Research Article

Comparative efficacy of herbal and synthetic amino acids for growth performance and hepatoprotective action in broiler chickens

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Abstract
A study was conducted to see the comparative effect of herbal product containing natural source of choline, methionine, lysine and biotin with synthetic amino acids on growth performance and hepatoprotection in broiler chickens. Seventy five healthy day old Vencob broiler chicks of nearly similar live body weight were equally divided into three groups, comprising twenty five chicks each. Group- I was positive control fed with the standard basal diet without any supplementation. Group-II was fed with basal diet supplemented with herbal phytoadditive (AV/CAP/18) @ 2Kg/tonne of feed (supplied by M/S Ayurvet Ltd., Baddi, H.P., India). Group-III was fed with basal diet supplemented with synthetic choline chloride (600gm/tonne), synthetic methionine (1kg/tonne), synthetic lysine (1kg/tonne) and biotin (150mg/tonne). At the end of sixth week, significantly higher live body weight (859.36, 1788.47 and 1804.14gm) with more economical FCR (2.27, 1.87 and 1.81), carcass yield (464.33, 1304.46 and 1344.98gm) from supplementation of herbal amino acids with equal competition as that of synthetic amino acids was observed. More satisfactory results were observed among total serum protein, serum albumin, serum globulin with lesser but within range values of serum cholesterol and triglycerides along with significantly lower SGPT and SGOT values showing normal satisfactory liver function from supplementation of herbal as well as synthetic amino acids as compared to control group. The treatment groups supplemented with herbal phytoadditive product have shown better serum immunoglobulin level. The gross and histopathological observations of Liver have shown necrotic and haemorrhagic lesions with fatty changes in control group, while group supplemented with herbal or synthetic amino acids were devoid of such lesions and were completely normal. It can be concluded that the herbal product (AV/CAP/18) can successfully replace synthetic amino acids (methionine, choline, lysine & biotin) in broiler feeds with better performance and fatty liver.

Keywords: Broilers, herbal, amino acids, performance, fatty liver.

1. Introduction
The health and productivity of poultry largely depends on optimum feed utilization, improved bodyweight, absence of disease and low mortality[1]. Poultry nutrition has improved a lot for past few decades. In spite of advances made on the nutritional aspects, a lot many nutritional problems are still remaining unsolved and serve as a challenge to investigators in this field worldwide. The broiler production is fastly grown area since last few decades. Genetic potentiality of broiler chickens is increasing day-by-day; hence reassessment of nutrient requirements is essential to bridge the gap between the genetic improvement and nutritional requirements[2]. In poultry ration, along with the vitamins and minerals, proteins have been playing critical role in development of musculature. One of the most important areas is amino acid nutrition. Amino acids are the basic constituents of living matter because they are building blocks of proteins and their proper balance in the diet is required to maintain protein quality, feed consumption, growth rate and carcass composition and as a result receiving considerable attention in industry[3]. Methionine, choline and lysine are universally recognized as the most limiting amino acids in broiler diets based on corn and soybean meal[2,4]. The supplementation of broiler feeds with these amino acids is very common in the poultry industry. Synthetic methionine and choline are listed among the prohibited synthetic substances and its usage has been questioned in organic farming practices[5]. The concept of organic farming recently came up with various objectives in general and to avoid residual effects of synthetic drugs in particular. Nearly 80% of world’s population relies upon herbal medicine for basic health care needs. Many herbs are rich source of these essential amino acids and also mimic the activity like that of methionine, choline or biotin and may be supplemented along with ration to replace synthetic feed. The present experimental trial was conducted to evaluate efficacy of polyherbal coded formulation AV/CAP/18 (supplied by M/S Ayurvet Limited, Baddi, India) in comparison to synthetic additives in improving overall growth and prevention of fatty liver in broiler chickens.

2. Materials and Methods
The study was conducted in the department of Animal Nutrition, College of Veterinary and Animal Sciences, Udgir, Dist. Latur, Maharashtra, India after approval from Committee for the purpose of control and supervision of experimentation on animals (CPCSEA). The mean maximum daily temperature recorded at the time of trial was 41±2°C and relative humidity (RH) 80.57 ± 1.50%.

