African Journal of Biotechnology Vol. 11(46), pp. 10617-10625, 7 June, 2012
Available online at http://www.academicjournals.org/AJB
DOI:10.5897/AJB11.4322

Full Length Research Paper

Comparative efficacy of herbal and synthetic methionine on performance of some haematological and biochemical parameters in domestic laying hens

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Accepted 18 April, 2012

A feeding trial was conducted to determine the bioefficacy of herbal methionine (HM) compared to synthetic methionine (SM) in the diets of domestic laying hens. The herbal methionine (Meth-o-Tas®) was supplied by Intas Pharmaceutical Limited, India. The HM and SM were added to a standard diet at 0.5 and 1.0 kg per ton and fed to 144 laying birds, 30 weeks of age, housed in 3 birds per cage unit. The birds were divided into 4 dietary treatments of 36 birds each and each treatment group was replicated 4 times with 9 birds per replicate. The trial lasted for 112 days and was divided into 4 periods of 28 days each. At the end of the feeding trial, 2 birds per replicate, representing 8 birds per treatment were sacrificed for the determinations of haematological and plasma biochemical profiles, liver weight and abdominal fat pad. Layers fed on diets supplemented with HM produced less ($P \leq 0.01$) eggs, had lower egg mass output ($P \leq 0.01$) and final body weight ($P \leq 0.05$) and poorer feed conversion efficiency ($P \leq 0.05$) than those layers fed on diets supplemented with SM. Feed intake was not affected ($P \geq 0.05$) by dietary methionine source. Exterior and interior egg quality characteristics such as egg weight, shell thickness, albumen weight and albumen height decreased ($P \leq 0.05$) with dietary supplementation of HM. Dietary supplementation of HM reduced ($P \leq 0.05$) abdominal fat pad. Total protein and albumin decreased ($P \leq 0.05$) with dietary supplementation of HM. Plasma and liver total cholesterol and triglyceride concentrations were lowered ($P \geq 0.05$) in birds fed on diets supplemented with HM. The activities of plasma and liver alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP) were not altered ($P \geq 0.05$) by HM supplementation. All haematological variables determined were not affected by HM supplementation. Under the conditions of this study, it was concluded that herbal methionine (Meth-o-Tas®) is not an effective substitute for synthetic methionine for optimum production performance.

Key words: Herbal methionine, synthetic methionine, performance, laying hens.

INTRODUCTION

Methionine, an indispensable amino acid, is needed for healthy and productive poultry. Methionine has main role in energy production, protein synthesis; it also helps in enhancing egg production with optimum egg size, overall growth performance, feed efficiency utilization and livability in broilers and layers (Binder, 2003; Aerni et al., 2005). Methionine is a potent donor of methyl groups, which contributes to the synthesis of many important substances including epinephrine, choline and creatinine (Bender, 1975). It is generally the first limiting amino acid in poultry diets.

A major cause of methionine deficiency is the fact that

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large amounts of vegetable protein supplements are used in feeds, in combination with low levels of animal and fish proteins. It is more economical to add methionine to poultry diets than more soybean meal or other natural protein to meet the requirements (Halder and Roy, 2007). Recently, the safety of such practice has been questioned and their use is becoming restricted in many regions of the world. Also, the cost of synthetic methionine has been on the increase with a resultant increase in the cost of finished feed. Recent reports have shown that there are other natural alternative supplements developed to replace synthetic methionine for maintaining animal performance and well being (Chattopadhyay et al., 2006).

Herbal methionine premixes, manufactured in India have recently found their way into animal feed industry. Methiorep® and Meth-o-Tas® are herbal methionine premixes that are available in animal feed market. They are phytoadditive containing herbal ingredients that mimic the activity of methionine. Under Indian conditions, herbal methionine (Methiorep®) has been found to replace DL-methionine very effectively when used in broiler rations (Chattopadhyay et al., 2006; Kalbande et al., 2009). However, Itoe et al. (2010) reported that Methiorep® is not substitute for synthetic methionine in broiler diets under Nigerian environment. There is little or no scientific report on the efficacy of herbal methionine on the performance of laying hens. Therefore, the present study was carried out to determine the comparative efficacy of herbal (Meth-o-Tas®) and synthetic methionine on the productive performance, haematological and biochemical parameters in domestic laying hens.

