

Haematological and histological characteristics of free ranged sasso chicken fed with non-conventional feedstuffs

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ABSTRACT

A study was conducted to evaluate the hematological, histological and anthelmintic effects of non-conventional feedstuff on ranged sasso chicken. A total of 150 sasso chicken were divided to 5 groups and fed with 4 formulated diets with 5% of betel, sword fern, oregano and tawa-tawa leaf meals and 1 control diet for 8 weeks. The study was conducted at ISU, College of Agriculture Student Instructional Unit. The study was laid out in a Completely Randomized Design (CRD). Results of the study show that in the hematological analysis, comparable data were seen in the WBC, RBC, Hemoglobin, Hematocrit and MCH. However, in the MCV, the birds fed with 5% tawa-tawa had the lowest result at 104.53fL but is within the normal range. Also, 5% betel leaf gave the highest result in platelet at 140.50 $10^9/L$ followed by 5% swordfern 80.50 $10^9/L$. Comparable data were observed in jejunal surface area of villi. However, significant difference was observed in the crypt depth. 5% tawa-tawa had the highest result at 26603.11 μm . The non-conventional feedstuff had no effect in the elimination of intestinal parasites. Use of non-conventional feedstuff as an additive to the poultry diet is suggested. However, to have a conclusive result on the histology of the intestines, it is recommended that samples of all the segment of the small intestines should be collected. It is also recommended that a concentrated solution of the local feedstuff such as leaf extract or methanol extract be used for further studies.

Key words: Sasso chicken, Free range, Non-conventional feedstuff, Hematology and histology of chicken

INTRODUCTION

Medicinal herbal and aromatic plants have been a gaining interest in recent years as feed additives in poultry. Medicinal herbs can alter the active compounds in final products depending on the part used such as the seed, leaf, root, or bark and the processing technique could be extraction with non-aqueous solvents (Khalid *et al.*, 2022; Ammar *et al.*, 2018).

Several studies in recent years have shown that the use of medicinal herbs as feed additives can improve the health, performance, and nutrient digestibility of sasso chickens (Yazdi *et al.*, 2014).

In order to evaluate the effects of plants on the health of animals, studies on the Haematological parameter measurement provides valuable information in human and animal medicine. Unfortunately, due to lack of information of such parameters, blood profile is not used widely in avian medicine (Krail and Suchy, 2000). Although there is limited information in this field, evaluation of blood profiles of sasso strains have been assessed by several studies (Hauptmanova *et al.*, 2002). Haematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. It serves as indicator of physiological, pathological and nutritional status of an animal (Okoruwa and Ikhimiyoa, 2014).

The perception of consumers on raw and cooked meat quality has created significant interest in increasing the understanding of digestive physiology and the dynamics of the gut microflora (Dibner and Richards, 2005). Physiological studies reveal that a functional gastrointestinal tract is vital for the digestion and absorption of nutrients required for the bird's maintenance and growth (Mateos *et al.*, 2002; Baurhoo *et al.*, 2009).

The small intestine is an important organ responsible for the digestion and absorption of nutrients from the diet. Any changes in its function affect the function of other organs and systems in the organism (Toman *et al.*, 2015). It also acts as the largest immunological organ in the body, as it is the first point of protection against exogenous pathogens that enter the body, preventing the pathogens from colonization and entering the host cells

and tissues (Choct, 2009). A balanced gut microorganism population, consisting of less pathogenic bacteria, and an increase in beneficial bacteria, may increase the availability of nutrients (Hashemi and Davoodi, 2010), presence of any intestinal parasite may decrease the absorption of nutrients and greatly effect the growth performance.

Tawa tawa leaves contain bioactive compounds like phenolics and flavonoids (Cruz, 2001). The bioactive components of Oregano leaves are tannins, flavonoids, phenols, thymol, carvacol and are rich in vitamins, calcium, copper, iron, magnesium and thiamine (Kumar *et al.*, 2017). Swordfern is rich in alkaloids, triterpenoids, flavonoids, vitamins and minerals (Cao *et al.*, 2017). Betel leaf contains biophenolics such as eugenol, peperols, hydroxychavicol and chavibetol (Kumar *et al.*, 2016). These compounds have anti oxidant properties, anti-inflammatory and antiviral properties which are used as herbal drugs or herbal medicine.

