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Research Article

Haematological and Serum Biochemical Parameters of Broiler Chickens Fed Varying Dietary Levels of Fermented Castor Oil Seed Meal (*Ricinus communis* L.) and Different Methionine Sources in South Western Nigeria

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ABSTRACT: In this experiment, the effect of varying dietary levels of fermented castor oil seed meal (FCSM) and different methionine sources (DL-methionine and herbal methionine) on haematological and serum biochemical parameters of broilers. A total of 240 one-day-old Anak broiler chicks were used in the experiment lasted 56 days. The dietary experiment was laid out as a completely randomized design in a 4 × 2 factorial arrangement consisting of 4 dietary levels of FCSM (0, 50, 100 and 150 g/kg) and 2 methionine sources (DL-methionine and herbal methionine). The birds were weighed and randomly distributed into 8 treatments with 3 replicates of 10 birds each. During the starter phase of the experiment, haemoglobin, red blood cell count, mean corpuscular haemoglobin concentration and eosinophil counts were higher (P<0.05) in birds fed diets supplemented with herbal methionine when compared with DL-methionine. Birds fed diets containing 100 and 150 g/kg FCSM supplemented with either DL-methionine or herbal methionine showed higher white blood cell values at the finisher phase. Dietary FCSM raised serum creatinine levels of broiler chickens at both starter and finisher phases. In conclusion, dietary inclusion of FCSM at higher levels of 100 and 150 g/kg had adverse effects on haematological and serum biochemical parameters of broiler chickens. Herbal methionine can successfully replace DL-methionine in the diets of broiler chickens. There is need for further research on effective processing techniques that can enhance the use of castor oil seed meal in monogastric animal rations.

Keywords: Broiler, Methionine, Haematology, Castor oil meal, Serum biochemistry

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Güney Batı Nijerya'da Değişik Düzeylerde Fermente Hint Yağı Tohumu Küspesi (*Ricinus communis* L.) ve Farklı Metiyonin Kaynakları İlave Edilen Rasyonlarla Beslenen Broyler Piliçlerin Hematolojik ve Serum Biyokimyasal Parametreleri

ÖZ: Bu çalışmada broyler rasyonlarına farklı düzeylerde fermente hint yağı tohumu küspesi (FHTK) ve metiyonin ilavesinin hematolojik ve serum biyokimyasal parametreleri üzerine etkisi belirlenmiştir. Toplam 240 adet, bir günlük yaşta Anak broyler civcivler kullanılan deneme ellialtı gün sürmüştür. Çalışmada, 4 farklı düzeyde FHTK (0, 50, 100 ve 150 g/kg) ve 2 farklı metiyonin kaynağından (DL-metiyonin ve bitkisel metiyonin) oluşan rasyonlar 4x2 faktöriyel düzende denenmiştir. Civcivler tartıldıktan sonra her biri 10 civcivden oluşan üç tekerrürlü 8 deneme grubuna tesadüfi olarak dağıtılmıştır. Denemenin başlangıcında bitkisel metiyonin ile beslenen piliçlerin hemoglobin, alyuvar sayısı, ortalama korpüsküler haemoglobin konsantrasyonu ve eozinofil sayıları, DL metiyonin ile beslenen civcivlerden daha yüksek bulunmuştur. DL metiyonin veya bitkisel metiyoninle birlikte 100 ve 150 g/kg FHTK içeren rasyonlarla beslenen piliçlerde akyuvar sayısı bitiş döneminde daha yüksek olmuştur. FHTK broyler piliçlerin serum kreatinin düzeylerini hem başlangıç hem de bitiş döneminde artırmıştır. Sonuç olarak, rasyonlara 100 ve 150 g/kg gibi yüksek düzeylerde FHTK ilavesi broylerlerde hematolojik ve serum biyokimyasal parametrelerini olumsuz etkilemiştir. Broyler rasyonlarında DL metiyonin yerine bitkisel metiyonin başarılı bir şekilde kullanılabilir. Hint yağı tohumu küspesinin tek mideli hayvanların rasyonlarında kullanımını artırabilecek etkin işleme teknikleri üzerinde ileri çalışmalara gereksinim vardır.

Anahtar Kelimeler: Broyler, Metiyonin, Hematolojik, Hintyağı unu, Serum biyokimyası

INTRODUCTION

With the increasing cost of conventional feed ingredients resulting from increased competition between man and livestock, novel protein feedstuffs are increasingly being sought for and research into their use are currently gaining priority and popularity (1). In many countries of the world, wide varieties of ingredients have been tested. Studies aimed at finding alternative protein sources include *Acacia sieberiana* seeds (2), velvet beans (*Mucuna spp*) (3), cotton seed cake (4), palm kernel extraction (5) and sorrel seeds (6) amongst others. Another unconventional protein feedstuff is castor oil seed meal (*Ricinus communis, L*). Attempts have been made to use castor oil seed as an alternative protein source for livestock, however, a major constraint identified is the presence of anti-nutritional factors (7; 8; 9). The potential of defatted castor bean meal in livestock feeds have been reported (10; 11). Castor bean cake contains about 32 to 48% crude protein depending on levels of decortications and deoiling (12) and the whole seed contains 2.9 to 3.28 kcal/kg true ME (13). Since most animals are vulnerable to castor oil seed poisoning with poultry birds still having a relative high tolerance (14), it is important to re-examine such claim. Oyawoye and Ogunkunle (15) stated that haematological indices such as packed cell volume, haemoglobin concentration, white blood cell and mean corpuscular volume are vital in monitoring feed toxicity