MATERIALS AND METHODS

The herbal methionine premix (Meth-o-Tas®) used for the study was supplied by Intas Pharmaceutical Limited, India. The composition of the HM as specified by the manufacturer includes the following: *Andrographic paniculata* (35%); *Zea mays* (25%); *Ocimum sanctum* (30%) and *Asparagus racemosus* (10%). The recommended inclusion rate is 1 kg per ton of layer's mash. The HM and SM were added to a corn-soy standard diet (Table 1), formulated to meet the nutrient requirements (NRC, 1994) of laying hens, at 0.5 and 1.0 kg per ton of feed.

A total of one hundred and forty-four (144) Haco black laying birds, 30 weeks of age were purchased from the Poultry Unit of the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. They were housed in a 3tier California type colony cages (43 × 41 cm) at three birds per cage placed in an open-sided poultry house. The cages were equipped with open galvanized feeders and aluminium water troughs. The birds were divided into 4 dietary treatments of 36 birds each. All animals received humane care and the principles outlined in the Helsinki declaration were adhered to strictly. Each treatment group was randomly replicated 4 times, with 9 birds per replicate. Feed and water were provided *ad libitum* throughout the experimental period. The duration of the experiment was 112 days (between 30 and 46 weeks of age) and was divided into 4 periods of 28 days each. Egg production was recorded daily and data were pooled into 28-day period. Feed consumption was determined at the end of each 28-day period. Individual body weights were obtained at the start and end of the experiment.

On the last three consecutive days of the 28-day periods, eggs gathered were used for exterior and interior egg quality determinations. The eggs were individually weighed on a sensitive weighing balance to determine average egg weights. Thereafter, they eggs were carefully broken out on a white tile (30 × 30 cm^2) with the blunt edge of a knife to determine the albumen, yolk, shell weights and shell thickness. The albumen of the broken egg was carefully separated from the yolk using a separator and the albumen and yolk weights were measured with a sensitive weighing balance. Albumen height was obtained through the measurement of the height of albumen at the widest expanse and midway between the yolk edge and the external egg with spherometer. The albumen height was converted to Haugh units using the formula described by Oluyemi and Robert (2000). The eggshells were air-dried for about 72 h and weighed to determine shell weight. The measurement of shell thickness was taken at the equator, broad and narrow ends, with a micrometer screw gauge (which had anvil jaws and calibrated in mm). The mean of the regions represented the shell thickness. Perfect shell was calculated by dividing the shell weight of the egg and multiplying by 100 (Chowdhury and Smith, 2001).

At the end of the experiment, 2 hens per replicate making 8 birds per treatment were slaughtered for biochemical liver and blood analyses. Livers were collected from the birds by careful removal of gall bladder, thoroughly washed and weighed and immediately stored at -20°C in individual sample bags until further analysis. Blood samples were collected from the birds for the analysis of haematological parameters, plasma proteins and enzymes. For haematological determinations, blood samples were collected into sample tubes containing anticoagulant as described by Lamb (1981). Plasma was harvested by centrifuging the blood samples at 3,000 rpm for 15 min in centrifuge machine. The heparinized plasma samples were stored at -20°C in sample tubes until further analysis. Haematological parameters determined included erythrocyte sedimentation rate, packed cell volume, red blood cell, haemoglobin, leucocyte, neutrophill, monocyte, basophil and eosinophil. Further preparations of liver samples for biochemical analysis were carried out according to Halder and Roy (2007).

Table 1. Gross composition of experimental diets.

