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Full Length Research Paper

Consequence of biotin as a nourish preservative on the development of *Gallus gallus domesticus*

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The study of broilers were divided in four groups; A, B, C and D fed on ration supplemented at concentrations of 0.15, 0.20, 0.25 and 0.30 mg/kg of feed; whereas, biotin was used in groups B, C and D. Group A was considered as control without use of biotin. The feed consumption was 4285, 4530, 4671 and 4688 g/bird in all groups. The biotin was used as feed additive at the rates of 0.15, 0.20, 0.25 and 0.30 mg/kg feed, respectively. Water intake was 7615, 7742, 7895 and 8039 g/bird, weight gain was 2025.80, 2130.10, 2220.00 and 2350.40 g/bird, feed conversion ratio was 2.115, 2.127, 2.104 and 1.994, carcass weight was 1228.30, 1312.50, 1433.90 and 1512.80 g/bird, heart weight was 9.50, 9.80, 10.20 and 10.40 g, gizzard weight was 33.00, 34.00, 37.40 and 38.60 g and liver weight was 40.30, 42.10, 43.50 and 43.80 g in groups A, B, C and D. The red blood cells (RBC) was 2.31, 3.22, 3.66 and 4.06 (million/cm^3), white blood cells (WBC) 24.77, 26.13, 28.04 (million/cm^3), and 27.73 and packed cell volume (PCV) 30.66, 35.66, 36.33 and 36.00% in groups A, B, C and D, respectively. The highest biotin level of 0.30 mg/kg feed remained superior in almost all the characters studied and is suggested to broiler farmers to use dietary biotin for getting maximum weight gain and subsequent higher carcass quantity in broiler, followed by greater net profit.

Key words: Vitamins, coenzyme, poultry production, RBC, WBC, PCV, chicks.

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INTRODUCTION

Livestock is one of the major sub-sectors of agriculture and the backbone of our economy, which contributes roughly one third in the total share of agriculture. About 35-38 million of the rural population is engaged to livestock to earn their livelihood through keeping 34.0 million cattle, 30 million buffaloes, 28.4 million sheep and 60.3 million goats. During last three years, the poultry meat production in Pakistan was 601, 652 and 707 thousand tons, showing a gradually increasing trend (GOP, 2010). The focus of the commercial poultry industry is the production of meat and eggs under intensive husbandry and chicken meat production (Soomro et al., 2013). Nearly all broilers and roasters are reared in confinement on litter floors, while breeders are confined in 1/3 litter and 2/3 slat houses. Chicken food consist of cereals, plants, animal, agro-based industrial, maize, sorghum, rice, fish meal, meat meal, cotton seed meal, gluten meal and minerals (Sahito et al., 2012). Whereas, Abbasi et al. (2013) got better production from broilers as well as from live stock animals of Sindh inPakistan due to better management and feed rations.

Biotin (Vitamin-H) was required in several enzymes particularly for *trans*-amination and decarboxylation of amino acids (Schultz, 2004). Vitamins are an essential component of a well-balanced diet and their major function is the metabolism and utilization of nutrients. Through research into the biological mechanisms of vitamin action, it has now been established that substantially higher intake of some vitamins may significantly influence the immune process in chickens (Siddique, 2004). In poultry, biotin is an essential coenzyme in carbohydrate, fat and protein metabolism.

Table 1. Application of different vaccines at experimental birds during 2012.

S/N	Vaccine	7th day	12th day	18th day	22nd day
1	Name of vaccine	BipestosNew Castle Disease + Infectious bronchitis (N.D.+1.B)	Gumboral C.T Infectious Bursal Disease	Hydro Pericardium syndrome (H.P.S)	Tad ndvacNew Castle Disease (N.D Lasota)
2	Batch no.	L.19452	L.78692	1854	2013022
3	Manufacturing date	6th Febuary, 2012	24th July, 2012	6th July 2012	1st Feb- 2012
4	Expiry date	6th August, 2013	24th July. 2013	After 60 days	1st September, 2012
5	Date of performance	14th July, 2012	28th July, 2012	25th July, 2012	29th July, 2012
6	Route	Intra-ocular	Intra-ocular	Sub-cut	Drinking water
7	Company	Merial	Merial	Sana Lab.	Tad Lohman

