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# MULTIFACTORIAL DISCRIMINANT ANALYSIS OF CEPHALIC MORPHOLOGY OF INDIGENOUS BREEDS OF SHEEP IN NIGERIA

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## ABSTRACT

The study aimed at classifying Nigerian indigenous breeds of sheep on the basis of their head conformation using multivariate analyses. Twelve cephalic traits of 1,200 sheep belonging to four breeds (Balami, Yankasa, Uda and WAD) were measured. The traits were subjected to principal component (PC) analysis to reduce data dimensionality. A discriminant canonical analysis was also applied to differentiate between the breeds. The cephalic index revealed that the sheep breeds are brachycephalic (short-headed) except Uda which was mesocephalic (medium-headed). The first PC reflected variables related to length and diameter amplitudes and explained 37.5 percent of the total variance. The second PC gave major relevance to skull, face and neck lengths of the sheep and contributed to 21.30 percent of the total variance, while the third PC loaded highly for skull length, head depth and neck length and contributed 17.35 percent of the total variance. The stepwise discriminant analysis revealed that skull width, head width, head length and head depth were the most discriminating variables to separate the four breeds of sheep.

**Key words:** sheep breeds; cephalic; discriminant analysis; multivariate; traits

## INTRODUCTION

In Nigeria, breeds of sheep are meat producing animals adapted to specific ecological zones of the country. They play agricultural, economic, cultural and religious roles. There are generally considered to be four breeds of sheep native to Nigeria. These are Balami, Uda, Yankasa and West African Dwarf (Ngere *et al*., 1979). Indigenous sheep breeds are an important storehouse of genetic material because of their ability to acclimatize to local, sometimes harsh environmental conditions, nutritional fluctuations across seasons and resistance to diseases and parasites (Kosgey and Okeyo, 2007). The Global Plan of Action for Animal Genetic Resources recognizes that a better understanding of the characteristics of indigenous livestock breeds is necessary for guiding decision making in the development of breeding programmes (FAO, 2007).

Sheep biodiversity has been described using morphological measurements or characterized using molecular data (Paiva *et al.*, 2011). According to Solomon *et al.* (2007), morphological description is an essential component of breed characterization that can be used to physically identify, describe and recognize a breed, and also to classify livestock breeds into broad categories. Historically, morphological studies, especially of the skull, were the major source of data used to characterize breeds (Kidd and Pirchner, 1971). Given their biometric nature, cephalic measurements and indices allow comparisons between breeds from very distant geographical areas, and permit research into

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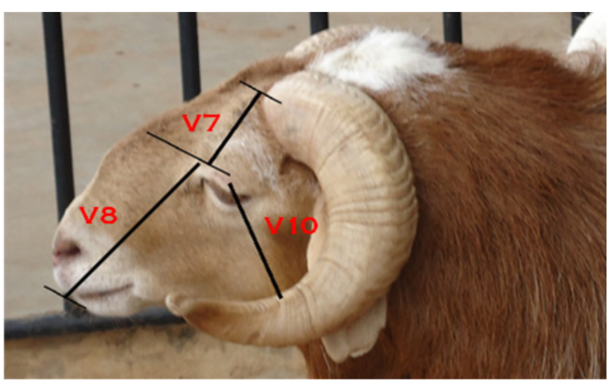
the distinctiveness of breeds based on cephalic evaluation (Pares and Jordan, 2008). According to Yunusa *et al.* (2013) incorporating more cephalic measurements in principal component analysis (PCA) models combined with biometry of cephalic anatomy will shed more light on the suitability of head measurements for breed classification. Skull morphometric studies within and across sheep breeds based on a relatively large number of specimens and employing multivariate analysis techniques are limited. Thus, this study sought to examine skull differences among indigenous breeds of sheep in Nigeria using their intraspecific variability by means of multivariate analyses via principal component and discriminant analyses.

## MATERIALS AND METHODS

The study was conducted in Ibadan, Oyo State, Nigeria. The city is the largest metropolitan geographical area in the country. In 2006, the total population of Ibadan was 2,550,593 while the average population density was 828 persons per km$^2$ (NPC, 2006). Due to its large population, Ibadan is a strategic location and hub for transactions in large number of livestock such as cattle, sheep, goats and chickens. Thus, all the breeds of sheep are found in Ibadan as representation of sampling frame for the study. A total of 1,200 sheep consisting of 300 animals from each breed were sampled from major small ruminant markets in Ibadan and households where sheep are raised. The sampling was conducted from November 2014 to June 2016.

**Data collection**

Twelve basic morpho-structural traits were taken with a flexible tape rule. Cephalic traits measured were skull width, head width, face width, skull length, head length, face length, head depth, neck length, neck width, horn length, horn width and horn space. All measurements were taken with calibrated tape rule. The measurements were taken following standard procedures and anatomical reference points as described by Parés *et al.* (2012), as shown in Figure 1. Cephalic index was estimated from the measured traits as the ratio of maximum


V1 – horn space, V2 – skull width, V3 – head width, V4 – face width, V5 – horn length, V6 – horn width, V7 – skull length, V8 – face length, V9 – head length (V4+V7), V10 – head depth, V11 – neck length, V12 – neck width

**Figure 1. Cephalic morphology of sheep**

width of the head multiplied by 100 and divided by maximum head length (Edilberto *et al.*, 2011). The cephalic index was used to categorize individuals as: dolicocephalic (long-headed) if the cephalic index value was less than 75.9; mesocephalic (medium-headed) if the cephalic index value ranged between 76 and 81 and brachycephalic (short-headed) if the cephalic index value was greater than 81.1 (Schlueter *et al.*, 2009).

**Statistical analyses**

Data were subjected to multivariate analyses, the data were submitted to preliminary univariate analyses using UNIVARIATE and FREQ of SAS (SAS INSTITUTE® 9.13, 2004). Data were analysed using simple descriptive statistics before it was submitted for principal component analysis (PCA). Stepwise discriminant procedure was applied using PROC STEPDISC. The CANDISC procedure was used to enable differentiation between the breeds, to estimate Mahalanobis distances and derive canonical functions. Differences among sheep breeds for cephalic traits were analysed using the following linear model:

$Y_{ij} = \mu + \alpha_i + e_{ij}$ where;

$Y_{ij}$ is the $j^{th}$ morphological variable for the $i^{th}$ breed of sheep,

$\mu$ the overall mean for each morphological variable for all sheep breeds,

$\alpha_i$ the effect of the $i^{th}$ sheep breed ($i^{th}$ = 4, for Yankasa, Uda, Balami, WAD)

$e_{ij}$ the residual error of null average and a constant variance.

## RESULTS AND DISCUSSION

Results of summary statistics of cephalic traits of the indigenous sheep are presented in Table 1. The results revealed a relatively moderate to high variability for the traits (Coefficient of variation (CV)). The highest CVs were obtained for horn space (52.71 %) and the lowest was recorded in head depth (15.77 %). The CV ranges between 16 % and 45.13 % were obtained for skull width, skull length, head width, face width, head length, face length, head depth, neck length, neck width and horn width.

Table 2 shows the effect of breeds on the cephalic traits of the sheep. Breeds of sheep differed significantly in the cephalic traits considered ($P < 0.05$). Results revealed significant differences between breeds in all variables. Uda and Balami had significantly ($p < 0.05$) higher mean values of the cephalic traits than Yankasa and WAD, with exception in skull length and horn space, which were significantly higher in WAD. Highest cephalic index was obtained in Balami (82.66 ± 11.98 %) followed by Yankasa (82.28 ± 11.65 %), WAD (82.16 ± 13.02 %) and Uda (78.35 ± 9.41 %). On average, the cephalic index reveals that Balami, WAD and Yankasa are brachycephalic (short-headed) breeds having cephalic

**Table 1. Summary statistics of cephalic traits of indigenous sheep breeds in Nigeria**

| Traits (cm) | Mean | Variance | Standard deviation | Coefficient of variation (%) | Range |
|---|---|---|---|---|---|
| Skull width | 20.92 | 2.52 | 1.50 | 16.00 | 9.00 |
| Head width | 32.24 | 9.87 | 3.14 | 19.35 | 27.90 |
| Face width | 27.73 | 8.63 | 2.94 | 21.40 | 16.50 |
| Skull length | 13.00 | 3.27 | 1.81 | 45.13 | 20.70 |
| Head length | 30.94 | 10.76 | 3.28 | 16.45 | 26.00 |
| Cephalic index (%) | 87.57 | 112.41 | 8.96 | 17.68 | 74.89 |
| Face length | 30.73 | 11.21 | 3.35 | 18.89 | 27.20 |
| Head depth | 29.51 | 5.98 | 2.45 | 15.77 | 21.00 |
| Neck length | 33.69 | 34.97 | 5.91 | 24.97 | 33.00 |
| Neck width | 50.37 | 82.12 | 9.06 | 24.91 | 62.40 |
| Horn length | 40.10 | 120.28 | 10.97 | 42.02 | 56.00 |
| Horn width | 26.21 | 15.34 | 3.92 | 27.56 | 28.50 |
| Horn space | 9.44 | 3.29 | 1.81 | 52.71 | 20.70 |

**Table 2. Effect of breeds on cephalic traits of indigenous sheep breeds in Nigeria**

| Traits (cm) | Balami | Uda | WAD | Yankasa |
|---|---|---|---|---|
| Skull width | 10.05 ± 1.37[b] | 10.74 ± 1.42[a] | 9.02 ± 1.62[c] | 9.97 ± 1.52[b] |
| Head width | 17.60 ± 3.10[a] | 17.53 ± 2.76[a] | 13.53 ±2.78[c] | 16.39 ± 2.55[b] |
| Face width | 14.65 ± 2.15[b] | 15.53 ± 3.21[a] | 10.98 ± 2.44[c] | 13.89 ± 2.32[b] |
| Skull length | 3.63 ± 0.65[b] | 4.17 ± 1.89[ab] | 4.29 ± 1.99[a] | 3.96 ± 2.06[ab] |
| Head length | 21.35 ± 2.11[b] | 22.41 ± 2.77[a] | 16.27 ± 2.76[d] | 20.03 ± 2.45[c] |
| Cephalic index (%) | 82.66 ± 11.98[a] | 78.35 ± 9.41[b] | 82.16 ± 13.02[ab] | 82.28 ± 11.65[ab] |
| Face length | 18.27 ± 2.51[b] | 19.70 ± 3.32[a] | 15.27 ± 2.65[c] | 17.86 ± 3.34[b] |
| Head depth | 16.38 ± 2.49[a] | 16.27 ±2.32[a] | 13.91 ± 1.84[c] | 15.55 ± 2.37[b] |
| Neck length | 25.36 ± 5.49[ab] | 26.86 ± 5.50[a] | 18.96 ± 5.01[c] | 23.90 ± 5.20[b] |
| Neck width | 39.29 ± 8.85[a] | 39.71 ± 9.34[a] | 30.22 ± 8.15[c] | 36.62 ± 7.80[b] |
| Horn length | 32.59 ± 9.19[a] | 30.20 ± 10.90[a] | 13.93 ± 6.42[c] | 8.41 ± 14.68[b] |
| Horn width | 15.78 ± 3.47[a] | 16.11 ± 3.62[a] | 10.21 ± 2.60[c] | 14.68 ± 3.31[b] |
| Horn space | 3.51 ± 2.54[ab] | 3.34 ± 1.55[ab] | 3.92 ± 1.65[a] | 3.18 ± 1.46[b] |

[abc]Means within the same row having different superscripts differ significantly ($p < 0.05$)

index values of circa 82 %. A sheep is said to be brachycephalic or brachycranial when its cephalic index is greater than 81.1 %. A brachycephalic individual is characterized with short and broad skull, flattened and widened occiput (Marchant *et al*., 2017). Uda sheep are mesocephalic (medium-headed) with cephalic index of 78 %, implying that the breed possess narrow or nearly oval skull. In previous studies on cephalic index, Sarma (2006) reported cephalic index of 41.95 for goats; 58.45 for puppies and 51.73 for lambs (Onar, 1999) and Karimi *et al.* (2011) reported cephalic index of 53.57 for Mehraban sheep.

Phenotypic correlation coefficients among cephalic traits of the sheep are presented in Table 3. Some traits were highly and positively correlated, while few traits were negatively correlated. High, positive and significant correlations were found between face length and face width (0.84), horn length and horn width (0.83), head length and face width (0.72), horn length and head length (0.70), horn length and neck width (0.70), horn

**Table 3. Phenotypic correlations among cephalic traits of indigenous sheep breeds in Nigeria**

| Traits (cm) | Skull width | Head width | Face width | Skull length | Head length | Face length | Head depth | Neck length | Neck width | Horn length | Horn width | Horn space |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Skull width | 1.00 | | | | | | | | | | | |
| Head width | 0.39 | 1.00 | | | | | | | | | | |
| Face width | 0.44 | 0.59 | 1.00 | | | | | | | | | |
| Skull length | 0.10 | 0.06 | 0.03 | 1.00 | | | | | | | | |
| Head length | 0.41 | 0.66 | 0.72 | -0.04 | 1.00 | | | | | | | |
| Face length | 0.44 | 0.55 | 0.84 | 0.48 | 0.61 | 1.00 | | | | | | |
| Head depth | 0.38 | 0.50 | 0.35 | 0.04 | 0.46 | 0.33 | 1.00 | | | | | |
| Neck length | 0.32 | 0.03 | 0.31 | 0.02 | 0.40 | 0.22 | 0.19 | 1.00 | | | | |
| Neck width | 0.40 | 0.48 | 0.53 | 0.07 | 0.57 | 0.50 | 0.57 | 0.28 | 1.00 | | | |
| Horn length | 0.39 | 0.53 | 0.61 | 0.03 | 0.70 | 0.55 | 0.56 | 0.45 | 0.70 | 1.00 | | |
| Horn width | 0.38 | 0.53 | 0.61 | 0.03 | 0.69 | 0.55 | 0.51 | 0.45 | 0.70 | 0.83 | 1.00 | |
| Horn space | 0.02 | -0.25 | -0.35 | -0.03 | -0.31 | -0.32 | -0.10 | -0.03 | -0.28 | -0.36 | -0.43 | 1.00 |

width and neck width (0.70). Result also revealed that horn space was the least correlated with all other traits. This implies that increase in any of the correlated traits will lead to corresponding increase in the other traits. There was no significant correlation between skull length and face width ($p > 0.05$). The correlation between skull length and neck length was not significant ($p > 0.05$), this implies that there is significant no relationship between these traits. However, horn space was negatively correlated with all other traits except skull width with no significant correlation ($p > 0.05$). Similarly, Karimi *et al.* (2011) reported a strong negative correlation between the cephalic index and the length and width of the skull in Mehraban sheep.

The percentage of total variance that best explained the data was summarized in the first three components. The three PCAs jointly explained 76.2 percent of the total variance formed by the traits. PC 1 axis could be linked to variables related to length and diameter amplitudes; PC2 gave a major relevance to skull, face and neck lengths of the sheep; whereas PC 3 axis gave a major relevance to skull length, head depth and neck length. Yunusa *et al*. (2013) reported that measurements that were associated with cranial development (head length and head width) tend to load on first component for Uda and Balami, which suggested them as classification traits for these sheep. Similar results were reported by Salako (2006) on immature Uda sheep, where all parameters considered but skull width, rump length and rump width loaded on the first component. The PC plot of scores for the sheep breeds is presented in Figure 2. Some breeds (Balami, Yankasa and Uda) overlapped, although the cephalic classification suggested different groupings.

The stepwise discriminant analysis indicated that four (skull width, head width, head length and head depth) out of twelve cephalic traits were effective at detecting the differences among the four breeds of sheep (Table 4). Head length was the most discriminating variable followed by head width, head depth and skull width. These variables were included in the final model as they were more informative.

The canonical (CAN) discriminant analysis identified three statistically significant ($p < 0.001$) canonical variables that accounted for 83.04, 13.54 and 3.42 percent of the total variation (Table 5). CAN 1 was dominated by head width and skull width. Skull width, head length and head width were highly correlated with CAN 2. Skull width and head width were highly correlated with CAN 3. In a study, conducted on Pyrean cattle by Parés *et al*. (2012), it was reported that variables that mostly contributed to the discrimination between breeds were face width and head depth.