2.1 Experimental design
Seventy five healthy day old Vencob broiler chicks of nearly similar live body weight were equally divided into three groups, comprising twenty five chicks each.

Group- I: positive control fed with the basal diet without any natural or synthetic source of choline, methionine, lysine & biotin.
Group-II: fed with basal diet supplemented with herbal formulation containing natural sources of choline, methionine, lysine & biotin (AV/CAP/18 @ 2Kg/tonne of feed (supplied by M/S Ayurved Ltd., Baddi, H.P., India)).

Group-III: fed with basal diet supplemented with synthetic choline chloride (600gm/tonne), synthetic methionine (1kg/tonne), synthetic lysine (1kg/tonne) and biotin (150mg/tonne) of feed.

AV/CAP/18 is a herbal ‘amino acids supplement’, added to feed of poultry and possesses a number of beneficial effects, including: increased growth performance, stimulation and rapid maturation of the immune system & many more. The product comprises of herbs viz. Cicer arietinum, Phaseolus mungo, Mucuna pruriens, Trigonella foenum graecum, Nigella sativa, Citrullus colocynthis & many more in a fixed concentration.

The chicks were vaccinated for Lasota and IBD vaccines on 7th and 14th day of age, respectively. The booster dose of IBD vaccine was given on 21st day. All the three groups were housed separately and maintained on ad libitum broiler starter and finisher ration and clean drinking water throughout the experiment. Measured quantity of feed was fed to chicks every day and the feed in balance was recorded after 24 hrs. Mean live body weight (g/chick/week) was computed at weekly intervals from 1st week to 6th week of study.

### Table 1. Composition of broiler starter and finisher ration (fg, in %)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Group I</th>
<th>Group II and III</th>
<th>Nutrient</th>
<th>Group I</th>
<th>Group II and III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starter</td>
<td>Finisher</td>
<td>Starter</td>
<td>Finisher</td>
<td>Starter</td>
</tr>
<tr>
<td>Maize</td>
<td>45.00</td>
<td>50.00</td>
<td>53.70</td>
<td>59.00</td>
<td>23.41</td>
</tr>
<tr>
<td>Jowar</td>
<td>06.00</td>
<td>06.00</td>
<td>05.00</td>
<td>05.00</td>
<td>2809</td>
</tr>
<tr>
<td>Sunflower Meal</td>
<td>11.00</td>
<td>05.00</td>
<td>02.00</td>
<td>02.00</td>
<td>01.00</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>33.00</td>
<td>33.00</td>
<td>33.00</td>
<td>33.00</td>
<td>125.37</td>
</tr>
<tr>
<td>Groundnut Meal</td>
<td>01.00</td>
<td>01.00</td>
<td>01.00</td>
<td>01.00</td>
<td>0.43</td>
</tr>
<tr>
<td>Lime Stone</td>
<td>05.50</td>
<td>05.50</td>
<td>05.50</td>
<td>05.50</td>
<td>0.43</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>01.50</td>
<td>01.50</td>
<td>01.50</td>
<td>01.50</td>
<td>0.43</td>
</tr>
<tr>
<td>Oil</td>
<td>02.00</td>
<td>02.00</td>
<td>02.00</td>
<td>02.00</td>
<td>0.43</td>
</tr>
<tr>
<td>Salt</td>
<td>00.50</td>
<td>00.50</td>
<td>00.50</td>
<td>00.50</td>
<td>0.43</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