Ingredient	Composition (kg)	
Maize	579.9	
Soybean meal	170	
Groundnut cake	90	
Wheat offal	45	
Bone meal	18	
Fish meal	12	
Limestone	80	
Lysine	0.1	
Methionine[a]	0.5	
Premix	2.5	
Salt	2	
Total	1000	
	Calculated analysis	**Proximate analysis**
Crude protein (%)	17.17	16.80
Crude fibre (%)	2.47	3.11
Moisture content	-	10.87
Crude fat (%)	4.00	-
Ash (%)	-	11.25
Calcium (%)	3.35	-
Phosphorus (%)	0.42	-
Lysine (%)	0.91	-
Methionine (%)[1]	0.32	-
Ether extract (%)	-	6.96
Metabolisable energy (Kcal/kg)	2701.10	-

[a]: Synthetic or herbal methionine; the quantity of corn was adjusted to accommodate methionine at 1.0 kg/ton.
[1]Methionine content varies, depending on the inclusion level of the herbal and synthetic methionine.

Plasma and liver samples were analyzed for proteins (total proteins and albumin), cholesterol, triglyceride concentrations and enzymes {alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP)}. Total protein, albumin, cholesterol, triglyceride, ALT, AST and ALP activities were determined in the Autoanalyzer Microlab 200, using commercial kits (Randox Laboratories Limited, Ardmore Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY). The globulin fraction was calculated by subtraction of albumin level from total protein level.

Proximate composition of the basal diet was carried out according to the method of Association of Official Analytical Chemists (AOAC) (2005). Data collected were subjected to one way analysis of variance (ANOVA) according to the General Linear Model Procedures of the science analysis system (SAS) (2008). When analysis of variance indicated a significant treatment effect, Duncan's multiple range test (Duncan, 1955) was used to compare the treatment means. The model included main effects of methionine source, methionine level and their interaction.

RESULTS

The productive performance of laying hens fed on diets supplemented with HM and SM is presented in Table 2. There was a significant effect of methionine source on egg production ($P \leq 0.01$) feed conversion efficiency ($P \leq 0.05$), and egg mass output ($P \leq 0.01$), final body weight ($P \leq 0.05$), abdominal fat content ($P \leq 0.05$) and liver weight ($P \leq 0.05$) of birds. Dietary methionine level also significantly ($P \leq 0.01$) influenced egg production. A source by level interaction ($P \leq 0.01$) was observed for egg production and egg mass output. Hen-day egg production decreased by 7.3% (79.94 ± 5.90 versus 74.14 ± 6.80%) and 18.3% (79.94 ± 5.90 versus 65.30 ±

Table 2. Production performance of domestic laying hens fed on diets supplemented with synthetic and herbal methionine.

Source	Level (ppm)	Rate of lay (%)	Daily feed intake (g/bird)	Feed conversion (kg/dozen eggs)	Egg mass (g/hen/day)[1]	Final body weight (kg)	Abdominal fat (% live weight)	Liver weight (% liver weight)
SM	0.5	79.94±5.70[a]	117.56±10.61	1.83±0.23[a]	46.82±7.71[a]	1.55±0.10[ab]	1.49±0.85[a]	2.34±0.27[b]
SM	1.0	79.61±8.74[a]	110.97±10.31	1.75±0.27[a]	47.57±10.12[a]	1.63±0.12[a]	2.19±1.28[ab]	2.09±0.23[b]
HM	0.5	74.14±6.80[b]	111.81±12.61	1.83±0.31[a]	42.08±8.77[b]	1.51±0.15[b]	1.20±1.02[ab]	2.70±0.39[a]
HM	1.0	65.30±5.66[c]	108.66±20.95	1.98±0.32[b]	35.85±9.80[c]	1.51±0.09[b]	0.88±0.39[b]	2.28±0.32[b]
Source								
SM		79.77[a]	114.26	1.78[a]	47.20[a]	1.59[a]	1.83[a]	2.21[b]
HM		69.72[b]	110.23	1.91[b]	38.97[b]	1.51[b]	1.04[b]	2.48[a]
Level								
0.5		77.04[a]	114.68	1.83	44.45	1.53	1.34	2.51[a]
1.0		72.45[b]	109.81	1.87	41.71	1.57	1.53	2.18[b]
Significance								
Source		**	NS	*	**	*	*	*
Level		**	NS	NS	NS	NS	NS	**
Source × level		**	NS	NS	**	NS	NS	NS

[abc]: Means with different superscript are significantly ($P \le 0.05$) different.
SM: Synthetic methionine; HM: herbal methionine.
NS: not significant at $P \le 0.05$; *: significant at $P \le 0.05$; **: significant at $P \le 0.01$.
[1]: Rate of lay (%) × Average egg weight.

