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Research Article
Effect of Sequential Feeding with Variations in Energy and Protein Levels on Performances of Sasso Broilers Under Hot and Humid Climate

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Abstract
Objective: This experiment was conducted to investigate the effect of two diets varying in energy and protein (one energy-high-protein-low and one energy-low-protein-high) on performances of Sasso broilers under tropics when offered sequentially. **Materials and Methods:** A total of 525 Sasso broiler chickens of 3 week of age were assigned into 3 treatments with 5 replicates of 35 birds each. The treatments were: broiler chicken fed (1) Basal diet (A group: control), (2) High energy and low protein diet (E^+P^- diet) in morning and low energy and high protein diet (E^-P^+ diet) in the afternoon (B group) and (3) E^-P^+ diet in morning and E^+P^- diet in the afternoon (C group). At 11 week of age, 30 chickens per treatment were slaughtered to collect blood, abdominal fat, gizzard, liver, heart, kidney, carcass, thigh, breast and intestine. Ultimate pH (pHu) and blood serum concentrations in glucose, triglycerides, total cholesterol, total protein, urea and creatinine were also assessed. **Results:** Results indicated that feed intake, water intake, body weight, body weight gain, mortality, feed conversion ratio and pHu were similar among treatment groups. However, intestinal length and carcass yield of chickens fed sequentially (B and C) were significantly higher ($p<0.05$) and their serum creatinine concentration was significantly lower ($p<0.05$)than those of control group. Abdominal fat, liver weight and serum triglycerides of chickens of B group were significantly lower ($p<0.05$) than those of A and C group. **Conclusion:** It was concluded that sequential feeding improved intestinal length, carcass yield weights and serum creatinine and triglycerides of Sasso chickens.

Key words: Sequential feeding, Sasso broilers, tropical climate, production performance, welfare

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Over the past two decades, global animal production has increased, especially in tropical and subtropical areas[1] with a great expansion of modern broiler industry[2]. Feed related costs are the main drivers of profitability of commercial poultry farms[3,4]. For instance, the broilers' energy requirements are responsible for 70% of the cost of the ration[5]. Intensive selection focused on post-hatch growth rate and feed conversion has increased meat yield in broilers[6]. Thus, fast-growing broilers fed with complete diet are reared to reach 2.5 kg of body weight at five to seven weeks of age[7]. The high growth rate of these broiler chickens is associated with health and meat quality disorders. The heart and lung of the birds, are hardly able to support the fast growth rate, thereby exposing them to hypoxia leading to the incidence of ascites and mortalities ranging from 5-7% and large-scale condemnation of carcass quality at slaughter[8,9].

Strategies to reduce these disorders have focused on manipulation of rearing environment, genetic and feeding. Sasso broilers are a slow growing chicken. Yakubu and Ari[10] reported low growth rate and low feed efficiency of Sasso broilers fed with complete diets. Improving feed efficiency is an important goal in their production[11]. Feeding methods such as skip a day, feed restriction, choice feeding and feed with low energy[12] have been used without solving the problems especially in tropical zone probably because of the harsh climatic conditions[13]. Alternating different diets during the day (termed 'sequential feeding') has been studied for nutritional purposes with fast growing chickens[14,15] and this has been reported to be effective in reducing mortality under acute heat challenge during the finishing period[16], improve welfare by reducing the occurrence of leg abnormalities[17,18] and to have no negative impact on weight[19]. Sequential feeding as a type of qualitative feed restriction has been reported to be effective in control of fast-growing bird's growth[17].

But it is questionable whether such feeding method plays a role in improving feed efficiency and growth rate in slow growing broiler chickens in hot and humid climate. The main objective of this study was to evaluate the effect of two unbalanced diets in energy and protein offered in sequential feeding on performances of Sasso broiler chickens under hot and humid climate.

MATERIALS AND METHODS

Experimental design: A total of 525 Sasso day old chicks were used for this study. During the first 3 week of age (starter phase), all chicks received the same starter diet (3100 kcal kg^{-1} of ME; 21% CP). At the beginning of growing phase (4th week), 525 chickens were weighed and assigned to three treatment groups of 175 chickens each with similar average body weights: A (control group) birds that were fed basal diet, B (the birds that were fed high energy and low protein diet (E^+P^- diet) in the morning and low energy and high protein diet (E^-P^+ diet) in afternoon and also, C birds which received E^-P^+ diet in the morning and E^+P^- diet in afternoon. Each treatment was divided into five replicates of 35 birds each. The birds were reared until 11 week of age. Feed was provided two times daily: 7.00 AM and 1.00 PM. Chickens were reared in an opened poultry house on deep litter system with a density of 5 birds m^{-2}. Water was supplied *ad libitum*. Light was provided at natural photoperiod of 12L/12D. The temperature recorded was between 25.2 and 33°C with average of 27.02°C while the relative humidity varied from 64-89% with average of 74.65%.

