the presence of coumarin that triggers the offensive or repulsive odour that emanates from Gliricidia forage (Arigbede et al., 2003). This corroborates earlier reports (Fasae *et al*., 2010) of nonchalant attitude of goats initially to the consumption of Gliricidia forage.

The average daily weight gain (ADG) of the experimental sheep varied (P<0.05) among the treatments, following the same trend with feed intake and reflecting better gain in weight with forage legume supplementation. This supports earlier reports on an increasing trend in weight gain as a result of the effect of forage legume supplementation of crop residues in various species of ruminants (Nguyen, 1998; Phimphachanhvongsod and Ledin, 2002; Fasae *et al*., 2010). Sheep on LL and GS supplementation had higher (P<0.05) ADG (33.57 and 30.89 g.day^{-1}, respectively) followed by sheep on EC (27.67) and least (P<0.05) values of 16.61 observed in sheep on the control treatment. The variations in ADG of the experimental sheep could therefore be attributed to variation in nutrient supply from the diets (Oddy and Sainz, 2002). Availability of digestible protein and energy reflected by the relatively low fibre levels of the supplementary forages could have provided ready nutrients for the synthesis of body tissues in the lower gut. This could be responsible for the higher weight gains and efficiency of feed utilization of sheep on the forage supplementary treatments.

However, the ADG values in this study are higher than the range of 23.33 to 28.57 g.day^{-1} as reported by Odeyinka (2001) for WAD goats fed forages of Leucaena and Gliricidia, but lower than that of the reports of Fasae and Alokan (2006) in Yankasa sheep fed maize offals diet with varying levels of *Leucaena leucocephala* leaf residues. The metabolic weight range of 8.23 to 13.95 kgW$^{0.75}$ observed in this study is higher than 5.88 to 7.11 as reported by Yusuf *et al*. (2010) for the same breed of sheep fed forages and concentrate.

The feed conversion ratio (FCR) also varied (P<0.05) among the treatments. Animals supplemented with LL had the best (P<0.05), which is statistically similar with sheep on GS diets. This is largely a reflection of the highest weight gain observed in animals on these treatments. Animals on the control treatment had the worst (P<0.05) FCR, indicating that the feed was not efficiently converted by the animals.

The apparent digestibility of various dietary combinations is shown in Table 3. Results showed that digestibility values differed (P<0.05) among the treatments. High protein contents of forage supplemented treatments played a significant role in improving the digestibility of nutrients over those of the control. This supports a report that digestion of feed in ruminant animals is highly influenced by the level of protein and fibre in the diet (Peyraud and Astigarraga, 1998). Thus, the control treatment with high fibre content could have contributed to its low digestibility.

Among the treatments, MC supplemented with LL and GS had the highest (P<0.05) digestibility values. This may be due to its higher protein content and also their low fibre content of these forages which reflected high DM intake by these animals. In addition, the 200 g of forage legume offered to sheep in this study could have encouraged digestibility of MC.

Table 3: Apparent digestibility (%) of maize cobs supplemented with forage legumes in West African Dwarf sheeps

Parameters	MC + EC	MC + LL	MC + GS	MC	SEM
Dry matter	69.78^{a}	77.94^{a}	73.12^{a}	60.07^{b}	4.32
Crude protein	67.70^{b}	77.94^{a}	71.21^{a}	51.24^{c}	5.36
Ether extract	74.44^{b}	76.60^{ab}	78.31^{a}	74.97^{b}	2.86
Ash	55.13^{b}	67.73^{a}	67.88^{a}	50.03^{b}	6.22
Neutral detergent fibre	66.12^{a}	69.48^{a}	67.20^{a}	51.54^{b}	2.74
Acid detergent fibre	62.24	60.22	61.17	63.35	2.14
Acid detergent lignin	54.21	50.11	50.96	55.21	2.01

a,b,c means with some superscripts within the same rows are not significantly different (P>0.05)
MC – Maize cob, EC – *Enterolobium cyclocarpum*, LL – *Leucaena leucocephala*, GS – *Gliricidia sepium*

The optimum dietary level of fodder leguminous trees and shrubs has been reported to be 30 % to 50 % of the ratio on DM basis (Stewart and Simon, 1994). This limits the secondary components that inhibit the digestibility and reduce the acceptability to animals at higher levels of inclusion (Reed *et al*., 1990).

Table 4 presents the mean haematology parameters of WAD Sheep fed maize cobs supplemented with forage legumes. The packed cell volume (PCV) was significantly (P<0.05) higher in sheep on LL supplementation compared to the other treatments but all were within the range 20.5 - 24.9 % as reported by Olayemi *et al.* (2000) for WAD sheep.

The red blood cells (RBC) values also differed (P<0.05) among treatments. The low values reported for sheep on the control treatment could be attributed to the nutritional status. Swenson (1990) reported that nutritional status of an animal can affect the RBC count. However, the RBC counts in animals fed the experimental diets do not suggest a susceptibility to anaemia related disease condition because the values fall within the normal range for healthy sheep.

The white blood cells (WBC) values observed in this study were within the normal range of 4 to $12x10^9$.L for sheep (Jain, 1993) reflecting that the animals were healthy while the haemoglobin concentration

Table 4: Haematology of West African Dwarf sheep fed maize cobs supplemented with forage legumes

Parameters	MC + EC	MC + LL	MC + GS	MC	SEM
Packed cell volume (%)	22.50^b	26.07^a	21.75^b	22.60^b	2.52
Haemoglobin (g.dL)	9.80^b	10.65^a	10.05^a	8.13^b	1.02
Red Blood Cell (10^{12}.L)	8.58^{ab}	10.11^a	9.65^a	7.38^b	0.92
White Blood Cell ($x10^9$.L)	11.00	12.94	11.70	10.80	1.07
Total protein (g.dL)	5.22^a	5.95^a	5.40^a	4.10^b	0.43

a,b,c means in the same superscripts within the same rows are not significantly different (P>0.05)
MC – Maize cob, EC – *Enterolobium cyclocarpum*, LL – *Leucaena leucocephala*, GS – *Gliricidia sepium*

was high and significantly (P > 0.05) influenced across treatments but fell within the normal range for sheep. High haemoglobin values have been shown to have an advantage in terms of the oxygen carrying capacity of the blood.

It has been reported that haematological and biochemical indices give insight into the production potential and help to monitor and evaluate incidence of diseases in animals (Orheruata and Aikhuomobhogbe, 2006). The result of haematological parameters in this study suggests that supplementation of maize cobs with forage legume do not pose health challenges.

CONCLUSION

Based on the experimental data, the supplementation of forage legume in maize cob diets did not show any adverse effect on feed intake, digestibility and haematological parameters of sheep. However, maize cobs supplementation with Leucaena and Gliricidia forage was the best in improving the feeding value of maize cobs, producing the optimum performance in sheep. It could therefore be concluded that forage legume could play a valuable role in supplying supplemental nitrogen to sheep fed maize cobs.