Generally, the study was conducted to determine the effects of Tawa-tawa, swordfern, oregano and betel leaf meals on the hematological values, its effects on the histology of the jejunum and its anthelmintic properties when given as an additive to ranged sasso chicken diet.

MATERIALS AND METHODS

Poultry production management

The experimental range type house was made of locally available materials such as galvanized iron, lumber, fish net, and black canvas. The experimental house was 18.0 x 12.0 meters in size and was divided into 15 pens measuring 5.5 x 3.0 meters each. Before the chicks arrive, the facility was cleaned and disinfected to prevent disease caused by harmful microorganisms. A total of one hundred fifty (150) sasso chicks was used in the study. They were purchased from a reliable hatchery.

The feed ingredients

The fresh leaves of various locally available feedstuffs were air dried and ground into powder before being carefully weighed using a digital weighing scale and thoroughly mixed with feed formulation using the appropriate amount of the various locally available feedstuff for each treatment.

Table 1. The nutrient and calculated analysis of starter formulated feed used in the study

Ingredients/Calculated analysis	T1	T2	T3	T4	T5
Betel leaf	-	5.00	-	-	-
Sword fern leaf	-	-	5.00	-	-
Oregano leaf	-	-	-	5.00	-
Tawa-tawa leaf	-	-	-	-	5.00
Yellow corn	55.19	56.00	56.09	55.80	55.44
Rice bran (D1)	5.00	-	-	-	-
Soybean meal	26.80	25.50	25.20	26.00	25.20
Fish meal (60)	5.00	5.00	5.00	5.00	5.00
Molasses	5.00	4.30	4.50	4.20	4.50
DL Methionine	0.04	0.05	0.06	0.06	0.06
Crude coconut oil	-	1.10	1.10	1.10	1.20
Dicaphos	1.07	1.15	1.15	1.14	1.10
Limestone	0.70	0.70	0.70	0.60	0.60
Salt	0.50	0.50	0.50	0.50	0.50
Min/Vit (Afsilin)	0.50	0.50	0.50	0.50	0.50
Toxin binder	0.20	0.20	0.20	0.20	0.20
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
Crude protein, %	20.11	20.12	20.10	20.10	20.10
M.E. (Kcal)	2835	2829	2829	2829	2835
Calcium, %	0.88	0.88	0.88	0.88	0.88
Phosphorus, %	0.42	0.42	0.42	0.42	0.42
Crude fiber, %	3.71	3.87	3.86	3.89	3.86
Lysine, %	1.12	1.06	1.05	1.07	1.08
Methionine, %	0.41	0.41	0.41	0.41	0.41

Table 2. The nutrient and calculated analysis of finisher formulated feed used in the study

Ingredients / Calculated analysis	T1	T2	T3	T4	T5
Betel leaf	-	5.00	-	-	-
Sword fern	-	-	5.00	-	-
Oregano	-	-	-	5.00	-
Tawa-tawa	-	-	-	-	5.00
Rice bran	10.0	5.00	5.00	5.00	5.00
Yellow corn	54.0	54.93	55.18	54.28	54.50
Soybean meal	26.8	25.50	25.20	26.00	25.20
Fish meal (60)	3.00	3.00	3.00	3.00	3.00
Molasses	5.00	3.80	3.80	3.70	3.70
DL Methionine	0.04	0.05	0.06	0.06	0.06
Coconut oil	-	1.25	1.20	1.35	1.30
Dicaphos	1.20	1.30	1.30	1.25	1.25
Limestone	0.60	0.60	0.60	0.60	0.60
Salt	0.30	0.30	0.20	0.30	0.30
Min/Vit	0.50	0.50	0.50	0.50	0.50
Toxin binder	0.20	0.20	0.20	0.20	0.20
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
CP %	18.7	18.8	18.85	18.81	18.79
M.E. Kcal.	2823	2828	2828	2824	2828
Calcium, %	0.79	0.798	0.796	0.790	0.798
Phosphorus	0.398	0.40	0.40	0.394	0.395
CF %	3.90	4.05	4.05	4.06	4.07
Lysine, %	1.02	0.96	0.96	0.97	0.98
Methionine	0.34	0.34	0.34	0.34	0.34

