JJBS

Volume 14, Number 2, June 2021
ISSN 1995-6673
Pages 205 - 211

Jordan Journal of Biological Sciences

Biochemical Effects of Low Crude Protein Diets Supplemented with Varying Methionine Concentrations

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Received May 2, 2020; Revised August 2, 2020; Accepted August 5, 2020

Abstract

The research investigated the consequences of decreased crude protein (CP) and methionine supplementation in diets on certain biochemical indices in broiler chicks. A total number of 135 newly hatched chicks were allotted to nine dietary treatments, 3 replicates of five birds each. Groups A-C served as the control with 20% CP and 0.6, 1.0 and 1.4% methionine supplementation. Groups D-F and G-I were placed on 17 and 14% CP diets respectively, with similar methionine supplementation as the control groups. The experiment lasted six weeks. Vital biochemical parameters studied include alkaline phosphatase, aminotransferases, superoxide dismutase, catalase, malondialdehyde, and organ-body weight ratios. Findings from the study indicated that there was a significant ($p<0.05$) reduction in alkaline phosphatase activities of tissues of birds placed on 17 and 14% CP as correlated with birds on the control. MDA concentration was significantly ($p<0.05$) elevated in the liver, kidney, and heart of broiler chicks fed diets with 14 and 17% CP at all methionine levels investigated when compared to control (20% CP). The low crude protein with varying methionine concentrations in broiler chick diets had no negative impact on birds' vital organs relative to whole body weight. These findings thus concluded that the functions and not size of the organs studied in broiler chicks were adversely affected at CP levels below 20%.

Keywords: Crude protein, methionine, lysine, gizzard, superoxide dismutase.

1. Introduction

Proteins are macromolecules that are made up of chains of amino acids; they play a key role in the structural build-up of body tissue (human and animal) and have a significant impact on growth as indices of performance in broiler birds (Pinchasov *et al*., 1990). Amino acids are structurally made up of a carbon framework, a carboxyl group, an amino group with an R-chain that distinguishes one amino acid from the other (Cheeke, 2005). Protein metabolism releases these amino acids that perform several useful functions such as the up growth of the architectural parts of muscles, enzymes, and antibodies that are specific in roles in the different parts of the body (Pond, 1995, Abbasi *et al*., 2014 and Van Emous *et al*., 2015).

Methionine content of plant-based poultry feeds is inadequate in meeting up with the requirement of avian (NRC, 1994). This non-sufficiency mostly hinders the biodiversity quality of protein in corn cum soybean-based diets (Meirelles *et al*., 2003, Lemme *et al*., 2005, Kim *et al*., 2006 and Matsushita, *et al*., 2007). The metamorphosis of methionine to S-adenosyl methionine enhances the DNA methylation process which eventually dictates cell growth and eventual specialization (Niculescu and Zeisel, 2002). The activities of skeletal muscle tissues and meat quality during the methylation process are also a function of methionine content of poultry diet (Liu *et al*., 2010).

Reducing feed cost while ascertaining the effectiveness of usage of reduced protein diet has been boosted with crystalline amino acids, in this case methionine, to meet the amino acid requirement in poultry birds as reported by (NRC, 1994) is always the major target of animal nutritionists. Excess nitrogen from high crude protein diets could pose a major health challenge to the environment. Hence, reducing the cost of production and environmental pollution is germane to the poultry industry (Keshavarz, 2003; Gunawardana et al., 2008 and Alagawany and Mahrose, 2014). Thus, for a safer environment, formulation of diets with well-balanced amino acids, in this case methionine, that precisely meet the needs of the birds becomes paramount. While ascertaining the adequacy of methionine to meet broiler birds' requirement, it is equally germane to monitor the biochemical enzyme activities of such diet at the organ level. It is imperative to be able to say categorically that the activities of the enzymes are being retained in the membrane or getting leaked out into the serum while feeding a low crude protein cum varying levels of methionine supplemented diets to broiler birds. This research, hence, evaluated some vital biochemical indices like alkaline phosphatase, alanine aminotransferase, catalase, superoxide dismutase, and malondialdehyde in vital tissues of broiler birds fed low protein with varying methionine concentrated diets.