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EFFECTS OF DIETARY SUPPLEMENTATION WITH COPPER SULPHATE AND COPPER PROTEINATE ON PLASMA TRACE MINERALS, COPPER RESIDUES IN MEAT TISSUES, ORGANS, EXCRETA AND TIBIA BONE OF COCKERELS

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ABSTRACT

The present study was carried out to investigate the effect of dietary supplementation with copper sulphate ($CuSO_4$) and copper proteinate (CuP) on plasma trace minerals, Cu residues in meat tissues, organs, excreta and tibia bone of cockerels using two hundred and forty (240) day-old cockerel chicks arranged in a 2 × 3 factorial arrangements involving 2 copper (Cu) sources ($CuSO_4$, CuP) supplemented with 3 dosages (0, 50 and 100 $mg.kg^{-1}$). There were 6 treatment groups of 40 birds each replicated 5 times with 8 birds each. Experimental diets were fed for starter (0-8 weeks) and grower phases (8-16 weeks) of the bird. Increasing Cu dosage in the ration for cockerel chicks (0-8 weeks) resulted in increased ($P<0.0001$) Cu intake and improved ($P<0.05$) Cu bioavailability in the serum. Chicks fed diet supplemented with CuP showed increased ($P<0.05$) serum Zn concentration and reduced ($P<0.05$) Cu intake when compared to those fed diet supplemented with $CuSO_4$. At the grower phase (8-16 weeks), cockerels fed control diet had reduced serum Cu, while those fed diet supplemented with Cu, showed increased ($P<0.05$) serum Cu concentration. Dietary supplementation with CuP resulted in reduced ($P<0.05$) Cu intake and increased ($P<0.05$) serum Cu concentration when compared with cockerels fed diet supplemented with $CuSO_4$. Residual Cu concentration in breast meat tissue increased ($P<0.05$) with increasing dietary dosage of Cu. Dietary supplementation with CuP resulted in reduced ($P<0.05$) excreta Cu concentration, hence reduced environmental Cu pollution unlike birds fed diet containing $CuSO_4$ which showed increased excreta Cu concentration. Dietary inclusion of CuP showed increased ($P<0.05$) liver, heart and tibia bone Cu concentration when compared with birds fed diet supplemented with $CuSO_4$. Cockerels fed diet supplemented with 100 $mg.kg^{-1}$ Cu sourced from CuP recorded the highest ($P<0.05$) liver, heart and tibia bone Cu concentration. It was concluded that dietary inclusion of 100 $mg.kg^{-1}$ Cu sourced from CuP is recommended for improved Cu bioavailability in blood, tissue, organs and tibia bone of cockerels. To achieve reduced environmental Cu pollution resulting from excreta of poultry birds, organic salts of Cu, such as CuP, is recommended as feed additive.

Key words: day-old cockerels; organic copper; plasma trace minerals; serum Cu levels; tibia bone

INTRODUCTION

Copper (Cu) is an essential trace element necessary for various enzymatic systems in the body (Lim and Paik, 2006). It is required for skin pigmentation, proper functioning of the central nervous system, immune and cardiovascular system (Jegede *et al*., 2011). Minimum daily requirement of Cu for most avian species was reported as 5-8 $mg.kg^{-1}$ (Leeson, 2009). Cu is normally added as a feed additive into the ration for poultry. However, due to its growth-promoting and antimicrobial effects, Cu has

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been included at rather high concentrations far above pharmacological levels in feed for poultry (Skrivan *et al*., 2002).

In the past, Cu has been included in poultry feeds in form of inorganic salts like sulphates, carbonates, chlorides and oxides. Continuous use of these inorganic mineral salts as feed additives has been implicated in environmental mineral pollution arising from the accumulation of poultry wastes (Maheshwari, 2013). Intake of inorganic salts of minerals was reported to breaks down in the gastrointestinal tract to form very reactive free ions which binds dietary molecules and hinders absorption (Close, 1998). Hence, the use of organic sources of Cu has been advocated. Inclusion of organic salts of copper in feed for broilers has been reported to lead to high efficacy and improved bioavailability than their inorganic counterparts (Jegede *et al.*, 2011).

Dietary inclusion of mineral salts has been reported to show pronounced mineral residues in organs and animal tissues (Idowu *et al.*, 2006). Residues of heavy metals in poultry meat consumed by humans can be of public health importance to man's health. Dietary inclusion of zinc salts into the ration for poultry showed significant effect on Zn residues of meat tissues (Shyam Sunder *et al*., 2008), tibia bone, liver, excreta and egg shell of laying hens (Sahin *et al*., 2002). Idowu *et al.* (2011) reported that broilers fed diet supplemented with Cu showed some residual effect of Cu in the resultant meat tissues produced. However, no study has been conducted on the effect of inclusion of Cu salts on mineral residues in meat tissue, organs, excreta samples and tibia bone of cockerels. This study, therefore, aims to investigate the effect of dietary supplementation with copper sulphate (inorganic Cu salt) and copper proteinate (organic Cu salts) on plasma trace minerals, copper residues in meat tissue, organs, excreta samples and tibia bone of cockerels.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Poultry Unit of the Teaching and Research Farm Directorate, Federal University of Agriculture, Abeokuta, Nigeria (Latitude 7° 13^{1} 49.46"N and Longitude 3° 26^{1} 11.98"E). Average temperature of 28.5 °C and mean annual rainfall of 1037 mm were recorded during the experimental period. The conduction of this study was agreed with the ethical policy and guideline of the Poultry Management Technical and Veterinary Committee of the university.

Cu sources

The Cu salts used in this study were Copper proteinate (CuP) and Copper sulphate ($CuSO_4 \bullet 5H_2O$). CuP was obtained from Alltech® Inc., USA (Alltech Inc. Kentucky, USA) and contained 10 % Cu (Bioplex Cu). Feed grade $CuSO_4$ containing 25 % Cu was purchased from Lucaris Limited, (Lucaris Nigeria Ltd, Lagos, Nigeria).

Experimental birds and dietary treatment

A total of 240 day-old cockerel chicks were assigned to 6 treatment groups of 40 birds each. Each treatment was replicated 5 times with 8 birds per replicate. The birds were managed intensively in a deep litter pen for 16 weeks. A basal diet containing no supplemental Cu (used as control) was formulated, each for the chick and growing phase of the birds. Six experimental diets were subsequently formulated in a 2 × 3 factorial arrangements involving two Cu sources ($CuSO_4$, CuP) supplemented with three dosages (0, 50 and 100 mg.kg^{-1}) for the chicks. The diets were formulated for the starter mash (0-8 weeks) and grower ration (8-16 weeks). Supplemental Cu was included into the basal diet containing basal Cu of 32.65 mg.kg^{-1} (chick diet) and 31.58 mg.kg^{-1} (grower diet) to formulate the respective experimental diets (Table 1). Diets were formulated to meet the NRC (1994) nutrient requirements of growing cockerels. Feed and water were supplied *ad libitum*. The experiment lasted for 16 weeks. The proximate compositions of the experimental diets were determined according to standard procedures (AOAC, 1995). Cu determination of feed was done using a Perkin Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK). Feed samples were ignited at 400 °C for 4 h in a muffle furnace; resultant ash was reconstituted using wet-ashing procedure.

Plasma Cu and trace minerals

Blood samples (about 2.5 ml per bird) were collected from the wing veins of 2 birds per replicate (n = 10 per treatment) at 56 and 112 days of the study into heparinized tubes. Plasma was harvested (by centrifuging at 3200 rpm for 10 min) and used for the estimation of plasma Cu, Zn, Mn and Fe using an atomic absorption spectrophotometer (Perkin Elmer A Analyst 100).

