

— **Effect of rubber seed meal on the meat composition, hepatic and serum enzyme activities of Japanese quails**

¹*Oluwatosin O. O. Kennedy, ²Iso E. Iso and ¹Kenneth P. Abasibong

¹Department of Animal Science, University of Calabar, Calabar, Nigeria

²Department of Animal Science, Cross River University of Technology, Obubra Campus, Nigeria

*oluwatosin.kennedyoko@gmail.com; oluwatosin.kennedyoko@unical.edu.ng

Abstract

This study evaluated the effect of dietary replacement of processed rubber seed meal (RSM) for soybean on the meat composition, hepatic and serum enzyme activities of growing Japanese quails. Boiled and toasted RSM were incorporated into the quail's diet as replacement for soybean meal at 50 and 100% each. Five experimental diets were formulated such that treatment 1, 2, 3, 4, and 5 contained 0% RSM, 50% Boiled RSM, 100% boiled RSM, 50% toasted RSM and 100% RSM as replacement for soybean meal, respectively. A total of 300, one-week old Japanese chicks were allotted to the five treatments, comprising of 60 chicks per treatment. Each treatment was further sub-divided into three replicates of 20 chicks per replicate. All birds were managed under the same environmental conditions as approved by the Animal Care and Ethical Committee of University of Calabar throughout the six weeks of experiment. At the end of the feeding trial, four birds were randomly picked per replicate (12 per treatment) and used for meat composition, hepatic and serum activity analyses. Data collected were subjected to the one-way ANOVA in a completely randomized design and significant means were separated using the least significant difference method. Results showed that increasing RSM up to 100% in quails diets had deleterious effects ($P < 0.05$) on the hepatic and serum enzyme activities of quail indicative of compromised health status, whereas their breast meat composition was not altered. This study concludes that for improved quail health and meat composition, up to 50% boiled rubber seed meal should be incorporated into quail diets.

Introduction

The shortage of good quality feeds has remained the major challenge to the development of the poultry industry in most developing countries.

Soybean meal is the main protein source ingredients used in formulation poultry ration. It contains 38-40% crude protein and 18-20% crude fat and it is an excellent source of lysine, tryptophan and threonine (Khatunet *al.*, 2015).

However, the cost of soybean meal is skyrocketing due to its competition for human and animal feeds therefore increasing the total feed cost and compromising the quality of available animal feeds (Olawoyin *et al.*, 2008).

Thus, to meet the animal protein demand while maximizing profit, crop residues, agro-industrial by-products and non-conventional feed resources which are locally available and do not compete with human nutrition are being evaluated to support livestock productivity (Ahaotu *et al.*, 2010; Ahaotu, 2018). One of such is the rubber seed meal (RSM) *Hevea brasiliensis*).

Rubber seed meal (RSM) is reported to have high levels of lysine and tryptophan, thus a complementary feed ingredient for maize in poultry and pig rations (Khatun *et al.*, 2015). According to Mmereole (2008), rubber seed meal contains 34.10% crude protein, 10.12% crude fat, 3.10% ash, 4.40% crude fibre contents with energy value of about 2520 kcal/kg. However, RSM utilization is limited due to the presence of a toxic factor, cyanogenic glucoside (Olawoyin *et al.*, 2008) which have negative effects such as reduction in palatability, digestibility and utilization of ration, intoxication, resulting in mortality or decreased production of animal and reduction in the quality of meat, egg, and milk products

due to the presence of hazardous residues (Ononiwu *et al.*, 2017).

Feed processing helps to enhance the feeding quality of agro-industrial by-products by reducing the level of toxicants, improving their nutrient value, acceptability of feed, and utilization by animals (Ahaotu *et al.*, 2016).

Researches have further indicated that hepatic and serum enzymes as well as their metabolites are responsive to nutritional influences involving novel feedstuffs in animal diets (Anurundu *et al.*, 2004; Yousuf, 2011). The resultant nutritional imbalances could lead to elevation or decreases in enzymes activities indicative of series of disease conditions. Thus, the need to evaluate the biochemical functioning of the animal when a novel feedstuff such as RSM is introduced (Abasiubong, 2006).

Nutritional studies on RSM have been concentrated on broilers (Mmereole, 2008; Ahaotu *et al.*, 2010a and b; Khatun *et al.*, 2015; Ahaotu, 2018), with limited data on its biochemical functions and effects on Japanese quails (Olawoyin *et al.*, 2008; Yousuf, 2011).

Therefore, the experiment was undertaken to evaluate the impact of processed rubber seed meal as replacement for soybean meal on the

meat composition, hepatic and serum enzyme activities of Japanese quails.