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2. Materials and Methods

2.1. Source of Chemicals and Reagents

Assay kits for various enzymes activities studied in this research were sourced from Randox Laboratories, County Antrim, UK. All other reagents were of analytical grade and were prepared according to standard procedure.

2.1.1. Experimental Diets and Management of Animals

Diets essentially rich in nutritional requirements of birds (Table 1) as well as suitable for the purpose of the research were prepared in the nutrition section of the Biochemistry and Nutrition unit of Department of Chemical Sciences, Fountain University, Osogbo. Broiler birds (arbor acre) used for the study were gotten from a reputable farm (ROS farm) in Osogbo. The birds were raised in a well brightened and well-oxygenated poultry unit where feed and water were served without restriction. The experimental protocol followed the regulations of the Animal Care and Use Committee, Fountain University, Osogbo, Nigeria. Vaccinations were carried out as at when due.

Table 1. Gross Composition (Kg/100 g) of Experimental Diets

Crude Protein (%)	20	20	20	17	17	17	14	14	14
Methionine (%)	0.6	1.0	1.4	0.6	1.0	1.4	0.6	1.0	1.4
Lysine (%)	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Maize	45.50	45.50	45.50	48.00	48.00	48.00	56.00	56.00	56.00
Soybean	32.00	32.00	32.00	21.00	21.00	21.00	12.00	12.00	12.00
Wheat offal	12.00	12.00	12.00	20.00	20.00	20.00	21.00	21.00	21.00
Palm oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Dicalcium phosphate	2.40	2.22	2.00	2.50	2.45	2.05	2.50	2.19	2.00
Limestone	2.22	2.00	1.82	2.35	2.00	2.00	2.09	2.00	1.79
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.30	0.70	1.10	0.34	0.74	1.14	0.38	0.68	1.18
Lysine	0.08	0.08	0.08	0.31	0.31	0.31	0.53	0.53	0.53
Total	100	100	100	100	100	100	100	100	100

CP-crude protein *Premix supplied the following information kg of diet: Vitamin A (12,500,000 I.U), Vit D3 (2,500,000 I.U), Vit E (40,000mg) Vitamin K3 (2,000mg), Vit B, (3,000mg), Vit B2 (5,500mg), Naicin (55,000mg), calcium panthothenate (11,500mg) Vit B6 (5000mg) Vit B12 (25mg), choline chloride (500, 000mg), folic acid (1,000mg), Biotin (80mg), Mn (120,000,mg), Fe (100,000mg), Zn (80,000mg), Cu (8,500mg), I (1,500mg) Co (300mg), Se (120mg).

2.1.2. Experimental Design

135 newly hatched broiler chicks were distributed to nine experimental dietary treatments, three replicates of five birds each. The experimental diets consisted of 20, 17 and 14 % crude protein (CP), each with 0.6, 1.0 and 1.4 % methionine (Table 1). A diet containing 20 % CP served as the control diet. All diets had a 1.2 % isolysine content. The study lasted six weeks.

2.2. Blood Collection and Tissue Homogenate Preparation for Analysis

Birds were starved a night preceding the day of the sacrifice (at the end of feeding trial) to empty the crop. Three birds per replicate were randomly selected and weighed, then 10 mL blood sample was aspirated via the jugular vein. Aspirated blood samples were centrifuged at 3000 revolutions per minute for 10 minutes using a Bench Centrifuge 90-1, Gallenkomp, England to get serum for the various analysis. The serum collected using Pasteur pipette was kept frozen at -20^{0}C and used within seven days of collection. Thereafter, the selected broiler birds were then sacrificed, de-feathered, reweighed to get dressing weight and then dissected to get precise organs of interest. The organs were cleaned with tissue paper to remove the attached debris. A known weight of each organ was then homogenized in an ice-cold 0.25 M sucrose solution according to the method of Ogbu and Okechukwu (2001) in order to retain the integrity of the tissues needed for analysis.

2.3. Enzyme Activities

The concentration of protein in the serum was analysed for, by the Biuret method as reported by Gornall *et al* (1949). Standard procedures for analysis of enzyme activities were strictly adhered to, as described for ALP by Wright *et al*. (1972); aminotransferases by Reitman and Frankel (1957); SOD by Amiceralli (1999); CAT by Beers and Sizer (1952) and MDA by Reilly and Aust (1978).