The Mahalanobis test established significant differences among the breeds of sheep. The distance between Balami and WAD was the longest while the shortest distance was

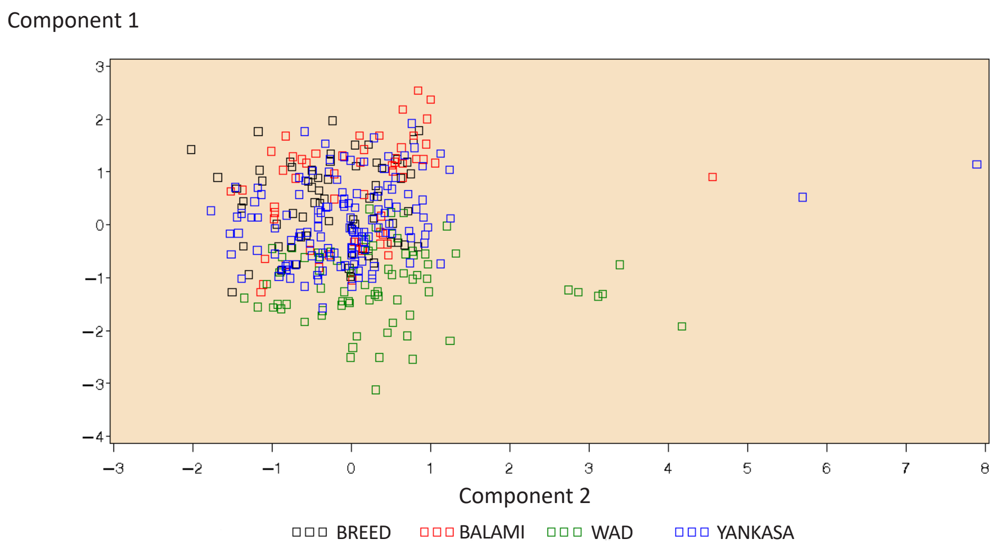



**Figure 2. Principal component plot of scores for morphometric data of four breeds of sheep**

**Table 4. Summary of stepwise selection of traits**

| Variables entered | Partial $R^2$ | F – value | Pr > F | Wilks' Lambda | Pr < Lambda | Average squared canonical correlation | Pr < ASCC |
|---|---|---|---|---|---|---|---|
| Skull width | 0.520 | 24.87 | <0.0001 | 0.730 | <0.0001 | 0.270 | <0.0001 |
| Head width | 0.717 | 71.00 | <0.0001 | 0.486 | <0.0001 | 0.513 | <0.0001 |
| Head length | 0.784 | 107.25 | <0.0001 | 0.385 | <0.0001 | 0.615 | <0.0001 |
| Head depth | 0.560 | 30.70 | <0.0001 | 0.686 | <0.0001 | 0.314 | <0.0001 |

**Table 5. Canonical correspondence analysis of breeds of sheep**

| Traits (cm) | Discriminant variates | | |
|---|---|---|---|
| | CAN 1 | CAN 2 | CAN 3 |
| Skull width | 0.630 | 1.000 | 1.000 |
| Head width | 1.000 | 0.717 | 1.000 |
| Head length | 0.305 | 0.784 | 0.046 |
| Head depth | 0.408 | 0.360 | 0.033 |
| Adjusted canonical correlation | 0.512 | 0.713 | 0.782 |
| Eigenvalue | 0.370 | 1.057 | 1.596 |
| Variance accounted for (%) | 83.04 | 13.54 | 3.42 |
| Cumulative variance (%) | 83.04 | 86.46 | 100 |

**Table 6. Mahalanobis distances between breeds of sheep**

| Breeds | Balami | Yankasa | Uda | WAD |
|---|---|---|---|---|
| Balami | 0 | | | |
| Yankasa | 1.19 | 0 | | |
| Uda | 0.91 | 0.50 | 0 | |
| WAD | 1.69 | 1.61 | 0.91 | 0 |

recorded between Uda and Yankasa. The distance between Uda and Balami was also close. There was a clear separation among the breeds of sheep. The significant differences in the distance indicated that differences among sheep breed populations are important for classification (Yakubu *et al.*, 2012). Separate grouping is an indication that different breeds of these sheep possess different cephalic qualities and characteristics. These differences might be attributed mainly to geographical origin of breeds. According to Mulyono *et al*. (2009), differences of origin distinguish phenotypic response based on potential for additive genes controlling body measurements.

## CONCLUSION

There are differences in the cephalic traits of Nigerian indigenous breeds of sheep. On the basis of the cephalic index, the sheep breeds are brachycephalic (short-headed), except Uda, which is mesocephalic (medium-headed). Traits such as head width, skull width, head length and head depth mostly contributed to the discriminating variables among the breeds of sheep.

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# EFFECT OF COMBINATIVE DIETARY ZINC SUPPLEMENTATION AND PLANT THYME EXTRACT ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN THE DIET FOR GROWING RABBITS

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## ABSTRACT

The aim of this study was to evaluate the effect of combinative orally administered zinc from organic source (Bioplex-Zn) and 0.1 % of *Thymus vulgaris* L. plant extract to feed mixtures for rabbits, applied into water during the growing period, on the selected parameters of nutrient digestibility. Feeding trial was conducted on 96 post-weaned rabbits, meat line P91 and M91 (aged 35 days, both sexes). They were randomly divided into 4 experimental groups (EG) with 24 animals in each group. The rabbits in the EG1 and EG3 were fed the same commercially available diet with no zinc additive. The feed mixture in the EG2 and EG4 was additionally administered at a dose of 33.3 g Bioplex-Zn per 100 kg each (this is addition of 50 mg $Zn.kg^{-1}$ feed mixture). The rabbits in the group EG3 and EG4 received 0.1 % of *Thymus vulgaris* L. plant extract applied into the water. Supplementation of the rabbit diets with zinc was done in order to determine its effects on live weight and consumption of feed per unit of live weight growth. Higher proportion of Zn in the mixture increased digestibility coefficients of fat, NDF, starch, organic matter, Mg, Na, K, Fe, Zn, Cu, Mn ($P < 0.05$) compared to EG1 with no zinc additive. Natural product of *Thymus vulgaris* L. extract consisted of flavonoids (54 %), diterpene (27 %) and phenolic acids (19 %), which are known to have an antimicrobial and antioxidant activity. The beneficial effect of combination of the *Thymus vulgaris* L. plant extract and zinc additive administration was manifested by the anti-coccidian action in rabbits. These results indicate that the addition of 50 mg $Zn.kg^{-1}$ of a mixture is sufficient to achieve optimal health, performance, increased feed conversion rate ($p < 0.05$) and average daily weight gain in broiler rabbits.

**Key words:** rabbits; Bioplex-zinc; plant extract; nutrient digestibility

## INTRODUCTION

Minerals have a special role in ensuring efficient growth, reproduction and immunocompetence in animals. The content of trace elements in the feed is governed by the nature of geochemical soil and plant species (Suttle, 2010). Zinc deficiency in animals is characterized by decreased feed intake, decreased growth (Ensminger *et al.*, 1990), low circulating levels of growth hormone (GH) and insulin-like growth factor-I and decreased hepatic production of insulin-like growth factor-I, GH receptor and GH-binding protein. Zinc positively affects feed utilization through participating in the metabolism of carbohydtrates, lipids and proteins (McDonald, 2000). Minerals activate enzymes which are essential cofactors of metabolic reactions and function as carriers of proteins, regulate digestion,

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respiration, water balance, muscle response and the neural transmissions, influence and maintain skeletal strength, balance pH and even mental balance, protect against disease, act as antagonists or synergists of other elements and play a vital role in the resistance, adaptation and evolution of new races and lines (Anke *et al.*, 1993; Szentmihalyi *et al.*, 1985; Haenlein, 1987; Underwood and Suttle, 1999).

Because many natural food ingredients show marginal Zn deficiency, this micronutrient is commonly supplemented to diets for livestock and poultry. Regardless of the fact that certain microelements are present in food in sufficient quantities, subclinical or clinical symptoms of their deficiency appear. This can be caused by their different and changeable availability, or the microelements are present in form that cannot be used. Obtained results showed that the presence of certain substances in food (phytic acid and oxalic acid), as well as interaction with other nutrients in the digestive tract, influences resorption mechanisms. Resorption of microelements is not dependent only on their content in food, but also on the animals' age, electrochemical reactions in the intestine and on the microelement form. Mineral salts, such as oxides, carbonates, chlorides and sulphates are most frequently used. Today, in addition to inorganic forms of minerals, the use of so-called „chelate" forms, i.e. organically bonded microelements, is becoming more frequent. Enteric diseases frequently occur in rabbits around the weaning period, leading to an extensive use of antibiotics. Therefore, new and safe antimicrobial agents are searched for to prevent and/or overcome infections. Prevention and treatment of clostridial infections by natural substances or phyto-additives are important because of animal mortality and economic losses (Chrastinová *et al.*, 2010; Chrastinová *et al.*, 2016; Marcin *et al.*, 2006). Natural products of plant origin (in this case the extract of *Thymus vulgaris* L.) are still a major part of traditional medicine. However, their effect on animal and human organisms is still not clear. Essential oils have recently emerged as alternatives to antibiotics in animal production (Simonová *et al.*, 2006; Takáčová *et al.*, 2012; Szabóova *et al.*, 2008).

The aim of this study was to examine the effects of combinative orally administered zinc from organic source. The effect of Bioplex-Zn and *Thymus vulgaris* L. plant extract at 0.1 % applied into drinking water on growth performance and selected parameters of nutrient digestibility in rabbits was the main goal of this study.

## MATERIAL AND METHODS

A total of 96 weaned rabbits (35$^{th}$ day of age, both sexes, meat line P91 and M91) were divided into 4 experimental groups (EG) with 24 animals in each group. The rabbits were kept in standard cages (0.61 m x 0.34 m x 0.33 m), two animals per cage. The cages allowed faeces separation. A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Temperature and humidity were recorded continuously by a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed air temperature in the building to be maintained within 24 ± 4 ˚C during the experiment. Relative humidity was 70 ± 5 %.

### Experimental procedures

The rabbits in the EG1 and EG3 groups were fed the same commercially available diet without zinc additive. The animals were fed complete pelleted feed (pellets of 3.5 mm in diameter) *ad libitum* and had free access to water.

The experimental diets were balanced for nutrient content and energy value (difference was in the content of zinc, Table 1). The contents of organic matter were not different. The study was carried out at the National Agricultural and Food Centre – Research Institute for Animal Production, Nitra, Slovak Republic in July and August 2017.

The diets were composed of 36 % dehydrated lucerne meal, 5.5 % extracted sunflower meal, 5.5 % extracted rapeseed meal, 9 % wheat bran, oats 13 %, malt sprouts 15 %, DDGS (dried distillers grains with soluble) 5 %, sodium chloride, mineral and vitamin mixture, barley 8 %, limestone 1 %. To the feed mixture in the EG2 and EG4, Bioplex-Zn (product of Alltech, USA) was additionally administered at a dose of 33.3 g per each 100 kg (this is addition of 50 mg $Zn.kg^{-1}$ into feed mixture). The rabbits in EG3 and EG4 groups received *Thymus vulgaris* L. (0.1 % of plant extract) applied into drinking water. Natural products of plant origin (in this case the extract of *Thymus vulgaris* L.) are still a major

**Table 1. Chemical composition of the experimental diets for growing rabbits**

| Nutrients in dry matter (g.kg$^{-1}$) | Basal diet | Basal diet supplemented with Bioplex Zinc |
|---|---|---|
| Crude protein | 172.54 | 172.55 |
| Crude fibre | 166.73 | 169.07 |
| Fat | 41.10 | 41.17 |
| N-Free Extract | 536.68 | 534.62 |
| Starch | 183.29 | 182.19 |
| Organic matter | 917.05 | 917.41 |
| ADF | 209.46 | 214.13 |
| NDF | 334.36 | 346.04 |
| Hemicellulose | 124.90 | 131.91 |
| Cellulose | 169.75 | 167.22 |
| Ash | 82.95 | 82.59 |
| Calcium | 6.52 | 6.64 |
| Phosphorus | 6.34 | 6.53 |
| Iron, mg. kg$^{-1}$ | 507.30 | 517.49 |
| Zinc mg. kg$^{-1}$ | 106.56 | 161.53 |
| Copper, mg. kg$^{-1}$ | 18.24 | 19.05 |
| Manganese, mg. kg$^{-1}$ | 133.66 | 125.38 |
| ME (MJ.kg$^{-1}$) | 10.99 | 10.84 |

Vitamin mixture provided per kg of the diet: Vit. A 1500000 IU; Vit. $D_3$ 125000 IU; Vit. E 5000 mg; Vit. $B_1$ 100 mg; Vit. $B_2$ 500 mg; Vit. $B_6$ 200 mg; Vit. $B_{12}$ 0.01 mg; Vit. K3 0.5 mg; biotin 10 mg; folic acid 25 mg; nicotinic acid 4000 mg; choline chloride 100000 mg; ADF - Acidodetergent fibre; NDF - Neutraldetergent fibre; ME - Metabolisable energy

part of traditional medicine. The extract of *Thymus vulgaris* L. contains especially flavonoids (54 %), diterpene (27 %) and phenolic acids (19 %), which are known to be antimicrobials and antioxidants.

All care and experimental procedures involving animals kept the guidelines stated in the Guide for the Care and Use of Laboratory Animals (1996), and the trials were accepted by the Ethical Commission at the Institute of Animal Physiology in Košice and by the Slovak Veterinary and Food Administration.

Body weight and feed consumption were measured every week during the experiment. Mortality and morbidity were also recorded in the groups daily, over the entire period of the experiment. The fattening experiment lasted for 42 days.

***In vivo* digestibility trial**

The digestibility test using the balance method was measured according to E.G.R.A.N. (2001). This method was developed within the European group on Rabbit Nutrition. Between 70-74 days of age, 4 rabbits (males, 2250 ± 100 g live body weight) from each group were housed individually in metabolic cages. The adaptation period for this diet was 28 days. The faeces were collected individually during 4 consecutive days according to the European reference method for rabbit digestion trials (Perez *et al*., 1995). Sampling of faeces was realized every 2 hours. Faeces were collected in bags during the daytime. Every day, in the morning, faeces were mixed with a handheld mixer, the average samples were pre-dried (at 60 °C for 36 h in a dryer) and grinded (1 mm screen) with laboratory grinder for chemical analysis. Chemical analyses were conducted according to AOAC (2000) for dry mater (DM), crude protein (CP), crude fibre (CF), crude fat, nitrogen free extract, ash and organic matter. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed sequentially (Van Soest *et al*., 1991) with a thermostable amylase pre-treatment. Starch was determined by polarimetric method on Polarimeter ADP 220 (Bellingham & Stanley Ltd., UK). For macro- and microelement analysis, samples were ashed at 550 °C, the ash was dissolved in 10 ml of HCl (1:3) and minerals were determined by the atomic absorption spectrometry (AAS) method, phosphorus content was determined by molybdovanadate reagent on Camspec M501. Mineralized samples were analysed for Ca, P, Mg, Na, K, Fe, Zn, Cu and Mn content. For mineral content determination, the spectrometer AAS iCE 3000 (Thermo, UK) was used.

Contents of mineral nutrients in feeds and faeces were determined in graphite cuvette through electrothermal atomization. Content of Ca was estimated at the wave length of 422.7 nm, Mg at 285.2 nm, Na at 589.0 nm, K at 766.5 nm, Fe at 248.3 nm, Zn at 213.9 nm, Cu at 324.8 nm, Mn at 279.5 nm and content of P at 410.0 nm as phosphomolybdenic yellow (Official Journal L 206, 29/07/1978, p.0043-0055). All the analyses were performed in triplicates. The nutrient digestibility was calculated according to the following formula:

Digestibility (%) = (Intake – Faecal Excretion) / Intake x 100

**The occurrence of *Eimeria* sp. oocysts in the faeces**

*Eimeria* sp. oocysts were enumerated in the faecal samples microscopically at the start of the experiment (day 0-1) and on the 42nd day of the experiment and expressed in counts of oocysts per 1 g of faeces (OPG). The samples were evaluated by the quantitative flotation technique - modified McMaster method (Ministry of Agriculture, Fisheries and Food, Manual of veterinary parasitological laboratory techniques, 1986).

Data were statistically evaluated using the SAS/STAT 1999-2001 statistical software (version 82). The results were quoted as the mean ± standard deviation (SD); statistical evaluation of the results was performed by a one-way ANOVA and Tukey's test. The significance level was set at $p < 0.05$.