During rearing, dead birds, weekly feed intake, daily water intake and weekly body weights were recorded. At the end of 11 week of age, blood samples were collected from 30 broilers per treatment for blood parameters determination. Thereafter, the birds were slaughtered and digestive organ weight, intestinal length, carcass weight and ultimate pH (pHu) were measured.

Experimental feed analyses: Experimental feeds (Table 1) were analyzed at the laboratory of Animal Nutrition of FIompiana FAmbolena Malagasy NORveziana (FIFAMANOR) in

Table 1: Ingredient contents and chemical compositions of experimental diets as formulated and fed

Ingredients	Control diet ET diet	Alternated diets (E^+P^-) diet	(E^-P^+) diet
Maize (kg)	52.50	59.00	42.00
Wheat bran (kg)	4.40	3.00	6.00
Soybean (kg)	31.50	28.00	40.00
Fish meal 40% (kg)	6.00	2.00	5.00
Lysine (kg)	0.20	0.20	0.20
Methionine (kg)	0.10	0.20	0.20
Oyster shell (kg)	1.50	1.80	1.80
Salt (kg)	0.30	0.30	0.30
Prémix 0,25%[1] (kg)	2.50	2.50	4.50
Palm oil (L)	1.00	3.00	0.00
Dietchemical compositions			
Dry matter (%)	90.02	90.23	89.97
Metabolizable energy (kcal kg^{-1})	3077.68	3222.10	2934.62
Crude protein (%)	21.57	18.84	24.27
Crude Fibre (%)	7.31	6.70	8.71

[1]Supplied per kilogram of diet; Vitamin A: 15,000 IU, Vitamin D3: 5 000 IU, Vitamin E: 100 mg, Vitamin K: 5 mg, Thiamin: 5 mg, Riboflavin: 8 mg, Pyridoxine: 7 mg, Vitamin B12: 0.02 mg, Niacin: 100 mg, Folic acid: 3 mg, Biotin: 0.3 mg, Calcium pantothenate: 25 mg, Choline: 550 mg, Manganese: 80 mg, Zinc: 90 mg, Iron: 50 mg, Copper: 20 mg, Iodine: 2 mg, Selenium: 0.2 mg, Cobalt: 0.6 mg, Butylated hydroxytoluene: 125 mg. ET: Control diet, (E^+P^-) Diet: High energy and low protein diet, (E^-P^+) Diet: Low energy and high protein diet

Madagascar. Dry matter (DM) was obtained by evaporation of water at 105°C. Nitrogen (N) content was estimated by the technique of Kjeldahl[20]. The percentage of crude protein (CP) was determined as N×6.25. Crude fibre (CF) was determined using the FibertecTecator (Fibertec System M6, 1020, hot extraction Unit)[21]. Metabolizable energy was estimated using the Table published by Sauvant *et al.*[22].

Digestive organ weight, intestinal length and meat ultimate pH (pHu): Carcass, abdominal fat, breast meat, gizzard, thigh, heart, liver, kidney and intestine were weighed to determine relative weights and intestinal length were measured. Breast meat was stored in a cooler at 4°C for 24 h. At the end of 24 h, the ultimate pH (pHu) of the breast muscle was measured. The measurement was done by inserting a glass electrode directly into the thickest part of the pectoral major muscle using a pH meter OARTON pH 700 (with precision of 0.01).

Biochemical profiles of blood samples: Blood samples were collected from 30 birds per treatment and 2 mL each sample was immediately centrifuged at 3000 rpm for 15 min to obtain serum. A volume of 2 μL of serum samples were stored in a freezer at -20°C until biochemical parameters analysis. Glucose, triglycerides, total cholesterol, total protein, urea and creatinine concentrations were evaluated by enzymatic procedures using the automated COBAS® systems. Serum triglycerides[23], total cholesterol[24], urea[25], creatinine[26], total protein[27] and glucose[28] levels were determined. All the samples were run in the same essay in order to avoid inter essay variability for each biochemical parameter.