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especially with feed constituents that affect blood formation. The physiological effects of diets on animals are best judged by chemical composition of the feed, performance of animals and cellular changes in the organs (16). The need to detoxify castor oil seed is essential especially when it is not of primary use to man. However, heat from sun drying after fermentation could destroy essential amino acids of which methionine is inclusive and consequently reduces the protein quality of the castor oil seed meal (8). Therefore, this study was carried out on the haematological and serum biochemical assessment of broiler chickens fed varying dietary levels of fermented castor oil seed meal (*Ricinus communis* Linn.) and supplementary methionine in South Western Nigeria.

MATERIALS and METHODS

Experimental location

The experiment was conducted at the Poultry Unit of Directorate of University Farms (DUFARMS) of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The location lies within the rainforest zone of South-Western Nigeria at longitude 7° 10', 37'N, latitude 3^o 26' 58'E and altitude 173 m above sea level. The climate is humid with a mean annual rainfall of 1037 mm. The mean annual temperature and humidity are 34.7°C and 82% respectively (17).

Collection of test ingredients and preparation of experimental diets

Castor oil seeds were obtained from a local market in Kogi State, Nigeria. The seeds were soaked in fresh water for 6 days in an air tight container to allow for natural fermentation. The fermented seeds were rinsed several times under a running tap of water to remove the undesired odour, sieved and sun dried for two days. The dried seeds were milled and incorporated into the experimental diets. DL-methionine (a combination of the natural form of L-methionine and its optical isomer D-methionine) was obtained from a commercial feed mill and herbal methionine (Herbo-Meth®) was obtained from a reputable veterinary store around the experimental location. Eight experimental broiler starter and finisher diets were formulated to meet the nutrient requirements of broiler chickens (18). The composition of the experimental diets is presented in Tables 1 and 2.

Experimental birds and management

All experimental procedures used were in accordance with the guidelines for care and use of animals in research (19). Two hundred and forty (240) day-old Anak broiler chicks were sourced from a reputable hatchery in Abeokuta, Ogun State. The chicks were subject to standard brooding for 2 weeks raised in deep litter pens of an open-sided poultry house. Normal prophylactic medication and vaccination were administered as and when due according to the recommendation prescribed by the hatchery. The birds were weighed and randomly distributed into 8 groups with three replicates of 10 birds each. The dietary experiment was laid out as a completely randomized design in a 4 × 2 factorial arrangement consisting of 4 dietary levels of FCSM (0, 50, 100 and 150 g/kg) and 2 methionine sources (DL-methionine and herbal methionine). The birds were housed in pens measuring 1.5 m × 1.5 m. Wood shavings were used as the litter material. Feed and fresh water were supplied *ad libitum* throughout the experiment.

Blood collection and analysis

At the end of weeks 4 and 8, 2.5 mls of blood was collected aseptically with sterile syringes and needles from the wing vein (brachial vein) of two birds per replicate into tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) as anti-coagulant.

Table 1 Ingredients and chemical composition of basal diets (as-fed basis; Weeks 0-4)

Ingredient (kg)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Maize	580	550	540	530	580	550	540	530
GNC	60	90	100	110	60	90	100	110
FCSM	0	50	100	150	0	50	100	150
Soybean meal	300	250	200	150	300	250	200	150
Fish meal	16	16	16	16	16	16	16	16
Bone meal	25	25	25	25	25	25	25	25
Oyster shell	15	15	15	15	15	15	15	15
Salt	2	2	2	2	2	2	2	2
*DL- Methionine	1	1	1	1	0	0	0	0
*Herbo-Meth	0	0	0	0	1	1	1	1
**Premix	2	2	2	2	2	2	2	2
Total	1000	1000	1000	1000	1000	1000	1000	1000
Calculated analysis								
Crude protein (%)	23.01	22.95	22.85	22.79	23.01	22.95	22.83	22.71
ME (kcal/kg)	3210	3170	3200	3205	3210	3170	3200	3205
Fibre (%)	3.9	4.2	4.3	4.6	3.9	4.2	4.3	4.6
Fat (%)	4.2	4.0	3.82	3.64	4.2	4.0	4.2	3.64
Ash (%)	2.88	2.7	2.44	2.39	2.88	2.7	2.44	2.39
Ca (%)	0.92	0.91	0.89	0.90	0.92	0.91	0.89	0.90
P (%)	0.45	0.44	0.47	0.45	0.45	0.44	0.47	0.45
Basal methionine (%)	0.50	0.48	0.45	0.43	0.50	0.48	0.45	0.43