Biotin is a vitamin, necessary to prevent deficiencies like perosis and deformed bones of chickens. Deficiencies have been reported in chickens; however, there is some evidence that certain conditions in chicks can increase the requirement for biotin. Biotin is being used for the prevention of bone deformities and other growth disorders in a synthetic form. Lee et al. (1995) reported biotin deficiency lesions (foot pads) in broilers and suggested that 300 µg per kg was the requirement for birds on a wheat-based diet. The observations on feed and water intake would be recorded on whole feed and water consumption on daily basis as well as weekly, while other observations associated with carcass quantity, weight of internal organs and feed efficiency were recorded on the basis of 10 birds randomly selected and slaughtered at the end of experiment. Finally, the better results obtained from the research on broiler growth through feed additive of biotin would be shared among the broiler farmer's community.

MATERIALS AND METHODS

The present study was performed to see the effect of biotin as a feed additive on the growth of broiler during July-August, 2012 at the Poultry Experiment Station, Department of Poultry Husbandry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, Sindh, Pakistan. The study was based on 200 broiler chicks, purchased from local chick distributor of Hyderabad. The chicks were weighed initially and observation was recorded as in weight of day old chicks. The chicks were housed in a hygienically prepared shed. The experimental chicks were divided in a randomized block design into four groups (A, B, C and D) having 50 chicks in each group. All groups were provided with rations 0.15, 0.20, 0.25 and 0.30 mg/kg of feed added with varying levels of biotin which was in solid form used in three groups such as; B, C and D which were also called as Vitamin-H. It was just not possible to supplement biotin on kilogram feed basis being micro element, so it was added as 10.0, 12.5 and 15.0 mg/50 kg bag, in groups, B, C and D, respectively.

Wooden dust was used as litter and spread after using dry limestone on floor. Chick-paper (Horka-200) was used to cover the litter and also to provide comfort to chicks during first week of the brooding with the temperature ranging from 40 to 45°C during first week and 5°C each week was reduced and maintained as house temperature (22°C). Humidity was maintained around 60%, light was given in a period of 24 h as artificial light. Initially broilers were offered broiler starter ration up to first three weeks followed by broiler finisher ration for rest of three weeks. At first day, sugar mixed water was offered to chicks for fleshing. Both feed and water were offered twice daily *Ad libitum*. The feed refusal from each group was collected and weighed daily at morning and in the evening. Feeding system was managed in such a way that initially it was offered to chicks in chick feeders, tube feeders then small feeders and large feeders were used when birds grew up. Same way was used for water intake; small drinkers/chick drinkers and large drinkers were used. The broilers were individually weighed weekly by weighing balance. Temperature and humidity was recorded by using Operating manual for Temperature and Humidity meter (Power supply: 1.5 v (AAA size) x1, Product dimension: 100x108x20 mm, weight: 160 g, storage: 20-60°C, 20-80% RH) made in China, respectively. To maintain the light in absence of electricity, automatic charging lighters, lamps and candles were used. For ventilation and reduction of house temperature, electric ceiling and electric exhaust fans were used. The flock was monitored strictly for any disease problems and vaccination was done against ND, Gambhoro and HPCS (Table 1).

The observations on feed and water intake were recorded on whole feed and water consumption basis daily as well as weekly, while other observations associated with carcass quantity, weight of internal organs and feed efficiency were recorded on the basis of 10 birds randomly selected and slaughtered at the end of experiment. The blood samples (3 samples in each

Table 2. Average feed consumption (g/bird) as affected by Biotin used as feed additive in broiler ration at different levels.

Weeks	Groups			
	A	B	C	D
W_1	156	168	158	160
W_2	430	458	489	490
W_3	620	655	690	695
W_4	885	914	947	948
W_5	1021	1078	1098	1100
W_6	1173	1257	1289	1295
Total	4285 c*	4530 b	4671 a	4688 a

*Different letters in the same raw means significant at p< 0.05

Table 3. Average water consumption (ml/bird) as affected by Biotin Supplementation at different proportions.