A formulated ration (Table 1 and 2) was used throughout the study. The ingredients are corn meal, rice bran, fish meal, salt, molasses, vitamins, limestone and ground various locally available feedstuff. After mixing, it was fed to the chicks with corresponding amount of various locally available feedstuffs for a period of time for 8 weeks.

Manual mixing of feeds was done. The ingredients with small amount will be mixed separately with the large amount. After thorough mixing of ingredients, the small amount and large amount was then mixed well with a shovel. For Treatment 1 pure formulated ration, Treatment 2, 5 % betel leaf meal, Treatment 3, 5 % sword

fern leaf meal, Treatment 4, 5 % oregano leaf meal and Treatment 5, 5% tawa-tawa leaf meal.

On the first week of feeding, the chicks were fed in an old newspaper after which a plastic feeder was used for feeding for the remaining weeks of the study. *Ad libitum* feeding was practiced throughout the study. Clean and fresh drinking water was given and was changed two times a day.

Experimental design and treatment

The birds were randomly distributed into 5 treatments; each treatment was replicated 3 times with the total of 15 experimental units with ten birds in every replication. The experiment was laid out using the Completely Randomized Design (CRD) with the following treatments:

The treatments were as follows:

T₁ – Pure formulated feeds

T₂ – Formulated feeds with 5% betel (leaf meal)

T₃ – Formulated feeds with 5% Sword fern (leaf meal)

T₄ – Formulated feeds with 5% oregano (leaf meal)

T₅ – Formulated feeds with 5% Tawa tawa (leaf meal)

Blood collection and haematological analysis

At the end of eight (8) weeks, two ranged sasso chicken were randomly selected from each replicate, starved of feed overnight but not water. From the selected birds, 0.5 ml blood sample was collected via the wing vein with a 3 ml sterile syringe. Once the sample was collected, it was immediately transferred to a microtube containing ethylene diaminetetraacetic acid (EDTA) as the anticoagulant to prevent the blood from clotting. The blood samples collected was brought to the AR Veterinary Clinic at Bulanao, Tabuk City, Kalinga for haematological assessment using a CBC machine. Blood values such as Hematocrit (Hct), Hemoglobin (Hgb), Platelet (Plt), Red blood cell count (RBC), White blood cell count (WBC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) were taken. Using the CBC machine minimizes the chance of errors occurring as compared to manual counting.

Sample collection of intestines

At 8 weeks of age, two ranged sasso chicken were randomly selected per treatment and fasted with feed for

six hours, and water was offered *ad libitum*. The ranged chicken was exsanguinated by severing the right common carotid artery and external jugular vein. After bleeding, scalding, plucking, and washing followed, the feet and head were removed. The internal organ was removed from the carcass and was placed in a container and was labeled.

The jejunal segment (most site of nutrient absorption) of the small intestine was identified for the collection. The collection site was approximately 5 inches after the duodenal loop for uniformity. Samples was obtained by making a transverse cut in the jejunum with a 3-cm thickness using a scalpel blade. This segment of the intestine was selected since the jejunum serves as the site where most nutrients are absorbed.

The collected samples were placed in a tissue cassette, labeled accordingly, and was placed in a glass container with 10% formalin. The formalin will serve as a fixing and preservative media until samples are ready for processing. Samples was sent to Pines City Hospital Pathology Department, Baguio City for further processing where they are prepared following the paraffin embedding technique, samples are cut into 5-micron thick using the microtome and was stained using hematoxylin-eosin stain. Stained slides are then labeled accordingly and were examined for villus height.