* calculated values

### Table 2. Per Kg composition of vitamins and trace minerals in feed

<table>
<thead>
<tr>
<th>Vitamin A - 2500 (IU)</th>
<th>Vitamin B12 - 0.015 (mg)</th>
<th>Vitamin B3 - 2500 (IU)</th>
<th>Copper - 15 (mg)</th>
<th>Vitamin E - 12 (mg)</th>
<th>Iron - 90 (mg)</th>
<th>Vitamin K - 1.5 (mg)</th>
<th>Manganese - 90 (mg)</th>
<th>Vitamin B1 - 1.5 (mg)</th>
<th>Iodine - 2 (mg)</th>
<th>Vitamin B2 - 0.25 (mg)</th>
<th>Zinc - 80 (mg)</th>
<th>Vitamin B5 - 1.0 (mg)</th>
<th>Selenium - 0.5 (mg)</th>
<th>Vitamin B6 - 2.5 (mg)</th>
<th>Cobalt - 0.5 (mg)</th>
<th>Vitamin B12 - 0.015 (mg)</th>
<th>Folic acid - 0.5 (mg)</th>
</tr>
</thead>
</table>

2.2 Parameters estimated

2.2.1 Feed Consumption

Measured quantity of feed was fed to chicks every day and the feed in balance was recorded after 24 hrs. The difference between the feed offered and balanced feed was worked out to know the actual feed consumed by each group on a particular day and expressed as g/day/group. In the similar manner, feed consumption as g/week/group was computed and at the end total feed consumption was calculated for 42 days.

2.2.2 Body Weight Gain

Ten chicks from each group were weighed individually on day 0 and at weekly intervals thereafter. Mean live body weight (g/chick/week) was computed at weekly intervals from 1st week to 6th week of study.

2.2.3 Feed Conversion Ratio (FCR)

The values of FCR of each group were calculated at weekly intervals on the basis of weekly live weights and weekly feed consumption.

2.2.4 Carcass yield: Live weights, dressed weight (carcass yield) of experimental birds of all groups were determined at the end of experiment.

2.2.5 Serum Biochemistry

The serum biochemical estimations were carried out in ten birds sacrificed at scheduled intervals from each group. The blood samples were collected directly from heart into tubes without anticoagulant for separation of serum. The serum samples were maintained at -20°C until analyzed. The individual serum samples were analyzed for total protein, albumin, globulin, SGOT, SGPT and Serum Immunoglobulin (HI Titre for IBD). The biochemical estimations were done by using Automatic Biochemical Analyzer ‘3000 revolution’ made by Tulip’s Diagnostic Pvt. Ltd., Mumbai.

2.2.6 Absolute Liver Weight: The liver was collected from the experimental birds and weighed. The mean weight was recorded in grams (g).

2.2.7 Pathological studies: The gross pathological lesions in the visceral organs of birds sacrificed at the end of experiment were recorded.

2.3 Histopathological Studies

Pieces of suitable thickness of liver were collected from the sacrificed birds of all the groups. The samples were preserved in 10 per cent neutral formal saline. The tissues were embedded in paraffin wax and processed as per the standard procedure for recording histochitectural alterations. The sections were cut at 3 to 5 μ thickness and were stained with Mayer’s haematoxylin and eosin for microscopic examination.

2.4 Recording of Data

All the results were analyzed statistically by analysis of variance to determine the means and standard error as per the methods described by Snedecor and Cochran.™
3. Results and Discussion

An experimental study in broiler birds for 42 days was conducted as per the experimental design and at the end of sixth week, the results were assessed on basis of effect on growth, feed efficiency, carcass traits, serum biochemical, gross and histo-pathological studies.