2.4. Statistical Analysis

Research values were described as means ± standard deviation (n=3). Analyses of Variance (ANOVA) accompanied by Tukey-Kramer test for nonconcurrences amongst means were utilized to ascertain any significant differences ($p<0.05$) between variables.

3. Results

3.1. Tissues Enzymes Assay

Observable ($p<0.05$) differences in the serum ALP activities of birds on 20% crude protein (CP) with varying methionine concentrations and those on other levels of crude protein (Table 2) were noticed. The same applies to all organs studied except the liver. Liver ALP portrayed no statistically significant ($p>0.05$) difference among the birds placed on 20 % and those on 17% CP, regardless of the varying methionine concentrations, but significantly different for those on 14% CP. Overall, various organ ALP enzyme activities decrease with a decrease in crude protein percentage cum increase in methionine concentrations.

Serum AST activities were noticeably ($p<0.05$) increased with a decrease in crude protein concentration of the diets (Table 3). Liver and heart AST were significantly ($p<0.05$) higher at 20% CP inclusion level with a corresponding decrease in the activities of the enzyme in

the serum at this level. In the same vein, liver and heart AST activities decreased noticeably ($p<0.05$), with a decrease in CP inclusion level, mostly at 14 and 17% regardless of the methionine variations, this correspondingly lead to increase in the enzyme activities of the serum at this level, except that at 17% CP and 0.6% methionine based diet, that had reduced serum activities competing favourably with birds on 20 % CP and 0.6-1.4% methionine inclusion level.

Though there were no observable ($p>0.05$) differences in the liver ALT enzyme activities amongst all experimental diets (control inclusive), numerically, as the crude protein decreases, liver ALT activities decrease with a concurrent elevation in the activities of the enzyme in the serum (Table 4).

Table 2. Specific activities of alkaline phosphatase (ALP- U/mg) in some organs and serum of broiler chickens fed with varying levels of crude protein and methionine supplemented diets

DIETARY TREATMENT	SERUM	LIVER	KIDNEY	HEART	GIZZARD	DUODENUM	PROVENTRICULUS
20%CP, 0.6%Met	0.49 ± 0.01^{d}	90.34±7.54^{a}	59.26±5.10^{a}	11.72±1.64^{a}	13.48±0.60^{a}	1.32±0.49^{a}	48.51±0.55^{a}
20%CP, 1.0%Met	0.49 ± 0.01^{d}	89.66 ±7.44^{a}	53.77±5.25^{a}	12.10±0.40^{a}	12.57±0.89^{a}	1.40±0.15^{a}	45.40±2.83^{a}
20%CP, 1.4%Met	0.50±0.09^{d}	85.67 ±8.98^{a}	55.08±6.12^{a}	12.02±2.02^{a}	16.84±1.45^{a}	1.37±0.05^{a}	45.24±1.44^{a}
17%CP, 0.6%Met	0.55 ± 0.05^{c}	87.45 ±6.45^{a}	44.76±4.64^{b}	7.78±0.91^{d}	10.64±1.44^{b}	0.57±0.04^{b}	40.81±0.91^{b}
17%CP, 1.0%Met	0.56±0.03^{c}	80.54 ±6.00^{a}	41.23±3.40^{b}	8.42±0.50^{c}	9.03±1.03^{b}	0.50±0.03^{b}	31.80±1.32^{b}
17%CP, 1.4%Met	0.56 ± 0.04^{c}	82.65 ±7.78^{a}	33.72±3.83^{c}	8.60±1.45^{c}	8.28±0.50^{b}	0.62±0.03^{b}	25.70±1.03^{b}
14%CP, 0.6%Met	1.96±0.03^{a}	68 .17 ±1.6^{b}	23.05±3.21^{d}	9.00±1.28^{b}	6.30±0.43^{c}	0.22±0.02^{c}	15.23±0.41^{c}
14%CP, 1.0%Met	1.88±0.09^{a}	69.20 ±2.38^{b}	18.03±1.45^{e}	9.58±0.94^{b}	6.80±1.94^{c}	0.25±0.01^{c}	12.45±0.49^{d}
14%CP, 1.4%Met	1.57±0.09^{b}	65.75 ±1.45^{c}	21.02±1.12^{d}	9.62±1.08^{b}	6.01±1.77^{c}	0.20±0.01^{c}	11.32±1.65^{d}

Values are mean±SD of 3 determinations. Enzyme activities are expressed as nmol $min^{-1}mg^{-1}$protein.
$^{a-d}$ Values carrying different superscripts for each organ are significantly different $P<0.05$

Table 3. Specific activities of Aspartate Transaminase (AST-U/mg) in the liver, heart, and serum of broiler chickens fed with varying levels of crude protein and methionine supplemented diets.