Measurement of intestinal villi

The histological sections were evaluated using standard light microscopy. Measurements were made from photomicrographs taken at 100× magnification. Major changes can occur in the intestine due to postmortem interval and fixation time and considerable variation in the quality of sections can occur. It is important to minimize these effects and to use only those regions of the intestinal sections presenting good morphology. Therefore, the length of both the villi and crypt regions three adjacent to each other were measured for each sample. From the same photomicrograph regions, measurements of areas for a determined length of the intestinal segment were made for corresponding total mucosa and crypt regions. Villi measurements were taken, the villus height or length was obtained by measuring the distance from the apex to the base of the villi.

Fecal sample collection and processing

Fecal samples were collected directly from the range where the birds are staying; random sampling was done to collect a good sample size. Debris like grass, feather and soil was removed. Sampling was done in the morning to prevent samples from desiccating. Collected samples were placed in a plastic cup with cover and were labeled accordingly and were placed in a cooler and were later transferred to a refrigerator to keep the sample cool and prevent the eggs from hatching.

The fecal samples were brought to the AR Veterinary clinic for further processing. A concentrated sugar solution was prepared using 1 liter of water and 400 grams of table sugar.

Samples were taken out from the cooler and were mixed thoroughly to make the samples homogenous and was weighed using a digital weighing scale, a 2-gram feces were collected and was placed in a 100 ml beaker. 50 ml sugar solution was added to the sample and was mixed thoroughly using a porcelain spatula. The solution was then filtered through a wire mesh into a separate beaker to separate the debris from the solution. The solution was set aside to rest and allow the parasite eggs to float to the surface before withdrawing a sub-sample. Using a pipette, samples were withdrawn from the surface of the solution and were transferred to the first compartment of the McMaster counting chamber. This was repeated until the second chamber was also filled with the sample. The counting chamber with the sample was allowed to rest for 5 minutes to allow the eggs to float to the surface and the debris to go to the bottom of the chamber.

Microscopic parasite evaluation

The method used to measure the Egg Per gram adapted by the researcher is the McMaster egg counting technique. The McMaster technique is used for demonstrating and counting helminth eggs in fecal samples. It is the most widely employed method for this purpose.

The McMaster chamber was placed under a microscope and was examined using 4X10 magnifications (scanner) for counting the parasite eggs. When a parasite egg is seen, the magnification was switched to 10X10 magnifications (low

power objective) to further identify the parasite egg. The Egg Per Gram (EPG) will be calculated based on the number of parasite ova observed under the counting chamber.

Identification of each parasite egg was observed under the low power objective at 100X. The parasite ova were examined for its morphology and are identified until the genus level only.

Data analysis

All data gathered was tabulated and analyzed using the Analysis of Variance (ANOVA) following the Complete Randomized Design (CRD) using the Statistical Tool for Agricultural Research (STAR). The Least Significant Difference was used to compare treatment mean difference.

RESULTS AND DISCUSSION

Haematological characteristics

Haematological values of poultry are influenced by sex, breed, climate, geographical location, season, day length, and times of day, nutritional status, life habit of species, and such other physiological factors (Talebi *et al.*, 2005). Results on the Haematological characteristics of sasso chicken fed with non-conventional feedstuff are shown in Table 3.

Tawa tawa leaves contain bioactive compounds like phenolics and flavonoids (Cruz, 2001). The bioactive components of Oregano leaves are tannins, flavonoids, phenols, thymol, carvacol and are rich in vitamins, calcium, copper, iron, magnesium and thiamine (Kumar *et al.*, 2017). Swordfern is rich in alkaloids, triterpenoids, flavonoids, vitamins and minerals (Cao *et al.*, 2017). Betel leaf contains biophenolics such as eugenol, piperols, hydroxychavicol and chavibetol (Kumar *et al.*, 2016). These compounds have anti-oxidant properties, anti-inflammatory and antiviral properties which are used as herbal drugs or herbal medicine.