3.1 Growth Parameters

In order to understand the performance of broiler birds during the experimental period, feed intake, their body weight gain and feed conversion ratio (FCR) were analyzed at weekly intervals. Significantly higher live body weight (859.36, 1788.47 and 1804.14g) with more economical FCR (2.27, 1.87 and 1.81), carcass yield (464.33, 1304.46 and 1344.93g) along with marked improvement in digestibility of nutrients from supplementation of herbal amino acids with equal competence as that of synthetic amino acids was observed. The results of body weight gain of the present study are well in confirmation with those reported by Wang et al17 who reported significant response in terms of body weight gain at day 21 and 42 in broilers supplemented with herbal methionine. In poultry ration along with the vitamins and minerals, amino acids play a critical role, amongst these methionine and lysine are essentially required for overall growth and performance5. The significant increase in mean final body weight and body weight gain in treatments may be attributed to the supplementation of essential amino acids in basal ration. Addition of methionine over and above the recommended requirement of broilers improves their performance in terms of body weight gain and food conversion efficiency10. The results in the present study are in corroborations with those reported by Halder and Roy15, Schutte and Pack14 that addition of herbal source of methionine along with feed improved performance in terms of body weight gain and feed efficiency in broilers. Herbs namely *Cicer aritinum*, *Phaseolus mungo*, *Mucuna pruriens* are rich source of proteins and essential amino acids6. The results are well in confirmation with those reported by Ozturkan et al19 that supplementation of choline, methionine and lysine to broilers improved performance in terms of feed efficiency and livability. Similar results were also given by Simone et al16 and Ohta and Ishibashi10 that supplementing herbal or synthetic sources of essential amino acids improved feed to body weight gain ratio in broilers.

3.2 Serum Biochemical studies

Significant improvement was observed in the values of total serum protein (2.98, 4.83 and 4.97gm/dl), serum albumin (1.40, 2.16 and 2.22gm/dl) and serum globulin (1.58, 2.66 and 2.76gm/dl) with low but within range values of serum cholesterol (148.38, 118.52 and 115.66mg/dl) and triglycerides (186.10, 160.05 and 163.39mg/dl). Significantly lower values of SGPT (20.97, 16.51 and 16.82 U/l) and SGOT (160.11, 131.76 and 133.62 U/l) were observed from supplementation of herbal as well as synthetic amino acid combination showing normal liver function as compared to control group. The addition of herbal amino acids supplement in the diet of poultry significantly contributed in reducing serum cholesterol, triglycerides & regulating the fat metabolism in broilers in addition to improvement in growth, performance & other biochemical parameters. The findings of this study are in corroborations with Halder and Roy15 who reported that supplementation of herbal methionine facilitate efficient lipid metabolism in liver and it may reduce the incidence of fatty liver in birds and JadHAV et al17 reported similar results in herbal choline supplementation in broilers.

Halder and Roy15 also found that supplementation of herbal methionine in diet markedly decreased liver triglycerides. Kaviarasann et al18 reported similar hepatoprotective, hypocholesterolemic & lipid lowering activities of some herbs in experimental chicken models. Data of liver enzymes indicated that use of herbal and synthetic products protected the liver function as evident by normalization of liver marker enzyme levels. Therefore, it can be concluded that supplementation of herbal amino acids in broiler rations elevate protein concentration in liver and lower per cent liver lipid which have beneficial effects to the birds. This finding indicates that supplementation of herbal amino acids facilitates efficient lipid metabolism in the liver and its transportation to body tissues and consequently, it may reduce the incidence of fatty liver in birds. This may be attributed to the efficacy of constituent herbs of AV/CAP/18 namely *Cicer aritinum*, *Phaseolus mungo*, *Mucuna pruriens*, *Trigonella foenum graecum*, *Nigella sativa*, *Citrus colocythis* & many more which arescientifically well proven for improving growth, productivity & hepatoprotective action17. It was observed that the geometric mean value of HI titre for IBD of treatment Group VI (238.86±12.80) Group V (238.86±12.80) were significantly high with marked improvement in resistance power which were found protective as compared to the control Group I (42.22±12.01). Tsiagbe et al18 showed that levels of methionine, higher than those required for growth performance; enhance the immune response of broilers.

3.3 Carcass yield

There is significant increase in the carcass yield of amino acid supplemented groups as compared to control group. Chattopadhay et al19 also observed significant difference in case of carcass yield among control and either herbal or synthetic methionine treated groups.

3.4 Absolute Liver weight

The mean values of liver weight of control Group I were significantly higher as compared to treatment groups (II & III) which might have resulted due to hepatopathy as the birds in control group were maintained on feed devoid of any amino acid supplementation.