Weeks	Groups			
	A	B	C	D
W_1	295	298	297	300
W_2	700	710	740	780
W_3	1120	1131	1158	1180
W_4	1500	1546	1600	1621
W_5	1850	1876	1900	1928
W_6	2110	2181	2200	2230
Total	7615 d*	7742 c	7895 b	8039 a

*Different letters in the same raw means significant at p< 0.05

group) were also collected and subjected to laboratory analysis for red blood cells (RBC), white blood cells (WBC) and packed cell volume (PCV). The data collected were tabulated and subjected to statistical analysis by using MSTATC Computer Software in General Linear Model, while the comparison within treatments were made by using LSD test such as mentioned by Snedcor and Cochran (1993).

RESULTS

Feed consumption

Average feed consumption of broilers (Table 2) was influenced significantly (P<0.01) with biotin supplementation as feed additive in broiler ration at different levels. The average feed intake was 4285, 4530, 4671 and 4688 g/bird in groups A, B, C and D, respectively. Feed consumption was significantly greater (4688 g/bird) in group D, while birds in group C and B consumed 4671 and 4530 g of feed per bird, respectively. However, the minimum feed consumption (4285 g/bird) was observed in group A (control). However, the differences between group C and D for feed consumption were statistically non-significant. The analysis of variance illustrated that feed intake of broilers increased significantly (P<0.01) with each increment of biotin supplementation in broiler ration as feed additive. The least significant difference (LSD) comparison test revealed that the differences when comparison was between groups A and B or B and C were statistically highly significant (P<0.01), while non-significant (P>0.05) between groups C and D.

Water consumption

Water intake of broilers (Table 3) was increased significantly (P<0.01) with increase in biotin supplementation as feed additive in broiler ration at different levels. Mean water intake was 7615, 7742, 7895 and 8039 g/bird in groups A, B, C and D, respectively. Water intake was significantly greater (8039 g/bird) in group D, while birds in group C and B consumed 7895 and 7742 g of water per bird, respectively. However, the minimum water intake (7615 g/bird) was observed in group A (control). However, the differences between

Table 4. Average live weight gain of broilers (g) as affected by supplementation of biotin at different levels.

Groups	Total Live body weight (g/bird)	Initial live body weight (g)	Weight gain (g/bird)
A	2073.80	48.00	2025.80 c*
B	2179.10	49.00	2130.10 bc
C	2269.00	49.00	2220.00 b
D	2400.40	50.00	2350.40 a

*Different letters in the same column means significant at p< 0.05

Table 5. Average feed conversion ratio of broilers as affected by supplementation of biotin at different levels.

Groups	Average feed consumption/bird (g)	Average weight gain (g/bird)	F.C.R.
A	4285	2025.80	2.115 b*
B	4530	2130.10	2.127 b
C	4671	2220.00	2.104 b
D	4688	2350.40	1.994 a

*Different letters in the same column means significant at p< 0.05

group C and D for water intake were statistically non-significant (P>0.05). The analysis of variance argued that water consumption of broilers increased significantly (P<0.01) with each increased level of biotin in broiler ration as feed additive. LSD comparison test showed that the differences either among treatments groups or when compared with control were statistically highly significant (P<0.01).

Weight gain (g/bird)

Weight gain is the key factor to be best considered as a feed composition which is appropriate, and economics of any research effort is based on weight gains. Mean weight gain of broilers (Table 4) was increased gradually with increase in biotin supplementation as feed additive in broiler ration at different levels. Mean weight gain was 2025.80, 2130.10, 2220.00 and 2350.40 g/bird in groups A, B, C and D, respectively. Weight gain was significantly (P<0.01) greater (2350.40 g/bird) in group D, while birds in group C and B gained 2220.00 and 2130.10 g weight per bird, respectively. The lowest weight gain (2025 g/bird) was recorded in group A (control).

The analysis of variance suggested that weight gain improved appreciably (P<0.01) with each increased level of biotin in broiler ration as feed additive. LSD comparison test revealed that the differences either among treatments groups or when compared with control were statistically significant with varying degree of significance.