## RESULTS AND DISCUSSION

The chemical composition of feeds (Table 1) gave an indication of the potential nutrient supply, but determination of digestibility provides an estimate of the nutrients available to the animal. Experimental animals did not show any health problems during the whole study period. Feeding was performed using balanced mixed feed according to feeding standards. The ability to discriminate among diets varying in Zn concentration has been described for several animal species and nutrients. Zn is important for the organism and has influence on the feed intake; however, there is a lack of data whether rabbits can discriminate among diets differing in mineral content to avoid Zn-deficiency. Enteric diseases frequently occur in rabbits around the weaning period, leading to an extensive use of antibiotics. Therefore, new and safe antimicrobial agents are searched for to prevent and / or overcome infections. Prevention and treatment of clostridial infections by natural substances or phyto-additives are important because of animal mortality and economic losses. The duration of the fattening experiment was 42 days. Body weight and feed consumption of rabbits were measured every week during the experiment. Among the experimental groups significant differences in feed intake, body weight and carcass value in fattening experiment (Table 2). Dietary supplementation of zinc to rabbits was carried out to determine its effects on live weight gain and consumption of feed per unit of live weight gain. The average daily weight gain was higher in all experimental groups: EG2 (40.91 g), EG3 (41.05 g), EG4 (41.51 g) comparing to the EG1 (basal diet without zinc additive - 38.55 g). Coccidiosis in rabbit breeds presents a serious health and economic problem (Vasilková *et al*., 2007; Pakandl, 2009). Between 7 and 8 weeks of age, mortality was registered in the group EG3, and only 2 rabbits were died by diarrhoea at 49 and 56 d of age. The overall mortality at the end of growing-fattening phase was 1 (EG1) vs. 2 rabbits (EG3), what could be caused by intake of the basal diet without zinc additive (Bioplex-Zn) and higher intake of starch in the diet (18.3 %) and 0.1 % concentration of thyme plant extract applied into water. Basal diet with supplementary Bioplex-Zn combined with 0.1 % thyme plant extract in water in a preventive way to improve intestinal health and reduce the need for non-sustainable practices. However, the general consensus of opinion is that overload of rapidly fermentable carbohydrates in the large intestine increases the likelihood of digestive disorders, at least in susceptible, recently weaned rabbits due to problems with enterotoxemia induced by *Clostridia* (Cheeke, 1987; De Blas *et al*., 1995; De Blas and Gidenne, 1998).

**Table 2. Effects of feed supplementation with zinc (additive Bioplex-Zn) and combinative administration with 0.1 % plant extract *Thymus vulgaris* on performance data of the fattening rabbits (mean ± SD)**

| Parameter (n = 24) | EG1 | EG2 | EG3 | EG4 |
|---|---|---|---|---|
| Initial weight (g) | 1161 ± 119 | 1114 ± 136 | 1071 ± 124 | 1114 ± 149 |
| Final weight (g) | 2779 ± 300 | 2830 ± 268 | 2796 ± 208 | 2859 ± 344 |
| Daily weight gain g.day$^{-1}$ | 38.55 ± 6.61 | 40.91 ± 7.50 | 41.05 ± 4.05 | 41.52 ± 5.55 |
| Feed intake g.day$^{-1}$ | 134 | 124$^{A}$ | 131 | 134 |
| Feed conversion ratio in g.g$^{-1}$ | 3.51 | 3.04$^{A}$ | 3.22$^{a}$ | 3.23$^{a}$ |
| Contrast to the basal diet (%) | | +13.39$^{A}$ | +9.17$^{a}$ | +7.98$^{a}$ |
| Mortality (n) | 1 | 0 | 2 | 0 |
| Carcass yield (%) | 55.65 ± 0.71 | 55.88 ± 2.23 | 54.92 ± 0.46 | 57.17 ± 0.71 |

EG1 - Basal diet without supplementary Zinc; EG2- Basal diet with supplementary with Bioplex- Zn at ; EG3- Basal diet without supplementary Zinc and with thyme plant extract 0.1 % in water; EG4- Basal diet with supplementary Bioplex-Zn combined with thyme plant extract 0.1 % in water; $^{a}$ = p ≤ 0.05; $^{A}$ = Significant difference at p ≤ 0.01

The chemical composition of feeds give an indication of the potential nutrient supply, but determination of digestibility provides an estimate of the nutrients available to the animal. The data of nutrient digestibility are summarized in Table 3.

Higher proportion of Zn in the mixture had influence on the increase of fat, NDF, starch, organic matter, Mg, Na, K, Fe, Zn, Cu, Mn digestibility coefficients (P < 0.05) compared with EG1 with no zinc additive. Lebas (1973; 1990) in the NZW rabbit breed determined 4 % better coefficients of digestibility for dry matter and organic matter than in the Californian rabbits; these coefficients of digestibility correspond to our results. Several authors (Rafay *et al*., 2009; Maertens, 1992; Lebas, 1989) specified these values of digestibility of basic nutrients as follows: crude protein - 75 %, crude fat - 65 % and crude fibre - 20 %. Digestibility coefficients of crude protein and crude fibre in our experiment were higher than published by Tůmová *et al*. (2004) and Ondruška *et al*. (2011). These authors carried out a balance experiment on meat rabbits and their digestibility values of presented nutrients were 77.2 % vs. 72.6 % (crude protein) and 10.7 % vs. 15.7 % (crude fibre). The effect of dietary zinc supplementation with a dose of 33.3 g Bioplex-Zn (organic substances) per each 100 kg on nutrient digestibility is presented in Table 3. The resulting digestibility coefficients for crude protein were in the range from 77.93 % to 82.48 %, which was similar to the data of Battaglini and Grandi (1988). The values of crude fibre digestibility (23.48 % - 29.60 %) and crude fat (83.92 % - 90.32 %) were higher in comparison to Bielański and Need Świadek (1993).

Studies worldwide have shown that in some countries, regarding the presence of certain minerals in the soil, there are different variations in terms of their deficit and surplus (Anke *et al*. 1988; 1993). Minerals have a special role in ensuring efficient growth and immunocompetence in animals. The content of trace elements in the feed is governed by the nature of geochemical soil and plant species. Addition of 3 % microalgae spirulina (*Arthrospira platensis*) and/or 2.5 % thyme (*Thymus vulgaris*) leaves in growing (7 weeks) dwarf rabbits did not prevent the animals from getting sick or dying during the 14 weeks of the study period, and there were no substantial effects on the growth performance and energy or nutrient digestibility (Dalle Zotte *et al*. 2013). Absorption of zinc occurs throughout the small intestine and usually in ranges from 5 % to 40 % for intake. Zinc absorption is reduced whenever diets are high in calcium or phytate. The values of zinc digestibility (1.52 % - 16.96 %) were lower in comparison to other herbivore species, e.g. goats. Similar relationships between the minerals are also observed in Cu deficiency, but they are less pronounced, what means that the absorption of Cu increases with Zn deficiency, but that the converse is not true (Memiši *et al*., 2014).

Different relationships between mineral absorption were observed with the goats that received bentonite, which increased the absorption

**Table 3. Effects of feed supplementation with zinc (additive Bioplex-Zn) and combinative administration with 0.1 % plant extract *Thymus vulgaris* on nutrient digestibility in % (mean ± SD)**

| Item (n=4) | EG1 | EG2 | EG3 | EG4 | t-test |
|---|---|---|---|---|---|
| Crude protein | 78.24 ± 1.04 | 82.48 ± 1.50 | 78.71 ± 0.84 | 77.93 ± 1.83 | a:b$^{+}$;b:c$^{++}$, d$^{+}$ |
| Fat | 83.92 ± 2.55 | 89.75 ± 0.64 | 84.82 ± 2.23 | 90.32 ± 0.69 | a:bd$^{+}$; c:d$^{++}$ |
| Crude fibre | 25.81 ± 0.62 | 29.60 ± 2.51 | 23.48 ± 0.81 | 25.77 ± 3.83 | a:bc$^{+}$; b:c$^{+}$ |
| ADF | 29.42 ± 3.43 | 37.74 ± 4.36 | 28.55 ± 1.88 | 28.71 ± 0.83 | a:bc$^{+}$; b:c$^{+}$d$^{+}$ |
| NDF | 34.40 ± 2.65 | 41.10 ± 3.91 | 36.23 ± 3.57 | 34.82 ± 1.35 | a:b$^{+}$, c, d |
| Starch | 93.97 ± 0.93 | 94.83 ± 0.96 | 96.84 ± 0.17 | 97.09 ± 0.08 | a:cd$^{++}$; b:cd$^{+}$ |
| N-Free Extract | 73.31 ± 1.88 | 79.41 ± 1.64 | 75.01 ± 1.10 | 74.41 ± 1.59 | b:cd$^{+}$ |
| Hemicellulose | 39.16 ± 5.62 | 43.45 ± 9.39 | 49.79 ± 7.15 | 31.78 ± 4.09 | a:d$^{++}$; b:d$^{+}$ |
| Cellulose | 32.41 ± 3.01 | 43.98 ± 9.07 | 24.15 ± 7.22 | 28.33 ± 1.17 | a:bd$^{+}$; b:cd$^{+}$ |
| Ash | 52.75 ± 2.93 | 62.43 ± 1.16 | 51.96 ± 1.08 | 51.83 ± 2.13 | b:a$^{+}$c$^{++}$d$^{+}$ |
| Organic matter | 65.90 ± 0.36 | 70.79 ± 1.80 | 65.76 ± 0.75 | 66.23 ± 1.00 | b:acd$^{+}$ |
| Calcium (Ca) | 59.41 ± 3.70 | 65.63 ± 4.75 | 44.20 ± 6.73 | 47.48 ± 3.66 | a:bcd$^{+}$; b:cd$^{+}$ |
| Phosphorus (P) | 40.48 ± 6.83 | 49.81 ± 5.75 | 37.40 ± 4.82 | 39.05 ± 2.08 | a:b$^{+}$; b:acd$^{+}$ |
| Magnesium (Mg) | 47.37 ± 6.38 | 53.42 ± 4.64 | 46.31 ± 3.17 | 50.83 ± 3.38 | b:c$^{+}$ |
| Sodium (Na) | 80.60 ± 5.02 | 84.18 ± 4.23 | 70.66 ± 13.49 | 82.42 ± 2.59 | b:d$^{+}$ |
| Potassium (K) | 84.15 ± 2.12 | 87.13 ± 1.27 | 85.29 ± 0.84 | 87.07 ± 4.18 | a:bd$^{+}$; b:c$^{+}$ |
| Iron (Fe) | 26.09 ± 4.12 | 37.18 ± 2.51 | 35.05 ± 3.81 | 25.93 ± 3.01 | a:bc$^{+}$; c:d$^{++}$ |
| Zinc (Zn) | 1.52 ± 0.86 | 2.87 ± 2.04 | 4.23 ± 2.13 | 16.96 ± 2.28 | a:c$^{+}$, d$^{++}$ |
| Copper (Cu) | 13.88 ± 4.11 | 31.84 ± 4.98 | 18.48 ± 2.65 | 20.69 ± 6.87 | a:b$^{+}$; b:cd$^{+}$ |
| Manganese (Mn) | 19.80 ± 2.06 | 21.23 ± 1.50 | 20.02 ± 1.64 | 19.55 ± 1.29 | b:c, d$^{+}$ |

$^{+}$ = $p \leq 0.05$; $^{++}$ = $p \leq 0.01$ Significant difference

of Fe but has decreased absorption of Cu and Zn (Schwarz and Werner, 1987; Siegert *et al*., 1986; Stefanidou *et al*., 2006). However, by addition of thyme plant extract at the concentration of 0.1 %, a significant decrease of digestibility coefficients of ADF, cellulose, Ca, P, Mg ($p \leq 0.05$) was recorded in EG3 compared to values in the EG1. It appears that the antagonistic effect of thyme plant also appeared without zinc additive in mixture at digestibility of crude fibre when a significant decrease in the EG3 (23.48 %) was recorded compared to the EG1 (25.81%). The key trace elements involved in animal feed are zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) (Andrieu *et al*., 2009). Zinc is the second most abundant trace element in mammals and birds, and forms a structural component of over 300 enzymes, where it may also be key to catalytic and regulatory activity. It plays and important role in anti-oxidant defence as an integral part of SOD. Manganese plays an important role in the body metabolism as an essential part of several enzymes. These elements have both negative and positive relationships within intermediary metabolism. Positive chemical bonds (Mn - Zn) are lesser than the antagonistic bonds (Cu - Zn). Absorption of Zn and Mn is inhibited also by Ca (Shajhalal *et al*., 2008; Wilde, 2006). Copper is a component of a several range of physiologically important metalloenzymes, lipid metabolism, anti-oxidant defence as an integral part of the essential enzyme - superoxide dismutase (SOD), immune function and carbohydrate metabolism (Wapnir, 1998). Occurrence of secondary Zn deficiency in animals with excessive content of Ca, P and phytic acid in feed rations is frequent. Deficiency of Mn in soil and plants is due to excessive liming of the soil.

The anti-coccidian effect was recorded in experimental groups EG2, EG3 and EG4 on the 42$^{nd}$ day compared to EG1 (Table 4). In EG1 290 OPG were counted, whereas in EG2 20 OPG were found, and in the EG3 - 40 OPG. The beneficial effect of combination of the *Thymus vulgaris* L. plant extract and zinc additive administration manifested as the anti-coccidian effect in rabbits. By improving

**Table 4. Comparison of the counts *Eimeria* sp. Oocysts in faeces of rabbits (expressed in counts of oocysts per 1 g (OPG) of faeces evaluated by the quantitative flotation technique)**

| Characteristic (n=5) | EG1 | EG2 | EG3 | EG4 |
|---|---|---|---|---|
| OPG | 290 | $20^{++}$ | $40^{++}$ | Neg.$^{++}$ |

EG1- Basal diet without supplementary Zinc; In the EG2 and EG4 the feed mixture was additionally administered as follows a dose of 33.3 g Bioplex-Zn, each per 100 kg. The rabbits in the group EG3 and EG4 received dose 0.1 % plant extract *Thymus vulgaris* were applied into supply water. $^{++}P < 0.01$

intestinal health, possibly through stimulation of the processes of nonspecific immunity, this feed additive may result in a lower exposure of animals to microbial toxins or other undesired metabolites (Plachá *et al*., 2013).

## CONCLUSION

The experimental results show that dietary supplementation of zinc to rabbits was carried out to determine its effects on growth of live weight and consumption of feed per unit of growth weight. No significant differences were observed in mean percentage of the carcass gain in tested variants. The beneficial effect of combination of the *Thymus vulgaris* plant extract and zinc additive administration was manifested by the anti-coccidian action in rabbits. Higher proportion of Zn in the mixture had influence on the increase of digestibility coefficients of fat, NDF, starch, organic matter, Mg, Na, K, Fe, Zn, Cu and Mn ($P < 0.05$) compared to EG1 with no zinc additive. On the basis of obtained results we can conclude that the total addition of 50 mg Zn per kg of mixture is sufficient to achieve optimal health and performance of the rabbits and increased feed conversion rate and average daily weight gain ($p < 0.05$). Consequently, this additive can enhance immune defence in critical situations, increase the intestinal availability of essential nutrients for absorption, and improve the growth of animals.

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# THE MIXED SILAGE OF MAIZE AND DENDROMASS AS A POTENTIAL FEED FOR WILD RUMINANTS

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## ABSTRACT

The aim of this study was to evaluate the mixed silage of maize and dendromass – twigs of broadleaf and conifer trees – as palatable feed for wild ruminants, rich in energy and of sufficient structural crude fibre content. In the experiment, we compared nutrient content of the M1 feed mixture (70 % maize + 30 % Dendro 1) and the M2 feed mixture (70 % maize + 30 % Dendro 2). Two variants of dendromass were used in the feed mixtures: Dendro 1 (75 % oak twigs, 25 % spruce twigs) and Dendro 2 (50 % oak twigs, 50 % spruce twigs). Spruce and oak twigs had higher dry matter content than maize. This was reflected also in the ensilaged mixtures made by combining maize and dendromass, in which the dry matter content was higher by 34.16 and 58.31 g per kilogram of fresh feed than in maize due to the addition of dendromass. Concentrations of crude protein and saccharides matched the character of the feed and were lower in the mixture of maize and dendromass compared to maize. As the crude fibre content in dendromass was 367.25 and 345.65 g.kg$^{-1}$ of dry matter and the entire fibre complex content was significantly higher than in maize, a significant increase in the content of all components of the fibre complex was determined. Fermentation process in the maize silage was more intense compared to mixture silages of maize and dendromass. It can be recommended to use the silage production of such mixtures to wild ruminant keepers in winter and at higher crude protein content also in summer. This method of biological protection of forests provides the necessary nutrients to the wild ruminants. Utilization of dendromass as feed is also considered as a partial recycling of logging waste.

**Key words:** maize; dendromass; silage; feed; wild ruminants

## INTRODUCTION

Damages caused by wild ruminants on forestry and agriculture in Slovakia are intensive. The population numbers of cloven-hoofed game in Slovakia reached their historic peaks. According to statistical data, the spring stock of main cloven-hoofed game species (before calving) in year 2015 was: roe deer – 106 906, red deer – 65 126, fallow deer – 15 807, mouflon – 13 350, and boar – 41 591 (Hunter Statistical Yearbook, 2015).

The Slovak legislative orders to hunters to feed game in such manner as to not cause damage to health of the animals (Directive NR SR no. 274/2009, 2009).