5.66%) when HM was used in laying hen diets at 0.5 kg and 1.0 kg per ton of finished feed, respectively. Feed conversion ratio and egg mass output decreased by about 11.6% (1.98 ± 0.23 versus 1.75 ± 0.27 kg feed/dozen eggs) and 24.6% (35.85 ± 9.80 versus 47.57 ± 10.12 g/hen/day), respectively when up to 1.0 kg per ton HM was added to laying hen diets. The final body weights followed the same pattern as described for egg production. Feed consumption was essentially not affected ($P \ge 0.05$) by the dietary treatments. Egg quality parameters as influenced by dietary supplementation of HM and SM are summarized in Table 3. Methionine source had a significant effect on egg weight, albumen weight and albumen height ($P \le 0.05$) and shell thickness ($P \le 0.01$). Level of methionine supplementation also had a significant ($P \le 0.05$) effect on egg weight. A source by level interaction was observed for egg weight ($P \le 0.05$) and egg shell thickness ($P \le 0.01$). Egg weight decreased by about8.1% (59.76 ± 4.41 versus 54.90 ± 5.33 g) when HM was added to laying hen diets up to 1.0 kg per ton of finished feed. Among the birds fed on SM, egg weights increased ($P \le 0.05$) by 3.3% (57.87 ± 5.66 versus 59.76 ± 4.41 g) when dietary level of supplementation increased from 0.5 to 1.0 kg per ton, but this was not applicable to those birds fed on HM which had their egg weights decreased ($P \le 0.05$) by the same magnitude (56.76 ± 5.61 versus 54.90 ± 5.33 g) with increasing level of supplementation. Though the numerical difference was small, eggshell thickness increased ($P \le 0.05$) with increasing level of SM in the diet.

Results of the haematological profiles of the

Table 3. Egg quality characteristics of domestic laying hens fed on diets supplemented with synthetic and herbal methionine.

Source	Level	Egg weight (g)	Albumen weight (g)	Albumen height (mm)	Haugh unit	Yolk weight (g)	Shell weight (g)	Shell thickness (mm)	Perfect shell[1]
SM	0.5	57.87±5.66[b]	36.85±4.51[a]	6.67±0.58[a]	81.93±3.90	14.58±1.57	5.55±0.55	0.32±0.042[b]	9.59±0.90
SM	1.0	59.76±4.41[a]	37.03±3.33[a]	6.68±0.55[a]	82.14±3.81	15.57±1.51	5.53±0.60	0.34±0.040[a]	9.25±1.05
HM	0.5	56.76±5.61[b]	36.61±4.65[a]	6.54±0.56[b]	81.92±3.83	14.01±1.63	5.24±0.96	0.32±0.049[b]	9.23±1.41
HM	1.0	54.90±5.33[c]	35.19±4.62[b]	6.56±0.51[b]	81.47±3.46	13.89±1.66	5.05±0.60	0.32±0.041[b]	9.20±0.99
Source									
SM		58.82[a]	36.94[a]	6.67[a]	82.04	15.07	5.54	0.33[a]	9.42
HM		55.83[b]	35.90[b]	6.55[b]	81.81	13.95	5.15	0.32[b]	9.22
Level									
0.5		57.32	36.73	6.61	81.93	14.30	5.40	0.32[b]	9.41
1.0		57.33	36.11	6.62	81.81	14.73	5.29	0.33[a]	9.23
Significance									
Source		*	*	*	NS	NS	NS	**	NS
Level		*	NS	NS	NS	NS	NS	**	NS
Source × level		*	NS	NS	NS	NS	NS	**	NS

[ab]: Means with different superscript are significantly ($P \leq 0.05$) different.
SM: Synthetic methionine; HM: herbal methionine.
NS: not significant at $P \leq 0.05$; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$.
[1](shell weight/egg weight) × 100.