DIETARY TREATMENT	SERUM	LIVER	HEART
20%CP, 0.6%Met	5.25±0.75^{d}	65.00±4.00^{a}	58.33±8.14^{a}
20%CP, 1.0%Met	6.75±0.86^{d}	58.11±3.95^{a}	57.25±0.75^{a}
20%CP, 1.4%Met	6.00±0.43^{d}	45.00±2.82^{b}	56.03±1.49^{a}
17%CP, 0.6%Met	6.50±0.50^{d}	51.33±4.03^{b}	47.00±3.00^{b}
17%CP, 1.0%Met	7.50±0.30^{c}	46.50±3.00^{b}	39.98±2.55^{c}
17%CP, 1.4%Met	8.75±0.73^{b}	45.25±4.25^{b}	36.50±3.50^{c}
14%CP, 0.6%Met	8.50±0.70^{b}	40.00±2.00^{c}	33.13±2.71^{c}
14%CP, 1.0%Met	8.90±0.30^{b}	33.50±3.50^{d}	29.33±2.89^{c}
14%CP, 1.4%Met	9.50±0.10^{a}	30.75±3.25^{d}	19.25±0.75^{d}

The results are mean ± SD of 3 determinations. Enzyme activities are expressed as nmol $min^{-1}mg^{-1}$protein.
$^{a-d}$ Values carrying superscripts different from the control for each organ are significantly different ($P<0.05$).

Table 4. Specific activities of alanine aminotransferase (ALT-U/mg) in the serum and liver of broiler chickens fed with varying levels of crude protein and methionine supplemented diets

DIETARY REATMENT	SERUM	LIVER
20%CP, 0.6%Met	2.00±0.20^{d}	21.67±1.15^{a}
20%CP, 1.0%Met	3.11±0.51^{c}	21.06±2.64^{a}
20%CP, 1.4%Met	3.33±0.25^{c}	21.33±0.76^{a}
17%CP, 0.6%Met	4.67±0.31^{a}	19.67±2.77^{a}
17%CP, 1.0%Met	3.67±0.04^{b}	20.00±1.00^{a}
17%CP, 1.4%Met	5.67±1.21^{a}	20.33±0.57^{a}
14%CP, 0.6%Met	4.67±0.51^{a}	18.67±3.21^{a}
14%CP, 1.0%Met	5.00±1.64^{a}	18.67±2.08^{a}
14%CP, 1.4%Met	6.67±1.51^{a}	19.67±2.32^{a}

Data are mean of three determinations ± SD. Specific enzyme activities are expressed as nmol min^{-1}mgprotein^{-1}.
$^{a-d}$ Values carrying different superscripts are significantly different ($P<0.05$).

3.2. Antioxidant enzymes assay

Statistically significant ($p<0.05$) difference occurred in all organs' SOD activities of birds on 20% CP with varying Met concentration when compared with other diets concentration levels in the study with the exception of the activity of the enzyme in the liver (Table 5). There was no significant difference ($p>0.05$) in the liver SOD activities of birds placed on 20% and 17% CP with varying Met concentration, but the duo was glaringly different when correlated with the performance of the enzyme on birds placed on 14% CP diet at various Met concentration.

Noticeable ($p<0.05$) differences in the CAT activities of birds on the various CP/Met supplemented diets (table 6) were observed. 20% CP / 0.6% Met was significantly ($p<0.05$) different from every other CP/Met combination. Overall, the activity of the enzyme reduces with a reduction in crude protein content at any methionine combination level.

Various organs malondialdehyde (MDA) level of birds on the control diet was noticeably ($p<0.05$) lower when compared with the values obtained for birds on other (experimental) diets signifying a more toxicity level in the studied organ at percentages lower than 20% (Table 7).