Statistical analyses: Data were analyzed with SAS statistical software package (SAS Version 6.124) using general linear model (GLM) procedure. All performances were subjected to one-way analysis of variance (ANOVA). Significant means (established at $p<0.05$) were separated using Tukey's test[29]. Analyses were performed according to the model as follows:

$$Y_{ij} = \mu + D_i + \varepsilon_{ij}$$

Where, Y_{ij} is the observation for dependent variables (treatment I and replication j). Treatment effect I $\left(\sum_{i=1}^{2}\right)$ is complete diet feeding and sequential feeding, j is the pen j in treatment I, Y_{ij} = Feed intake, nutrient intake and digestibility, feed intake, water intake, body weight, body weight gain, feed conversion ratio, organ weight and intestine length, pHu and ε_{ij} is the residual. Dunnet multiple comparison test was used to compare differences between serum concentrations of glucose, triglyceride, total cholesterol, total protein, urea and creatinine. Also, Pearson correlation coefficient was used to analyze mortalities. The significant effect of feeding system on variables was reported when $p<0.05$. Mean values of all parameters are presented in Table with their standard error.

RESULTS

Feed intake and water intake: Feed intake in the morning, feed intake in the afternoon, total daily feed intake and total daily water intake are presented in Table 2. Total daily feed intake and water intake was similar among all treatments throughout the experimental period. But, feed intake in the morning and feed intake in the afternoon of the birds was not similar. The chickens significantly consumed more ($p<0.05$) feed in the morning than in the afternoon.

Body weight, weight gain and mortality: Table 2 shows the body weight, weight gain and feed conversion ratio. Overall, chickens' weights increased with age. At 11 wk of age, body weight of chickens was similar compared with the control group (A). Also, weight gain followed the same trend. In addition, there was no difference in mortality across all groups ($p>0.05$).

Table 2: Effect of sequential feeding system on feed, water intake and growth parameters in Sasso broiler chickens

	Treatments			
Parameters	A	B	C	p-value
Morning feed intake	34.69 ± 3.99^{b}	65.00 ± 7.26^{a}	54.99 ± 7.22^{ab}	0.009
Afternoon feed intake	58.77 ± 8.07^{a}	23.07 ± 3.55^{b}	33.91 ± 5.19^{b}	0.001
Total daily feed intake (g)	94.75±1.1	88.90±5.27	88.08±5.72	NS
Daily water intake (mL)	0.34±0.03	0.33±0.03	0.33±0.03	NS
Body weight at 11 week (g)	1757.00±45.08	1687.00±29.69	1750.00±24.58	NS
Body weight gain during 4-11 week (g)	191.10±7.26	179.20±3.56	187.20±2.78	NS
Mortality (%)	2.52±0.15	3.29±0.67	2.84±0.42	NS

[a,b]Means within row values with different superscript differ significantly ($p<0.05$), NS: Non significant. A: Bird fed complete diet, B birds fed $E^{+}P^{-}$ diet in morning and $E^{-}P^{+}$ diet in the afternoon, C birds fed $E^{-}P^{+}$ diet in morning and $E^{+}P^{-}$ diet in the afternoon

Table 3: Effect of sequential feeding system on nutrients intake and feed conversion ratio in Sasso broiler chickens

	Treatments			
Parameters	A	B	C	p-value
Daily energy intake (kcal)	263.10±37.53	243.30±35.61	246.10±33.82	NS
Crude protein intake (%)	1.85±0.26	1.71±0.26	1.72±0.24	NS
Feed conversion ratio	3.57±0.14	3.48±0.09	3.36±0.18	NS

NS: Non significant, A: Bird fed complete diet, B: Birds fed E^+P^- diet in morning and E^-P^+ diet in the afternoon, C: Birds fed E^-P^+ diet in morning and E^+P^- diet in the afternoon

Table 4: Effect of sequential feeding system on digestive organ weight, intestine length and meat ultimate pH (pHu)

	Treatments			
Parameters	A	B	C	p-value
Abdominal fat (%)	1.01±0.17^a	0.51±0.15^b	1.09±0.33^a	0.02
Gizzard (%)	2.04±0.17	2.06±0.18	2.12±0.09	NS
Liver (%)	2.51±0.08^a	2.10±0.08^b	2.49±0.13^a	0.04
Kidney (%)	0.21±0.01	0.20±0.01	0.20±0.01	NS
Heart (%)	0.59±0.07	0.56±0.05	0.54±0.02	NS
Small intestine weight (%)	5.85±0.26	4.85±0.33	5.25±0.32	NS
Small intestine length (cm)	105.56±3.16^b	122.93±4.03^a	128.60±5.24^a	0.004
pHu	5.97±0.13	5.85±0.03	6.00±0.04	NS