Feed conversion ratio

Feed conversion ratio denotes the amount of feed utilized for producing a unit of weight gain. Mean feed conversion ratio of broilers (Table 5) was improved significantly under higher biotin supplementation levels. Mean feed conversion ratio worked out was 2.115, 2.127, 2.104 and 1.994 in groups A, B, C and D, respectively. Feed conversion ratio was significantly (P<0.01) superior (1.994) in group D, while birds in group C and A recorded 2.104 and 2.115 feed conversion ratio, respectively. Relatively poor feed conversion ratio (2.127) was recorded in group B where the broilers were fed on ration added biotin at the rate of 0.25 mg/kg feed. The analysis of variance indicated that feed conversion ratio improved significantly (P<0.01) when broilers were fed on rations supplemented with different levels of biotin. LSD comparison test revealed that the differences among A and B or B and C were statistically non-significant (P>0.05), while highly significant when first three groups were compared with group D.

Carcass weight (g/bird)

Mean carcass weight of broilers (Table 6) increased significantly with increase in biotin as feed additive in broiler ration at different levels. Carcass weight was 1228.30, 1312.50, 1433.90 and 1512.80 g/bird in groups A, B, C and D, respectively. Carcass weight was significantly (P<0.01) higher (1512.80 g/bird) in group D,

Table 6. Average carcass weight (g/bird) of broilers as affected by supplementation of biotin at different levels.

Groups	Live body weight (g/group)	Average carcass weight (g/bird)
A	2073.80	1228.30 d*
B	2179.10	1312.50 c
C	2269.00	1433.90 b
D	2400.40	1512.80 a

*Different letters in the same column means significant at p< 0.05

Table 7. Average weight of giblets (g) of broilers as affected by supplementation of biotin at different levels.

Groups	Heart weight	Gizzard weight	Liver weight
A	9.50 b*	33.00 b	40.30 b
B	9.80 ab	34.00 b	42.10 ab
C	10.20 a	37.40 a	43.50 a
D	10.40 a	38.60 a	43.80 a

*Different letters in the same column means significant at p< 0.05

while birds in group C and B recorded 1433.90 and 1312.50 g carcass weight per bird, respectively. The lowest carcass weight (1228.30 g/bird) was recorded in group A (control). The analysis of variance indicated that carcass weight improved significantly (P<0.01) when biotin was supplemented in broiler ration at different rates. LSD comparison test revealed that the differences either among treatments groups or when compared with control were statistically highly significant (P<0.01).

Heart weight (g)

Heart weight of broilers (Table 7) increased significantly with increase in biotin as feed additive in broiler ration at different levels. Heart weight was 9.50, 9.80, 10.20 and 10.40 g in groups A, B, C and D, where the biotin was used as feed additive at concentrations of 0.15, 0.20, 0.25 and 0.30 mg/kg feed, respectively. Significantly maximum heart weight (10.40 g) was recorded in broilers of group D, closely followed by group C (10.20 g), while birds in group B recorded 9.80 g heart weight and the lowest (9.50 g) weight was recorded in control group A. The analysis of variance indicated that heart weight improved significantly (P<0.05) when biotin was supplemented in broiler ration at different rates. LSD comparison test revealed that the differences either among treatments groups or when compared with control were statistically significant (P<0.05) with the exception of differences between group C and D, where non-significant differences were observed.

The results further showed that gizzard weight of broilers increased significantly with increase in biotin supplementation at different levels in ration. Gizzard weight was 33.00, 34.00, 37.40 and 38.60 g in different groups, respectively. Significantly maximum gizzard weight (38.60 g) was recorded in broilers of group D, closely followed by group C (37.40 g), while birds in group B recorded 34.00 g gizzard weight and the lowest (33.00 g) weight was recorded in group A (control). The analysis of variance suggested that gizzard weight increased significantly (P<0.05) when biotin was supplemented in broiler ration at different levels. LSD comparison test revealed that the differences between group C and D or between A and B were statistically non-significant.