White blood cell counts (WBC): The above table shows a comparable result across the different treatments. The results range from 24.10 to 26.92 $\times 10^9/L$ which is within the normal range for chicken. Normal WBC counts in chickens ranged from 20 to 30 $\times 10^3/mm^3$ (Astuti *et al.*, 2014). This is a sign that the chickens are healthy.

Table 3. Haematological characteristics of the ranged sasso chicken

Treatments	Haematological parameters						
	WBC x10 ⁹ /L	RBC x10 ¹² /L	HGB g/L	HCT %	MCV fL	MCH pg	PLT 10 ⁹ /L
T1	24.10	2.04	8.87	22.53	110.90 ^a	43.68	25.33 ^b
T2	26.08	2.66	12.90	29.28	110.13 ^a	48.28	140.50 ^a
T3	26.34	2.29	11.37	25.48	111.40 ^a	49.13	80.50 ^{ab}
T4	26.92	2.16	9.28	23.78	110.02 ^a	42.82	23.00 ^b
T5	25.19	2.33	9.72	24.35	104.53 ^b	41.63	28.67 ^b
ANOVA	ns	ns	ns	ns	**	ns	**
C.V. (%)	5.63	14.13	16.11	14.32	1.61	8.46	52.91
LSD 0.01					4.54		81.60

Note: Means with the same letter are not significantly different. ns= not significant, **= Highly significant at 1% level

Red blood cell counts (RBC)

The RBC values of the ranged Sasso chicken were comparable with each other with a mean ranging from 2.04 to 2.66 x10¹²/L. Astuti *et al.* (2014) reported that the normal haematological values for RBC in broiler chicken is 2.5-3.5 x 10⁶/mm³. In this study, the RBC indices were still within the usual range for chickens.

Haemoglobin concentration (Hgb)

The results revealed comparative values across the different treatments on the ranged chicken fed with locally available feedstuff, they have an average haemoglobin value ranges from 8.87 to 12.90 g/L, and these figures are classified normal.

Hematocrit (%)

Similar with other blood parameters, hematocrit results of the ranged sasso chicken were not affected by the different locally available feedstuff. The range of the average number of broilers hematocrit in this study ranged from 22.53 to 29.28 %. According to Sgavioli *et al.*, 2019, normal range hematocrit values in broilers range from 24-43%.

Mean corpuscular volume (MCV)

The MCV value ranges from 104.53 to 111.40 fL. Among the treatments, Treatment 5 has the lowest value at 104.53 which is significantly different to the first 4 treatments. MCV value in treatments 4, 2, 1 and 3 obtained higher value as compared to treatment 5. Londok and Rompis (2021) MCV normal values in chicken ranged from 90-140 fl. Although treatment 5 has the lowest value, it is still within the normal range. This is influenced by the ambient temperature (Biswas *et al.*, 2011). In addition, chickens in tropical countries are

susceptible to seasonal fluctuations in hot temperatures, and MCV can rise as a result of heat stress (Mohamed *et al.*, 2012).

Mean corpuscular haemoglobin (MCH)

The result showed that the values are comparable from the different treatments. The MCH value ranges from 41.63 to 49.13 pg. The normal MCH of chickens obtained in this present study is similar to the findings Londok and Rompis (2021) ranged from 33-49 pg. Pandian *et al.* (2012) said that MCH was used to determine the RBC's Hb content, whereas the MCHC value was used to determine the RBC's Hb concentration

Platelet

In terms of the platelet of the ranged sasso chicken, significant differences were observed from the different treatments. The formulation with Betel leaf (T2) has the highest value at 140.50 x10⁹/L followed by treatment 3 (T3) with swordfern at 80.50 x10⁹/L. Treatments 1 (control), Treatment 4 (T4) with oregano and Treatment 5 (T5) with tawa-tawa, were comparable to each other.

Histological characteristics

The surface area of villi and crypt depth of the jejunum of ranged Sasso chicken fed with locally available feedstuff diet is presented in Table 4.