Copper residues in meat tissues, organs and excreta sample

At the end of the study, two birds were selected from each replicate (n = 10 per treatment). Birds whose weight is a representative of the average weight of birds contained in each replicate were selected, slaughtered, de-feathered and dissected to separate

Table 1: Gross composition of basal diet

Ingredients	Chick starter mash (0-56 days)	Grower mash (57-112 days)
Maize	48.00	45.00
Soybean meal	15.00	11.00
Groundnut cake	11.00	8.00
Fish meal (72 % Crude protein)	2.00	1.00
Wheat offal	19.20	30.30
Bone meal	1.50	1.50
Oyster shell	2.50	2.50
Salt (NaCl)	0.25	0.25
*Premix (Cu free)	0.25	0.25
Methionine	0.20	0.10
Lysine	0.10	0.10
Total	100.00	100.00
Determined Analysis		
Crude protein (%)	22.04	17.20
Crude fibre (%)	4.26	4.75
Metabolizable energy ($MJ.kg^{-1}$)[a]	11.53	11.21
Ether extract (%)[a]	4.01	4.53
Calcium (%)[a]	1.45	1.30
Phosphorus (%)[a]	0.54	0.45
Lysine (%)[a]	0.91	0.75
Methionine (%)[a]	0.54	0.42
Cu level in basal diet ($mg.kg^{-1}$)[a]	32.65	31.58

Vitamins/mineral premix (Godomix©) included at the rate of 2.5 kg per ton of feed contains the following: Vitamin. A: 3.200.000 IU, vitamin D: 640.000 IU, vitamin E: 2000 mg, vitamin k3: 800 mg, vitamin B1: 2000 mg, vitamin B2: 6000 mg, vitamin. B6: 5000 mg, vitamin B12: 25 mg, niacin: 6000 mg, panthotenic acid: 20,000 mg, folic acid: 1000 mg, biotin: 8 mg, manganese: 30,000 mg, iron: 20,000 mg, zinc: 20,000 mg, copper: 0 mg, cobalt: 80 mg, iodine: 480 mg, selenium:40 mg, choline: 800,000 mg BTH: 25,000 mg. Supplementary dietary Cu was added to the basal diets at 0, 50 and 100 $mg.kg^{-1}$ Cu concentrations.
[a] Calculated value

the thigh, breast meat and organs (liver, kidney and heart). About 10 g cut sample from the thigh (*Biceps femoris*) and breast (*Pectoralis major*) muscle of each bird were collected (Jensen, 1984). Liver, heart and kidney samples were also collected and stored at -20 °C prior to analysis. For estimation of excreta Cu residues, two birds were randomly selected from each replicate at day 112 and housed individually in metabolic cages. Two days of acclimatization period were allowed for the birds in cages followed by 2 days of excreta collection. The samples of fresh excreta collected per replicate (n = 10 per treatment) were stored at - 20 °C until analysis.

Prior to laboratory analysis, samples of meat, organ and excreta were dried (100 °C for 36 h) and digested using a modified wet-ashing procedure (James, 1996). The ash was reconstituted in 5 ml of HCl solution and analysed for Cu using a Perkin-Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK).

Cu residues in tibia bone

The right tibia of slaughtered birds was removed by cutting with a sharp knife. This was cleaned from adhering meat tissues and cartilage, placed into hexane for 48 h to remove fat and dried in an oven for 24 h until constant weight. The tibiae were weighed and ashed (at 600 ± 5 °C) for 4 h. Ash sample (0.2 g) of each tibia bone (n = 10 per treatment) was solubilized in 5 ml of 50 % HCl, mineral extract filtered into a volumetric flask and diluted using deionized water to a required volume. Cu concentration was determined

using the Perkin Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK).

Statistical analysis

Data generated were analyzed as a two-factor model (Cu levels × Cu source) consisting of two Cu sources (WMD and CGD) supplemented with 3 dosages (0, 50 and 100 mg.kg^{-1}). Data were analysed using the general linear models procedure of the SAS (SAS Institute, 2000) to determine the main effects (Cu levels, Cu source) and their interaction (Cu levels × Cu source). Significant differences were considered at $P < 0.05$.

RESULTS

Cu intake and plasma trace minerals

Main effect of Cu levels and source on Cu intake and plasma trace minerals of cockerels fed diet supplemented with $CuSO_4$ or CuP is shown in Table 2. Cu intake ($P<0.0001$) and serum Cu ($P<0.05$) of cockerel chicks (0-8 weeks) increased with increasing dietary dosage of Cu. However, feed intake, serum Zn, Mn and Fe concentration of the chicks were not affected by varying Cu dosage used in this study. Chicks fed diet supplemented with $CuSO_4$ had higher ($P<0.05$) Cu intake and lower ($P<0.05$) serum Zn than those fed with diet supplemented with CuP.

Cockerels fed control diet had the least ($P<0.05$) feed intake and serum Cu concentration. Cu intake increased ($P<0.001$) with increasing dietary dosage of Cu supplemented. All cockerels fed diet supplemented with Cu, irrespective of the dosage, showed high ($P<0.05$) serum Cu concentration. Grower cockerels (8-16 weeks) fed diet supplemented with CuP had lower feed intake ($P<0.05$), Cu intake ($P<0.0001$) and higher ($P<0.05$) serum Cu concentration than their counterparts fed with $CuSO_4$.

Interaction effect of Cu levels and source on Cu intake and plasma trace minerals of cockerels fed diet supplemented with $CuSO_4$ and CuP is shown in Table 3. Cockerel chicks fed diet supplemented with 100 mg.kg^{-1} Cu (irrespective of the source) recorded the highest ($P<0.0001$) Cu intake, whilst chicks fed diet, supplemented with 100 mg.kg^{-1} Cu sourced from CuP, had the highest ($P<0.05$) serum Cu concentration. Chicks fed control diet and groups fed diet supplemented with CuP (irrespective of Cu dosage) recorded the highest ($P<0.05$) serum Zn. Grower cockerels fed diet

Table 2: Main effect of Cu levels and source on Cu intake and plasma trace minerals of cockerels fed diet supplemented with copper sulphate or copper proteinate

Parameters	Main effect of Cu levels					Main effect of Cu source			
	0	50 mg.kg^{-1}	100 mg.kg^{-1}	Pooled SEM	P- value	$CuSO_4.5H_2O$	CuP	Pooled SEM	P- value
0-8 weeks									
Total feed intake (kg per bird)	2.20	2.30	2.53	0.10	0.750	2.43	2.25	0.09	0.900
Total Cu intake (mg per bird)	71.67^{c}	189.74^{b}	334.93^{a}	22.76	<0.0001	202.77^{a}	194.79^{b}	26.65	0.027
Serum Cu (µg.mL^{-1})	0.19^{c}	0.31^{b}	0.54^{a}	0.09	0.044	0.25	0.44	0.02	0.070
Serum Zn (µg.mL^{-1})	1.65	1.40	1.45	0.10	0.065	1.30^{b}	1.70^{a}	0.97	0.044
Serum Mn (µg.mL^{-1})	0.07	0.09	0.08	0.002	0.700	0.06	0.08	0.001	0.073
Serum Fe (µg.mL^{-1})	1.53	1.53	1.62	0.11	0.990	1.56	1.56	0.09	0.075
9-16 weeks									
Total feed intake (kg per bird)	2.92^{b}	3.36^{ab}	3.66^{a}	0.99	0.042	3.63^{a}	2.99^{b}	0.97	0.044
Total Cu intake (mg per bird)	92.24^{c}	274.24^{b}	481.58^{a}	47.66	<0.0001	303.16^{a}	262.21^{b}	65.60	<0.0001
Serum Cu (µg.mL^{-1})	0.18^{b}	0.40^{a}	0.49^{a}	0.09	0.040	0.20^{b}	0.51^{a}	0.08	0.041
Serum Zn (µg.mL^{-1})	1.30	1.43	1.31	0.04	0.140	1.20	1.49	0.15	0.095
Serum Mn (µg.mL^{-1})	0.11	0.10	0.09	0.010	0.085	0.10	0.10	0.008	0.105
Serum Fe (µg.mL^{-1})	1.73	1.78	1.73	0.14	0.099	1.73	1.75	0.11	0.990

Means in the same row having different letters are different significantly ($P<0.05$)

supplemented with CuP showed increased (P<0.05) serum Cu when compared with groups supplemented with $CuSO_4$.