laying hens fed on diets supplemented with HM and SM are presented in Table 4. Statistical analysis showed that there was no significant ($P \geq 0.05$) effect of methionine source, level and source with level interaction on all the parameters determined. Table 5 shows the plasma biochemical profiles of domestic laying hens as influenced by HM and SM supplementation. The concentrations of total protein and albumin were higher ($P \leq 0.05$) in birds fed on diets supplemented with SM than their counterparts fed on HM supplemented diets. Total protein and albumin concentrations were 17.9 and 26.1%, respectively higher than those birds on HM supplemented diets. Although there was a trend for lower cholesterol and diets supplemented with HM, the differences were not significant ($P \geq 0.05$) from those fed on SM supplemented diets. Feeding diets containing HM did not significantly (P triglyceride concentrations in layers fed ≥ 0.05) alter the activities of plasma enzymes determined (Table 5). Plasma ALT (34.75 ± 5.27 versus 28.75 ± 6.58 IU/L), AST (53.00 ± 14.71 versus 45.13 ± 13.42 IU/L) and ALP (42.63 ± 8.19 versus 38.13 ± 8.18 IU/L) had their activities not significantly altered by addition of HM up to 1.0 kg per ton to laying hen diets. Liver biochemical profiles (Table 6) followed the same pattern as described for plasma biochemical properties. Essentially, theconcentrations of total protein and albumin were higher ($P \leq 0.05$) in layers fed on SM supplemented diets than those layers fed on HM supplemented diets. There was also a trend for lower ($P \geq 0.05$) cholesterol and triglyceride concentrations in layers fed on HM supplemented diets. Liver enzymes activities were not

Table 4. Haematological profiles of domestic laying hens fed on diets supplemented with synthetic and herbal methionine.

Source	Level ppm	ESR	PCV	RBC	HB	LYM	NEU	MONO	BGS	EOS
SM	0.5	2.63±0.74	28.00±1.60	236.13±7.80	9.33±0.53	60.0±2.76	23.63±1.06	13.13±2.64	2.38±0.46	0.88±0.35
SM	1.0	2.25±1.91	21.75±13.61	181.00±112.95	7.23±4.53	46.50±3.31	17.88±11.10	8.13±5.19	1.88±0.53	0.63±0.74
HM	0.5	3.00±1.60	27.38±2.88	229.13±21.55	9.10±0.96	60.25±3.34	24.75±1.28	12.00±2.27	2.25±0.52	0.75±0.46
HM	1.0	2.88±1.36	27.75±2.19	234.63±10.07	9.23±0.75	59.88±28.78	24.13±2.10	12.88±2.42	2.50±1.25	0.63±0.52
Source										
SM		2.43	24.87	208.56	8.27	53.25	20.75	10.62	2.12	0.75
HM		2.93	27.56	231.88	9.16	60.06	24.43	12.43	2.37	0.68
Level										
0.5		2.81	27.68	232.63	9.21	60.12	24.18	12.56	2.31	0.81
1.0		2.56	24.75	207.81	8.22	53.18	21.00	10.50	2.18	0.62
Significance										
Source		NS	NS	NS	NS	NS	NS	NS	NS	NS
Level		NS	NS	NS	NS	NS	NS	NS	NS	NS
Source × level		NS	NS	NS	NS	NS	NS	NS	NS	NS

ESR: Erythrocyte sedimentation rate (mm/hr); PCV: packed cell volume (%).
RBC: red blood cell x 10^6/mm^3; HB: haemoglobin (g/100ml); LEU: leucocyte; NEU: neutrophill (%).
MONO: monocyte; BAS: basophil; EOS: eosinophil.

significantly (P ≥ 0.05) altered in layers fed on HM supplemented diets compared to those layers fed on SM supplemented diets.