Table 5. Specific activities of Superoxide Dismutase (SOD-U/ml) in some vital organs of broiler chickens fed with varying levels of crude protein and methionine supplemented diets.

DIETARY TREATMENT	LIVER	KIDNEY	HEART	DUODENUM	GIZZARD	PROVENTRICULUS
20%CP, 0.6%Met	0.55 ± 0.04^{a}	0.67 ± 0.15^{a}	0.42 ± 0.07^{a}	0.37 ± 0.06^{a}	0.43 ± 0.01^{b}	0.69 ± 0.02^{a}
20%CP, 1.0%Met	0.51 ± 0.03^{a}	0.53 ± 0.08^{ab}	0.41 ± 0.55^{a}	0.25 ± 0.05^{bc}	0.48 ± 0.02^{a}	0.69 ± 0.03^{a}
20%CP, 1.4%Met	0.58 ± 0.01^{a}	0.48 ± 0.01^{b}	0.39 ± 0.09^{a}	0.25 ± 0.02^{b}	0.41 ± 0.01^{bc}	0.66 ± 0.03^{a}
17%CP, 0.6%Met	0.58 ± 0.08^{a}	0.39 ± 0.02^{c}	0.37 ± 0.10^{b}	0.28 ± 0.03^{b}	0.26 ± 0.01^{e}	0.47 ± 0.09^{b}
17%CP, 1.0%Met	0.54 ± 0.04^{a}	0.39 ± 0.01^{c}	0.32 ± 0.00^{b}	0.18 ± 0.03^{c}	0.21 ± 0.01^{f}	0.30 ± 0.04^{c}
17%CP, 1.4%Met	0.51 ± 0.04^{a}	0.37 ± 0.02^{c}	0.28 ± 0.03^{c}	0.31 ± 0.08^{ab}	0.27 ± 0.01^{e}	0.46 ± 0.15^{b}
14%CP, 0.6%Met	0.31 ± 0.04^{c}	0.35 ± 0.04^{cd}	0.28 ± 0.03^{c}	0.11 ± 0.01^{d}	0.36 ± 0.05^{c}	0.30 ± 0.05^{c}
14%CP, 1.0%Met	0.41 ± 0.03^{b}	0.33 ± 0.04^{d}	0.23 ± 0.08^{cd}	0.17 ± 0.04^{c}	0.39 ± 0.01^{c}	0.22 ± 0.11^{d}
14%CP, 1.4%Met	0.39 ± 0.05^{bc}	0.21 ± 0.00^{e}	0.20 ± 0.02^{d}	0.12 ± 0.05^{cd}	0.35 ± 0.20^{cd}	0.24 ± 0.25^{d}

The results are mean ± SD of 3 determinations. Enzyme activities are expressed as nmol $min^{-1}mg^{-1}$protein.

[a-d] Values carrying superscripts different from the control for each organ are significantly different ($P<0.05$).

Table 6. Specific activities of catalase (CAT- U/ml) in the kidney and heart of broiler chickens fed with varying levels of crude protein and methionine supplemented diet

DIETARY TREATMENT	LIVER	KIDNEY	HEART
20%CP, 0.6%Met	37.21 ± 1.21^{a}	42.13 ± 4.60^{a}	52.42 ± 4.62^{a}
20%CP, 1.0%Met	33.67 ± 2.23^{b}	35.89 ± 3.19^{ab}	45.10 ± 1.05^{b}
20%CP, 1.4%Met	30.18 ± 3.18^{b}	31.07 ± 4.76^{b}	42.72 ± 4.83^{b}
17%CP, 0.6%Met	25.13 ± 2.38^{c}	37.83 ± 2.30^{ab}	36.48 ± 1.01^{c}
17%CP, 1.0%Met	22.01 ± 3.49^{c}	38.27 ± 3.76^{ab}	31.99 ± 1.24^{d}
17%CP, 1.4%Met	28.08 ± 2.92^{c}	35.86 ± 3.33^{ab}	30.26 ± 3.53^{d}
14%CP, 0.6%Met	18.82 ± 1.55^{d}	24.67 ± 1.80^{c}	25.68 ± 1.13^{e}
14%CP, 1.0%Met	11.82 ± 1.25^{e}	17.90 ± 3.45^{d}	23.09 ± 1.61^{ef}
14%CP, 1.4%Met	13.08 ± 1.12^{e}	15.13 ± 1.49^{d}	21.82 ± 1.25^{f}

The results are mean ± SD of 3 determinations. Enzyme activities are expressed as nmol $min^{-1}mg^{-1}$protein.