### Table 1. Overall performance and biochemical profile of the birds at day 42.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Live Body Weight (g)</td>
<td>859.36</td>
<td>1788.47</td>
<td>1804.14</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>810.16</td>
<td>1739.67</td>
<td>1755.84</td>
</tr>
<tr>
<td>Total feed intake (g)</td>
<td>1951.00</td>
<td>3273.48</td>
<td>3274.00</td>
</tr>
<tr>
<td>Feed Conversion Ratio (FCR)</td>
<td>2.27</td>
<td>1.83</td>
<td>1.81</td>
</tr>
<tr>
<td>Carcass yield (g)</td>
<td>464.33</td>
<td>1304.46</td>
<td>1344.98</td>
</tr>
<tr>
<td>Total Serum Protein (g/dl)</td>
<td>2.98</td>
<td>4.83</td>
<td>4.97</td>
</tr>
<tr>
<td>Serum Albumin (g/dl)</td>
<td>1.40</td>
<td>2.16</td>
<td>2.22</td>
</tr>
<tr>
<td>Serum Globulin (g/dl)</td>
<td>1.58</td>
<td>2.66</td>
<td>2.76</td>
</tr>
<tr>
<td>Serum Cholesterol (mg/dl)</td>
<td>148.38</td>
<td>118.52</td>
<td>115.66</td>
</tr>
<tr>
<td>Serum Immunoglobulins (HI titre)</td>
<td>73.52</td>
<td>238.86</td>
<td>238.86</td>
</tr>
<tr>
<td>Serum Triglycerides (mg/dl)</td>
<td>186.10</td>
<td>160.05</td>
<td>163.39</td>
</tr>
<tr>
<td>SGPT (U/l)</td>
<td>20.97</td>
<td>16.51</td>
<td>16.82</td>
</tr>
<tr>
<td>SGOT (U/l)</td>
<td>160.11</td>
<td>131.76</td>
<td>133.62</td>
</tr>
<tr>
<td>Absolute Liver weight (g)</td>
<td>42.05</td>
<td>34.96</td>
<td>35.54</td>
</tr>
</tbody>
</table>

Means with different superscripts differ significantly (P<0.05)
3.5 Gross and histopathological studies

The gross and histopathological observations of liver supplemented with herbal amino acid and synthetic amino acid have shown completely normal, within range and satisfactory results as compare to control group (Figure 1, 2, 3 & 4). Enlarged liver with necrotic, haemorrhagic lesions and fatty changes in liver were observed in control group. There was marked discolouration with abnormal pale colour in control group. While group supplemented with herbal or synthetic amino acids were devoid of such lesions and were completely normal.

Figure-1 and Figure-2 both showing enlarged Liver of affected birds from control groups with marked discolouration and abnormal pale colour.

Figure-3 and Figure-4 both showing enlarged Liver of affected birds from control groups with necrotic foci and pin-point petechial haemorrhages.

The histopathological evaluation of liver section revealed fatty changes along with congestion and prominent sinusoids in the hepatocytes of birds in case of control group (Figure 5 & 6). While the liver section of treatment groups were normal without any change. Results revealed protective effect of polyherbal formulation on liver functions. Supplementation of product may eliminate the possibility of occurrence of fatty liver syndrome or other hepatopathic effects.

Fig 5: section of liver showing fatty changes in control group

Fig 6: section of liver showing congestion, prominent sinusoids and fatty changes in hepatocytes of control group.
4. Conclusion

Results of present study demonstrate significant improvement in growth performance and hepatoprotective action of herbal amino acid supplement AV/CAP/18. It can be concluded that the herbal product (AV/CAP/18) can successfully replace synthetic amino acids (methionine, choline, lysine & biotin) in broiler feed. This may be attributed to the efficacy of constituent herbs of AV/CAP/18 namely *Cicer arietinum*, *Phaseolus mungo*, *Mucuna pruriens*, *Trigonella foenum graecum*, *Nigella sativa*, *Citrullus colocynthis* & many more that mimics the activity like that of synthetic amino acids and are found to be rich source of methionine, choline & lysine which are scientifically well proven for improving growth, productivity & hepatoprotective action.

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References