GNC-Groundnut cake, FCSM- Fermented castor oil seed meal, * represents DL-methionine or herbal methionine (herbo-meth) supplementation, **Vitamin/Mineral Premix contains the following per kg diet, Vit. A: 4.000.000IU, Vit D: 80000, Vit. B_{12}: 25 mg, Niacin: 6000 mg, Vit. E: 4000, Vit.K_3: 800 mg, Vit.B_3:100 mg, Vit. B_2: 600mg, Vit. B_6: 5000 mg, Panthotenic acid: 2000 mg, Folic acid: 200 mg, Biotin: 8 mg, Manganese: 30000, Iron: 800 mg, Zinc: 2000 mg, Cobalt: 80 mg, Iodine: 400 mg, Selenium: 40 mg, Choline: 8000 mg

Table 2 Ingredients and chemical composition of basal diets (as-fed basis; Weeks 4-8)

Ingredients (kg)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Maize	580	580	570	572	580	580	570	572
GNC	65	65	75	73	65	65	75	73
FCSM	0	50	100	150	0	50	100	150
Soybean meal	250	200	150	100	250	200	150	100
Wheat offal	65	65	65	65	65	65	65	65
Bone meal	24	24	24	24	24	24	24	24
Oyster shell	10	10	10	10	10	10	10	10
Salt	2	2	2	2	2	2	2	2
**Premix	2	2	2	2	2	2	2	2
*DL-methionine	2	2	2	2	0	0	0	0
*Herbo-Meth	0	0	0	0	2	2	2	2
Total	1000	1000	1000	1000	1000	1000	1000	1000
Calculated analysis								
Crude protein (%)	20.23	20.31	20.23	20.21	20.23	20.20	20.23	20.21
ME (kcal/kg)	3220	3100	3225	3200	3220	3100	3225	3200
Fibre (%)	3.9	4.2	4.5	4.7	3.9	4.2	4.5	4.7
Fat (%)	4.2	4.0	3.82	3.64	4.2	4.0	4.2	3.64
Ash (%)	2.88	2.7	2.44	2.39	2.88	2.7	2.44	2.39
Ca (%)	0.90	0.92	0.89	0.91	0.90	0.92	0.89	0.91
P (%)	0.43	0.46	0.44	0.51	0.43	0.46	0.44	0.51
Basal methionine (%)	0.42	0.45	0.43	0.42	0.42	0.45	0.43	0.42

GNC-Groundnut cake, FCSM- Fermented castor oil seed meal, * represents DL-methionine or herbal methionine (herbo-meth) supplementation, **Vitamin/Mineral Premix contains the following per kg diet, Vit. A: 4.000.000IU, Vit D: 80000, Vit. B_{12}: 25 mg, Niacin: 6000 mg, Vit. E: 4000, Vit.K_3: 800 mg, Vit.B_3:100 mg, Vit. B_2: 600mg, Vit. B_6: 5000 mg, Panthotenic acid: 2000 mg, Folic acid: 200 mg, Biotin: 8 mg, Manganese: 30000, Iron: 800 mg, Zinc: 2000 mg, Cobalt: 80 mg, Iodine: 400 mg, Selenium: 40 mg, Choline: 8000 mg

Haematological analysis of red blood cell, white blood cell, haemoglobin concentration, packed cell volume and white blood cell differential counts were determined according to the method described by Jain (20). The mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were calculated (21) respectively. Another 2.5 ml of blood samples were collected into tubes without Ethylene Diamine Tetra Acetic Acid (EDTA) according to the procedure of Baker and Silverton (22). Serum metabolites such as total serum protein, albumin, globulin, creatinine, cholesterol, glucose and urea nitrogen were analyzed. The blood samples for serum analysis were allowed to clot before centrifuging at 1100-1300 rpm for 15 minutes in a micro-centrifuge to obtain serum that is free from cell debris. After this, separated sera were decanted into bijoh bottles and stored at -20^{o}C until they were analyzed. The biochemical analysis was done using a spectrophotometer (Sentinel, Italy). The levels and activities of liver enzymes such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were also determined according to the enzymatic colorimetric method described by Schmidt and Schmidt (23) using a commercial diagnostic reagent kits (Randox Laboratories Ltd., UK).

Statistical analysis: All the data generated in the study were subjected to analysis of variance according to the general linear model procedure in a 4 × 2 factorial arrangements in a completely randomized design using SAS (24) software package. Treatment means were compared using Duncan's Multiple Range Test (25) at 5% level of significance.