DISCUSSION

Previous studies on the bioefficacy of herbal methionine premixes in poultry diets have been concentrated on broiler chickens and most of these studies were conducted in India where the products are manufactured. Chattopadhyay et al. (2006), Halder and Roy (2007) and Kalbande et al. (2009) demonstrated that broiler chickens fed on diets supplemented with herbal methionine (Methiorep®) had similar performance in terms of body weight and weight gain and feed conversion efficiency with those birds fed on DL-methionine. Even the feed conversion ratio of broilers fed on 15 g herbal methionine/kg diet was significantly better than that of broilers fed on 10 g DL-methionine/kg diet (Chattopadhyay et al. 2006). Using the same herbal methionine and dietary inclusion levels, Itoe et al. (2010) showed that broiler chickens fed on supplemental herbal methionine were inferior in weight gain and feed conversion efficiency to those birds fed on supplemental DL-methionine. According to the manufacturer, the benefits associated with HM with respect to laying hens include: increased egg production, optimum egg size and improved growth performance and livability of birds.

However, the results presented in this study clearly revealed that layers fed on diets supplemented with HM (Meth-o-Tas®) produced less eggs, had lower egg mass output and final body weight and poorer feed conversion efficiency than those layers fed on SM supplemented diets. Production performance was further affected when dietary inclusion of HM was increased to 1.0 kg per ton of finished diet. This suggests that the herbal methionine under investigation is probably deficient in methionine content. Dietary supplementation of HM significantly reduced abdominal fat pad. Earlier study by Chattopadhyay et al.

Table 5. Plasma biochemical profiles of domestic laying hens fed on diets supplemented with synthetic and herbal methionine.

Source	Level	Total protein (g/100m)	Albumin (g/100ml)	Globulin (g/100ml)	Cholesterol (mg/100ml)	Triglyceride (mg/100ml)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
SM	0.5	3.69±0.66[ab]	1.46±0.44[ab]	2.23±0.78	51.75±8.90	61.58±8.90	34.75±5.27	53.00±14.71	42.63±8.19
SM	1.0	4.45±0.92[a]	1.75±0.46[a]	2.70±0.63	51.68±9.77	66.75±9.62	32.75±6.25	56.88±12.12	44.50±9.75
HM	0.5	3.46±0.35[ab]	1.24±0.14[ab]	2.22±0.41	46.13±4.61	58.63±6.78	30.38±3.38	49.00±11.24	38.38±9.85
HM	1.0	3.23±0.62[b]	1.15±0.09[b]	2.08±0.63	44.13±7.77	55.88±6.52	28.75±6.58	45.13±13.42	38.13±8.18
Source									
SM		4.07[a]	1.61[a]	2.47	51.71	64.17	33.75	54.94	43.57
HM		3.34[b]	1.19[b]	2.15	45.13	57.25	29.56	47.06	38.25
Level									
0.5		3.58	1.35	2.23	48.94	60.11	32.56	51.00	40.51
1.0		3.84	1.45	2.39	47.90	61.32	30.75	51.01	41.32
Significance									
Source		*	*	NS	NS	NS	NS	NS	NS
Level		NS	NS	NS	NS	NS	NS	NS	NS
Source x level		NS	NS	NS	NS	NS	NS	NS	NS

[ab]: Means with different superscript are significantly (P ≤ 0.05) different.
SM: Synthetic methionine; HM: herbal methionine; ALT: alanine transferase ; AST: aspartate transferase; ALP: alkaline phosphatise.
NS: not significant at P ≤ 0.05; * :significant at P ≤ 0.05.

(2006) showed that herbal methionine decreased fat deposition in broiler chickens. There is evidence in the literature to demonstrate that fortification of laying hen diets with methionine increases egg weight. Shafer et al. (1998), Narvaez-Solatre et al. (2005), Liu et al. (2005) and Zeweil et al. (2011) reported that egg weight increased with increasing level of methionine intake by laying hens. Observed increase in egg weight with increasing level of SM supplementation of laying hen diets corroborated these earlier results.