Materials and methods

The feeding trial was conducted at Poultry unit of the Teaching and Research farm, University of Calabar, Nigeria. All biochemical analyses were conducted at the Laboratory of the Department of Biochemistry, University of Calabar, Nigeria. Calabar is located within the tropical rain forest zone of Nigeria and has a land mass of 233.2 square mile (604m²). Calabar lies between latitude 4⁰5-8⁷N and 415⁰39⁷N, and longitude 8⁰17⁷E and 10⁰43⁷E of the Equator. Its Relative humidity is 55-99% with an elevation above sea level of 99 meters. The annual temperature and rainfall ranges between 25⁰C-30⁰C and 1260-3500mm, respectively (Google Earth, 2017).

Collection and preparation of rubber seeds

Rubber seeds were obtained from PAMOL Nigeria Ltd. at Akampa, Cross River State, Nigeria. The seeds were carefully dehulled and decorticated thereafter divided into two groups for further processing. The first group was boiled at 105⁰C for 45 mins. Then sundried to constant weight for seven days. The second group was toasted for 30 mins. at 120⁰C per 2kg, both groups were then milled (using 2mm sieve hammer mill) to form boiled rubber seed

meal (BRSM) and toasted rubber seed meal (TRSM), respectively. Each meal was used as replacement for soybean meal at 50% and 100% while the control diet contained no rubber seed meal (Table 1). A total of five experimental diets were formulated.

Experimental animal and management

A total of 300, one-week old Japanese quails (*Coturnix coturnix japonica*) was purchased from an agent of VOM Hatchery, Jos-Plateau State, Nigeria. The chicks were randomly distributed to the five dietary treatments comprising of T₀ (without RSM), T₁ (50% BRSM), T₂ (100% BRSM), T₃ (50% TRSM) and T₄ (100% TRSM) with three replicates of 20 chicks each such that each treatment had 60 chicks. The chicks were brooded in respective pens at a temperature of 32 – 35⁰C during 1st week, 29.5 – 32⁰C during 2nd week, and 27-29.5 °C during 3rd week with the help of electric light for maintaining proper temperature.

Feed and water were provided ad libitum throughout the six weeks of experiment and all groups were managed under the same environmental conditions in line with the approved guidelines of the Animal Care and Ethical Committee of the University of Calabar.

At the sixth week, four birds were randomly picked per replicate (12 per treatment) and used for the determination of meat composition, hepatic and serum enzyme activities. Each quail was euthanized with chloroform in a glass chamber, dissected, then blood and liver samples were obtained.

3ml of blood sample was collected from the heart into plain vacutainer and centrifuged at 4,000 rpm for 20 minutes at 4⁰C to obtain serum. 5g of Liver tissue was collected from each quail, they were pooled per treatment then liver sample was collected.

Meat sample was collected from the deboned breast muscle, wrapped in foiled paper, properly labelled and immediately placed in a refrigerator (4⁰C) for 24 hours.

Chemical analyses

The feed ingredients and samples of quail breast muscles were analyzed to determine their proximate components according to the methods described by AOAC (2004). Serum and hepatic enzyme; aspartate amino transferase (AST), Alanine amino transferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) analyses were carried out using the standard Randox kit. All analyses

were conducted at the Biochemistry Laboratory, Department of Biochemistry University of Calabar.

Statistical analysis

All data obtained were subjected to the one-way analysis of variance in completely randomized design (CRD). The analyses were performed using SPSS statistical software (SPSS, 2011). The meat sample was converted to percentage of live weights prior to statistical analysis. Significant means were separated using the least significant differences (LSD) at 5% level of significance (Steel *et al.*, 1997).

Results and discussion

Table 2 presents the chemical composition of quail breast meat as influenced by rubber seed meal. No significant ($P>0.05$) differences were observed between treatments on the nutrient composition of quail breast muscle. The dry matter contents of the breast muscle (24.50 – 28.09%) were within range (24.46 – 25.79%) previously reported (Yalcin *et al.*, 2005).

Values for ash and crude protein were higher than those of Yalcin *et al.* (2005) but comparable to values obtained by Kirkpinar and Oguz (1995). Result further indicated slight improvement ($P>0.05$) in the crude protein content of the breast muscles of quails

fed processed RSM especially those fed 50% BRSM. Also, fat content in the breast muscle decreased in quails fed boiled RSM compared to those on the control or Toasted RSM diets. These observations were in line with earlier reports of Olawoyin *et al.* (2008) that there were no detrimental effects of RSM replacement for soybean meal on the breast muscle of quail meat.

Significant ($P>0.05$) elevated values were observed between treatments on the serum enzymes (ALT, ALP and LDH) and liver enzymes (ALP and LDH) activities as shown in Table 3. Serum enzyme activities were generally higher than those obtained from the liver enzymes. These results indicated that there were mild to moderate liver disease conditions in quails fed Rubber seed meal compared to those on Soybean meal. The total replacement of soybean meal with either boiled or rubber seed meal tends to exert more functional demand on the liver for detoxification processes. This in turn might provoke adverse effects on the liver and influences abnormal enzymatic activities and disease conditions.