Cu residues in meat tissues, organs, excreta and tibia bone

Main effect of Cu levels and source on residues of Cu in meat tissues, organs, excreta samples and tibia bone of cockerels fed diet supplemented with $CuSO_4$ and CuP is shown in Table 4. Thigh meat Cu level increased (P<0.05) with increasing dietary dosage of Cu. Control-fed birds had the least (P<0.05) thigh and breast meat Cu. However, Cu source showed no effect (P>0.05) on Cu residues of thigh and breast meat. Liver, heart, excreta and tibia bone Cu levels increased (P<0.05) with increasing dietary Cu dosage. Cockerels fed diet supplemented with CuP showed higher (P<0.05) liver, heart, tibia bone Cu and reduced (P<0.05) excreta Cu than birds fed with $CuSO_4$.

Table 5 shows the interaction effect of Cu levels and source on residual Cu concentration in meat tissues, organs, excreta samples and tibia bone of cockerels fed diet supplemented with $CuSO_4$ or CuP. CuP supplementation resulted in increased (P<0.05) thigh meat Cu. Control-fed birds showed the least (P<0.05) thigh meat Cu. Cockerels fed diet supplemented with 100 $mg.kg^{-1}$ Cu from CuP had the highest (P<0.05) liver and heart Cu concentration. Highest (P<0.05) excreta Cu was obtained from cockerels fed diet supplemented with 100 $mg.kg^{-1}$ Cu from CuSO4. Birds fed diet supplemented with 100 $mg.kg^{-1}$ Cu from CuP also showed the highest (P<0.05) tibia bone Cu.

DISCUSSION

Cu intake and plasma minerals

Improved feed intake of growing cockerels obtained in the present study following increasing Cu dosage agreed with previous work of Chowdhury *et al.* (2004) that dietary supplementation with Cu salts stimulated feed intake of poultry birds. Previous findings also confirmed improved feed efficiency following dietary supplementation of Cu at higher pharmacological levels (Baker *et al*., 1991).

Increased plasma Cu of cockerels obtained with increasing dietary Cu dosage agreed with previous findings that dietary Cu levels showed direct influence on plasma Cu (Luo and Dove, 1996). Supplementation with CuP in the present study, however, showed higher bioavailability (as reflected in increased plasma Cu), when compared with birds fed with $CuSO_4$. Previous studies confirmed that organic Cu was more absorbed than inorganic Cu (Baker *et al*., 1991).

Table 3: Interaction effect of Cu levels and source on Cu intake and plasma trace minerals of cockerels fed diet supplemented with copper sulphate or copper proteinate

Parameters	$CuSO_4.5H_2O$			Cu Proteinate			Pooled	
	0	50 $mg.kg^{-1}$	100 $mg.kg^{-1}$	0	50 $mg.kg^{-1}$	100 $mg.kg^{-1}$	SEM	P - value
0-8 weeks								
Total feed intake (kg per bird)	2.37	2.41	2.50	2.02	2.18	2.55	0.09	0.060
Total Cu intake (mg per bird)	77.38^{d}	199.29^{b}	331.63^{a}	65.95^{f}	180.18^{c}	338.23^{a}	46.50	<0.0001
Serum Cu ($\mu g.mL^{-1}$)	0.18^{c}	0.22^{c}	0.35^{b}	0.20^{c}	0.40^{b}	0.72^{a}	0.09	0.043
Serum Zn ($\mu g.mL^{-1}$)	1.60^{a}	1.20^{b}	1.10^{b}	1.70^{a}	1.60^{a}	1.80^{a}	0.75	0.042
Serum Mn ($\mu g.mL^{-1}$)	0.06	0.08	0.07	0.07	0.09	0.08	0.002	0.095
Serum Fe ($\mu g.mL^{-1}$)	1.55	1.52	1.60	1.50	1.54	1.64	0.10	0.059
9-16 weeks								
Total feed intake (kg per bird)	3.41^{ab}	3.65^{a}	3.83a	2.43^{c}	3.07^{b}	3.49^{ab}	0.95	0.041
Total Cu intake (mg.bird)	107.75^{e}	297.77^{c}	503.95^{a}	76.73^{f}	250.70^{d}	459.21^{b}	52.44	<0.0001
Serum Cu ($\mu g.mL^{-1}$)	0.18^{b}	0.20^{b}	0.22^{b}	0.18^{b}	0.60^{a}	0.75^{a}	0.09	0.035
Serum Zn ($\mu g.mL^{-1}$)	1.20	1.40	1.00	1.40	1.45	1.62	0.095	0.064
Serum Mn ($\mu g.mL^{-1}$)	0.11	0.09	0.09	0.10	0.10	0.09	0.01	0.070
Serum Fe ($\mu g.mL^{-1}$)	1.75	1.80	1.65	1.70	1.75	1.80	0.10	0.099

Means in the same row having different letters are different significantly (P <0.05)

The interaction of plasma trace minerals with Cu supplementation noted in the current study was also reported in previous studies on rat (Van Campen and Scaife, 1967). Increased Cu concentration was reported to trigger improved Fe retention (Prohaska, 1991). Reduced plasma Zn concentration obtained in this study with increasing $CuSO_4$ supplementation corroborated previous findings that Cu and Zn mutually hamper the absorption of each other (Gipp *et al*., 1974).

Copper residues in meat tissues, organs and excreta sample

The Cu concentration values obtained in the thigh and breast meat in this study was within the range reported in the literature (Ledoux *et al.*, 1991). Increased tissue Cu concentration recorded with increasing Cu dosage confirmed the assertion that

Table 4: Main effect of Cu levels and source on Cu concentration in meat tissues, organs, excreta samples and tibia bone of cockerels fed diet supplemented with copper sulphate or copper proteinate

Parameters	Main effect of Cu levels					Main effect of Cu source			
	0	50 mg.kg^{-1}	100 mg.kg^{-1}	Pooled SEM	P- value	$CuSO_4.5H_2O$	CuP	Pooled SEM	P- value
Meat tissue Cu									
Thigh (mg.100g^{-1})	2.10^{c}	3.40^{b}	3.73^{a}	0.97	0.025	2.97	3.18	0.21	0.060
Breast (mg.100g^{-1})	1.85^{b}	2.15^{ab}	2.55^{a}	0.92	0.042	2.20	2.17	0.18	0.055
Cu in Organs									
Liver (mg.100g^{-1})	3.53^{c}	4.98^{b}	6.45^{a}	1.01	0.044	4.51^{b}	5.46^{a}	1.20	0.044
Kidney (mg.100g^{-1})	2.10	2.25	2.10	0.11	0.090	2.10	2.20	0.10	0.106
Heart (mg.100g^{-1})	2.30^{c}	2.80^{b}	3.83^{a}	0.95	0.019	2.18^{b}	3.77^{a}	0.98	0.029
Excreta Cu (mg.100g^{-1})	3.11^{c}	4.73^{b}	5.90^{a}	0.99	0.040	5.20^{a}	3.96^{b}	0.95	0.035
Tibia bone Cu (mg.100g^{-1})	4.66^{c}	5.85^{b}	8.10^{a}	1.42	0.030	5.52^{b}	6.89^{a}	1.36	0.022