Supplementary feeding of wild ruminants is one of the possible methods to reduce damages to the forests caused by wild ruminants. When the nutritional requirements of the animals are covered by supplementary feeding, the need to cover these requirements by forest tree browsing is decreased. Several authors (Missbach, 1975; Ueckermann *et al.*, 1977; Pheiffer and Hartfiel, 1984; Putman and Staines, 2004; Rajský *et al.*, 2008) demonstrated that proper supplementary feeding markedly decreased the rate of the forest

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tree browsing. Importance of proper supplementary feeding to decrease bark browsing by deer in forests is supported in the reports of Reimoser (2004) and Konôpka and Hell (2004). At present, the advantages and disadvantages of supplementary feeding of wild game are being discussed, for example, by Katona *et al*. (2014) and Milner *et al*. (2014).

The papers of Webster *et al*. (2001), Jeon *et al*. (2003), Galló *et al*. (2017) and others describe the utilization of ensilaged feeds in wild ruminant nutrition. Maize is an easy to ensilage roughage, and high-quality maize silage is a good source of structural as well as non-structural carbohydrates (Vršková and Bencová, 2011). Quality of maize silage is well-investigated (Bíro *et al*., 2016; Tyrolová *et al*., 2016; Rajčáková *et al*., 2013). However, it differs from the traditional food sources for wild ruminants by its nutritional characteristics. Twigs of broadleaves and conifer tree species represent natural food for free-living ruminants. The ensilaged mixture of maize and twigs from forest tree species, as a potential feed for ruminants, will enrich the maize silage with structural fibre and other nutrients by addition of this natural food. This is a prerequisite for attractive feed rich in energy and with adequate content of structural crude fibre. Questions of nutrition and impact on the environment are interesting in connection with high population density of free-living ruminants. There is little relevant information on ensilaging mixtures of maize and dendromass.

The aim of this study was to evaluate the possibility of ensilaging a mixture of maize and dendromass – twigs of broadleaf and conifer trees, and to monitor the influence of the fermentation process on the nutritional value of the produced ensilaged feed mixtures.

## MATERIAL AND METHODS

In the first part of the experiment, fresh mixtures were prepared by combining whole maize plants and 50-70 cm long twigs of oak and spruce. Maize was harvested at the stage of wax ripeness and it represented 70 % of the mixture (fresh matter content). The remaining 30 % (fresh matter content) was dendromass – twigs of oak and spruce. Twigs harvesting was matched to the date of maize harvesting, in first week of September. Maize and twigs were cut to a size below 3 cm and the mixtures were homogenized.

Two variants of dendromass were used in the mixtures: Dendro 1 (75 % oak twigs, 25 % spruce twigs) and Dendro 2 (50 % oak twigs, 50 % spruce twigs). Two variants of a mixture were prepared: mixture 1 (M1) (70 % maize and 30 % Dendro 1) and mixture 2 (M2) (70 % maize and 30 % Dendro 2).

Samples of maize, Dendro 1 and Dendro 2, as well as samples of the M1 and M2 were analysed. By the organic analysis of all samples the following parameters were determined: dry matter, crude protein, crude fibre, saccharides, ash, fat and starch, according to the Decree of the MP SR no. 2145 /2004-100 (2004); acid detergent and neutral detergent fibre – according to Van Soest *et al*. (1991).

In the second part of the experiment, experimental silages were produced. Maize, M1 and M2 were filled into 1.7-liter glass laboratory silos. No ensilaging additives were used. Each variant of the silage was produced six times. The filled experimental silos were stored in a dark room with stable temperature at 22 ± 1 °C. During the fermentation process, changes in weight were monitored, and based on those, loses of dry matter weight were calculated in percentages. After 180 days from ensilaging the experiment was terminated.

In addition to the basic parameters of organic analysis like in fresh matter in first part of experiment, the following parameters of fermentation process were also determined for the silage samples: pH in the aqueous extract was determined using electrometric method, lactic acid and total volatile fatty acid content was determined by gas chromatography and alcohol content by micro-diffusion method. All chemical analyses were performed according to the Decree of the MP SR no. 2145 /2004-100 (2004).

## RESULTS AND DISCUSSION

In the experiment, the nutrient contents and fermentation processes were compared among maize, dendromass, mixture 1 (70 % maize and 30 % Dendro 1) and mixture 2 (70 % maize and 30 % Dendro 2).

Nutrient content in the maize plants, dendromass and in mixtures composed by combining maize and dendromass before ensilaging is presented in Table 1. Spruce and oak twigs had higher dry matter content. This was reflected also in the feed mixtures of maize and dendromass for ensilaging, in which the dry matter content was determined to be higher by 34.15 and 58.31 g per kilogram of fresh feed, than in maize, due to the added dendromass. Concentrations of crude protein and saccharides corresponded with the character of the feed, and they were lower in the feed mixtures compared to maize. As the crude fibre content in dendromass was 367.25 (Dendro 1) and 345.65 $g.kg^{-1}$ (Dendro 2) of dry matter and the entire fibre complex content was significantly higher than in maize, a significant increase in the content of all components of the fibre complex was revealed. Due to the lower ratio of maize, starch content was significantly lower in the feed mixtures compared to maize. Of the other monitored nutrients, an increase in the concentration of fat and ash in the feed mixtures compared to maize should be mentioned. Based on calculations it was determined that nitrogen-free extract content was lower in the feed mixtures compared to maize.

In the feed mixtures for ensilaging, twigs of oak and spruce were used. Twigs of forest trees are the natural food source for wild ruminants, and their nutritional content is sufficient as maintenance feed for wild ruminants. Wild ruminants prefer in their nutrition the youngest parts of twigs, which also have the highest content of utilizable nutrients. Each species of wild ruminants is adapted to a qualitatively different food source, which allows for utilization of all natural food sources and lowers competition (Hofmann, 1995). Further from the end of the twig the biomass is older and, therefore, also the nutritional value is lower due to the increasing crude fibre content and increasing levels of indigestible components.

Roe deer requires feed with high concentration of nutrients and lower fibre content, therefore, it consumes only 3-5 cm long ends of twigs and occasionally, depending on the thickness of the twig, only buds. In case of thin twigs, for example from a willow or a beech, roe deer can consume twigs up to 7-10 cm long (Rajský *et al*., 2017). In comparison, red deer consumes longer spring shoots, up to 20-25 cm long, therefore, also biomass with higher crude fibre content. Rajský *et al*. (2015) determined high nutritional content of the dry matter in twigs (20-25 cm long) consumed by wild ruminants. The following values of a crude protein content were determined: oak 12.47 %, spruce 12.84 %, willow 15.63 %, maple 13.55 %,

**Table 1. The nutrient content of tested biomass before the production of silage in $g.kg^{-1}$ dry matter**

| Parameters | Maize | M 1 | M 2 | Dendro 1 | Dendro 2 |
|---|---|---|---|---|---|
| Dry matter in $g.kg^{-1}$ FW | 287.32 | 321.48 | 329.01 | 485.98 | 457.69 |
| Organic matter | 952.51 | 951.93 | 946.49 | 947.81 | 940.39 |
| Crude protein | 78.37 | 75.02 | 78.28 | 63.50 | 76.29 |
| Crude fibre | 190.41 | 230.67 | 223.72 | 367.25 | 349.56 |
| ADF | 213.47 | 278.04 | 265.61 | 447.63 | 429.86 |
| NDF | 512.64 | 573.41 | 564.02 | 602.00 | 528.99 |
| Hemicelluloses | 299.17 | 295.37 | 298.41 | 154.37 | 99.13 |
| Nitrogen-free extract | 659.14 | 619.83 | 613.65 | 489.37 | 474.28 |
| Total sugars | 110.25 | 90.45 | 94.44 | 39.78 | 39.82 |
| Reduced sugars | 106.32 | 88.36 | 83.95 | 31.78 | 38.84 |
| Starch | 245.50 | 167.43 | 192.31 | 0.00 | 0.00 |
| Fat | 24.59 | 26.41 | 30.84 | 27.68 | 40.25 |
| Ash | 47.49 | 48.07 | 53.51 | 52.19 | 59.61 |

FW – fresh weight, ADF – acid detergent fibre, NDF – neutral detergent fibre; M 1 – mixture 1: 70 % maize + 30 % Dendro 1; M 2 – mixture 2: 70 % maize + 30 % Dendro 2; Dendro 1 – dendromass mixture in rations of 75 % oak twigs and 25 % spruce twigs; Dendro 2 – dendromass mixture in rations of 50 % oak twigs and 50 % spruce twigs beech 11.71 %, European aspen 17.68 %, elderberry 28.23 %. The authors further determined the fat content in the dry matter of oak (2.32 %), fir (5.21 %), spruce (4.35 %), silver poplar (4.33 %), European aspen (4.05 %) and elderberry (3.48 %). Mineral content was also high in the dry matter of select trees: elderberry - 9.99 %, ash - 8.67 %, oak - 6.48 % and spruce - 3.32 %. End parts of twigs of broadleaf and conifer trees generally provide high nutritional value to wild ruminants.

In the experiment, twigs longer than 20-25 cm were used – 50-70 cm long – therefore also the crude protein content in the dry matter was lower (Dendro 1 - 6.35 %, Dendro 2 - 7.63 %) compared to the abovementioned values. If younger tree parts were used, the crude protein content in the silages would be higher as well.

Fermentation process without a silage additive in the maize silage was more intense compared to ensilaged mixtures M 1 and M 2, which were also made without silage additive (Table 2). This is proven by the lower pH value and higher lactic acid content with the differences being highly significant. High significance of the differences between the experimental silages was determined also in the alcohol content and propionic acid content. Total content of total volatile fatty acids reached similar levels in all silages and no significant differences were determined. The maize silage, evaluated as Class I, was considered as a high quality silage. The addition of dendromass to maize at the ratio of 30 % increased the dry matter content in the ensilaged matter, which influenced also the fermentation. The concentration of fermentation products in M 1 and M 2 was decreased. Despite that, according to the evaluation of the fermentation process, M 1 was evaluated to be of Class I, as high quality silage too and M 2 to be of Class II, as good quality silage (The Decree of the MP SR no. 39/1/2002-100, 2002).

Successful fermentation process was reflected also in the nutritional composition of the resulting silages. Measured values are presented in Table 3. Similarly to the original matter, also in the resulting silages the level of fibre complex and ash was higher in the feed mixtures than in maize silage and the starch content, as well as nitrogen-free extract content, was lower. Determined differences were statistically highly significant. Losses of dry matter during fermentation were higher in all silages, determined to be from 8.14 to 9.98 %. Although the lowest losses were determined for maize silage, there was no statistically significant difference.

Ensilaged mixture 1 with the higher oak ratio and ensilaged mixture 2 with balanced ratios of oak and spruce are utilizable in nutrition of wild ruminants in winter, when their crude protein requirements decrease. Fat content of M 2 was higher due to higher spruce ratio compared to M 1.

**Table 2. Parameters of fermentation process in tested silages**

| Parameters n = 6 | Maize | | M 1 | | M 2 | |
|---|---|---|---|---|---|---|
| | x̄ | SD | x̄ | SD | x̄ | SD |
| pH | $3.74^{a}$ | 0.02 | $4.13^{b}$ | 0.09 | $4.27^{b}$ | 0.06 |
| Acids in $g.kg^{-1}$ dry matter | | | | | | |
| - lactic | $67.05^{a}$ | 1.60 | $45.31^{b}$ | 3.12 | $36.26^{c}$ | 3.21 |
| - acetic | 18.85 | 1.08 | 18.10 | 1.23 | 19.69 | 1.76 |
| - propionic | $0.18^{a}$ | 0.02 | $0.52^{b}$ | 0.14 | $1.18^{c}$ | 0.24 |
| - butyric + isob. | $1.08^{a}$ | 0.32 | $1.34^{ab}$ | 0.38 | $1.69^{b}$ | 0.20 |
| - valeric + isov. | $0.14^{a}$ | 0.02 | $0.23^{a}$ | 0.05 | $0.05^{b}$ | 0.01 |
| - capronic + isoc. | $0.10^{a}$ | 0.01 | $0.03^{b}$ | 0.01 | $0.03^{b}$ | 0.01 |
| Total volatile fatty acids | 20.35 | 1.35 | 20.22 | 1.58 | 22.64 | 2.83 |
| Total acids | $87.40^{a}$ | 1.34 | $65.53^{b}$ | 6.09 | $58.90^{b}$ | 5.85 |
| Alcohol in $g.kg^{-1}$ dry matter | $5.17^{a}$ | 0.33 | $6.26^{b}$ | 0.56 | $7.89^{c}$ | 0.64 |

Different superscripts within a row mean statistical difference ($P \leq 0.01$); $^{ab}$ vs $^{a,b}$ is not different

**Table 3. The nutrient content of tested silages in g.kg$^{-1}$ dry matter**

| Parameters n = 6 | Maize | | M 1 | | M 2 | |
|---|---|---|---|---|---|---|
| | x̄ | SD | x̄ | SD | x̄ | SD |
| Dry matter v g.kg$^{-1}$ FW | 266.60$^{a}$ | 2.69 | 295.71$^{b}$ | 4.38 | 299.75$^{b}$ | 5.09 |
| Dry mater losses in % | 8.14 | 1.03 | 9.23 | 1.36 | 9.98 | 1.58 |
| Organic matter | 953.31$^{a}$ | 0.32 | 948.66$^{b}$ | 1.61 | 946.71$^{b}$ | 2.34 |
| Crude protein | 79.14 | 1.17 | 78.05 | 2.09 | 80.30 | 0.89 |
| Crude fibre | 204.03$^{a}$ | 5.94 | 260.16$^{b}$ | 3.55 | 272.19$^{c}$ | 5.84 |
| ADF | 252.37$^{a}$ | 4.56 | 304.08$^{b}$ | 8.77 | 330.71$^{c}$ | 6.12 |
| NDF | 497.59$^{a}$ | 6.67 | 534.16$^{b}$ | 10.52 | 558.65$^{c}$ | 8.75 |
| Hemicelluloses | 245.22$^{a}$ | 3.13 | 230.08$^{b}$ | 5.94 | 227.94$^{b}$ | 3.27 |
| Nitrogen-free extract | 639.77$^{a}$ | 5.22 | 580.65$^{b}$ | 7.82 | 561.78$^{c}$ | 4.56 |
| Total sugars | 3.93$^{a}$ | 1.26 | 1.46$^{b}$ | 0.01 | 3.82$^{a}$ | 0.55 |
| Reduced sugars | 2.01$^{ab}$ | 1.13 | 1.01$^{a}$ | 0.01 | 2.43$^{b}$ | 0.95 |
| Starch | 313.77$^{a}$ | 4.33 | 231.63$^{b}$ | 11.33 | 203.53$^{b}$ | 23.16 |
| Fat | 30.37 | 1.12 | 29.80 | 0.91 | 32.44 | 0.70 |
| Ash | 46.69$^{a}$ | 0.32 | 51.34$^{b}$ | 1.61 | 53.29$^{c}$ | 2.34 |

FW – fresh weight, ADF – acid detergent fibre, NDF – neutral detergent fibre; M 1 – mixture 1: 70 % maize + 30 % Dendro 1, M 2 – mixture 2: 70 % maize + 30 % Dendro 2; Different superscripts within a row mean statistical difference (P ≤ 0.01); $^{ab}$ vs $^{a,b}$ is not different

This data corresponds with the knowledge that twigs of conifer trees have higher fat content in dry matter compared to broadleaf trees. The starch content in the mixture silages was 231.63 (M 1) and 203.53 (M 2) g.kg$^{-1}$ of dry matter, which provides sufficient energy to wild ruminants. This energy in combination with crude fibre at 260.16 (M 1) and 272.19 (M 2) g.kg$^{-1}$ of dry matter in effective structural form, which predetermines this feed for *ad libitum* intake.

The topic of dendromass silage production was previously discussed by Montes Pérez *et al*. (2015). The authors evaluated ensilaging of *Brosimum alicastrum* and *Leucaena leucocephala* trees for the purposes of feeding white-tailed deer and they determined negative causality between the crude fibre content and silage intake. Sánches *et al*. (2007) obtained good results by feeding dairy goats with a silage containing tree species of *Albizia lebeck* and *Piscidia piscipula*. Hatt and Clauss (2001) utilized *Salix alba* and *Populus Canadensis* dendromass in the Rotterdam ZOO. Mbatha and Bakera (2018) describe the use of dendromass silage in farming of wild ungulates in Southern regions of Africa. They determined good fitness of the animals and, in addition to their nutritional value, the silages served also as a source of water.

One of the factors, which may limit the increase of the dendromass ratio in feed mixtures, is the content of anti-nutrients, such as condensed tannins, alkaloids and terpenes. Nde and Philile (2017) state, that tannin content in feeds has negative effect on ruminant feed intake and digestion. However for farmed wild ruminants, which have limited access to browsing of trees, dendromass silages represent a source of the aforementioned anti-nutrients, which are at certain dozes beneficial to their health (Mbatha and Bakare, 2018). Tannins, for example, have a potential to mitigate iron overload disorder in wild ungulates (Lavin, 2012). Influence of condensed tannins, applied by feeding *Acacia nilotica*, *Eblica officinalis*, *Syzygium cuminii*, *Grewia optiva*, *Mangifera indica* dendromass, on elimination of digestive tract parasite *Haemonchus contortus* in goats in India, is described by Azad *et al*. (2017). Browse is an important dietary item of browsers, thus, insufficient nutrients due to deficient browse supply may result in diseases, abnormalities, under-performance and eventually death (Clauss *et al*., 2008). Rogosic *et al*. (2007) described adaptation of microbes in the gut of a wild ruminants to tannins.