The observations in this present study are consistent to earlier findings by Olawoyin *et al.* (2008) and Yousuf, (2011) that beyond 50% RSM replacement for soybean meal the health

status of Japanese quails might be compromised. Ahaotu (1999) and Ahaotu *et al.* (2010a) reported that as incorporation levels of RSM increased to 30% growth performance decreased. Whereas the weights of some digestive organs such as gizzard, proventriculus, liver, spleen, kidney and heart significantly increased (Ahaotu, 2018) due to additional load exerted during the digestive process.

ALP is known to be a good indicator of bone growth and the elevated value obtained in quails fed 100% RSM could suggest negative effects of osteoblastic activity (Schalm *et al.*, 1975). ALT: ALP ratio is used as a disease diagnostic tool. The higher ratios in RSM fed quails compared to ratio obtained in the control may suggest a cholestasis condition as earlier observed (Uboh, 2004). The increased LDH activity in quails also indicated the presence of anti-nutrients in the RSM meal. These results imply that though no clinical incidences of diseases were recorded, sub-clinical liver damages were evident especially at 100% RSM replacement.

Conclusion

This study concludes that 50% boiled rubber seed meal could conveniently replace soybean in quail diet without any adverse effects on the meat composition, hepatic and serum enzyme

activities. It is therefore recommended that RSM must be properly processed for feed formulation and it should not be included beyond 50% in quail diets.

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Table 1. Composition of experimental diets %

Ingredient %	Control 0%	50% BRSM	100%B	50%	100%
	RSM		RSM	TRSM	TRSM
Maize	48.71	48.71	48.71	48.71	48.71
Soybean meal	23.82	11.91	-----	11.91	-----
RSM	-----	11.91	23.82	11.91	23.82
Fish meal	7.92	7.92	7.92	7.92	7.92
Wheat offal	10.00	10.00	10.00	10.00	10.00
Dcp	3.00	3.00	3.00	3.00	3.00
Oyster shell	6.00	6.00	6.00	6.00	6.00
*Vitamin/mineral premix	0.25	0.25	0.25	0.25	0.25
Salt	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100
Calculated nutrient					
ME, kcal/kg	2539	2769	30002	2798	3057
Crude protein	22.00	19.40	16.80	19.38	16.78
Analyzed nutrient					
Crude protein	21.51	18.62	16.58	19.19	16.70

*Composition per 2kg of mixture contains: Vitamin A- 15,000,000 iu, Vitamin D – 3,000,000 iu., Vitamin e- 15,000 iu, Vitamin K- 2.5g, Vitamin B₁- 1g, Vitamin B₂- 10g., Vitamin B₁₂- 4g, Folic acid – 2g, Biotin – 0.1g, Niacin – 70g, BHT – 125g, CalciumD-Pantotenic acid – 20g

RSM – Rubber seed meal, BRSM – Boiled rubber seed meal, TRSM - Toasted rubber seed meal

Table 2. Rubber seed meal and its influence on breast muscle of quails

Carcass composition , %	Control 0% RSM	50% BRSM	100% BRSM	50% TRSM	100% TRSM	SEM
Dry matter	24.15	24.65	25.29	24.54	27.09	0.21
Crude protein	46.84	48.16	47.23	47.40	47.41	0.10
Crude fat	5.52	4.72	4.82	5.67	5.89	0.04
Ash	6.20	5.77	6.18	5.72	5.93	0.07
NFE	41.44	41.35	41.77	41.21	40.77	0.12

NS - Not Significant (P>0.05)

RSM – Rubber seed meal

BRSM – Boiled rubber seed meal

TRSM - Toasted rubber seed meal

Table 3. Rubber seed meal and its influence on hepatic and serum enzyme activities in quails

Enzyme activity, IUL	Control 0% RSM	50% BRSM	100% BRSM	50% TRSM	100% TRSM	SEM
Hepatic enzymes						
AST	56.49	62.79	50.89	65.84	62.28	2.46
ALT	4.06	7.01	7.07	7.01	7.20	1.05
ALP	41.04 ^c	52.44 ^b	56.58 ^b	69.00 ^a	38.92 ^c	0.15
LDH	45.39 ^c	75.64 ^{ab}	41.26 ^c	88.02 ^a	66.68 ^b	0.07
Serum enzymes						
AST	112.92	70.77	70.72	86.92	89.04	2.45
ALT	23.78 ^a	6.26 ^d	7.88 ^d	16.52 ^b	10.46 ^{bc}	0.05
ALP	431.72 ^c	483.46 ^b	621.21 ^a	352.62 ^d	498.87 ^b	0.06
LDH	115.4 ^b	226.93 ^b	264.06 ^a	210.43 ^{ab}	218.68 ^{ab}	0.05

^{a, b, c} Means indicate significant (P<0.05) differences across rows

RSM – Rubber seed meal. BRSM – Boiled rubber seed meal, TRSM - Toasted rubber seed meal, AST – Aspartate amino transferase, ALT - Alanine amino transferase. ALP - Alkaline phosphatase, LDH - lactate dehydrogenase