Means in the same row having different letters are different significantly (P <0.05)

Table 5: Interaction effect of Cu levels and source on Cu concentration in meat tissues, organs, excreta samples and tibia bone of cockerels fed diet supplemented with copper sulphate or copper proteinate

Parameters	$CuSO_4.5H_2O$			Cu Proteinate			Pooled	
	0	50 mg.kg^{-1}	100 mg.kg^{-1}	0	50 mg.kg^{-1}	100 mg.kg^{-1}	SEM	P - value
Meat tissue Cu								
Thigh (mg.100g^{-1})	2.01^{c}	3.20^{b}	3.70^{a}	2.18^{c}	3.60^{a}	3.76^{a}	0.99	0.044
Breast (mg.100g^{-1})	1.90^{b}	2.20^{ab}	2.50^{a}	1.80^{b}	2.10^{ab}	2.60^{a}	0.89	0.027
Cu in Organs								
Liver (mg.100g^{-1})	3.52^{c}	4.96^{b}	5.06^{b}	3.53^{c}	5.00^{b}	7.84^{a}	0.94	0.019
Kidney (mg.100g^{-1})	2.10	2.20	2.00	2.10	2.30	2.20	0.09	0.107
Heart (mg.100g^{-1})	2.20^{c}	2.10^{c}	2.25^{c}	2.40^{c}	3.50^{b}	5.40^{a}	0.97	0.032
Excreta Cu (mg.100g^{-1})	3.17^{d}	5.18^{b}	7.25^{a}	3.05^{d}	4.28^{c}	4.55^{bc}	1.02	0.033
Tibia bone Cu (mg.100g^{-1})	4.60^{c}	5.50^{bc}	6.45^{b}	4.72^{c}	6.20^{b}	9.75^{a}	1.64	0.022

Means in the same row having different letters are different significantly (P <0.05)

dietary Cu levels directly influence tissue Cu residues (Komprada *et al*., 1999). Increased thigh and breast meat Cu obtained with increased Cu dosage could be attributed to low level of copper clearance in the muscle tissue of birds (Aoyagi and Baker, 1993).

Increased liver Cu of cockerels obtained with increasing dietary Cu levels implied high liver Cu accumulation. Previous studies also confirmed that liver Cu concentration is influenced by variations in dietary Cu concentrations (Luo *et al*., 2005). Liver is a reliable response criterion of Cu status and relative bioavailability. Increased liver Cu concentration obtained with cockerels fed diet supplemented with CuP implied higher bioavailability. Chowdhury *et al.* (2004) reported high liver Cu concentration in broilers fed with organic Cu compared to those receiving inorganic Cu.

Excreta Cu concentration followed a different pattern unlike the trend obtained for liver Cu. Increasing dietary Cu from $CuSO_4$ resulted in increased excreta Cu, whilst inclusion of CuP showed reduced excreta Cu. Reduced excreta Cu with birds fed with CuP agreed with previous findings that inclusion of inorganic Cu resulted in reduced excreta Cu and environmental Cu pollution (Chowdhury *et al.*, 2004). Reduced excreta Cu was also obtained with metal-amino acid chelates in comparison to inorganic Cu salts (Lee *et al*., 2001). This low excreta Cu concentration in CuP-fed group could be linked to improved retention of Cu.

Dietary supplementation with CuP increased tibia bone Cu with the highest value obtained with cockerels fed with 100 $mg.kg^{-1}$ Cu from CuP. Banks *et al*. (2004) also reported that birds fed with Cu-lysine had highest tibia Cu compared with those fed with copper citrate or sulphate. Cao *et al.* (2000) showed that tibia bone Zn was higher in birds supplemented with organic Zn compared with those supplemented with inorganic Zn.

CONCLUSION

The findings of this study showed that dietary inclusion of up to 100 $mg.kg^{-1}$ CuP improved Cu bioavailability in the blood, meat tissues, organs and tibia bone of cockerels. To achieve reduced environmental Cu pollution resulting from excreta of poultry birds, organic salts of Cu, such as CuP, is recommended as feed additive.

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INVESTIGATION ON THE USABILITY OF SOME MATHEMATICAL MODELS IN *IN VITRO* GAS PRODUCTION TECHNIQUES

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ABSTRACT

The aim of this study was to investigate the usage of some mathematical models in *in vitro* gas production techniques. With this aim, the Logistic, Orskov, Verhulst, Janoscheck, Weibull, Bridges, Mitscherling, Monomolecular and Von Bertalanffy models were used, respectively. The goodness of fit of these models to *in vitro* gas production data was examined by using various criteria, such as Mean Square Error (MSE), Adjusted coefficient of determination ($\bar{R}^2$), Accuracy factor (AF) and Bayesian Information Criterion (BIC). However, autocorrelation and the distribution of residuals were examined with the Durbin Watson and Shapiro Wilks tests, respectively. Although the Verhulst and Logistic models showed a lower goodness of fit according to the other models, these models were found to be suitable for *in vitro* gas production studies in the result of all the criteria and tests. As a result, it was determined to be suitable for the *in vitro* gas production technique of other models except for the Orskov model.

Key words: mathematical model; Orskov model; *in vitro*; animal nutrition

INTRODUCTION

Mathematical models have been used for a long time to determine the kinetics of digestion of forage. The first mathematical model in ruminant nutrition is the study to determine the dry matter digestibility of forage obtained by the *in vitro* method by Axelsson (1939) with the estimated regression equation. McMeekan (1943) added the standard errors of the regression equation and the correlation coefficient to this model. However, due to substantially high standard errors of the studies further work was carried out in those years to reduce the standard error (Kivimae, 1960). However, the demand for mathematical models increased in following years with the invention of *in vitro* and *in situ* techniques (Tilley *et al.*, 1960; Tilley and Terry,1963; Mcleoad and Minson,1969; Menke *et al.*, 1979). Recently, many mathematical modeling studies have been carried out to describe production data better (France *et al.*, 2005; Sahin *et al.*, 2011; Wang *et al.*, 2011). Forage digestion kinetics can be estimated more accurately by means of these models.

The aim of this study is to investigate the usability of some mathematical models in *in vitro* gas production techniques. For this purpose, nine models were discussed and tried to determine new models which are not scanned and reached in earlier *in vitro* studies except for the Orskov model. Furthermore, a new form of Logistic and Monomolecular models are discussed in this study. The models used in this study are Logistic, Verhulst, Janoschek, Weibull, Bridges, Mitscherling, Monomolecular and Von Bertalanffy, respectively.Various goodness of fit criteria were used to identify similarities and differences between these models.

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MATERIAL AND METHODS

The data set used in this study consists of dry matter digestibility values of each of three replications of four different legume forage crops (white clover - *Trifolium repens* L., red clover - *Trifolium pretense* L., common vetch - *Vicia sativa* L. and yellow sweet clover - *Melilotus officinalis* L.) obtained by using *in vitro* technique and taken at the hours 3, 6, 24, 48, 72 and 96 from Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Animal Science, and used with written permission of the researchers related.

In this study, the equation of Orskov model which is selected for control and of other models are given in Table 1 and also parameter meanings are given in Table 2.