Comparable data were observed in jejunal surface area of villi; however, a significant difference was observed in the crypt depth of the ranged Sasso chicken. Nonetheless, the ranged Sasso chicken fed with 5% oregano leaf meal have the greatest surface area of villi among treatments with a mean value of 37593.63 μm (T4) while in Treatment 1, sasso in the control group obtained the lowest surface

area of villi with a mean value of 26554.86 μm . An increase in villi length results in an increase in surface area, and therefore more area for absorption of nutrients to take place (Parsaie *et al.*, 2007; Saeid *et al.*, 2013).

In terms of jejunum crypt depth, significant differences among treatments were observed. Statistically, birds in treatment 5 fed with Tawa – tawa leaf meal obtained a deeper crypt depth mean value of 26603.11. However, birds in treatments 4, 2, 3 and 1 obtained comparable crypt depth with mean value of 18760.77, 17554.26, 12909.45 and 12030.28, respectively.

An increase in crypt depth and a decrease in villi height can lead to increase secretions into the gastrointestinal tract, resulting in diarrhea, a decrease in disease

resistance, and a decreased animal performance (Parsaie *et al.*, 2007; Catalá-Gregori *et al.*, 2008). Deep crypts are a sign of a high turnover of cells along the villi, and high demand on the crypts to produce new cells for villi growth (Xu *et al.*, 2013). Enterocytes are damaged by pathogen bacteria in the digestive tract, which leads to an increase in crypt depth (Parsaie *et al.*, 2007). High demand for tissue turnover results in an increase in the energy requirements for maintenance of the digestive tract (Choct, 2009). Shallower crypts are associated with a lower tissue turnover and therefore less demand for new tissue. This also results in fewer enterocytes in the secretory stage, therefore fewer secretions, and more villi enterocytes along the longer villi with absorptive functions, resulting in better nutrient absorption (Saeid *et al.*, 2013).

Table 4. Histological evaluation of jejunal intestinal surface area of villi and crypt depth of the free ranged sasso chicken

Treatments	Jejunum	
	Surface area of villi (μm)	Crypt depth (μm)
T ₁ - Control	26554.86	12030.28 ^b
T ₂ - Formulated Ration with 5% BLM	26596.89	17554.26 ^b
T ₃ - Formulated Ration with 5% SFLM	33010.88	12909.45 ^b
T ₄ - Formulated Ration with 5% OLM	37593.63	18760.77 ^b
T ₅ - Formulated Ration with 5% TLM	35824.95	26603.11 ^a
ANOVA	ns	**
C.V. (%)	17.27	16.37
LSD 0.01		7441.42

Note: Means with the same letter are not significantly different. ns= not significant, **= Highly significant at 1% level

Previous studies have already confirmed that longer intestinal villi indicate an improved ability to absorb nutrients in the intestine (Caspary, 1992; Awad *et al.*, 2006). In addition, it has been proven that longer villi are associated with active cell mitosis, which provides a greater absorptive potential of villi for various nutrients (Onderci, 2006). Deeper intestinal villi crypts indicate a rapid metabolism of tissue in order to allow the renewal of the intestinal villi, if there is a need for its regeneration (Hamedi *et al.*, 2011). Lowering the height of the villi or reducing crypt depths of intestinal villi may lead to a reduction in the absorption of nutrients (Saeid *et al.*, 2013).

Fecal egg count

Table 5 shows the result of the McMaster egg count for the EPG of the samples on the fourth week and eighth week. On

the 4th week of collection, Treatments 1, 2 and 5 were positive for parasite ova. This is due to the life cycle of roundworm where the prepatent period is 3-4 weeks. The prepatent refers to the time the bird ingests the infective stage of the parasite until the time it is able to reproduce and produce eggs. Thus, in the first 4 weeks, the parasite is still developing into adults thus the interpretation of the result for the first test gives us a negative to low result.