[a-d] Values carrying superscripts different from the control for each organ are significantly different ($P<0.05$).

Table 7. Malondialdehyde (MDA- n mol/ml) concentrations in some vital organs of broiler chickens fed with varying levels of crude protein and methionine supplemented diets

DIETARY TREATMENT	LIVER (x10-9)	KIDNEY (x10-9)	HEART (x10-9)	GIZZARD (x10-9)	DUODENUM (x10-9)	PROVENTRICULUS (x10-9)
20%CP, 0.6%Met	2.47 ± 0.32^{c}	2.03 ± 0.50^{c}	0.84 ± 0.02^{e}	5.15 ± 0.50^{e}	8.42 ± 0.05^{f}	7.19 ± 1.20^{d}
20%CP, 1.0%Met	2.21 ± 0.33^{c}	2.69 ± 0.01^{c}	0.87 ± 0.09^{e}	8.68 ± 0.31^{d}	10.01 ± 0.31^{e}	5.50 ± 3.90^{d}
20%CP, 1.4%Met	2.39 ± 0.13^{c}	2.76 ± 0.25^{c}	0.90 ± 0.05^{e}	11.37 ± 0.79^{c}	13.70 ± 0.60^{d}	7.18 ± 0.11^{d}
17%CP, 0.6%Met	3.05 ± 0.14^{b}	16.90 ± 2.09^{ab}	5.24 ± 0.12^{b}	11.20 ± 1.90^{c}	17.30 ± 2.00^{a}	13.40 ± 1.00^{c}
17%CP, 1.0%Met	3.17 ± 0.32^{b}	18.70 ± 2.90^{a}	8.07 ± 1.24^{a}	15.10 ± 2.40^{b}	14.48 ± 0.09^{c}	13.20 ± 2.80^{c}
17%CP, 1.4%Met	3.30 ± 1.21^{b}	17.20 ± 1.76^{a}	8.30 ± 0.99^{a}	20.30 ± 1.30^{a}	14.20 ± 1.10^{bc}	16.20 ± 4.10^{c}
14%CP, 0.6%Met	5.40 ± 0.48^{a}	14.00 ± 1.92^{b}	1.60 ± 1.45^{de}	9.63 ± 0.30^{d}	14.69 ± 0.04^{b}	21.10 ± 1.40^{b}
14%CP, 1.0%Met	5.40 ± 0.97^{a}	15.8 ± 1.57^{b}	2.06 ± 0.21^{d}	13.40 ± 1.10^{b}	16.80 ± 2.30^{ab}	25.10 ± 1.60^{a}
14%CP, 1.4%Met	5.10 ± 0.46^{a}	17.60 ± 1.90^{a}	3.61 ± 0.06^{c}	17.20 ± 2.70^{a}	18.60 ± 2.50^{a}	27.30 ± 2.10^{a}

The results are mean ± SD of 3 determinations. Malondialdehyde concentrations are expressed as nmol mg^{-1} protein.

[a-d] Values carrying superscripts different from the control for each organ are significantly different ($P<0.05$).

3.3. Organ to Body Weight Ratio

The formulation of the experimental diet did not affect all organs that were studied (Table 8). No significant differences ($p>0.05$) between the various organs to body ratio amongst all crude protein/methionine concentrations under investigation.

Table 8. Organ to body weight ratio (%) of broiler chickens fed with varying levels of crude protein and methionine supplemented diets ($x10^{-2}$).