^{a,b}Means within row values with different superscript differ significantly (p<0.05), NS: Non significant. A: Bird fed complete diet, B: Birds fed E^+P^- diet in morning and E^-P^+ diet in the afternoon, C: Birds fed E^-P^+ diet in morning and E^+P^- diet in the afternoon

Table 5: Effect of sequential feeding system on meat yield of broilers

	Treatments			
Parameters	A	B	C	p-value
Carcass yield (%)	63.91±0.85^b	69.07±1.66^a	69.06±1.15^a	0.02
Breast yield (%)	16.22±0.63	17.62±0.75	17.24±0.56	NS
Thigh yield (%)	21.95±0.38	22.17±0.70	21.47±0.38	NS

^{a,b}Means within row values with different superscript differ significantly (p<0.05). NS: Non significant. A: Bird fed complete diet, B: Birds fed E^+P^- diet in morning and E^-P^+ diet in the afternoon, C: Birds fed E^-P^+ diet in morning and E^+P^- diet in the afternoon

Energy intake, protein intake and feed conversion ratio: As shown in Table 3, sequential feeding method did not significantly affect energy intake, protein intake and feed conversion ratio of Sasso broiler chickens. The birds had similar (p>0.05) energy and protein intake and showed no differences in feed conversion ratio.

Digestive organ weight, intestinal length and meat ultimate pH (pHu): Table 4 shows the effect of sequential feeding system on digestive organs, abdominal fat, intestinal weight, intestinal length and meat ultimate pH (pHu). No significant difference was noticed in gizzard, heart and kidney weight (p>0.05) between the control and the other treatment groups. Also, meat ultimate pH (pHu) did not differ among the birds (p>0.05). However, significant reduction (p<0.05) was observed in abdominal fat and liver weight of chickens of treatment B group. Moreover, intestinal length of chickens fed sequentially was significantly longer (p<0.05) than those in the control group.

Breast yield, thigh yield and carcass yield: As shown in Table 5, broiler chickens across the treatment groups had similar (p>0.05) breast yield and thigh weight but significant differences (p<0.05) were observed in carcass yield. Carcass yield of A group was significantly lower (p<0.05) than those of B and C groups.

Biochemical profiles of chickens: Table 6 shows the effect of sequential feeding system on biochemical profiles of broiler chicken. Total cholesterol, total protein, glucose and urea serum concentrations were not significantly different in group B and C compared to control group (A). Nevertheless, serum triglycerides of birds in B group was significantly lower (p<0.05) than those of A and C group whose values were similar. Also, serum creatinine of broiler chickens fed sequentially were significantly lower (p<0.05) than those of control group.

DISCUSSION

The present study aimed to investigate the response of slow growing broiler chickens to two unbalanced diets in energy and protein under hot and humid climate. Broiler chickens offered sequential feeding significantly consumed more feed in the morning than in the afternoon but daily feed intake was similar. Also, daily water intake was not significantly

Table 6: Effect of sequential feeding system on biochemical profiles of broiler chickens

	Treatments			
Parameters	A	B	C	p-value
Total cholesterol (g L^{-1})	0.82±0.28	0.87±0.12	1.06±0.06	NS
Total protein (g L^{-1})	29.55±3.28	30.72±2.00	32.57±1.12	NS
Glucose (g L^{-1})	2.36±0.19	2.12±0.19	2.37±0.04	NS
Urea (g L^{-1})	0.02±0.001	0.02±0.002	0.02±0.002	NS
Creatinine (g L^{-1})	0.06±0.003[a]	0.04±0.006[b]	0.04±0.003[b]	0.007
Triglyceride (g L^{-1})	0.87±0.11[a]	0.56±0.04[b]	0.87±0.05[a]	0.012

[a,b]Means within row values with different superscript differ significantly ($p<0.05$). NS: Non significant. A: Bird fed complete diet, B Birds fed E^+P^- diet in morning and E^-P^+ diet in the afternoon, C: Birds fed E^-P^+ diet in morning and E^+P^- diet in the afternoon