Statistical Analyses

Determination of the goodness of fit

The goodness of fit of each model is evaluated by using Mean Square Error (MSE), Adjusted coefficient of determination ($\overline{R}^2$), Bayesian Information Criterion (BIC). Formulas of these criteria are:

$$\text{Mean Square Error (MSE)} = \text{MSE } \frac{1}{n}\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2$$

$$\text{Adjusted coefficient of determination} = \overline{R}^2 = 1 - \frac{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2/(n-k)}{\sum_{i=1}^{n}(Y_i - \bar{Y})^2/(n-1)}$$

$$\text{Accuracy Factor} = AF = e\left[\sqrt{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2/n}\right]$$

$$\text{Bayesian Information Criterion} = \text{BCI} = nln\left(\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2/n\right) + kln(n)$$

and these expressions refer to;

E : Exponential expression (2.7182),
$\hat{Y}_i$: Predictive value,
Y_i : Observed value,
$\bar{Y}$: Mean of observed values,
n : Sample size,
ln : Natural logarithm.

Examination of errors

Shapiro Wilk (SW) test is used to determine whether the errors in the models are distributed normally (West, 1999). Durbin Watson (DW) statistics was used to determine whether there is an autocorrelation among the errors in models (Lopez *et al.*, 2004).

All models are fitted by using SAS (8.0) package program NLIN command and Levenberg Marquardt algorithm (SAS, 1999). It is tested by one-way analysis of variance (One Way ANOVA) whether MSE, $\overline{R}^2$, AF and BIC criteria are statistically different from each other. Tukey's multiple comparison technique was used for the purpose of determining the difference between the criteria which is significant (Pearse and Hartley, 1966). The level of significance is taken as $P < 0.05$.

RESULTS AND DISCUSSION

The values of model parameters are given in Table 3. Similar results are received when Orskov model which is used for control, and parameter values of other models, are compared. As a result, it has been determined that the parameter values of the Orskov model are compatible with the parameter values of new models. The results of the DW test of the models are also given in Table 3. There has not been any autocorrelation in model errors according to the DW test ($P > 0.05$). The results of DW test has come out to be non-significant, which is in accordance with the findings of Lopez *et al.* (1999) and Uckardes *et al.* (2013), who reported that the model does not include the systematic error and therefore the model could be used.

The SW test has been used to determine whether errors in the models are normally distributed and the results are given in Table 4. As a result of this test, the errors in all models show a normal distribution ($P > 0.05$). There is no systematic deviation in the errors of models for both of the results of DW and SW tests;

Table 1: The models used in the study

Models	Equations
1. Logistics	$Y = a / (1 + e^{b-ct})$
2. Orskov	$Y = a + b (1 - e^{ct})$
3. Verhulst	$Y = a / (1 - be^{ct})$
4. Janoscheck	$Y = a - (a - b)e^{- ctd}$
5. Weibull	$Y = a - be^{-ctd}$
6. Bridges	$Y = a + b (1 - e^{- (ctd)})$
7. Mitscherling	$Y = a (1 - be^{-ct})$
8. Monomolecular	$Y = a - be^{- ct}$
9. Von Bertalanffy	$Y = a - (a - b)e^{-ct}$

e: exponential

Table 2: The models used in the study and parameter expressions

Models	Parameter Expressions				
	Initially obtained amount of gas or the amount of digestion	Slowly obtained amount of gas or the amount of digestion	Speed of the amount of slowly obtained gas or digestion speed	Total production amount of gas or amount of digestion	Key characteristic of the curve / *Shape parameter*
1. Logistics	ln(a/d-1) (*)	a- ln(a/d-1)	c	a	b
2. Orskov	a	b	c	a+b (*)	-
3. Verhulst	a/(1-b) (*)	a- a/(1-b) (*)	c	a	b
4. Janoscheck	b	a-b	c	a	d
5. Weibull	a-b	b	c	a	d
6. Bridges	a	b	c	a+b (*)	d
7. Mitscherling	a(1-b) (*)	a- a(1-b) (*)	c	a	-
8. Monomolecular	a-b (*)	b	c	a	-
9. Von Bertalanffy	b	a-b	c	a	-

- : unavailable (*): it was obtained from the equation

thus these models are determined to be suitable for *in vitro* gas production studies.

The results of the goodness of fit criteria are given in Table 5. While the results of analysis of variance of BIC values of models of common vetch forage crops has come out to be non-significant ($P > 0.05$), the results of analysis of variance models of other forage crops have been found to be significant ($P < 0.05$; $P < 0.01$; $P < 0.001$). Statistically significant differences have been found between MSE and $\overline{R}^2$ values of the models which are used for four different legume forage crops ($P < 0.05$; $P < 0.01$). MSE values of the Verhulst model and also in some cases the Logistic models have been higher than the other models. However, the results of MSE values of other models are similar to each other. A similar situation is observed in the values of $\overline{R}^2$. Although $\overline{R}^2$ values are significant as a result of analysis of variance of yellow sweet clover forage crop, there has not been any difference as a result of the Tukey test. While the Verhulst model and also in some cases the Logistic model has given low results in terms of the values of $\overline{R}^2$, higher values of $\overline{R}^2$ are obtained from the other models. According to Lopez *et al*. (2004) and Korkmaz *et al.* (2011) are the values of MSE being high and values of $\overline{R}^2$ are being low; it shows that the model does not provide a good fit to the data set.

The results of AF are similar with that of $\overline{R}^2$ (Table 5). The results of the analysis of variance of yellow sweet clover forage crop are significant, but no differences are found as a result of the Tukey test. However, the AF results of the Logistic and Verhulst models of goodness of fit are lower as compared to other models. Lopez *et al.* (2004) reported that AF values which are low show that the model shows a good fit to the data set.

BIC criteria has a slightly different evaluation than other criteria. BIC has a more strict attitude toward the number of parameters than the other criteria (Alzahal *et al*., 2007). The result of the analysis of variance of common vetch forage crop is non-significant ($P>0.05$).

As a result, the Verhulst and Logistic models have showed lower performance than the other models in some cases in terms of the goodness of fit criteria. Therefore, it can be misleading to make an emphasis that these models should not be used. Wang *et al.* (2011) have reported that the models which have low performances increased goodness of fit by model modification.

Models may differ from each other in terms of behaviours (structure) and the number of parameters. A model might have a more flexible structure than another model or a model which is having less number of parameters, can fit more easily. In this study, comparing to other models, the Weibull, Janoscheck and Bridges models have the parameters which shape the curve and it is more difficult to make them fit. However, these models have a more flexible structure than other models. Although having more number of parameters seems like a disadvantage, this feature can be evaluated

Table 3: Parameter values of legume forage crops and Durbin Watson test results ($\bar{X} \pm S_{\bar{x}}$)