## CONCLUSION

We evaluated the possibility of ensilaging a mixture of oak and spruce twigs and maize under laboratory conditions. The addition of dendromass to maize increased the dry matter content, fibre complex content, fat and ash content in the mixture silage and decreased the starch and total sugar content when compared to maize. Despite this, the fermentation process in the resulting mixture silages was adequate, and it is possible to recommend the silage production of such mixtures to game keepers in winter and at higher crude protein content also in summer, when the female nutritional requirements increase. This method allows to meet the nutritional requirements of the wild ruminants. Also utilization of dendromass for feeding purposes can serve as a method of logging waste recycling. The results indicate that it is possible to consider also other variants of ensilaged feed mixtures.

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# EFFECT OF PHYTOBIOTICS (MIXTURE OF GARLIC, GINGER AND CHAYA LEAF) ON GROWTH PERFORMANCE, HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF PULLET CHICKS

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## ABSTRACT

The aim of this study was to investigate the effect of mixture of garlic, ginger and Chaya leaf (hereafter termed "Phytomix") at graded levels on the growth performance, haematological indices and serum biochemistry of pullet chicks fed corn-soybean-based diets. Six hundred, 1-day-old females of ISA Brown pullet chicks were assigned to five treatment groups, each group was replicated six times (20 birds in each). Birds were fed basal diet supplemented with Phytomix at different levels: 0.0, 2.5, 5.0, 7.5 and 10.0 g.kg$^{-1}$ throughout the experimental period, which lasted for 56 days. Phytochemical screening revealed the presence of tannins, polyphenols, terpenes, saponins, flavonoids, alkaloids and cardiac glycosides in garlic, ginger and Chaya leaf, while the cyanides were found only in Chaya leaf. Highest quantities of bioactive substances were found in Chaya leaf followed by ginger and garlic. Pullet chicks, fed the diets supplemented with phytomix, had higher ($P < 0.05$) weight gain, reduced feed intake, better FCR and improved liveability when compared to the control diet. In addition, phytomix supplementation showed a linear improvement in growth performance of pullet chicks. Dietary phytomix inclusion significantly ($P < 0.05$) influenced the RBC and WBC of pullet chicks with highest values obtained in birds fed the diets containing 5.0 and 10.0 g.kg$^{-1}$, respectively. Inclusion of phytomix in the diets of pullet chicks produced significant ($P < 0.05$) effect on all serum chemistry parameters measured, except creatinine and AST ($P > 0.05$). It was concluded that dietary phytomix supplementation up to 10.0 g.kg$^{-1}$ had positive effect on growth performance and health status of pullet chicks.

**Key words:** pullet chicks; growth; blood indices; phytobiotics; Chaya leaf

## INTRODUCTION

The increasing awareness and the search for high-quality, safe animal products led to the growing interest in the use of phytobiotics as feed additives. Phytobiotics are known to exhibit antioxidant, anti-proliferate, anti-carcinogenic, anti-inflammatory and immunomodulatory, anti-diarrheic, hypolipidemic, detoxifying, digestion-stimulating, and flavoring properties (Grashorn, 2010). The bioactive substances of several plant parts (bark, leaves, stem, roots, fruits, flower, seeds) and their extracts, which are responsible for such nutritional and medicinal benefits, have been screened, and there is a wide variation in qualitative and quantitative composition of secondary metabolites both within and among such phytogenics (Kuti and Konoru, 2006).

Garlic (*Allium sativum* L.) of Alliaceae family has been reported to contain bioactive constituents

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such as alliin, ajoene, diallylsulfide, flavonoids, saponins, enzymes (allinase), B-vitamins and minerals (Suleria *et al.*, 2015). Ginger (*Zingiber officinale* Roscoe.), of Zingiberacea family contains active ingredients including essential oils (zingiberene, zingiberol, D-camphor), Shogaols, Diarylheptanoids, Gingerols, Paradol, Zerumbone, 1-Dehydro-(10) gingerdione, Terpenoids and Ginger flavonoids, which are responsible for its pungency and anti-serotonin effect, for enhanced gastrointestinal function and mucin synthesis, as well as other biological activities (Ganguly, 2017). Chaya leaf (*Cnidoscolus aconitifolius* (Mill.) Johnston), of Euphorbiaceae family, has good record of being used ethno-medically for prophylaxis and curative treatment of ailments such as diabetes, obesity, kidney stones, insomnia, gout, hemorrhoids, scorpion stings, acne and the eye problems. Its efficacy as anti-diuretic, laxative, appetite and digestion-stimulant has been proven (Jensen, 1997; Donkoh *et al.*, 1999). However, only few studies quantified the phytochemicals presence in these selected phytobiotics, thus the need for this study.

Studies on the use of garlic and ginger, solely or as a mixture, as feed additives in poultry feeding have been well-documented. Elagib *et al.* (2013) reported that 3 % dietary garlic supplementation resulted in increased feed intake, higher body gain and better feed utilization. Ademola *et al.* (2009) also observed higher body gains and improved FCR in broiler chickens fed garlic and ginger mixture. Ademola *et al.* (2012) reported that mixture of ginger and garlic produced positive effects on growth performance of pullet growers, hen-day production and egg weight of layers, whereas Bamidele and Adejumo (2012) reported non-significant effect on growth performance of pullet growers fed diets containing mixture of ginger and garlic.

However, there is a paucity of available literature on the combined use of garlic, ginger and chaya as feed additives for pullet chicks. In this study, we hypothesized that synergistic effects of bioactive ingredients present in garlic, ginger and Chaya leaf will enhance growth performance and improve health status of pullet chicks. This study, therefore, attempts to quantify the phytochemical constituents of garlic, ginger and Chaya leaf, and also assess the response of pullet chicks to graded levels of Phytomix (garlic, ginger and Chaya leaf mixture) in terms of growth, haematology and serum biochemical indices.

## MATERIALS AND METHODS

### Ethical Approval

The study protocol and procedures were conducted in accordance with the Animal Care and Use Review Committee guidelines of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria.

### Location of the Study

This trial was conducted at the Poultry Farms and Agricultural Science Laboratory of the Department of Agricultural Education, School of Vocational Education, Federal College of Education, Abeokuta, Ogun State, Nigeria.

### Collection and Preparation of Phytobiotics

Garlic bulbs and ginger rhizomes used in this study were grown in Nasarawa State of Nigeria due to the prevailing favourable growing conditions. Garlic bulbs (Soft neck variety) were harvested at maturity (5 months), while ginger rhizomes (UG 1 variety) were harvested at full maturity of 9 months. Thereafter, they were transported to Abeokuta, Ogun State. Fresh Chaya leaf samples (*C. aconitifolius*) were harvested from 2-years-old Chaya plants, which were planted by stem cuttings, at the Crop Production Farm near Osiele village in Abeokuta. They were identified at the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria. Garlic bulbs were separated into cloves and peeled. Ginger rhizomes were cleaned and manually chopped to air drying. Chaya leaves were thoroughly washed with clean water and chopped to 2-3 cm in size to air drying. Garlic, ginger and Chaya leaf samples were air dried at temperature between 25.50 – 28.30 °C (Mean ± SD; 26.58 ± 1.02 °C) for 2-3 weeks until the desired level of dryness was obtained. Thereafter, they were milled individually to powder and stored in air-tight bags until phytochemical analysis and incorporation into the formulated diet.

### Phytochemical Screening

Phytochemical screening for major constituents was performed using standard qualitative procedures as previously described (Trease and Evans, 1989; Mordi and Akanji, 2012). The test for tannins was carried out by dissolving 0.5 g of the dried powdered plant extract in 20 mL of distilled water, then filtered and 0.1 % ferric chloride reagent was added to the filtrate. For cardiac glycosides, 0.5 g of extract was added to 2 mL of acetic anhydrate plus $H_2SO_4$ using killer kiliani test. The test for alkaloids was carried out by adding 0.5 g of an aqueous extract into 5 mL of 1 % HCl, boiled and filtered. Then Mayer's reagent was added. The extract was subjected to frothing test for the identification of saponin. Haemolysis test was then performed on the frothed extracts in water to remove false positive results. The extract was also tested for free glycoside-bound anthraquinones by adding of 5 g of the extract to 10 mL of benzene, then filtered and ammonia solution was added. The presence of flavonoids was determined using 1 % aluminum chloride solution in methanol-concentrated HCL, magnesium turnings and potassium hydroxide solution.

**Management of Birds and Diets**

A total of six hundred (600) 1-day-old female, ISA Brown pullet chicks were purchased from a reputable hatchery in Abeokuta, Nigeria. They were randomly allocated on weight equalization basis to five dietary groups, each having six replicates with 20 birds per replicate. Basal diet (Corn-soybean based) was formulated to meet the nutrient requirement of pullet chicks (Table 1). Proximate analysis of basal diet (crude protein, crude fibre, ether extract and ash) was carried out using the standard procedure of the Association of Official Analytical Chemists (AOAC, 1995). Selected phytobiotics (garlic, ginger and Chaya leaf) were mixed in equal proportion on weight-for-weight basis to form "Phytomix" and the graded levels

**Table 1. Composition of experimental basal diet for pullet chicks (0 – 8 weeks)**

| Ingredients | Composition ($g.kg^{-1}$) |
|---|---|
| Maize | 520.00 |
| Soybean meal (45% CP) | 270.00 |
| Palm kernel cake (PKC) | 60.00 |
| Groundnut cake (GNC) | 20.00 |
| Wheat offal | 71.50 |
| Bone meal | 20.00 |
| Limestone | 30.00 |
| L-Lysine HCl | 1.20 |
| DL-Methionine | 1.80 |
| Vitamin/Mineral Premixa | 2.50 |
| Industrial Salt (NaCl) | 2.50 |
| Enzyme (Fullzyme®)[b] | 0.25 |
| Toxin binder (Zerotox®)[b] | 0.25 |
| Total | 1000 |
| Determined analysis ($g.kg^{-1}$, except ME)[c] | |
| Metabolizable Energy ($kcal.kg^{-1}$)[d] | 2785.50 |
| Crude protein | 205.30 |
| Ether extract | 37.20 |
| Crude fibre | 41.60 |
| Total ash | 295.00 |

[a]Provided vitamin-mineral premix per 2.5 kilogram of diet: 12,000,000 IU vitamin A; 2,500,000 IU vitamin $D_3$; 30,000 IU vitamin E; 2,000 mg vitamin $K_3$; 2,250 mg vitamin $B_1$; 6,000 mg vitamin $B_2$; 4,500 mg vitamin $B_6$; 15 mcg vitamin $B_{12}$; 40,000 mg niacin; 1,500 mg folic acid; 50 mcg biotin; 15,000 mg pantothenic acid; 300,000 mg choline chloride; 125,000 mg antioxidant (butylhydroxytoluene); 80,000 mg Mn; 50,000 mg Zn; 5,000 mg Cu; 20,000 mg Fe; 1,000 mg I; 200 mg Se; 500 mg Co; Produced by Rotinol International Ltd., Nigeria; [b]Manufactured by Biofeed Technology Inc., Canada, [c]Mean values of 3 replicates; [d]ME was estimated from the proximate values

of 0.0, 2.5, 5.0, 7.5 and 10.0 g.kg$^{-1}$ were included in the basal diet to form five experimental diets. Birds were fed one of five diets for the experimental period of 56 days. The birds had unrestricted access to feed offered in mash form during the study. Biosecurity measures and vaccination programme were strictly adhered to.

**Data Collection and Analysis**

**Growth Performance**

Bodyweight of the birds per replicate was measured on a weekly basis in order to compute weight gain. Daily feed intake was also measured as the difference between the feed offered and leftovers in order to estimate feed conversion ratio. A record of mortality was kept as it occurred. Liveability was computed as proportion of live birds left after the experiment.

**Haematology and Serum Biochemistry Indices**

At 56$^{th}$ day, blood samples (2.5 ml each) were collected from two birds in each replicate (*n = 12* per treatment) with needle and syringe through the jugular vein directly into EDTA-containing bottles and plain bottle for the determination of haematological indices and serum chemistry, respectively, using standard procedures, as described by Weiss and Wardrop (2010) and Fafiolu *et al.* (2014).

**Statistical Analysis**

The generated data were subjected to a One-way Analysis of Variance (ANOVA) in a Completely Randomized Design using General Linear Model procedure of SAS for Windows 9.1.3 version (SAS, 2007). Significant means among the treatments were compared according to Tukey's HSD test at 95 % probability level. Orthogonal polynomials were used to assess the linear and quadratic effects of varying levels of garlic, ginger and Chaya leaf mixture.

## RESULTS AND DISCUSSION

**Phytochemical Analysis of Garlic, Ginger and Chaya Leaf**

Phytochemical screening revealed presence of tannins, polyphenols, terpenes, saponins, flavonoids, alkaloids and cardiac glycosides in garlic, ginger and Chaya leaf, while cyanides were found only in Chaya leaf. Highest quantities of bioactive substances were found in Chaya leaf, followed by ginger and garlic (Table 2). This suggests that Chaya leaf holds a potential to offer better nutritional and health benefits as feed additive and functional food compared to garlic and ginger. Tannins are known to promote the healing of wounds and burns and also to possess anti-diarrhoeal and anti-haemorrhagic agent (Mordi and Akanji, 2012). Presence of polyphenols and terpenes would ensure better oxidative stability by scavenging the reactive oxygen species (ROS) and free radicals that could damage DNA of cells, thereby regulating enzyme systems that play crucial role in the *in vivo* redox homeostasis at the cellular level (Muthusamy and Sankar, 2015). Also, this probably suggests that there could be a delayed lipid peroxidation process in fresh and processed poultry products obtained from birds fed diets containing these bioactive substances (Botsoglou *et al.*, 2003). Phenols have

**Table 2. Phytochemical analysis of garlic, ginger, and Chaya leaf**[#]

| Items (mg.100 g$^{-1}$) | Garlic | Ginger | Chaya leaf |
|---|---|---|---|
| Tannins | 0.90 | 1.70 | 7.80 |
| Polyphenols | 69.13 | 118.04 | 184.07 |
| Terpenes | 1.80 | 3.90 | 4.60 |
| Saponins | 86.15 | 157.05 | 321.00 |
| Flavonoids | 2.10 | 3.30 | 3.80 |
| Alkaloids | 128.11 | 327.00 | 526.01 |
| Glycosides | 39.00 | 112.85 | 126.72 |
| Cyanides | 0.00 | 0.00 | 0.596 |

[#]Mean values on 3 replicates

been noted to moderate enzymatic activities that cause inflammation, thereby suggesting its anti-inflammatory properties (Muthusamy and Sankar, 2015).

Saponins helps to fight parasitic infections in plants, and supports the immune system to confer antimicrobial protection against viruses and bacteria in man and animals (Tende *et al.*, 2014). It also plays an active role in the urea metabolism by lowering the activity of the intestinal and faecal urease, thus, causing reduced $NH_3$ formation, thereby enhancing kidney formation. Moreover, saponins exert haemolytic, hypocholesterol and cardiac depressant properties, thus, implying healthy cardiac function and reduced risk of cardio-vascular diseases (Muthusamy and Sankar, 2015). The steroidal saponins, alongside with organosulfur constituents in garlic, are direct antioxidants and show cholesterol-lowering effects, which may result in reduced risk of coronary and cardiovascular diseases. Tende *et al.* (2014) reported that anti-hypercholesterol effects improved serum lipid profile in Wistar rats, as well as cardio-protective effect on isolated perfused (rabbit) heart, caused by single and combined doses of administered ginger and garlic. These effects could be attributed to the inherent organosulfur constituents and steroidal saponins. Flavonoids, due to their high anti-oxidative activities, were known to exert anti-allergic, anti-inflammatory, anti-microbial, anti-proliferative and anti-carcinogenic effects, while alkaloids have been noted for their detoxifying, analgesic, anti-spasmodic, anti-bacterial and hypotensive properties (Akachukwu *et al.* 2014).

Relative variation in quantitative values of phytochemical constituents obtained in this study, compared to those reported by Akachukwu *et al.* (2014), could be attributed partly to the differences in plant growing conditions, age at harvest, processing methods (air-dried vs oven-dried), and extraction methods. Moreover, cyanide content determined in chaya leaf (5.96 $mg.kg^{-1}$) will probably not produce any chronic health disorder in pullet chicks because of its lower concentration when compared to the recommended safe cyanide level (10 mg HCN equivalents/g fresh weight) in food products containing cyanogens (FAO/ WHO, 1991) and its dietary inclusion level.