different compared with control group suggesting that such feeding strategy could modulate feed intake and water intake[14,15]. Daily water intake is an important indicator for the bird's health, welfare, feed digestion, livability, performance and metabolism[30]. Many studies have shown that for healthy birds, feed intake increase with water intake[31-33]. In the present study, in agreement with the findings of Fanatico *et al.*[34], all treatments had less than 5% mortality although DeBasilio *et al.*[16] found significant differences in mortality. Moreover, chickens in all the treatment groups had similar body weights and body weight gains. This observation could be linked to the similar energy and protein intake of the chickens as shown in Table 3 in agreement with findings of Fosoul *et al.*[35] in 48H cycle. Thus, similar growth performance indicates that sequential feeding strategy did not impact negatively on the chicken's growth performance. Indeed, sequential feeding may influence feed intake (especially protein) of chickens depending on their growth rate[15]. This is in agreement with Forbes and Shariatmadari[36] and Forbes[37]. On the contrary, Bouvarel *et al.*[19], Leterrier *et al.*[18] and Bizeray *et al.*[17] observed significant reduction in growth performances of chickens fed sequential feeding than those of control group. In addition, significant impact was noticed on abdominal fat and liver weight of chickens of B group.

Abdominal fat is a reliable parameter for assessing total body fat content because it is directly linked to total body fat content in avian species[38]. In general, decreasing dietary energy levels down-regulate the number of enzymes such as fatty acid synthase, which consequently reduces body fat deposits through depression of novo lipogenesis in the liver[39] and such less intense activity might have induced lower liver weight of chickens of B group. Feed restriction reduces fat deposition by impeding enzymes involved in hepatic lipogenesis and also by increasing fatty acid oxidation[39]. Besides, sequential feeding as a type of qualitative feed restriction might play a role in fat deposition[35]. As a result of the data of B group of broiler chicken, it could be suggested that the process described above might also be able to explain a significant reduction of their blood triglycerides. Moreover, in avian species, most fatty acids are synthesized in the liver and transported via low-density lipoproteins or chylomicrons for storage in adipose tissues as triglycerides[40].

Valuable information on the health status and health disorders could be obtained by the biochemical blood parameters such as glucose, triglycerides, total cholesterol, total protein, urea and creatinine. In the present study, serum creatinine of broiler chickens fed sequential feeding (B and C group) was significantly lower than those of A group. Creatinine is an important indicator of protein metabolism. It is a by-product of phosphocreatine breakdown in skeletal muscle. Its concentration has a correlation with muscle mass, age, physical activity and diet[41-43]. Broiler chickens fed sequentially showed lower serum creatinine concentration and higher carcass weight than those of control group. This suggests an efficient use of protein in the birds. In human for example, oral creatinine ingestion has been shown to increase athletic performance[41]. With regard to small intestine, its length was significantly higher in chickens of sequential feeding groups (B and C group), suggesting a morphological and histological alteration[44]. The efficiency of utilization of dietary protein in poultry depends partly on the features of the gastrointestinal tract[45]. The small intestine, especially crypts and villi of the absorptive epithelium, plays a significant role in the final phase of nutrient digestion and assimilation[46].

Generally, the low feed efficiency of slow growing chickens as observed in the present trial was confirmed by Castellini *et al.*[47] and Gordon and Charles[48]. Interestingly, apart from carcass yield, sequential feeding did not significantly affect breast meat and thigh muscle of the chickens. The increase in small intestine observed in sequentially fed chickens (B and C group) may have increased their absorptive surface area[35] and then carcass yield.

Although, in present study, feed conversion ratio of birds was similar and carcass yield was significantly improved. For clear understanding, this deserves further investigations in order to conclude safely about the influence of such feeding strategy on intestinal transit. Similar observation was made by Leterrier *et al.*[18] but, these findings contradict with Bouvarel *et al.*[19] who observed that sequential feeding increased feed conversion ratio. Meat quality of broilers can be estimated quickly by determining the pH-value of breast muscles[49]. Van Laack[50] reported significant correlations between muscle pH value and poultry meat quality. The ultimate pH (pHu) was similar across the treatments in the present study. Previous studies revealed that the highest quality products of broiler breast meat usually tend to fall within ultimate pH range (5.7-6.0)[51-53]. Thus, irrespective of feeding strategies used in this study, all the chickens had quality meat.

CONCLUSION

Sasso broiler chickens fed sequentially had higher carcass yield and significant lower serum creatinine concentration. The sequential feeding significantly affected abdominal fat, liver weight and serum triglycerides concentration. Overall, strategy of feeding high energy low protein ($E^+P^–$) diet in the morning and low energy high protein (E^-P^+) diet in afternoon could be used to reduce fat deposition and strategy of feeding ($E^–P^+$) diet in the morning and ($E^+P^–$) diet in afternoon could be used to improve carcass weight of Sasso broiler chickens in tropical areas.

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