In the 8th week of collection, all of the samples came back positive for eggs because the parasites inside the birds are all mature and are able to produce eggs. The parasites identified were *Ascaridia galli* and *Capillaria* spp. Both parasites are roundworm but have different life cycle, the life cycle of *Ascaridia* spp. is direct and does not require an intermediate host, as for *Capillaria* spp., it needs an earthworm for its life.

Table 5. Average fecal egg count of ranged sasso chicken fed with non-conventional feedstuff

Treatments	EPG on 4 th week	EPG on 8 th week
T1	50	400
T2	50	250
T3	0	350
T4	0	200
T5	50	200

The phytochemicals found in plants that have anthelmintic effects are the polyphenols, tannin, flavonoids and saponins which acts synergistically to kill worms. A study conducted by Justin *et al.*, 2019 showed that by using the ethanolic extract by concentrating the flavonoids and other phytochemicals found in plants will result in their anthelmintic activity. The plants used in the study do have flavonoid and tannins but were only fed to the experimental animals on low amounts, thus not meeting the higher concentration in order for the phytochemicals to be effective.

The results imply that despite adding the locally available feedstuff on the diet of the sasso chicken, it can be concluded that it has no effect on the elimination of the intestinal parasites of the experimental birds.

CONCLUSION

The study evaluated the effects of non-conventional feedstuffs, namely betel leaf, swordfern leaf, oregano leaf, and tawa-tawa leaf meals, on the hematological characteristics, intestinal histology, and anthelmintic activity in free-range Sasso chickens. Results revealed that the inclusion of these locally available feed resources generally produced comparable hematological values in terms of white blood cell (WBC), red blood cell (RBC), hemoglobin, hematocrit, and mean corpuscular hemoglobin (MCH).

However, significant differences were observed in mean corpuscular volume (MCV) and platelet count. Birds fed with 5% tawa-tawa leaf meal recorded the lowest MCV value (104.53 fL), although the value remained within the normal physiological range for chickens. Meanwhile, birds fed with 5% betel leaf meal exhibited the highest platelet count ($140.50 \times 10^9/L$), followed by those fed with 5% swordfern leaf meal ($80.50 \times 10^9/L$), suggesting possible hematopoietic effects of these plant materials.

In the histological evaluation of the jejunum, comparable results were observed in the villi surface area among treatments. Nevertheless, significant variation was noted in crypt depth, where birds fed with 5% tawa-tawa leaf meal showed the deepest crypts (26,603.11 μm). This finding may indicate increased intestinal tissue turnover and regenerative activity.

The fecal egg count results further indicated that the non-conventional feedstuffs used in the study had no significant effect on the elimination of intestinal parasites in free-range Sasso chickens. Although the plants contain bioactive compounds with reported antiparasitic properties, the concentration included in the diets may not have been sufficient to produce effective anthelmintic activity.

Overall, the study demonstrated that locally available plant-based feed additives can be utilized in poultry diets without adverse effects on the hematological and intestinal health of free-range Sasso chickens.

FUTURE WORKS

Future studies should focus on the use of concentrated extracts of betel leaf, swordfern leaf, oregano leaf, and tawa-tawa leaf in order to maximize the potential effects of their bioactive compounds. Methanolic or ethanolic extracts may provide higher concentrations of phytochemicals such as flavonoids, tannins, phenolics, and saponins, which could enhance both hematological and anthelmintic responses.

Further biochemical characterization of these locally available feed resources is also recommended to identify and quantify their active compounds. This would help explain their observed effects on platelet count, mean corpuscular volume, and intestinal morphology.

To obtain more comprehensive histological data, future research should include the collection and examination of all segments of the small intestine, including the duodenum, jejunum, and ileum. This would provide a clearer understanding of the influence of non-conventional feedstuffs on intestinal development and nutrient absorption.

Additional studies are likewise recommended to evaluate the anthelmintic efficacy of concentrated leaf extracts against intestinal parasites in free-range chickens. Moreover, the use of methanolic extracts of these feed resources as dietary supplements in native chicken production systems should also be explored.

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