RESULTS

The effects of varying dietary fermented castor oil seed meal (FCSM) levels and methionine supplementation on haematological and serum biochemical parameters of starter broiler chickens (weeks 0-4) are presented in Table 3. Haemoglobin concentration and red blood cell were highest (P<0.05) in birds fed 150 g/kg FCSM and lowest in those fed 0 g/kg FCSM. Mean corpuscular volume was higher in birds fed 0 and 150 g/kg FCSM (P<0.05). Birds fed from 0 - 100 g/kg FCSM had comparably high mean corpuscular haemoglobin and globulin values. Mean corpuscular haemoglobin concentration was highest in birds fed 100 g/kg FCSM and lowest in those fed 150 g/kg FCSM. The lowest white blood cell, highest total protein and albumin were obtained in birds fed 0 g/kg FCSM (P<0.05). Birds fed from 50 -150 g/kg FCSM had comparably high creatinine values. Birds fed diets containing herbal methionine recorded higher haemoglobin concentration, red blood cell, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and eosinophils than those fed DL-methionine (P<0.05).

The effects of interaction of varying dietary fermented castor oil seed meal (FCSM) levels and methionine supplementation on haematological and serum biochemical parameters of starter broiler chickens (weeks 0-4) are presented in Table 4. Birds fed 150 g/kg FCSM supplemented with DL-methionine and those fed from 50 - 150 g/kg FCSM supplemented with herbal methionine had comparably high packed cell volume values.

Table 3 Effects of varying dietary FCSM levels and methionine supplementation on haematological and serum biochemical parameters of starter broiler chickens (weeks 0-4)

Parameters	Dietary levels of FCSM (g/kg)				SEM	Methionine sources		SEM
	0	50	100	150		DL-M	HM	
Packed cell volume (%)	27.83	28.83	29.00	31.33	1.18	28.0	30.50	0.84
Haemoglobin (g/dl)	9.38^{c}	10.27^{b}	9.70^{bc}	11.05^{a}	0.20	9.13^{b}	11.07^{a}	0.15
Red blood cell ($\times 10^{12}$/l)	1.46^{c}	1.57^{b}	1.52^{bc}	1.82^{a}	0.02	1.48^{b}	1.70^{a}	0.01
MCV (fl)	19.19^{a}	19.31^{a}	18.59^{b}	18.84^{b}	0.10	19.10	18.87	0.07
MCH (pg)	64.16^{ab}	65.28^{a}	63.46^{ab}	60.88^{b}	1.14	61.85^{b}	65.04^{a}	0.81
MCHC (g/dl)	33.37^{bc}	33.80^{b}	34.14^{a}	32.37^{c}	0.62	32.39^{b}	34.50^{a}	0.44
White blood cell ($\times 10^{12}$/l)	3.10^{c}	4.97^{ab}	5.48^{a}	4.23^{b}	0.34	4.14	4.75	0.24
Heterophils (%)	66.17	62.64	63.50	60.17	2.47	62.25	61.69	1.75
Lymphocytes (%)	30.83	33.62	32.50	35.50	2.05	34.58	34.00	1.45
Eosinophils (%)	2.83	3.07	3.17	3.33	0.32	2.75^{b}	3.42^{a}	0.23
Monocytes (%)	0.17	0.67	0.83	1.00	0.26	0.42	0.89	0.19
Glucose (mg/dl)	137.83	135.37	139.17	145.50	8.85	140.20	138.8	6.26
Total protein (g/l)	68.28^{a}	63.16^{b}	61.33^{b}	60.67^{b}	1.27	62.89	63.83	0.90
Albumin (g/l)	42.33^{a}	38.49^{b}	37.45^{b}	37.30^{b}	1.23	39.76	38.02	0.87
Globulin (g/l)	25.95^{a}	24.67^{ab}	23.88^{ab}	23.37^{b}	0.79	23.13	25.81	0.56
Uric acid (mg/dl)	40.67	39.00	36.83	41.00	2.97	38.67	40.08	2.10
Creatinine (mg/dl)	0.60^{b}	0.74^{a}	0.71^{ab}	0.71^{ab}	0.04	0.71	0.67	0.03
AST (IU/L)	125.33	123.83	116.83	117.17	5.60	115.40	126.20	3.96
ALT (IU/L)	29.83	34.17	29.83	30.17	1.67	30.67	31.33	1.18
ALP (IU/L)	48.17	45.17	48.17	47.83	1.87	47.92	46.75	1.32

$^{a-c}$Mean values in the same row having different superscripts are significantly different (P<0.05), FCSM: Fermented castor oil seed meal, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, SEM: Standard Error of Mean, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, DL-M: DL-methionine, HM: Herbal methionine

Table 4 Effects of interaction of varying dietary FCSM levels and methionine supplementation on haematological and serum biochemical parameters of starter broiler chickens (weeks 0-4)