**Growth Performance**

Influence of diets supplemented with phytomix on growth performance of pullet chicks is shown in Table 3. Final weight and daily weight gain increased progressively ($P < 0.05$) with increase in phytomix level (linear, $P = 0.014$). Feed intake reduced ($P < 0.05$) as phytomix supplementation increased, however, the least value was observed in birds fed the diet containing 5.0 $g.kg^{-1}$ phytomix (linear, $P = 0.003$). Feed conversion ratio (FCR) of pullet chicks decreased significantly ($P = 0.0049$) as phytomix supplementation increased from 0 to 10.0 $g.kg^{-1}$ (linear, $P < 0.001$). Phytomix supplementation had positive effect ($P < 0.05$) on liveability of pullet chicks as no mortality was recorded in birds fed the diet supplemented with 10.0 $g.kg^{-1}$ phytomix. The improvement in body gain of pullet chicks fed phytomix could be due to its positive effect on palatability and digestion stimulation by endogenous

**Table 3. Growth performance of pullet chicks (0 – 8 weeks) fed diet supplemented with phytobiotics (mixture of chaya leaf, garlic and ginger powder)#**

| Items | Level of phytobiotics inclusion ($g.kg^{-1}$) | | | | | Pooled | Significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 2.5 | 5.0 | 7.5 | 10.0 | SEM | *P*-value | Linear | Quadratic |
| Initial weight (g) | 34.40 | 34.43 | 34.50 | 34.10 | 34.13 | 0.126 | 0.7845 | 0.815 | 0.799 |
| Final weight (g) | 379.33[b] | 379.41[b] | 389.69[ab] | 420.59[a] | 434.21[a] | 7.748 | 0.0139 | 0.014 | 0.734 |
| Daily weight gain (g) | 6.16[c] | 6.16[c] | 6.34[bc] | 6.90[b] | 7.14[a] | 0.139 | 0.0365 | 0.014 | 0.738 |
| Daily feed intake (g) | 44.01[a] | 42.05[ab] | 38.84[b] | 41.43[ab] | 39.41[ab] | 0.629 | 0.0287 | 0.003 | 0.434 |
| FCR | 7.16[a] | 6.86[ab] | 6.12[bc] | 6.01[bc] | 5.52[c] | 0.184 | 0.0049 | <0.001 | 0.723 |
| Livability (%) | 93.33[c] | 95.00[bc] | 95.00[bc] | 98.33[ab] | 100.00[a] | 0.766 | 0.0067 | 0.001 | 0.418 |

#Mean values on 6 replicate pen, Values in the same row having different superscripts differ @ $P < 0.05$, FCR = Feed conversion ratio, SEM = standard error of the mean enzyme secretion, which may enhance nutrient role for development of the digestive system, muscle tissue accretion, immune system and to improve the growth performance. Ginger is known to contain a protein digesting enzyme (zingibain), which is supposed to improve digestion. Moreover, dietary inclusion of ginger into animal diets caused notable increase in the amount of pancreatic and intestine lipase (Ganguly, 2017). According to Muthusamy and Sankar (2015), phytobiotics could also increase the output of digestive enzymes from the pancreas, gut mucosa and increased bile flow for improved nutrient metabolism.

Reduced ($P < 0.05$) feed intake and improved FCR in birds fed phytomix is an indication of better feed utilization efficiency. Chick phase (0-6 weeks) is a critical period for development of digestive tract organs and the immune system. Efforts to deliver the rightful needed dietary nutrients and substrates to enhance biosynthesis of intestinal epithelial cells and to boost cell-mediated and humoral immune system will go along to reduce birds' susceptibility to infectious diseases, physiologic and environmental stress throughout their lifetime. These findings indicate that the level of inclusion and synergistic interaction between phytochemical constituents of phytobiotics determines their resultant efficacy in birds. These results are in concert with the data of Ademola *et al.*, (2012), who reported that mixture of ginger and garlic induced positive effects on growth performance of pullet growers, hen-day production and egg weight of layers. In addition, Ademola *et al.* (2009) and Oleforuh-Okoleh *et al.* (2015) observed higher body gains and improved FCR in broiler chickens fed garlic and ginger mixture. Elagib *et al.* (2013) also noted that dietary garlic supplementation led to the higher body gain and best feed utilization efficiency in broiler chickens. Liveability was positively ($P < 0.05$) influenced by the treatments. This is in agreement with Donkoh *et al.*, (1999), who reported decreased mortality rate in broiler chickens fed the diet containing chaya leaf meal (CLM) in two trials. Decrease in mortality rate with increased phytomix addition confirmed their antioxidant, anti-inflammatory and anti-microbial properties. Garlic, ginger and Chaya leaf are known to contain appreciable amount of vitamin C, a potent antioxidant, which prevents or reduces cellular damage. The metabolic activities of allicin and other organosulfur compounds, present in garlic, inhibit the activity of inflammatory enzymes in the body, have greater antioxidant potential and enhance the activity of thymus gland to stimulate the proliferation of the body T-cells and interleukin-2 (IL-2), which plays active role in the immune system function (Suleria *et al.*, 2015). Ganguly (2017) had earlier reported that ginger is able to regulate the activities of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, thereby prevent or delay lipid peroxidation. Therefore, the synergy of bioactive

**Table 4. The haematological indices of pullet chicks (0 – 8 weeks) fed diet supplemented with phytobiotics (mixture of chaya leaf, garlic and ginger powder)#**

| Items | Level of phytobiotics inclusion (g.kg$^{-1}$) | | | | | Pooled | Significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 2.5 | 5.0 | 7.5 | 10.0 | SEM | *P*-value | Linear | Quadratic |
| PCV (%) | 25.50 | 25.50 | 28.00 | 24.50 | 26.50 | 0.431 | 0.0773 | 1.000 | 0.513 |
| Hb (g.100 ml$^{-1}$) | 8.55 | 8.60 | 9.45 | 8.00 | 8.80 | 0.165 | 0.0575 | 0.668 | 0.933 |
| RBC (x 10$^{9/L}$) | 2.09$^{b}$ | 2.28$^{ab}$ | 2.69$^{a}$ | 2.34$^{ab}$ | 2.43$^{ab}$ | 0.067 | 0.0331 | 0.984 | 0.180 |
| WBC (x 10$^{12/L}$) | 9.25$^{b}$ | 9.25$^{b}$ | 11.00$^{ab}$ | 10.00$^{ab}$ | 12.25$^{a}$ | 0.372 | 0.0146 | 0.104 | 0.002 |
| Neutrophils (%) | 12.00 | 17.50 | 21.00 | 17.50 | 18.50 | 1.429 | 0.4148 | 0.234 | 0.189 |
| Lymphocytes (%) | 86.00 | 80.50 | 76.50 | 81.00 | 78.00 | 1.472 | 0.3240 | 0.155 | 0.262 |
| Monocytes (%) | 1.00 | 1.50 | 1.00 | 0.00 | 1.50 | 0.272 | 0.4413 | 0.867 | 0.485 |
| Eosinophils (%) | 1.00 | 0.50 | 1.00 | 1.50 | 1.50 | 0.235 | 0.7019 | 0.175 | 0.551 |
| Basophils (%) | 0.00 | 0.00 | 0.50 | 0.00 | 0.00 | 0.072 | 0.0723 | 1.000 | 0.260 |

#Mean values on 12 birds/treatment, Values in the same row having different superscripts differ @ $P < 0.05$, SEM = standard error of the mean substances in garlic, ginger and chaya may support the chicks' protective mechanisms against oxidative damage and enhance their immune system, thereby suppressing their susceptibility to infections.

**Haematology**

Dietary phytomix inclusion significantly (P < 0.05) influenced the RBC (red blood cells) and WBC (white blood cells) of pullet chicks with highest values obtained in birds fed the diets containing 5.0 and 10.0 g.kg$^{-1}$, respectively (Table 4). In addition, WBC of pullet chicks increased quadratically ($P$ = 0.002) as phytomix supplementation increased. Increased RBC implies that dietary phytomix enhanced haematopoietic process, morphology and osmotic fragility of erythrocytes produced with no any adverse effect on physiological and metabolic functions of the body. This confirms the ability of the phytochemical constituents present in garlic, ginger and chaya to improve the health status of the pullet chicks. Donkoh *et al*., (1999) reported increased RBC in broiler chickens fed diets containing chaya leaf meal (CLM). Tende *et al*., (2014) reported that RBC activates the signalling of cardio-protective process by converting the organosulfur compounds in garlic to hydrogen sulphide ($H_2S$), an endogenous cardio-protective vascular cell signalling molecule, thus stimulating blood circulation. Also, ginger extract was found to improve blood flow in animals due to significant (dose-dependent) decrease in the arterial blood pressure and cardio-depressant effect on the rate and force of spontaneous contractions (Tende *et al*., 2014; Suleria *et al*., 2015). Improved blood circulation will not only increase oxygen carrying capacity of the cells for better nutrient transport throughout the body system, but will also prevent fat deposition in the arteries, thereby reducing the chance of cardio-vascular diseases.

Relative higher WBC counts obtained in birds fed the diets containing phytomix indicates enhanced host's immune system by stimulating antibody production as well as creation of the unfavourable environment for pathogenic microbes thereby confirming their anti-microbial properties. This confirms the immuno-modulatory properties of garlic, ginger and chaya leaf, which is an indication, that the birds could perform their phagocytic functions for optimum immunity levels and higher disease resistance (Fafiolu *et al*., 2014). In agreement, Oleforuh-Okoleh *et al*. (2015) reported that aqueous extract of garlic and ginger induced positive effect on PCV (packed cell volume) and WBC of broiler chickens. Non-significant (P< 0.05) effect of phytomix on cell counts indicates no signs of lymphopenia, heterophilia and leukocytosis.

**Table 5. Serum biochemical indices of pullet chicks (0 – 8 weeks) fed diet supplemented with phytobiotics (mixture of chaya leaf, garlic and ginger powder)[#]**

| Items | Level of phytobiotics inclusion (g.kg$^{-1}$) | | | | | Pooled | Significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 2.5 | 5.0 | 7.5 | 10.0 | SEM | *P*-value | Linear | Quadratic |
| Glucose (mg.100 ml$^{-1}$) | 148.25$^{c}$ | 156.25b$^{c}$ | 185.80$^{a}$ | 175.50$^{ab}$ | 186.75$^{a}$ | 4.640 | 0.0012 | <0.001 | 0.133 |
| Total protein (g.L$^{-1}$) | 56.70$^{cd}$ | 53.55$^{d}$ | 64.80$^{ab}$ | 69.75$^{a}$ | 61.05$^{bc}$ | 1.628 | <0.0001 | <0.001 | 0.009 |
| Albumin (g.L$^{-1}$) | 39.20$^{b}$ | 36.70$^{b}$ | 37.95$^{b}$ | 46.35$^{a}$ | 41.30$^{ab}$ | 0.990 | 0.0006 | 0.002 | 0.680 |
| Globulin (g.L$^{-1}$) | 17.50$^{c}$ | 16.85$^{c}$ | 26.85$^{a}$ | 23.40$^{ab}$ | 19.75$^{bc}$ | 1.089 | 0.0004 | 0.020 | 0.001 |
| A:G | 2.25$^{a}$ | 2.24$^{a}$ | 1.43$^{b}$ | 1.98$^{ab}$ | 2.11$^{a}$ | 0.098 | 0.0119 | 0.249 | 0.115 |
| Urea (mg.100 ml$^{-1}$) | 7.50$^{a}$ | 7.40$^{a}$ | 6.90$^{a}$ | 5.40$^{ab}$ | 4.25$^{b}$ | 0.392 | 0.0039 | 0.833 | 0.026 |
| Creatinine (mg.100 ml$^{-1}$) | 0.55 | 0.55 | 0.65 | 0.55 | 0.50 | 0.016 | 0.1245 | 0.153 | 0.153 |
| AST (IU.L$^{-1}$) | 98.65 | 108.30 | 113.80 | 126.65 | 114.90 | 4.172 | 0.3336 | 0.102 | 0.317 |
| ALT (IU.L$^{-1}$) | 12.40$^{c}$ | 17.60$^{b}$ | 13.65$^{c}$ | 12.80$^{c}$ | 22.65$^{a}$ | 1.047 | <0.0001 | <0.001 | <0.001 |
| AST/ALT | 7.95$^{ab}$ | 6.15$^{b}$ | 8.56$^{ab}$ | 9.90$^{a}$ | 5.08$^{b}$ | 0.544 | 0.0086 | 0.514 | 0.031 |

[#]Mean values on 12 birds/treatment, Values in the same row having different superscripts differ @ P < 0.05, A:G = Albumin/globulin ratio, SEM = standard error of the mean.

**Serum Biochemistry**

Serum biochemical indices of pullet chicks fed the diets supplemented with phytomix are shown in Table 5. Inclusion of phytomix into the diets of pullet chicks produced significant ($P < 0.05$) effect on all serum biochemistry parameters measured, except ($P > 0.05$) creatinine and AST. Serum glucose increased linearly ($P < 0.001$) as phytomix inclusion increased from 0 to 10.0 $g.kg^{-1}$. The highest total protein (linear, $P < 0.001$; quadratic, $P = 0.009$) was obtained in birds fed the diets containing 7.5 $g.kg^{-1}$. In a similar manner, the highest albumin (linear, $P = 0.002$) was obtained in birds fed the diets containing 7.5 $g.kg^{-1}$, while the highest globulin value (linear, $P = 0.020$; quadratic, $P = 0.001$) was obtained in birds fed the diets containing 5.0 $g.kg^{-1}$.

Higher serum glucose level may indicate enhanced energy metabolism due to stimulation of endogenous digestive enzymes by phytomix and due to the release of adequate substrate (glucose) needed for mechanical work and body maintenance. This could be attributed to higher weight gain observed in chicks fed the diets containing 10.0 $g.kg^{-1}$ phytomix.

Assessment of serum proteins in poultry birds is important because they play crucial role in the maintenance of colloid osmotic pressure, mobility of dietary nutrients, minerals and hormones, as a rapid substitute for indispensable amino acids, as well as biosynthesis of enzymes and immune system (Piotrowska *et al*. 2011). Albumin carries and delivers important nutrients to body cells. It is important for tissue growth and healing as it supplies appropriate amino acids needed for tissue proteins synthesis in the period of quick somatic growth of birds (Piotrowska *et al*. 2011), and also binds to toxins and free radicals, thus minimizing oxidation process and preventing cell damage. In this present study, the feeding with a phytomix above 5 $g.kg^{-1}$ produced higher albumin in birds indicating that such higher concentration of garlic, ginger and chaya leaf would be required to exert their wound healing and antioxidant effect. Also, higher total protein and albumin could be a pointer to enhanced liver functions by phytomix, since plasma proteins are synthesized predominantly by the liver. Higher globulin, observed in birds fed 5 $g.kg^{-1}$ phytomix and above, implies improved host's immune system and could be attributed to reduced mortality observed in birds fed such diets. The present finding is strongly supported by Oleforuh-Okoleh *et al*. (2015), who reported significant increase in serum total protein, albumin and globulin of broiler chickens fed aqueous extract of garlic and ginger.

Serum urea concentration reduced ($P < 0.05$) significantly (quadratic, $P = 0.026$) as phytomix level increased. Urea concentration provides insight to diagnosis of renal damage or functionality of the kidney. Reduced ($P < 0.05$) serum urea concentration in pullet chicks confirms that the selected phytobiotics exhibited digestive-stimulating effect, which translated to better digestion, absorption and utilization of protein in dietary treatment groups, which invariably led to improved protein utilization without any harmful effect on the functionality of liver and kidney.

Higher ALT value (linear, $P < 0.001$; quadratic, $P < 0.001$) was recorded in chicks fed the diet containing 10.0 $g.kg^{-1}$ phytomix. Alanine aminotransferase (ALT) is a liver enzyme that catalyzes the transfer of amino groups from L-alanine to α–ketoglutarate in order to produce hepatic metabolite oxaloacetate (Kim *et al*., 2008). ALT also plays an important role in biotransformation and detoxification of various toxicants, ROS, endo- and xerobiotics. Higher ALT values in birds fed 10 $g.kg^{-1}$ phytomix could be attributed to higher serum glucose level. Kim *et al*. (2008) had reported a positive correlation between serum glucose level and the ALT activity. Kim *et al*. (2008) noted that the serum ALT activity may be affected by a number of factors that are not associated with hepatic necrosis, and that the elevated ALT values, which are the five-times less than the upper limit of the normal range, non-persistent, and without any clinical symptoms, may not be worrisome. Therefore, the synergistic effect of bioactive substances in selected phytobiotics improved the health status of pullet chicks.