Unfortunately, little information has been published on the effects of herbal methionine supplements on the biochemical profiles of domestic chickens (Prabhakaran et al., 1996). Non-significant differences were observed in virtually all the haematological variables determined for birds fed on diets supplemented with both SM and HM. This is in agreement with the results of Rekhatel et al. (2010) who showed that herbal methionine had no significant effect on haematological profiles of broiler chickens. The implication is that dietary herbal methionine has no detrimental effect on survivability of chickens. Rajurker et al. (2009) also reported that herbal methionine supplement (Methiorep®) is totally safe and has no adverse effect even when used at the highest limit dose of 5 g/kg body weight of male Wistar rats. Total plasma and liver protein, albumin and globulin concentrations observed are within the normal range reported for chickens (Prabhakaran et al., 1996). There are differences in total plasma and liver protein and albumin concentrations; birds fed on diets supplemented with HM had higher total protein and albumin concentrations than those fed on diets supplemented with SM. These results are contrary to those of Halder and Roy (2007) and Rekhatel et

Table 6. Liver biochemical profiles of domestic laying hens fed on diets supplemented with synthetic and herbal methionine.

Source	Level	Total protein (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)	Cholesterol (mg/100ml)	Triglycerides (mg/100ml)	ALT (IU/L)	AST (IU/L)
SM	0.5	3.94±1.06[ab]	1.63±0.53[a]	2.31±0.68	54.88±13.62	61.00±8.91	40.38±6.27	56.00±12.51
SM	1.0	4.71±0.86[a]	1.75±0.50[a]	2.96±0.63	54.00±10.16	66.88±10.30	46.63±5.10	61.38±11.16
HM	0.5	3.77±0.37[b]	1.31±0.32[b]	2.46±0.28	50.50±9.63	62.75±6.02	38.00±5.45	53.00±11.01
HM	1.0	3.59±0.64[b]	1.36±0.39[b]	2.23±0.36	49.43±7.89	58.57±8.73	39.00±6.38	56.57±14.42
Source								
SM		4.33[a]	1.69[a]	2.64	54.44	63.94	43.50	58.69
HM		3.69[b]	1.33[b]	2.35	50.00	60.80	38.50	54.79
Level								
0.5		3.86	1.48	2.39	52.69	61.88	39.19	54.50
1.0		4.19	1.57	2.60	51.72	62.73	42.82	58.98
Significance								
Source		*	*	NS	NS	NS	NS	NS
Level		NS	NS	NS	NS	NS	NS	NS
Source × level		NS	NS	NS	NS	NS	NS	NS

[ab]: Means with different superscript are significantly ($P \leq 0.05$) different.
SM: Synthetic methionine; HM: herbal methionine; ALT: alanine transferase; AST: aspartate transferase.
NS: not significant at $P \geq 0.05$; *: significant at $P \leq 0.05$.

al. (2010) who did not observe any significant effect of HM supplementation on total protein and albumin concentrations of broiler chickens.

In terms of plasma and liver cholesterol and triglyceride concentrations, though not statistically significant, the findings of the present study can be well correlated to those reported by Halder and Roy (2007), Kalbande et al. (2009) and Rekhatel et al. (2010) that herbal methionine proved better than DL-methionine in lowering total cholesterol and triglyceride concentrations and according to the authors, this may be attributed to the hypo-cholesterolemic activity of certain constituent herbs in herbal methionine formulation. The constituent herbs may have also facilitated efficient fatty acid oxidation in the liver and its transportation to the body tissues and consequently, it may reduce the incidence of fatty liver in birds. Generally, the activities of plasma and liver AST, ALT and ALP observed are within the normal range for chickens (Marjanovic et al., 1975). This suggests that supplementation of laying hen diets with herbal methionine did not inhibit the activities of plasma enzymes or cause any liver dystrophy or other vital organs abnormalities where these enzymes are secreted. These results corroborate those of Halder and Roy (2007), Kalbande et al. (2009) and Rekhatel et al. (2010) who reported that the plasma concentrations of ALT, AST and ALP were not affected by supplementation of broiler diets with herbal methionine.

Under the conditions of this study, it is concluded that herbal methionine (Meth-o-Tas®) is not an effective substitute for synthetic methionine for optimum production performance.

Abbreviations

HM, Herbal methionine; SM, synthetic methionine; ALT, alanine transferase; AST, aspartate transferase; ALP, alkaline phosphatise.

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