Methionine sources	+DL-methionine				+ Herbal methionine				SEM
FCSM levels (g/kg)	0	50	100	150	0	50	100	150	
Packed cell volume (%)	29.33^{abc}	25.33^{c}	26.00^{bc}	31.33^{ab}	26.33^{bc}	32.33^{a}	32.00^{a}	31.33^{ab}	1.67
Haemoglobin (g/dl)	9.07^{d}	9.20^{d}	7.73^{e}	10.53^{bc}	9.70^{cd}	11.33^{ab}	11.67^{a}	11.57^{a}	0.29
Red blood cell ($\times 10^{12}$/l)	1.41^{d}	1.45^{cd}	1.25^{e}	1.83^{a}	1.52^{c}	1.69^{b}	1.79^{a}	1.83^{a}	0.03
MCV (fl)	19.52^{a}	19.32^{a}	18.43^{c}	19.13^{ab}	18.85^{bc}	19.30^{a}	18.74^{bc}	18.54^{c}	0.14
MCH (pg)	64.47^{a}	63.60^{a}	61.87^{ab}	57.48^{b}	63.85^{a}	66.96^{a}	65.06^{a}	64.28^{a}	1.61
MCHC (g/dl)	33.02^{a}	32.91^{a}	33.55^{a}	30.05^{b}	33.88^{a}	34.69^{a}	34.72^{a}	34.69^{a}	0.87
White blood cell ($\times 10^{12}$/l)	2.87^{bc}	3.01^{bc}	4.33^{b}	2.60^{c}	3.33^{bc}	3.18^{bc}	6.63^{a}	5.87^{a}	0.48
Heterophils (%)	63.66	63.00	60.00	66.33	65.67	61.67	66.00	65.00	3.49
Lymphocytes (%)	33.67^{ab}	33.66^{ab}	37.33^{a}	30.33^{b}	31.00^{b}	35.33^{a}	28.66^{b}	30.33^{b}	2.90
Eosinophils (%)	2.67	2.67	2.67	3.00	3.00	3.33	3.67	3.67	0.46
Monocytes (%)	0.00^{b}	0.67^{ab}	0.00^{b}	1.00^{ab}	0.33^{b}	0.67^{ab}	1.67^{a}	1.00^{ab}	0.37
Glucose (mg/dl)	130.67	141.33	138.00	150.67	145.00	129.40	140.33	140.33	12.52
Total protein (g/l)	68.23^{a}	63.00^{ab}	60.67^{b}	59.67^{c}	68.33^{a}	63.33^{ab}	62.00^{b}	61.67^{b}	1.79
Albumin (g/l)	43.03^{a}	40.21^{ab}	37.80^{ab}	38.01^{ab}	41.63^{ab}	36.77^{b}	37.10^{b}	36.59^{b}	1.74
Globulin (g/l)	25.20^{ab}	22.79^{b}	22.87^{b}	21.66^{b}	26.70^{a}	26.56^{a}	24.90^{ab}	25.08^{ab}	1.11
Uric acid (mg/dl)	37.33	41.33	36.33	39.67	44.00	36.67	37.33	42.33	4.20
Creatinine (mg/dl)	0.56^{b}	0.66^{ab}	0.63^{ab}	0.67^{ab}	0.63^{ab}	0.82^{a}	0.79^{a}	0.75^{a}	0.06
AST (IU/L)	123.00	125.33	104.00	109.33	127.67	122.33	129.67	125.00	7.92
ALT (IU/L)	30.00^{ab}	35.00^{a}	25.67^{b}	32.00^{ab}	29.67^{ab}	33.33^{ab}	34.00^{a}	28.33^{ab}	2.37
ALP (IU/L)	46.67	45.67	51.00	48.33	49.33	44.67	45.33	47.33	2.64

$^{a-e}$Mean values in the same row having different superscripts are significantly different (P<0.05), FCSM: Fermented castor oil seed meal,MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, SEM: Standard Error of Mean, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase

Table 5 Effects of varying dietary FCSM levels and methionine supplementation on haematological and serum biochemical parameters of finisher broiler chickens (weeks 4-8)

Parameters	Dietary levels of FCSM (g/kg)				SEM	Methionine sources		SEM
	0	50	100	150		DL-M	HM	
Packed cell volume (%)	31.90^{a}	29.65^{ab}	26.50^{b}	26.83^{b}	1.13	28.84	28.59	0.80
Haemoglobin (g/dl)	9.80^{a}	8.80^{ab}	7.62^{b}	7.37^{b}	0.59	8.09	8.70	0.41
Red blood cell (×10^{12}/l)	1.70^{a}	1.51^{b}	1.45^{b}	1.41^{b}	0.08	1.51	1.53	0.06
MCV (fl)	19.26	19.67	18.94	18.80	0.49	19.34	19.00	0.35
MCH (pg)	57.84	58.44	52.80	53.57	4.40	54.25	57.07	3.11
MCHC (g/dl)	30.05	29.82	27.85	28.62	2.31	28.19	29.98	1.64
White blood cell (×10^{12}/l)	2.46^{c}	3.33^{bc}	4.50^{b}	6.05^{a}	0.48	4.05	4.12	0.34
Heterophils (%)	64.10	62.33	63.46	63.83	2.47	62.75	63.42	1.75
Lymphocytes (%)	32.23	33.34	32.21	32.17	2.05	33.67	32.00	1.45
Eosinophils (%)	3.00	3.33	3.33	3.00	0.29	2.75^{b}	3.58^{a}	0.02
Monocytes (%)	0.67	1.00	1.00	1.00	0.32	0.83	1.00	0.23
Glucose (mg/dl)	165.67	161.50	152.83	158.50	7.59	161.70	157.60	5.37
Total protein (g/l)	56.00	55.33	62.50	63.17	4.57	56.58	61.92	3.23
Albumin (g/l)	34.50	35.83	37.67	35.67	3.12	34.00	37.83	2.21
Globulin (g/l)	21.50	19.50	24.83	27.50	2.78	22.58	24.08	1.97
Uric acid (mg/dl)	48.17^{c}	48.25^{c}	64.83^{b}	72.67^{a}	1.98	58.08	58.83	1.40
Creatinine (mg/dl)	1.08^{b}	1.17^{ab}	1.17^{ab}	1.45^{a}	0.10	1.34	1.07	0.07
AST (IU/L)	138.50	143.17	140.67	140.55	6.06	138.60	142.90	4.29
ALT (IU/L)	15.15	16.28	13.81	15.47	0.84	15.60	14.75	0.60
ALP (IU/L)	43.50	48.83	40.67	41.67	3.26	44.67	42.67	2.30