## CONCLUSION

Phytochemical analysis showed that highest quantities of bioactive substances (tannins, polyphenols, terpenes, saponins, flavonoids, alkaloids, and cardiac glycosides) were found in Chaya leaf followed by the ginger and garlic. Pullet chicks fed diets, supplemented with phytomix, had significantly ($P < 0.05$) higher weight gain, reduced feed intake, better FCR and improved liveability when compared with the control diet. In addition, phytomix supplementation showed a linear improvement in growth performance of pullet chicks. Dietary phytomix supplementation, in this study, led to the increased RBC and WBC formation. Phytomix inclusion resulted in higher concentration of serum glucose, total protein, albumin and globulin, as well as reduced serum urea concentration in pullet chicks.

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# EFFECT OF MANURE AND HARVESTING AGE ON PHYSICAL AND CHEMICAL PROPERTIES OF *PENNISETUM* HYBRID SILAGE

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## ABSTRACT

This study was conducted to investigate the combined effect of manure type and harvesting age on physical properties and chemical composition of silage produced from *Pennisetum* hybrid forage. The manure types were from cattle, poultry, swine, goat, while the harvesting times were 4 and 8 weeks after cutback (WAC). Colour results varied significantly ($P < 0.05$). Dry matter, ether extract, crude fibre, ash, crude protein (CP), neutral detergent fibre, acid detergent fibre and lignin contents of the silage varied significantly ($P < 0.05$). Higher CP content (91.0 $g.kg^{-1}$) of the silage was obtained from the interactive effect of poultry or goat manure and 4 WAC harvesting age of *Pennisetum* hybrid. Higher ash content (330.0 $g.kg^{-1}$) was recorded for the combined effect of goat manure and 4 WAC on the *Pennisetum* silage, while the least (70.0 $g.kg^{-1}$) occurred in grass without manure application sampled 8 WAC. Higher Ca (7.14 $g.kg^{-1}$) was recorded in silage from swine manure and 4 WAC harvesting age effect. Phosphorus content of the silage was higher (2.57 $g.kg^{-1}$) with poultry manure and 8 WAC effect, while the least (1.01 $g.kg^{-1}$ and 1.98 $g.kg^{-1}$) were from no manure effect at both harvesting ages. Crude protein content of silage made from the *Pennisetum* hybrid fertilized with either poultry or goat manure and harvested at 4 WAC was the best. Physical properties of the silage were generally of acceptable/desirable grades. With or without the manure application, the minerals of the silage were generally higher than the recommendation of ruminants.

**Key words:** colour; fibre; organic manure; proximate; regrowth; silage

## INTRODUCTION

Natural grazing lands constitute the major feed resources in the developing countries, especially in Africa countries by providing more than 90 % of animal feed requirements either in the form of grazing resources or conserved forages (Kitaba and Tamir, 2007).

Inadequate supply of both quantity and quality feed is responsible for low livestock productivity (Peters, 1980). Ruminant production in Sub-Sahara Africa is seriously affected by seasonal availability of quality forages. The problem of pasture shortage is more severe in the dry season when ruminants subsist on very poor quality crop residues which results in correspondingly low level of production performance (Areegbe *et al.*, 2012). Grazing of ruminants have been used as palliative in the past, but could not satisfactorily address the problems of dry season feeding as animal losses are often recorded as a result of stress associated with prolonged search for green herbage that are usually of very poor quality (Mohammed, 1990).

Forage conservation in form of silage has been reported as a sustainable means of supplementing feed for ruminants in the dry season (Babayemi and Igbekoyi, 2008). Forage conservation is,

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therefore, promoted with the main objective of ensuring that quality feed is available for animals throughout the season. Silage making helps to secure feeds during seasons of high production for conservation and for later use in periods of relative shortage. In dealing with lack of feed during the dry season forage conservation especially as silage is considered to be the preservation technique with the greatest potential for protein rich foliage (Man and Wiktorsson, 2002).

The *Pennisetum* hybrid grass, which is derived from the cross of a short day photoperiod sensitive "maiwa" cultivar of bulrush millet or the pearl millet (*Pennisetum thyphoides* Schum) and the common elephant grass (*Pennisetum purpureum*), is known with high nutritive value and acceptability of the "maiwa" and the dry matter yield potential of elephant grass. Therefore, the objectives of this study was to investigate the effect of manure type and harvesting age on physical characteristics and chemical composition of silage produced from the *Pennisetum* hybrid grass.

## MATERIALS AND METHODS

### Experimental site

The field experiment was conducted at the Teaching and Research Farm; the chemical analyses were carried out at the Department of Pasture and Range Management Laboratory, Federal University of Agriculture, Abeokuta, Nigeria. The experimental site lies within the savanna agro-ecological zone of South-Western Nigeria (latitude: 7 °N, longitude 3.5 °E) (Google Earth, 2011). It experiences average annual rainfall of 1037 mm in a bimodal distribution pattern, with mean monthly temperature which ranges between 25.70 °C in July and 30.20 °C in February (Google Earth, 2011; OORBDA, 2011).

### Land preparation, organic manure collection, analysis, application and planting of grass

A total land area, measuring 846 m$^2$ used for the experiment, was divided into three parcels with each parcel subdivided into equal plots of 12 m$^2$, and each plot was cleared, followed by ploughing and harrowing. After land preparation and before planting, soil samples were randomly collected from the plots at the depth of 0-15 cm using soil auger. The soil samples were bulked per replicate and thoroughly mixed, a sub-sample of 300 g was taken to determine the pre-planting nutrient status of the soil (Table 1).

Animal manures from cattle, poultry, goat and swine used for the study were collected from the Teaching and Research Farm. Representative samples were taken from the heap, oven-dried to a constant weight at 65 °C, thereafter milled through

**Table 1. Physico-chemical properties of the composite soil samples taken at 0-15 cm depth from the experimental site before planting**

| Chemical properties | Values |
|---|---|
| pH | 7.03 |
| Total nitrogen (%) | 0.11 |
| Organic carbon (%) | 1.29 |
| C:N ratio | 28.38 |
| Available P (mg.kg$^{-1}$) | 53.87 |
| Acidity (cmol.kg$^{-1}$) | 0.13 |
| CEC | 1.79 |
| Exchangeable cations (cmol.kg$^{-1}$) | |
| Sodium (Na) | 0.80 |
| Potassium (K) | 0.20 |
| Calcium (Ca) | 2.77 |
| Magnesium (Mg) | 2.72 |
| Particle size | |
| Sand (%) | 77.93 |
| Silt (%) | 17.33 |
| Clay (%) | 4.73 |

**Table 2. Mineral composition of animal manures**

| Nutrients | Cattle | Swine | Poultry | Goat |
|---|---|---|---|---|
| N (g.kg$^{-1}$) | 15.60 | 16.90 | 30.20 | 15.3 |
| P (g.kg$^{-1}$) | 6.90 | 6.30 | 10.60 | 8.70 |
| K (g.kg$^{-1}$) | 7.30 | 7.60 | 10.30 | 8.90 |
| Ca (g.kg$^{-1}$) | 21.20 | 31.60 | 37.20 | 24.20 |
| Mg (g.kg$^{-1}$) | 11.70 | 19.20 | 17.30 | 12.50 |
| Na (g.kg$^{-1}$) | 1.10 | 1.60 | 2.10 | 1.30 |
| Fe (mg.kg$^{-1}$) | 615.00 | 651.00 | 631.00 | 576.00 |
| Zn (mg.kg$^{-1}$) | 54.80 | 81.20 | 75.40 | 38.60 |
| Cu (mg.kg$^{-1}$) | 29.10 | 27.30 | 32.70 | 23.80 |
| Mn (mg.kg$^{-1}$) | 322.00 | 260.00 | 218.00 | 257.00 |

a 1 mm sieve and were separately analysed to determine the mineral composition in each manure type (Table 2). Thereafter, the remaining collected manures, from which samples were taken, were broadcast to individual plots according to their treatments. After the application of manures, the plots were left for two weeks for the mineralization to take place before planting of the grass at 1 m × 1 m intervals. Eight weeks after planting, the grasses were cut back to 10 cm above ground level to allow uniform regrowth.

**Experimental design**

The study was a 5 × 2 factorial experiment arranged in a completely randomized design, which comprised of five manure types (cattle, poultry, swine, goat and no-manure as control) and two harvesting times (4 and 8 weeks after cutback). The experiment was replicated thrice.

**Harvesting of forage materials**

Samples of the grass fertilized with animal manures were harvested at 4 and 8 weeks after cutback (WAC) 15 cm above the ground level. The samples were chopped into pieces of 2-3 cm in length, wilted for 4 hours to reduce their moisture contents at a temperature of 23.4 °C and relative humidity of 71.2 % before ensiling. The forages were carefully packed into laboratory bottle silos (960 ml) after mixing thoroughly following the method described by Yokota *et al.* (1995) and were ensiled for a period of 6 weeks at an ambient temperature of 26 °C. At the expiration of the ensiling, the bottle silos were opened for determination of physical properties according to the procedures of Bates (1998). Also, samples of 300 g were taken from each of the silos and oven-dried at 65 °C to a constant weight. The dried samples were thereafter milled through a 1 mm sieve for chemical analyses.

**Chemical analyses**

The approximate composition of the silage was determined according to the procedure of AOAC (2000). Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were determined using the method of Van Soest *et al.* (1991). The mineral compositions for Calcium, Potassium, Phosphorus and Magnesium were determined using Atomic Absorption Spectrophotometer (Fritz and Schenk, 1979). All the values after chemical analyses are in the dry matter form.

**Statistical analysis**

The data collected were subjected to a two-way analysis of variance and the treatment means were separated using Duncan Multiple Range Test of SAS (1999) package.

## RESULTS AND DISCUSSION

Table 3 presents the effect of manure type and harvesting age on the physical properties of silage produced from *Pennisetum* hybrid. Odour, moisture and mouldiness percentages were similar ($P > 0.05$), while the colour results were significantly

**Table 3. Effect of manure type and harvesting age on the physical properties of silage produced from *Pennisetum* hybrid grass**

| Manure type | Harvesting age | Colour | Odour | Moisture | Mouldiness |
|---|---|---|---|---|---|
| | ← | | % | | → |
| Cattle | 4 WAC | 8.00[ab] | 16.67 | 7.33 | 9.00 |
| Swine | 4 WAC | 8.67[ab] | 19.33 | 7.67 | 9.00 |
| Goat | 4 WAC | 8.33[ab] | 20.00 | 8.00 | 9.00 |
| Poultry | 4 WAC | 7.00[b] | 17.67 | 7.33 | 9.33 |
| Control | 4 WAC | 7.67[ab] | 16.33 | 8.00 | 8.67 |
| Cattle | 8 WAC | 7.33[ab] | 25.33 | 6.33 | 9.00 |
| Swine | 8 WAC | 8.33[ab] | 24.67 | 8.00 | 9.00 |
| Goat | 8 WAC | 8.33[ab] | 25.00 | 6.67 | 9.00 |
| Poultry | 8 WAC | 9.00[a] | 25.67 | 8.00 | 9.00 |
| Control | 8 WAC | 9.00[a] | 24.33 | 8.33 | 9.00 |
| SEM | | 0.14 | 0.19 | 1.04 | 0.20 |

[a,b:] Means in same column with different superscripts are significantly ($p < 0.05$) different; WAC: weeks after cutback

different ($P < 0.05$). The colour ratings (7.00-9.00 %) of this study fell within the range of 5-8 (green to yellowish-green) and 9-12 (yellow to brownish), which are respectively regarded as desirable and acceptable ranges for silage (Bates, 1998). 't Mannetje, (1999) has reported that good silage usually preserves the original colour of the pasture or any forage. Interactive effect of poultry manure application and 4 WAC on the silage made recorded the least percentage in the colour (7.00 %) rating. The green/yellowish-green colour was closed to the original colour of the grass, which was an indication of good quality silage that was well preserved (Oduguwa *et al.*, 2007). Odour ratings (16.33-25.67 %) of this study were within the ranges of acceptable (11-23 %) and desirable (24-28 %) silage odour (Bates, 1998). Moisture constituents of this study were within the range of acceptable (6-8 %), reported in the silage physical evaluation sheet of Bates (1998). Most mouldiness values of this study were within the range of no-mould as reported by Bates (1998).

The interactive effect of manure type and harvesting age on the approximate composition and fibre fractions of silage produced from *Pennisetum* hybrid is presented in Table 4. The results of the dry matter (DM), ether extract (EE), ash and crude protein (CP) contents of the silage were significantly different ($P < 0.05$). Results of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) contents of the silage were significantly different ($P < 0.05$). The CP content (91.00 g.kg$^{-1}$) of the silage made from grasses fertilized with poultry or goat manures and harvested at 4 WAC recorded significantly higher value than 74.00 g kg$^{-1}$ reported for *Panicum maximum* cv. Ntchisi, when ensiled at 4 WAC (Babayemi, 2009). Similarly, the CP content of silage from grass, harvested at 4 WAC (63.00 - 79.30 g.kg$^{-1}$) with or without manures application, were higher than 52.00 g.kg$^{-1}$ reported for *Panicum maximum* cv. Ntchisi when ensiled at 8 WAC and sampled after 47 days (Babayemi, 2009). Better silage made from this study could be linked to manure application which enhanced the known quality of *Pennisetum* hybrid grass. The CP contents of the silage in this study surpassed the threshold of 60 g kg$^{-1}$ required by rumen microbes to build their body protein (Van Soest, 1994). Hence, intake of forages by ruminants and rumen microbial activities would be positively affected. Therefore, the silage would provide adequate nitrogen requirement by rumen microorganisms to maximally digest the main components of dietary fibre leading to the production of volatile fatty acid (Trevaskis *et al.*, 2001; Lamidi and Ogunkunle, 2016) which, in turn, facilitates microbial protein synthesis (Lamidi and Aina, 2013).

*Pennisetum* hybrid grass that were fertilized with goat, poultry or swine manure, produced silage of higher CP content at 4 WAC, when compared to the silage made from unfertilized grasses. The NDF, ADF and ADL contents of *Pennisetum*

**Table 4. Effect of manure type and harvesting age on the chemical composition (g.kg$^{-1}$ DM) of silage produced from *Pennisetum* hybrid grass**

| Manure type | Harvesting age | DM | CP | EE | Ash | NDF | ADF | ADL |
|---|---|---|---|---|---|---|---|---|
| | | g.kg$^{-1}$ DM | | | | | | |
| Cattle | 4 WAC | 566.00$^{ab}$ | 81.70$^{ab}$ | 80.00$^{bc}$ | 130.00$^{c}$ | 560.00$^{bcd}$ | 360.00$^{ab}$ | 160.00$^{abcd}$ |
| Swine | 4 WAC | 480.00$^{c}$ | 86.30$^{a}$ | 90.00$^{bc}$ | 120.00$^{c}$ | 600.00$^{abcd}$ | 360.00$^{ab}$ | 100.00$^{f}$ |
| Goat | 4 WAC | 504.00$^{b}$ | 91.00$^{a}$ | 163.30$^{a}$ | 330.00$^{a}$ | 560.00$^{bcd}$ | 346.70$^{b}$ | 130.00$^{e}$ |
| Poultry | 4 WAC | 500.00$^{b}$ | 91.00$^{ab}$ | 73.30$^{bc}$ | 243.30$^{b}$ | 540.00$^{cd}$ | 340.00$^{b}$ | 126.70$^{ef}$ |
| Control | 4 WAC | 492.00$^{c}$ | 81.70$^{ab}$ | 140.00$^{a}$ | 136.70$^{c}$ | 540.00$^{cd}$ | 400.00$^{a}$ | 150.00$^{bcde}$ |
| Cattle | 8 WAC | 570.00$^{ab}$ | 79.30$^{ab}$ | 60.00$^{c}$ | 80.00$^{bc}$ | 660.00$^{a}$ | 400.00$^{a}$ | 170.00$^{ab}$ |
| Swine | 8 WAC | 572.00$^{ab}$ | 74.70$^{ab}$ | 80.00$^{bc}$ | 80.00$^{bc}$ | 660.00$^{a}$ | 280.00$^{c}$ | 140.00$^{cde}$ |
| Goat | 8 WAC | 600.00$^{a}$ | 63.00$^{b}$ | 70.00$^{bc}$ | 90.00$^{bc}$ | 520.00$^{b}$ | 360.00$^{ab}$ | 133.30$^{de}$ |
| Poultry | 8 WAC | 622.00$^{a}$ | 72.30$^{ab}$ | 100.00$^{b}$ | 90.00$^{bc}$ | 620.00$^{abc}$ | 380.00$^{ab}$ | 163.30$^{abc}$ |
| Control | 8 WAC | 600.00$^{a}$ | 74.70$^{ab}$ | 90.00$^{bc}$ | 70.00$^{c}$ | 640.00$^{ab}$ | 400.00$^{a}$ | 180.00$^{a}$ |
| SEM | | 1.12 | 2.20 | 6.40 | 19.90 | 11.80 | 7.30 | 4.90 |

$^{a-f:}$ Means on same column with different superscripts varied significantly (p > 0.05); WAC: weeks after cutback; DM: Dry matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin

hybrid ensiled at 4 and 8 WAC were higher than the results reported for unfertilized *Panicum maximum* cv. Ntchisi, ensiled at 4th and 12th week after cutback (Babayemi, 2009). Higher NDF content (660.00 g.kg$^{-1}$) was obtained from silage made from grass fertilized with cattle and swine manures and harvested at 8 WAC, while the least value (52.00 %) was in silage made from grass fertilized with goat manure at 8WAC. Majority of the NDF values recorded in this study were below the 650 g.kg$^{-1}$, suggested as the limit at which intake of tropical feeds by ruminants would be limited (Eastridge, 2006).