DIETARY TREATMENT	LIVER	KIDNEY	HEART	GIZZARD	PROVEN-TRICULUS	DUODENUM
20%CP, 0.6%Met	2.50±0.01[a]	0.38±0.02[a]	0.58±0.02[a]	1.90±0.50[a]	0.40±0.10[a]	0.60±0.10[a]
20%CP, 1.0%Met	2.45±0.11[a]	0.35±0.02[a]	0.57±0.01[a]	2.00±0.30[a]	0.50±0.10[a]	0.70±0.10[a]
20%CP, 1.4%Met	2.50±0.01[a]	0.37±0.01[a]	0.58±0.00[a]	2.30±0.30[a]	0.40±0.10[a]	0.50±0.10[a]
17%CP, 0.6%Met	2.49±0.10[a]	0.37±0.01[a]	0.57±0.00[a]	2.30±0.40[a]	0.50±0.10[a]	0.70±0.10[a]
17%CP, 1.0%Met	2.51±0.02[a]	0.36±0.03[a]	0.57±0.03[a]	2.60±0.50[a]	0.50±0.10[a]	0.50±0.20[a]
17%CP, 1.4%Met	2.50±0.01[a]	0.38±0.02[a]	0.58±0.00[a]	3.20±0.30[a]	0.60±0.10[a]	0.70±0.20[a]
14%CP, 0.6%Met	2.53±0.01[a]	0.38±0.06[a]	0.56±0.09[a]	1.80±0.60[a]	0.50±0.10[a]	0.80±0.10[a]
14%CP, 1.0%Met	2.49±0.18[a]	0.36±0.03[a]	0.57±0.01[a]	1.90±0.70[a]	0.40±0.10[a]	0.50±0.10[a]
14%CP, 1.4%Met	2.50±0.01[a]	0.38±0.09[a]	0.56±0.08[a]	2.50±0.20[a]	0.50±0.01[a]	0.80±0.20[a]

The results are mean ± SD of 3 determinations.

[a] Values carrying same superscripts with the control for each organ and serum are not significantly different ($P>0.05$).

4. Discussion

Alkaline phosphatase is an omnipresent membrane-bound glycoprotein that initiates the disintegration of phosphate monoesters at fundamental pH values. Tissue nonspecific alkaline phosphatase is found in various tissues in the human body. It plays a significant role in metabolism, especially in liver functionality and bone growth. It facilitates the breakdown of protein (Hada *et al*., 1978 and Husni *et al*., 2012). It had earlier been reported that significantly increased levels of ALP enzyme activities of birds on experimental diets as compared to the activity of the same enzyme for birds on the control diet might indicate damage to the liver (Arslan *et al*., 2003 and Saeid *et al*., 2014). This study reported a significant ($p<0.05$) decrease in ALP enzyme activities of liver and other organs of interest as correlated with the control, hence no detrimental aftermath on the activities of organs studied as it has to do with ALP activities. Serum ALP enzyme activities, on the other hand, demonstrated a progressive increase as crude protein percentages decrease. This is an indication that at reduced crude protein percentage (mostly below 20%), enzyme activities in the serum increase. This might signify more toxic enzyme activities of the serum at reduced crude protein percentage (below 20%). This aligned with the work of Samantha *et al*; (2018) that proclaimed highest blood serum ALP activity levels in broiler birds given methionine over or above the requirement as suggested by NRC, (1994) recommendation.

Aspartate aminotransferase (AST) is an aminotransferase enzyme that initiates the metamorphosis of aspartate and carbonyl compound to oxaloacetate and glutamate. It is found in all tissues save bone, with zenithal levels mostly found in the liver and skeletal muscle (Evans, 2009). The aminotransferases (AST and ALT) are normally restricted within liver cells and are discharged into the blood when liver cells are impaired. Hence, elevated levels of AST or ALT mostly signify liver impairment (Hrapkiewic and Medina, 2007 and Madu and Nadro, 2017). It had earlier been reported that the activities, most of the liver enzymes that are associated with the breaking down of amino acids decline with a flat protein-based diet and invariably increases with an elevated protein-based diet (Muramatsu *et al*., 1971; Roudbaneh *et al*., 2013 and Ospina-Rojas *et al*., 2014. This negates the work of Elham *et al*; (2010) which reported the highest serum AST concentration for birds on the control diets as against other experimental diets, probably because the birds used were challenged alongside and fed with methionine and threonine concentration over and above NRC, (1994) recommendation. This is buttressing the fact that at reduced CP inclusion level, mostly below 20%, most enzymes got leaked into the serum in broiler birds.