The results represented that, the liver weight of broilers increased significantly in response to biotin supplementation at different levels in broiler ration. Liver weight was 40.30, 42.10, 43.50 and 43.80 g in groups A, B, C and D, where the biotin was supplemented at the rates of 0.15, 0.20, 0.25 and 0.30 mg/kg feed, respectively. Significantly maximum liver weight (43.80 g) was recorded in broilers of group D, followed by group C (43.50 g), while birds in group B recorded 42.10 g liver weight and the lowest (40.30 g) liver weight was recorded in group A (control).

The analysis of variance revealed that liver weight increased significantly (P<0.05) when biotin was used as feed additive in broiler ration at different levels. LSD comparison test revealed that the differences between group C and D were statistically non-significant, while significant when these groups were compared to rest of the groups including control.

Table 8. Haemotological examination of blood of broilers as affected by supplementation of biotin at different levels.

Groups	RBC (million/cm^3)	WBC (million/cm^3)	PCV (%)
A	2.31 d*	24.77	30.66
B	3.22 c	26.13	35.66
C	3.66 b	28.04	36.33
D	4.06 a	27.73	36.00

*Different letters in the same column means significant at p< 0.05

Table 9. Mortality percentage of broilers fed on ration containing Biotin at varying proportions.

Treatments	Total number of birds	Number of birds survived	Number of birds dead	Mortality (%)
A	50	47	3	6.00
B	50	48	2	4.00
C	50	48	2	4.00
D	50	46	4	8.00
Total mortality	200	189	11	5.50

Red blood cells (RBC), white blood cells (WBC) and pecked cell volume (PCV)

The results of the haemotological examination of red blood cells (RBC), white blood cells (WBC) and pecked cell volume (PCV) in broilers (Table 8) fed on ration, contained biotin as feed additive at different levels and indicate that RBC was affected significantly, while non-significant effect on WBC and PCV were recorded due to supplementation dietary biotin in broiler ration at different levels. The values for RBC are 2.31, 3.22, 3.66 and 4.06 million/cm^3, WBC; 24.77, 26.13, 28.04 and 27.73 million/cm^3 and PCV; 30.66, 35.66, 36.33 and 36.00% in groups A, B, C and D, where biotin was used as feed additive at the rates of 0.15, 0.20, 0.25 and 0.30 mg/kg feed, respectively. The values for blood examination were within the normal range and hence no adverse effect of biotin even at higher level was found on haemotological values of broilers.

Mortality rate

The mortality of broilers in different groups fed on rations with dietary biotin at different levels was also worked out and is presented in Table 9. It is obvious from the results that on experimental chicks there was no adverse effect of biotin used as feed additive at increasing levels and the mortality that occurred was assumed to be incidental or natural. The mortality recorded was 6.00, 4.00, 4.00 and 8.00% in groups A, B, C and D, where the broilers were fed on rations which contained biotin as feed additive at the rates of 0.15, 0.20, 0.25 and 0.30 mg/kg feed, respectively. The overall mortality remained 5.50%.

DISCUSSION

The biotin 0.25 mg/kg feed used as feed additive in broiler ration proved to be the maximum level to affect feed consumption; further increase in biotin supplementation (0.30 mg/kg feed) did not affect feed consumption of the broilers. The increase in feed consumption with increased level of biotin was associated with the fact that biotin is a coenzyme in carbohydrates and involved in conversion of protein and carbohydrates to fat, thus increases appetite and in result feed intake increases. Results are also in conformity to those of Odoyo (1997) who experienced increased feed intake by broilers when biotin supplementation increased in ration; while Tuncer et al. (1999) reported that with higher biotin supplementation levels, the overall broiler performance was improved along with increased feed consumption. Santin et al. (2000) experienced positive impact of biotin when used as feed additive in broiler ration and reported increased feed intake with increased biotin levels.

The water intake by the broilers was continuously increasing with increase in use of biotin as feed additive and those broilers that were fed on ration containing highest level of biotin supplementation (0.30 mg/kg feed) took maximum quantity of water. The increase in water intake was mainly associated with the increase in feed intake, because when broilers took greater amount of

feed, obviously they needed more water for its digestibility. Moreover, biotin being known as a coenzyme in carbohydrates is involved in conversion of protein. These results are fully supported by the findings of Odoyo (1997) who argued that broilers consumed greater quantities of water, and fed on rations containing higher levels of biotin as feed additives.