The interactive effect of manure type and harvesting age on the mineral composition of silage produced from *Pennisetum* hybrid is in Table 5. Calcium (Ca), Potassium (K), Phosphorus (P) and Magnesium (Mg) contents of the silage were significantly different (P < 0.05). Higher Ca (7.14 g.kg$^{-1}$) content was observed in the silage made from grass

**Table 5. Effect of manure type and harvesting time on the mineral composition (g.kg$^{-1}$ DM) of silage produced from *Pennisetum* hybrid grass**

| Manure type | Harvesting age | Calcium | Phosphorus | Potassium | Magnesium |
|---|---|---|---|---|---|
| | | g.kg$^{-1}$ DM | | | |
| Cattle | 4 WAC | 6.90$^{bc}$ | 2.27$^{c}$ | 22.70$^{f}$ | 1.26$^{a}$ |
| Swine | 4 WAC | 7.14$^{a}$ | 2.37$^{bc}$ | 33.20$^{b}$ | 1.16$^{b}$ |
| Goat | 4 WAC | 6.60$^{d}$ | 2.32$^{bc}$ | 20.20$^{g}$ | 1.27$^{a}$ |
| Poultry | 4 WAC | 7.06$^{ab}$ | 2.46$^{ab}$ | 34.10$^{a}$ | 1.26$^{a}$ |
| Control | 4 WAC | 7.10$^{ab}$ | 1.98$^{d}$ | 24.00$^{de}$ | 1.17$^{b}$ |
| Cattle | 8 WAC | 6.99$^{bc}$ | 2.37$^{bc}$ | 24.16$^{d}$ | 1.17$^{b}$ |
| Swine | 8 WAC | 6.84$^{cd}$ | 2.28$^{c}$ | 23.40$^{e}$ | 1.26$^{a}$ |
| Goat | 8 WAC | 6.46$^{e}$ | 2.46$^{ab}$ | 24.47$^{d}$ | 1.06$^{c}$ |
| Poultry | 8 WAC | 6.67$^{d}$ | 2.57$^{a}$ | 25.70$^{c}$ | 1.14$^{b}$ |
| Control | 8 WAC | 6.78$^{cd}$ | 1.01$^{e}$ | 20.00$^{g}$ | 1.26$^{a}$ |
| SEM | | 0.42 | 0.80 | 0.45 | 0.14 |

$^{a-d:}$ Means in same column with different superscripts are significantly (p < 0.05) different; WAC: weeks after cutback that was fertilized with swine manure at 4 WAC. The highest (34.10 g.kg$^{-1}$) and least (20.20 g.kg$^{-1}$) K contents were observed in silages that were made from the grass fertilized with poultry and goat manures, respectively, at 4 WAC. The phosphorus content of the silage, obtained from the grass fertilized with poultry manure, was significantly higher (2.57 g.kg$^{-1}$) at 8 WAC. However, the lowest (1.01 g.kg$^{-1}$ and 1.98 g.kg$^{-1}$) P contents were recorded from the silage made from grasses without manure application at 8 and 4 WAC, respectively. The range of P in this study fell within the normal requirements for growing cattle (1.1 - 4.8 g.kg$^{-1}$) (Minson, 1990). The highest Mg content (1.27 g.kg$^{-1}$) was measured in the silage made from grass that was fertilized with goat manure and harvested at 4 WAC. The Mg contents of the silage in this study were lesser than the recommended range 2.0 g.kg$^{-1}$ DM for ruminants in the tropics (McDowell and Arthington, 2005). There is a need to strengthen a *Pennisetum* hybrid silage with Magnesium in order to eliminate their deficiency in animals.

## CONCLUSION

The physical properties (colour, odour, moisture and moldiness) of *Pennisetum* hybrid silage were generally of acceptable/desirable grades for high quality silage. Crude protein contents of the silage from the grass, fertilized with either poultry or goat manures and harvested at 4 WAC, were better than the other treatments. Hence, application of manure to the grass and harvesting at tender age will provide good silage for ruminants, especially during dry seasons, when quality and quantity of forages are very low.

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# EFFECT OF HUMIC SUBSTANCES ON THE REPRODUCTION PARAMETERS OF FARMED BROWN HARE : SHORT COMMUNICATION

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## ABSTRACT

The aim of the study was to evaluate the effect of humic acids on the reproduction parameters of farmed brown hares and the possibility of their utilisation as prevention of gastrointestinal tract diseases when feeding green fodder. Animals were divided into three groups, 4 breeding pairs per group. Brown hares in the control group were fed granulated feed mixture without additives. The hares in experimental groups were fed granulated feed mixture with HUMAC Natur additive, purchased from Humacon, at 1 % concentration. The experimental group 2 was fed also green fodder during the entire experiment (April - September) in addition to the granulated feed mixture. In the experimental group 1, the ratio of live born leverets was significantly higher by 19.54 % ($P \leq 0.05$) and the ratio of weaned leverets was higher by 9.74 % compared to the control group, but no significant differences were found. The significantly highest mortality (12.50 %; $P \leq 0.001$) before weaning (28 days of age) was determined in the experimental group 1. In the experimental group 2 was the highest ratio of weaned leverets (89.19; no significant differences was found) and no increase in mortality, as a result of feeding green fodder, was determined.

**Key words:** brown hare; *Lepus europaeus*; reproduction; humic substances

## INTRODUCTION

Rapid decrease in the population numbers of wild brown hare in Europe led to expansion of its farming as one of the possible methods of strengthening the decimated populations in the intensely managed agricultural environment. However, due to the hare's behavioural characteristics as a wild game animal, brown hares farmed in cages are under increased stress and therefore also express increased sensitivity to various pathogens. Most often, losses are caused by proliferation of coccidia in the digestive track, enterocolitis and decreased resistance to bacterial infections due to stress.

As a preventive measure, fodder feed and hay were excluded in favour of feeding exclusively granulated feed mixtures. Despite this, coccidiosis is present in the farming systems and many breeders choose to treat with anticoccidial medicaments. Many farms in fact feed the hares granulated feed mixtures with added anticoccidial medicaments throughout the year. However, it is necessary to point out that the primary objective of brown hare farming should be production of animals that can adapt after release to the new environment with natural sources of nutrition. When animals from farms that use feed mixtures with added medicaments are used for restocking, mortality is high already shortly after release. One of the causes is the digestive system not adapted to the large quantities of green fodder and the collapse of "virgin" organism's immunity system.

In recent years, humic substances are ever more frequently utilized in animal nutrition. Humic substances are among the most widespread

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organic compounds. They belong to natural organic compounds, which are created by chemical and biological decomposition of organic matter, mainly plants and animals. Humic compounds are naturally present primarily in sediments, soils, peat, brown coal, lignin and some other materials (Veselá *et al*., 2005).

At present, the prevalent opinion among scientist is that humic substances have their origin in lignin. Biodegradation of lignin creates polyphenols, which likely play key role in the creation process of humic substances and are considered their precursors (Peña-Méndez *et al*., 2005).

Due to their high absorption capacity and ability to bind microbial toxins, fungal toxins and other toxic compounds, such as ammonia and polychlorinated biphenyl (PCB), dioxins, etc. humates were in the past used primarily for reduction of ammonia in stables for large livestock (Ndayegamiye and Cote, 1989; Shi *et al*., 2001).

Humic acids have significant influence on decreasing the amount of pathogens in the digestive track and function as prevention and treatment for coccidiosis in livestock. Effective utilization of antibacterial, antiviral and anticarcinogenic impact of humates in veterinary practice and animal nutrition is well-described (Thiel *et al*., 1977; Huck*et al*., 1991; Yamada *et al*., 1998; Joone *et al*., 2003).

The objective of our study was to evaluate the effect of humates, supplemented in granulated feed mixture as the only feed and in combination with green fodder, on the reproduction parameters of farmed brown hare.

**Table 1. Composition of the feed mixture used in control and experimental groups**

| | |
|---|---|
| Protein (%) | 15.00 |
| Fibre (%) | 17.00 |
| Ash (%) | 10.00 |
| Ca (%) | 0.80 |
| P (%) | 0.50 |
| Na (%) | 0.14 |
| vit. A (I.E. $kg^{-1}$) | 8000 |
| vit. D2 (I.E. $kg^{-1}$) | 800 |
| vit. E ($mg.kg^{-1}$) | 40 |

## MATERIAL AND METHODS

The experiment was conducted at a registered brown hare farm (reg. number - SK-FCH-NR- 468) at the NPPC – Research Institute for Animal Production Nitra.

Brown hares (*Lepus europaeus*, Pall.), kept in outdoor cages throughout the year were used in the experiment. Duration of the experiment was 6 months (began: 1.4.2017, finished: 30.9.2017).

Breeding pairs in the first or second reproduction year were selected for the experiment, due to variability of reproduction parameters increasing significantly in older animals.

The breeding pairs were kept in standard cages for brown hare farming sized 2 x 1.8 m. The leverets were weaned into cages of the same size at the age of 28 days. As the cages were situated outdoors, the reproduction cycle was influenced by the climatic conditions, which might have influenced the reproduction parameters as compared to other seasons.

Total of 12 breeding pairs of brown hare were included into the experiment and divided into 3 groups, 4 pairs per group:

- Control group: feed mixture with no additive
- Experimental group 1: 1.00 % additive in the feed mixture
- Experimental group 2: 1.00 % additive in the feed mixture + green fodder

Granulated feed mixture was supplemented in storage feeders *ad libitum* to all groups. During the entire experiment, the hares in the experimental groups were fed experimental feed mixture with added feed supplement – HUMAC Natur (Humacon, s.r.o., Košice, Slovakia) with high ratio of humic acids at 1 % concentration. Nutrient composition of the feed mixture is presented in Table 1.

The evaluated commercial feed additive (Humacon, s.r.o., Košice, Slovakia) is a brown and black powder on the basis of oxihumolit. It is biologically and pharmacologically highly active 100 % natural substance. Usually, it is applied in small doses to feed mixtures for all animal species. Application of this additive prevents diseases (especially diarrhea) or corrects existing disorders due to its wide-range effect on the organism as a whole. It has detoxical, antiseptic and fungicidal effects. The active ingredients in the additive are humic acids, fulvic acids, minerals, trace elements and carboxymethylcellulose complex with humic acids. Composition of the additive is presented in Table 2.

In the experimental group 2, green fodder was fed at least 6 times a week at dose of 0.5 - 1.00 kg depending on the number of reared leverets. Due to the necessity to use fresh green fodder, green fodder was not supplemented when the weather conditions (rainy days) prevented it. Selected as green fodder was a mixture of red clover, white clover, alfalfa, *Lotus corniculatus*, meadow fescue, red fescue, orchard grass and perennial ryegrass.

During the entire experiment, the animals had *ad libitum* access to drinking water supplied through stainless steel drinker.

In the experiment, selected reproduction parameters were monitored: ratio of live born leverets (%), mortality before weaning at 28 days of age (%), and ratio of weaned leverets (%).

**Statistics**

Statistical analysis of the results was performed using $\chi^2$ test and the t-test with the level of significance at P-values of less than 0.05; 0.01 and 0.001. The result are presented as means ± standard deviation. The statistical package SAS 9.1 (SAS, 2003) was used for the analysis.

**Table 2. Composition of the HUMAC Natur additive in 100 % dry mater**

| | |
|---|---|
| Humic acids (%) | 62.00 |
| Unbound humic acids (%) | 48.00 |
| Fulvic acids (%) | 9.00 |
| Minerals and trace elements (%) | 9.00 |

## RESULTS AND DISCUSSION

Of the total of 12 breeding pairs included in the experiment, one pair from the control group was excluded due to the female's infertility. Infertility in one animal in a pair is encountered on average in 5 to 10 % of farmed brown hares. Similar ratio of infertile animals was determined in the evaluation of farms in 2016 (Sládeček *et al.*, 2016).

Table 3 shows the results for reproduction parameters in each group. The total number of litters in the control group was 12, on average 4 ± 0.00 litters per breeding pair. Similar number of litters was determined in experimental group 1, where the average number of litters per breeding pair was 4 ± 0.00 as well. The lowest number of litters was determined in the experimental group 2: total of 15 litters, which represents 3.75 ± 0.50 litters per breeding pair on average.

In the control group, 37 leverets were born during the experiment, at average 12.33 ± 3.06 per breeding pair. Of the total number of born leverets, 21.62 % were dead born. In the experimental group 1, 48 leverets were born, 12 ± 1.63 per breeding pair at average, with a large number of live born leverets (97.92 %). Compared to the control group, the number of dead born leverets was lower by 19.54 % (P ≤ 0.05).

Similarly, lower number of dead born leverets was determined also in the experimental group 2, where from the total of 37 born leverets only 3 leverets were dead born (8.11 %). Other authors also determined lower mortality on different species of animals. Árvayová *et al.* (2012) determined in experiments on rabbits lower mortality before weaning by 0.76 % in the experimental group fed

**Table 3. Comparison of the reproduction parameters among the groups**

| | Total number of litters | Number of born: Total (pc) | Number of born: Live born (%) | Number of born: Dead born (%) | Mortality before weaning (%) | Weaned leverets (%) | Significance of differences |
|---|---|---|---|---|---|---|---|
| Control | 12 | 37 | 78.38 | 21.62 | 2.70 | 75.68 | |
| Exper. 1 | 16 | 48 | 97.92$^{*}$ | 2.08$^{***}$ | 12.50$^{***}$ | 85.42 | $\chi^2$ test |
| Exper. 2 | 15 | 37 | 91.89 | 8.11$^{**}$ | 2.70 | 89.19 | |

$^{*}$P ≤ 0.05; $^{**}$P ≤ 0.01; $^{***}$P ≤ 0.001

feed mixture enriched with humates. Increase in live weight gain by 5-7 % and decrease in mortality by 3-5 % was determined by Stepchenko *et al.* (1991) in broiler chickens (up to 22 days old) after application of peat-based supplements at 0.25 % concentration in feed.

The highest mortality (12.50 %; P ≤ 0.001) before weaning (28 days of age) was determined in the experimental group 1, where losses appeared in four litters with higher number of leverets. Mortality during rearing in the control group and the experimental group 2 was at the same level of 2.70 %, which represents one leveret dying in each group during the experimental period.

Additive of Humacid 60 (natural humic substances) at 0.5 % concentration, applied to weaned piglets at the age of 25 to 70 days, had a positive influence on weight gain and decrease of mortality rate and added at 0.7 % concentration to complete feed mixtures for broiler chickens, it increased the growth intensity, while decreased feed consumption and mortality rate (Vaško *et al.*, 2007).

The evaluation of the number of weaned leverets out of the total number of born leverets shows positive impact of the tested additive on this parameter. While in the control group the ratio of weaned leverets was only 75.68 %, in the experimental group 1 this ratio was higher by 9.74 % and in the experimental group 2 - higher by 13.51 %, however these results were not significantly different.

Significant effect of humate application was noticed in relation to higher number of live born leverets in the experimental group 1 (P ≤ 0.05) and lower number of dead born leverets in experimental group 1 (P ≤ 0.001) and experimental group 2 (P ≤ 0.01).

According to Dabovich *et al.* (2003) a humin acid product Promax has nutraceutical properties in that it stimulates neutrophil activity, which may protect against bacterial pathogens and reduce mortality during acute bacterial infection. Islam *et al.* (2005) found that humin acid has many beneficial effect like antibacterial, antiviral and anti-inflammatory in animals, improves immune system, stress management and reduces odour in faeces. It also has positive effect on liver functioning. Ultimately reduces mortality and increases growth in poultry. Edmonds *et al.* (2014) describes that mortality was numerically reduced (-36 %) from broilers fed the diets supplemented with humin acid compared with control diet.

In our experiment, we have not confirmed a positive effect of the application of humates in relation to mortality before weaning.

## CONCLUSION

The results of our study show positive influence of humic substances on the reproduction parameters of farmed brow hare. In the experimental groups supplemented with humates (HUMAC Natur) the ratios of both live born leverets and weaned leverets was higher than in the control group.

The results suggest the possibility of successful feeding with green fodder in combination with complete granulated feed mixtures with humic additive. The highest ratio of weaned leverets was in the experimental group 2. In this group no increased mortality due to enterocolitis or coccidiosis, as a result of feeding green fodder feed, was determined.

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