Alanine aminotransferase (ALT), also an aminotransferase enzyme is obtained in serum and organ tissues, most importantly in the liver, though appreciable amounts also originated in the kidney, skeletal muscle, and myocardium. The enzyme is raised in serum under conditions of notable cellular loss and is always an indication of liver functionality. Nutritional intake, restraint, and drug prescription may affect plasma ALT in rodents (Evans, 2009). The observed results of the ALT are in line with the research findings of Elham *et al*; (2010) which reported a non-significant effect on liver ALT activities especially on day 28 when birds were fed methionine and threonine above NRC, (1994) recommendation.

Superoxide dismutase (SOD) is a pristine detox enzyme and the utmost competent oxidant inhibitor in the cell. It is a crucial endogenic oxidant inhibitor enzyme that fights against free radicals. It activates the neutralization of superoxide ion (free radicals), thereby relinquishing the presumably pernicious superoxide anion that is not very risky (Hercberg *et al*., 2004; Halliwell *et al*., 2005; Yuan *et al*., 2010 and Dysken *et al*., 2014). The observed results of the ALT supported the work of Subbaiah *et al*; (2011) that proclaimed a reduction in the SOD activities of birds infected with Newcastle Disease Virus (NDV) and given dietary methionine content over and above the recommendation of NRC, (1994). On the other hand, it negated the research outcome of Jan *et al*; (2018) which reported increased activities of SOD in the liver of turkeys fed increased dietary methionine contents over 40% above NRC, (1994) recommendation.

Catalase (CAT) is an accepted oxidation inhibitor enzyme found almost in all living tissues that uses a non-metallic bivalent element, that is, the most sufficient element in the earth's surface. The enzyme utilizes iron or manganese as an alloy and disproportionates the degeneration or diminution of the simplest peroxide to water and oxygen, hence finalizing the cleansing procedure mimicked by superoxide dismutase (Chelikani *et al*., 2004). The observed result in this regard is in line with the research outcome of Jan *et al*; (2018) that reported a surge in the activity of the enzyme in the intestinal wall and liver of turkey fed increase methionine content above NRC, (1994) recommendation.

Malondialdehyde (MDA) is an extremely dangerous secondary product formed partly by lipid oxidation derived free radicals. It is an enzymatic assay globally used for determining oxidative stress in the biomedical field where lipid peroxidation is a chain phenomenon giving rise to the formation of various active compounds which eventually result in cellular damage (Slatter *et al*., 2000). The results of the MDA are in accord with the finding of Subbaiah *et al*; (2011) that acclaimed a surge in the level of MDA of birds infected with Newcastle Disease Virus (NDV) and given dietary methionine content over and above the recommendation of NRC. A reduction in the MDA level in the liver, but a surge in the activity of the enzyme in the intestinal wall of turkey fed increased dietary methionine content above NRC, (1994) recommendation (>40%) was earlier reported by Jan *et al*; (2018). Hepatic MDA concentration in this study was significantly influenced by crude protein/methionine combination at below 20% and 1.4% respectively. This does not align with the research outcome of Jan *et al*; (2018) which claimed that higher methionine concentrations in the diet did not influence the hepatic MDA combination.

Organ to body weight ratio is usually an indication of impending toxic effect, in this case of experimental diet on vital organs monitored (Olayode *et al*., 2019). The biochemical reason that warrants analyzing for relative organ weight is that mostly, organ weight changes are normally relative weight-wise to total body weight. Hence, they are usually examined to find out whether the size of the organ has changed, especially as a subset of the weight of the whole animal, which will eventually serve as an indicator of the adverse effect of drugs or experimental diets on the target organ (Nigatu *et al*., 2017). Vital organs have been reported to obtain their nutritional requirement irrespective of other body performance demands (Cengiz and Küçükersan, 2010; Ospina-Rojas *et al*., 2014 and Shirzadegan *et al*., 2015). This research finding negates the work of Amr (2015) which reported a markedly highest weight of proventriculus, heart and liver of birds on the control diet (22.1% CP, 1.18% lysine and 0.52% methionine) as compared with other experimental diets when birds were fed with exuberance methionine and lysine in the presence or absence of L-Carnitine.

5. Conclusion

In conclusion, enzyme activities and not the size of the organs studied in broiler chicks were adversely affected at CP levels below 20%, up till 14%, even with methionine supplementation.

Acknowledgments

The author hereby acknowledges the input of laboratory staff of Fountain University Osogbo for their input during laboratory analysis.

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