Models	Parameter				DW
	a	b	c	d	
Common vetch					
1. Logistics	367.27 ± 10.78	0.723 ± 0.029	0.093 ± 0.005	-	Ns
2. Orskov	90.64 ± 6.390	283.97 ± 5.259	0.054 ± 0.003	-	Ns
3. Verhulst	365.37 ± 8.348	-2.06 ± 0.033	0.094 ± 0.001	-	Ns
4. Janoscheck	376.23 ± 11.695	83.94 ± 6.439	0.064 ± 0.003	0.950 ± 0.001	Ns
5. Weibull	376.57 ± 11.726	294.067 ± 5.459	0.066 ± 0.003	0.940 ± 0.001	Ns
6. Bridges	85.35 ± 6.428	290.50 ± 5.373	0.062 ± 0.003	0.960 ± 0.001	Ns
7. Mitscherling	374.60 ± 11.529	0.759 ± 0.009	0.054 ± 0.003	-	Ns
8. Monomolecular	374.60 ± 11.529	283.97 ± 5.59	0.054 ± 0.003	-	Ns
9. Von Bertalanffy	374.60 ± 11.529	90.64 ± 6.387	0.054 ± 0.003	-	Ns
White Clover					
1. Logistics	374.20 ± 9.133	0.709 ± 0.040	0.081 ± 0.001	-	Ns
2. Orskov	97.84 ± 6.929	285.90 ± 4.325	0.046 ± 0.001	-	Ns
3. Verhulst	368.33 ± 7.890	-2.14 ± 0.071	0.095 ± 0.005	-	Ns
4. Janoscheck	385.87 ± 9.559	91.69 ± 6.958	0.054 ± 0.001	0.950 ± 0.001	Ns
5. Weibull	386.33 ± 9.586	295.97 ± 4.457	0.056 ± 0.001	0.940 ± 0.001	Ns
6. Bridges	93.99 ± 6.958	292.43 ± 4.391	0.053 ± 0.001	0.960 ± 0.001	Ns
7. Mitscherling	383.73 ± 9.494	0.746 ± 0.012	0.046 ± 0.001	-	Ns
8. Monomolecular	383.73 ± 9.464	285.90 ± 4.325	0.046 ± 0.001	-	Ns
9. Von Bertalanffy	383.73 ± 9.494	97.84 ± 6.929	0.046 ± 0.001	-	Ns
Red Clover					
1. Logistics	357.73 ± 17.246	0.866 ± 0.023	0.099 ± 0.003	-	Ns
2. Orskov	76.21 ± 5.303	289.37 ± 12.236	0.054 ± 0.002	-	Ns
3. Verhulst	358.37 ± 17.010	-2.318 ± 0.054	0.094 ± 0.003	-	Ns
4. Janoscheck	366.53 ± 18.273	69.52 ± 5.012	0.064 ± 0.002	0.950 ± 0.001	Ns
5. Weibull	367.53 ± 17.636	299.50 ± 12.689	0.066 ± 0.002	0.940 ± 0.001	Ns
6. Bridges	70.93 ± 5.073	295.93 ± 12.546	0.062 ± 0.002	0.960 ± 0.001	Ns
7. Mitscherling	365.57 ± 17.522	0.792 ± 0.005	0.054 ± 0.002	-	Ns
8. Monomolecular	365.57 ± 17.522	289.37 ± 12.236	0.054 ± 0.002	-	Ns
9. Von Bertalanffy	365.57 ± 17.522	76.21 ± 5.303	0.054 ± 0.002	-	Ns
Yellow Sweet Clover					
1. Logistics	348.00 ± 11.117	0.664 ± 0.044	0.096 ± 0.004	-	Ns
2. Orskov	90.69 ± 1.790	263.60 ± 12.200	0.057 ± 0.002	-	Ns
3. Verhulst	348.17 ± 12.479	-1.937 ± 0.039	0.095 ± 0.004	-	Ns
4. Janoscheck	355.70 ± 11.374	84.346 ± 2.007	0.067 ± 0.002	0.950 ± 0.001	Ns
5. Weibull	356.00 ± 11.374	273.067 ± 12.610	0.069 ± 0.002	0.940 ± 0.001	Ns
6. Bridges	85.68 ± 1.959	269.633 ± 12.433	0.065 ± 0.002	0.960 ± 0.001	Ns
7. Mitscherling	354.33 ± 11.375	0.743 ± 0.012	0.057 ± 0.002	-	Ns
8. Monomolecular	354.33 ± 11.375	263.60 ± 12.200	0.057 ± 0.002	-	Ns
9. Von Bertalanffy	354.33 ± 11.375	90.69 ± 1.790	0.057 ± 0.002	-	Ns

DW: Durbin Watson; Ns: P>0.05

Table 4: Shapiro Wilk W test results for errors in legume forage crops

Models	Common vetch		White clover		Red clover		Yellow sweet clover	
	W	SL	W	SL	W	SL	W	SL
1. Logistics	0.904	0.357	0.854	0.134	0.873	0.197	0.878	0.220
2. Orskov	0.858	0.147	0.894	0.297	0.927	0.524	0.884	0.243
3. Verhulst	0.911	0.400	0.871	0.912	0.872	0.193	0.892	0.287
4. Janoscheck	0.857	0.142	0.902	0.342	0.915	0.403	0.887	0.260
5. Weibull	0.857	0.141	0.901	0.338	0.933	0.574	0.887	0.257
6. Bridges	0.857	0.142	0.900	0.329	0.931	0.561	0.886	0.256
7. Mitscherling	0.862	0.159	0.895	0.301	0.927	0.524	0.891	0.279
8. Monomolecular	0.858	0.147	0.894	0.297	0.927	0.524	0.884	0.243
9. Von Bertalanffy	0.585	0.147	0.894	0.297	0.927	0.523	0.883	0.242

W>SL = P > 0.05; SL: Level of significance of difference

as an advantage. Zwitering *et al.* (1990) reported that a model which has less number of parameters should be preferred rather than the one which has more parameters in choosing the model. They stated that the reason why they proposed this preference was that the relationship between parameters may increase due to increase in number of parameters and therefore the model may fit with more difficulty. Moreover, these researchers reported that the model which has less parameters is simpler and therefore easier to use and because the less parameter solution is more stable since the parameters are less correlated.

However, Wang *et al.* (2011) reported that increased flexibility of the model and the number of parameters by adding a parameter shapes the curve in the logistic model. Schofield *et al.* (1994) turned Logistic and Gompertz models into a dual-phase structure by adding parameters to them and thus increase the effectiveness of the models. According to France *et al*. (2005) and Calabro *et al.* (2004), the reason of these studies is to show that dependence on only one or a few models should be avoided since different digestive curves can be obtained depending on the amount of organic matter forage material (quickly or slowly degradable) or in case of using different species of ruminants. Therefore, the models which have flexible structure, can outperform more in some cases as compared to the models which are more stable like Orskov model.

However, models can provide calculation of some parameters considered to be important for digestion, for example, active digestibility, the amount of gas production at a required time. Sahin *et al.* (2011) reported that they had obtained t_{25}, t_{50}, t_{75} and t_{95} times of exponential models. Besides, France *et al.* (2000) reported that they had obtained a general formula of t_p for the Generalized Michaelis-Menten, Generalized Mitscherlich and Logistic models. But these researchers emphasized that there is no analytic solution for these equations; thus they should be evaluated as numeric. These results show the slow but continuous increase in sigmoidal models. Uckardes (2013) noticed that the Mitscherlich model is modified by adding a new biologically meaningful parameter to describe the degradation kinetics and also developed new theoretical approaches to the modified Mitscherlich model regarding the description of *in situ* nylon bag and *in vitro* gas production techniques.

CONCLUSION

In conclusion, having a large number of models should not cause a confusion in deciding which model to use. That is because, reaction of the model will also be different depending on the feed materials used. While one model shows a very good fit to the data set in one study (due to the feed material), it may exhibit low performance in another study. Therefore, it might be necessary to select an appropriate model for the data set and having a large number of models referring to variety in a way rather than being a disadvantage.

As a result of this study, it is concluded that these models other than the Orskov model can be used to estimate *in vitro* gas production kinetics by using different forage crops.