[a-c]Mean values in the same row having different superscripts are significantly different (P<0.05), FCSM: Fermented castor oil seed meal, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, SEM: Standard Error of Mean, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, DL-M: DL-methionine, HM: Herbal methionine

Table 6 Effects of interaction of varying dietary FCSM levels and methionine supplementation on haematological and serum biochemical parameters of finisher broiler chickens (weeks 4-8)

Methionine type	+ DL-methionine				+Herbal methionine				SEM
Level of FCSM (g/kg)	0	50	100	150	0	50	100	150	
Packed cell volume (%)	32.21^{a}	29.48^{ab}	26.67^{b}	27.00^{ab}	31.58^{a}	29.82^{ab}	26.33^{b}	26.67^{b}	1.60
Haemoglobin (g/dl)	9.23^{ab}	8.50^{ab}	7.43^{b}	7.20^{b}	10.37^{a}	9.10^{ab}	7.80^{ab}	7.53^{b}	0.83
Red blood cell (× 10^{12}/l)	1.67	1.54	1.38	1.45	1.73	1.47	1.52	1.38	0.11
MCV (fl)	19.52	19.90	19.00	18.95	19.00	19.44	18.91	18.65	0.70
MCH (pg)	55.50	55.06	53.71	52.73	60.18	61.82	51.89	54.41	6.22
MCHC (g/dl)	28.49	27.83	28.30	28.15	31.61	31.80	27.40	29.09	3.27
White blood cell (× 10^{12}/l)	2.43^{b}	3.30^{b}	4.40^{ab}	6.07^{a}	2.48^{b}	3.37^{b}	4.60^{ab}	6.03^{a}	0.68
Heterophils (%)	63.66	62.00	60.00	61.33	64.00	61.67	66.00	65.00	3.49
Lymphocytes (%)	33.67^{ab}	33.67^{ab}	36.67^{a}	35.34^{a}	32.00^{ab}	33.91^{ab}	28.66^{b}	30.33^{b}	2.90
Eosinophils (%)	2.67	3.00	3.00	2.33	3.33	3.67	3.67	3.67	0.41
Monocytes (%)	0.67	1.33	0.33	1.00	0.67	0.67	1.67	1.00	0.46
Glucose (mg/dl)	176.67	157.33	151.33	161.33	154.67	165.67	154.33	155.67	10.73
Total protein (g/l)	50.00	56.67	61.00	58.67	62.00	54.00	64.00	67.67	6.46
Albumin (g/l)	29.67	37.00	36.00	33.33	39.33	34.67	39.33	38.00	4.41
Globulin (g/l)	20.33	19.67	25.00	25.33	22.67	19.33	24.67	29.67	3.93
Uric acid (mg/dl)	49.33^{b}	46.67^{b}	63.67^{a}	72.67^{a}	47.00^{b}	49.67^{b}	66.00^{a}	72.67^{a}	2.80
Creatinine (mg/dl)	1.26^{ab}	1.19^{ab}	1.42^{ab}	1.56^{a}	0.89^{b}	0.91^{b}	1.15^{ab}	1.35^{ab}	0.15
AST (IU/L)	135.33	145.35	132.33	141.33	141.67	141.00	149.00	139.77	8.57
ALT (IU/L)	15.37	16.47	13.98	16.60	14.93	16.09	13.64	14.33	1.19
ALP (IU/L)	43.00	48.33	41.00	46.33	44.00	49.33	40.33	37.00	4.61

[a-d]Mean values in the same row having different superscripts are significantly different (P<0.05), FCSM: Fermented castor oil seed meal, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, SEM: Standard Error of Mean, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase

Birds fed from 50 - 150 g/kg FCSM supplemented with herbal methionine recorded similar high haemoglobin concentration values. Birds fed 150 g/kg FCSM supplemented with DL-methionine and those fed 100 and 150 g/kg FCSM supplemented with herbal methionine showed similar high red blood cell values. Similar mean corpuscular volume values were obtained in birds fed 0, 50 and 150 g/kg FCSM supplemented with DL-methionine and birds fed 50 g/kg FCSM supplemented with herbal methionine. Similar mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were obtained in birds fed from 0 - 100 g/kg FCSM supplemented with DL-methionine and all treatments fed from 0-150 g/kg FCSM supplemented with herbal methionine. White blood cell count was lowest in birds fed 150 g/kg FCSM supplemented with DL-methionine and highest in those fed 100 and 150 g/kg FCSM supplemented with herbal methionine. Similar lymphocytes were obtained in birds fed from 0-100 g/kg FCSM supplemented with DL-methionine and birds fed 50 g/kg FCSM supplemented with herbal methionine. Monocytes were comparable in birds fed 50 and 150 g/kg FCSM with DL-methionine supplementation and birds fed from 50 - 150 g/kg FCSM supplemented with herbal methionine. Similar total protein was observed in birds fed 0 and 50 g/kg FCSM with either DL-methionine or herbal methionine supplementation. Albumin was similar in birds fed from 0-150 g/kg FCSM with DL-methionine supplementation and those fed 0 g/kg FCSM supplemented with herbal methionine. Comparable globulin values were obtained in birds fed 0 g/kg FCSM with DL-methionine supplementation and all treatments fed from 0-150 g/kg FCSM supplemented with herbal methionine. Creatinine was comparable in birds fed from 50 - 150 g/kg FCSM with DL-methionine supplementation and all treatments fed from 0-150 g/kg FCSM supplemented with herbal methionine. Alanine aminotransferase activity was similar in birds fed 0, 50 and 150 g/kg FCSM with DL-methionine supplementation and all treatments fed from 0-150 g/kg FCSM supplemented with herbal methionine.

The effects of varying dietary fermented castor oil seed meal (FCSM) levels and methionine supplementation on haematological and serum biochemical parameters of finisher broiler chickens (weeks 4-8) are presented in Table 5. Packed cell volume and haemoglobin concentration were comparably highest in birds fed 0 and 50 g/kg FCSM. Red blood cell was highest in birds fed 0 g/kg FCSM (P<0.05). White blood cell was highest in birds fed 150 g/kg FCSM (P<0.05) and lowest in those fed 0 g/kg FCSM (P<0.05). Birds fed 0 and 50 g/kg FCSM had lower (P<0.05) uric acid values and those fed 150 g/kg FCSM had higher values. Creatinine was lowest in birds fed 0 g/kg FCSM (P<0.05) among all treatments. Birds fed diets containing herbal methionine recorded higher eosinophils than those fed DL-methionine (P<0.05).

The effects of interaction of varying dietary fermented castor oil seed meal (FCSM) levels and methionine supplementation on haematological and serum biochemical parameters of finisher broiler chickens (weeks 4-8) are presented in Table 6. Birds fed 0, 50 and 150 g/kg FCSM supplemented with DL-methionine and those fed 0 and 50 g/kg FCSM supplemented with herbal methionine had comparably high packed cell volume values. Birds fed 0 and 50 g/kg FCSM supplemented with DL-methionine recorded similar haemoglobin concentration values with birds fed from 0 - 100 g/kg FCSM with herbal methionine supplementation. Birds fed 100 and 150 g/kg FCSM supplemented with either DL-methionine or herbal methionine showed high white blood cell values. Lymphocytes were comparably high in birds fed from 0-150 g/kg FCSM with DL-methionine supplementation and those fed 0 and 50 g/kg FCSM supplemented with herbal methionine. Uric acid was high in birds fed 100 and 150 g/kg FCSM supplemented with either DL-methionine or herbal methionine. Creatinine was comparable in birds fed from 0-150 g/kg FCSM with DL-methionine supplementation and those fed 100 and 150 g/kg FCSM supplemented with herbal methionine