The results showed a linear trend of effectiveness for weight gain in response to supplementation of biotin as feed additive in broiler ration. This increase in weight gain with each increased level of biotin was mainly associated with increased feed intake, which obviously was converted into weight gain. Hou and Huang (1995) concluded that weight gain of broilers significantly increased when given ration supplemented with biotin at higher rates up to 300 µg/kg feed, while Lee et al. (1995) reported increased body weight with increased biotin levels (300 µg/kg feed). Similarly, Oloyo and Ogunmdede (1998) used biotin as feed additive and obtained higher weight gains in broilers when biotin used up to 240 µg/kg feed. The above findings of the earlier researchers are well in accordance with the results of present study, where the maximum weight gain was obtained when biotin was used at 300 µg/kg feed as feed additive in broiler ration.

The trend of effectiveness for feed conversion efficiency showed that higher biotin level as feed additive in broiler ration up to 0.30 mg/kg feed was linearly effective to influence feed efficiency positively, while lower biotin levels did not affect feed efficiency of broilers. The findings reported by Balios and Poupoulis (1996) further confirmed the results of the present investigation and concluded that feed conversion ratio improved with increasing levels of biotin. Similarly, Tuncer et al. (1999) and Santin et al. (2000) were of the experience that increasing biotin supplementation in feed significantly improved the feed efficiency of broilers. It is evident from the trend of the results that increasing biotin as feed additive in broiler ration linearly influenced the carcass weight. This increase was mainly associated with increase in weight gain and that was the result of increased feed intake. Odoyo (1997) obtained significantly higher carcass quantity in result of 320 µg/kg feed biotin when used as feed additive. Similarly, Buda (2000) experienced significantly higher carcass values under higher biotin supplementation in broiler feed. The above findings of the earlier researchers are well comparable with the results of the present investigation, where the maximum carcass weight has been obtained when biotin was used as feed additive in broiler ration at the rate of 300 µg/kg feed.

It is obvious from the results regarding weight of giblets (liver, gizzard and heart) that when biotin was used as feed additive in broiler ration, the effects on liver, gizzard and heart weights were statistically significant ($P<0.05$). However, a similarity and association was noticed that in all the three giblets, the differences in groups C and D were statistically non-significant. This shows that biotin supplementation beyond 0.25 mg/kg did not affect the weight of giblets. However, the overall results for liver, gizzard and heart clearly showed that effect of biotin as feed additive was significant on their weights. Similar results have been reported by Oloyo (1995) who found increased liver weight with 200 µg/kg feed biotin supplementation in broiler ration, while Odoyo (1997) reported increased weight of internal organs of broilers with increase in dietary supplementation of biotin. Moreover, Santin et al. (2000) also found increase in liver weight with increase in biotin concentration in feed.

Significant effect on RBC was observed and little increase in WBC and PCV was also found, but all the values either for RBC, WBC or PCV were within the normal ranges. None of the values of any of the haemotological observation was beyond the normal range. Thus, it can be concluded that biotin supplementation did not affect abnormally the haemotological values of broilers. The results reported by Balios and Poupoulis (1996) reported that blood serum and other values were not affected by biotin supplementation in broiler feed even up to 400 µg/kg feed. Lechowski and Nagorna (1995) and Oloyo and Ogunmodede (1998) reported that blood lipids were significantly affected by dietary biotin, but values were within the normal range. There was no adverse effect of biotin used as feed additive at increasing levels on mortality, and the mortality occurred was assumed to be incidental or natural. Hence, biotin supplementation in broiler ration is considered to be more suitable and beneficial as indicated in the production performance of broilers and due to use of biotin, the broiler produced the better net profit.

Conclusion

It was concluded that all the production parameters were affected significantly and positively when biotin was used as feed additive in broiler ration. The highest biotin level of 0.30 mg/kg feed remained superior in almost all the characters studied and is suggestible for the broiler farmers to use dietary biotin at the rate of 0.30 mg/kg feed (300 µg/kg feed) for getting maximum weight gain and subsequent higher carcass quantity in broilers that provided the greater net profit for the farmers.

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