Table 5: MSE, AF and BIC analysis of variance and tukey test results of nine different models of four different forage crops ($\bar{X} \pm S_{\bar{x}}$)

Models	Common vetch MSE	White clover MSE	Red clover MSE	Yellow sweet clover MSE
1. Logistics	$475.5^{ab} \pm 82.5$	$365.8^{b} \pm 16.9$	$272.5^{bc} \pm 64.3$	$481.0^{b} \pm 48.9$
2. Orskov	$215.1^{ab} \pm 52.2$	$146.3^{a} \pm 12.3$	$91.9^{ab} \pm 25.9$	$248.2^{a} \pm 44.4$
3. Verhulst	$490.3^{b} \pm 91.3$	$426.4^{b} \pm 18.6$	$380.9^{c} \pm 63.4$	$485.5^{b} \pm 48.9$
4. Janoscheck	$187.2^{ab} \pm 47.91$	$124.3^{a} \pm 11.1$	$76.7^{a} \pm 21.3$	$211.8^{a} \pm 33.5$
5. Weibull	$181.8^{a} \pm 47.1$	$120.2^{a} \pm 10.8$	$73.9^{a} \pm 20.4$	$208.5^{a} \pm 35.1$
6. Bridges	$202.4^{ab} \pm 58.3$	$128.6^{a} \pm 11.3$	$79.6^{a} \pm 22.3$	$215.1^{a} \pm 31.8$
7. Mitscherling	$215.1^{ab} \pm 52.2$	$146.3^{a} \pm 12.3$	$91.9^{ab} \pm 25.9$	$248.2^{a} \pm 44.4$
8. Monomolecular	$215.1^{ab} \pm 52.2$	$146.3^{a} \pm 12.3$	$91.9^{ab} \pm 25.9$	$248.2^{a} \pm 44.4$
9. Von Bertalanffy	$215.1^{ab} \pm 52.2$	$146.3^{a} \pm 12.3$	$91.9^{ab} \pm 25.9$	$248.2^{a} \pm 44.4$
Significance Level	**	***	***	***
Models	$\bar{R}^2$	$\bar{R}^2$	$\bar{R}^2$	$\bar{R}^2$
1. Logistics	$0.9527^{b} \pm 0.006$	$0.9646^{b} \pm 0.002$	$0.9726^{b} \pm 0.005$	$0.9417^{a} \pm 0.011$
2. Orskov	$0.9787^{a} \pm 0.004$	$0.9858^{a} \pm 0.001$	$0.9911^{a} \pm 0.002$	$0.9696^{a} \pm 0.008$
3. Verhulst	$0.9512^{b} \pm 0.006$	$0.9587^{b} \pm 0.002$	$0.9694^{b} \pm 0.007$	$0.9412^{a} \pm 0.011$
4. Janoscheck	$0.9815^{a} \pm 0.004$	$0.9880^{a} \pm 0.001$	$0.9925^{a} \pm 0.002$	$0.9741^{a} \pm 0.006$
5. Weibull	$0.9821^{a} \pm 0.004$	$0.9884^{a} \pm 0.001$	$0.9928^{a} \pm 0.002$	$0.9745^{a} \pm 0.006$
6. Bridges	$0.9800^{a} \pm 0.005$	$0.9876^{a} \pm 0.001$	$0.9925^{a} \pm 0.002$	$0.9738^{a} \pm 0.006$
7. Mitscherling	$0.9787^{a} \pm 0.004$	$0.9858^{a} \pm 0.001$	$0.9911^{a} \pm 0.002$	$0.9696^{a} \pm 0.008$
8. Monomolecular	$0.9787^{a} \pm 0.004$	$0.9858^{a} \pm 0.001$	$0.9911^{a} \pm 0.002$	$0.9696^{a} \pm 0.008$
9. Von Bertalanffy	$0.9778^{a} \pm 0.004$	$0.9858^{a} \pm 0.001$	$0.9911^{a} \pm 0.002$	$0.9696^{a} \pm 0.008$
Significance Level	***	***	***	*
Models	AF	AF	AF	AF
1. Logistics	$1.083^{b} \pm 0.0072$	$1.075^{b} \pm 0.0096$	$1.066^{b} \pm 0.0106$	$1.083^{a} \pm 0.0125$
2. Orskov	$1.052^{a} \pm 0.0075$	$1.047^{a} \pm 0.0062$	$1.036^{a} \pm 0.0081$	$1.055^{a} \pm 0.0121$
3. Verhulst	$1.083^{b} \pm 0.0020$	$1.071^{b} \pm 0.0116$	$1.069^{b} \pm 0.0110$	$1.083^{a} \pm 0.0080$
4. Janoscheck	$1.048^{a} \pm 0.0075$	$1.043^{a} \pm 0.0057$	$1.033^{a} \pm 0.0072$	$1.052^{a} \pm 0.0115$
5. Weibull	$1.048^{a} \pm 0.0078$	$1.043^{a} \pm 0.0059$	$1.032^{a} \pm 0.0072$	$1.051^{a} \pm 0.0120$
6. Bridges	$1.049^{a} \pm 0.0075$	$1.044^{a} \pm 0.0062$	$1.033^{a} \pm 0.0072$	$1.052^{a} \pm 0.0120$
7. Mitscherling	$1.052^{a} \pm 0.0075$	$1.047^{a} \pm 0.0062$	$1.035^{a} \pm 0.0076$	$1.058^{a} \pm 0.0158$
8. Monomolecular	$1.052^{a} \pm 0.0075$	$1.047^{a} \pm 0.0062$	$1.036^{a} \pm 0.0081$	$1.055^{a} \pm 0.0121$
9. Von Bertalanffy	$1.052^{a} \pm 0.0075$	$1.047^{a} \pm 0.0062$	$1.036^{a} \pm 0.0081$	$1.055^{a} \pm 0.0121$
Significance Level	***	***	***	*
Models	BIC	BIC	BIC	BIC
1. Logistics	44.87 ± 1.162	$43.22^{b} \pm 0.319$	$40.81^{a} \pm 1.566$	$45.08^{a} \pm 0.683$
2. Orskov	39.14 ± 1.603	$36.77^{a} \pm 0.576$	$33.02^{a} \pm 1.945$	$40.31^{a} \pm 1.174$
3. Verhulst	45.06 ± 1.239	$44.30^{b} \pm 0.307$	$41.71^{a} \pm 1.590$	$45.15^{a} \pm 0.679$
4. Janoscheck	40.07 ± 1.688	$37.57^{a} \pm 0.606$	$33.72^{a} \pm 1.919$	$41.19^{a} \pm 1.044$
5. Weibull	39.85 ± 1.708	$37.33^{a} \pm 0.612$	$33.46^{a} \pm 1.906$	$41.06^{a} \pm 1.109$
6. Bridges	40.50 ± 1.890	$37.81^{a} \pm 0.599$	$33.97^{a} \pm 1.928$	$41.32^{a} \pm 0.979$
7. Mitscherling	39.14 ± 1.603	$36.77^{a} \pm 0.576$	$33.02^{a} \pm 1.945$	$40.31^{a} \pm 1.174$
8. Monomolecular	39.14 ± 1.603	$36.77^{a} \pm 0.576$	$33.02^{a} \pm 1.945$	$40.31^{a} \pm 1.174$
9. Von Bertalanffy	39.14 ± 1.603	$36.77^{a} \pm 0.576$	$33.02^{a} \pm 1.945$	$40.31^{a} \pm 1.174$
Significance Level	Ns	***	*	*

Ns: P>0.05; *:P<0.05; **P<0.01; ***: P<0.001; AF: Accuracy factor; MSE: Mean Square Error; BIC: Bayesian Information Criterion

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