DISCUSSION

During the starter phase of the experiment, haemoglobin concentration, red blood cell, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and eosinophils were higher in birds fed diets supplemented with herbal methionine when compared with DL-methionine. Rekhatel et al. (26) reported that herbal methionine had no significant effect on haematological profiles of broiler chickens. Igbasan et al. (27) also observed no significant difference in virtually all haematological variables of birds fed diets supplemented with both synthetic methionine and herbal methionine (Metio-tas). The packed cell volume values (25.33 to 32.33 %) obtained in this study were slightly lower than the normal range of 30.00 to 35 % reported by Campbell et al. (28) for domestic chickens. The haemoglobin concentration values which ranged from 7.20 to 11.67 g/dl fell within the reference values of 6.0 to 13.0 g/dl for domestic chickens (29). Red blood cell values (1.25 to 1.83×10^{12}/l) were lower than the reference values of 2.11-2.88×10^{12}/l for broiler chickens (30). Starter broiler chickens fed diets containing 150 g/kg FCSM supplemented with herbal methionine or DL-methionine recorded higher values than the control birds in terms of haematological parameters such as packed cell volume, haemoglobin concentration and red blood cell. This may be attributed to the increased production of red blood cells in these birds as they tried to develop immunity to the toxic effect of residual ricin. Radeleff (31) reported that ricin as a protein is capable of inducing immunity in animals consuming it in small quantities over a period of time. The mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values reported in the starter phase of the present study were higher than the values obtained by Mustapha et al. (9) when broiler chickens were fed replacement levels of boiled and fermented castor seed meal. Similar and high mean corpuscular volume values were obtained in birds fed 0, 50 and 150 g/kg FCSM supplemented with DL-methionine and those fed 50 g/kg FCSM supplemented with herbal methionine. Similar and high mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were obtained in birds fed from 0-100 g/kg FCSM supplemented with DL-methionine and all treatments fed from 0-150 g/kg FCSM supplemented with herbal methionine. The increase in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration could be an indication of anaemia as suggested by Bush (32). Ojediran et al. (33) also reported an increase in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration as a sign of anaemia in broiler chickens fed differently processed *Jatropha* kernel meal. Mitruka and Rawnsley (34) reported that mean corpuscular volume is vital in determining the cell size of erythrocytes and therefore, an important factor in determining the ability of birds to withstand prolonged oxygen starvation. The white blood cell values obtained across the treatments slightly decreased at the finisher phase compared to the starter phase. An increase in white blood cell count is indicative of toxic condition (32). At the starter phase, white blood cell count was lowest in birds fed 150 g/kg FCSM supplemented with DL-methionine and highest in those fed 100 and 150 g/kg FCSM supplemented with herbal methionine. Birds fed diets containing 100 and 150 g/kg FCSM supplemented with either DL-methionine or herbal methionine showed higher white blood cell values at the finisher phase. White blood cell is known to play an important role in antibody production and recognition of foreign bodies (35). The above observation in white blood cell count suggests a defensive mechanism against the possible presence of residual ricin or infection in the birds. This corroborates the finding of Oso et al. (36) who reported a significant increase in white blood cell count of cockerel chicks as FCSM inclusion increased up to 150 g/kg in the diet. The lymphocytes (28.66 - 37.33 %) and monocytes (0.00-1.67%) which are agranulocytes of the white blood cell were lower than the reference values of 47.2 to 85.0 % and 0.06 to 5.0 %, respectively for healthy domestic chickens (37). Just as observed at the starter phase, finisher broiler chickens fed 100 and 150 g/kg FCSM supplemented with herbal methionine recorded lower lymphocytes. This could possibly be due to the presence of residual toxin or infection in the birds. Lymphocytes secrete antibodies that bind to foreign micro-organisms in body tissues and mediate their destruction. Monocytes are effective in the direct destruction of pathogens and clean-up of cellular debris from sites of infection (38). The high total protein observed in birds fed 0 and 50 g/kg FCSM with either DL-methionine or herbal methionine supplementation could be attributed to good protein reserve reflecting the ability of the birds to store protein for tissue development. Plasma protein is an indication of protein reserve in the body that can reflect the ability of chicken to store reserve protein even after the animal has reached its maximum capacity for depositing tissue (39). The trend of serum albumin was similar to that of total protein, however, serum globulin was inconsistent with total protein. Serum albumin and globulin depend on availability of dietary protein (40). High uric acid noted in birds fed 100 and 150 g/kg FCSM supplemented with either DL-methionine or herbal methionine could be attributed to high quality of protein fed (41) and higher amino acid breakdown as a consequence of higher protein supply. High creatinine was observed in birds fed from 50 - 150 g/kg FCSM with DL-methionine supplementation and all treatments fed from 0-150 g/kg FCSM supplemented with herbal methionine. A marked increase in serum creatinine suggests functional damage to the kidney. Creatinine is one of the most sensitive biochemical markers employed in the diagnosis of renal damage because it is excreted through the kidney (14). Alanine aminotransferase values were similar in birds fed 0, 50 and 150 g/kg FCSM with DL-methionine supplementation and all treatments fed from 0-150 g/kg FCSM supplemented with herbal methionine. Alanine aminotransferase is a key enzyme in the biotransformation and detoxification of various toxicants, reactive oxygen species and endo - and xenobiotics (14). Alanine aminotransferase activity as an index has been used as an indicator of liver function and elevated levels are monitored in liver malfunction (42).

CONCLUSION

It was concluded that dietary inclusion of fermented castor oil seed meal at higher levels of 100 and 150 g/kg has adverse effects on haematological and serum biochemical parameters of broiler chickens. Herbal methionine (Herbo-meth) can successfully replace DL-methionine in the diets of broiler chickens. There is need for further research on effective processing techniques that can enhance the use of castor oil seed meal in monogastric animal rations.

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