Effect of difference methionine sources on performance of broilers challenged with *Salmonella Pullorum.*

Trial: 07 CAU

1. Summary

The aim of this study was to compare the effects of different methionine sources on performance of broilers challenged with enteric pathogens namely Salmonella *pullorum* cvcc 518. The hypothesis of an antimicrobial function of HMTBA, comparable with those of organic acids, was tested. Growth performance clearly showed that challenged birds presented lower growth performances, mainly due to lower feed intake without clear effect on FCR. The effect of Methionine sources allowed significant and numerical higher feed intake and better growth without affecting feed conversion with HMTBA compared to DLM, both in controlled and challenged conditions. The bacterial analyses and the gut content pH measurement showed evidences for acidifying properties of HMTBA that allowed a numerical decrease in Salmonella and E. coli numbers in the Gastro Intestinal Tract. Taking these results into account, the use of HMTBA as Methionine source could be associated with “organic acid like” functions.

2. Material and method

The effect of methionine hydroxyl-analogue and DL-methionine when broilers were challenged with Salmonella *pullorum* cvcc518 was evaluated according to the following design:

2 treatments x 2 conditions (challenged & unchallenged) x 6 replicates of 10 broilers

Table 1. Experimental design

Treatments	T1	T2	T3	T4
Conditions	Unchallenged		Challenged (S. *Pullorum* cvcc 518)	
Starter phase 0-21 days				
Methionine sources*	DL-Methioine 0.27%	DL-HMTBA 0.3%	DL-Methioine 0.27%	DL-HMTBA 0.3%
Grower phase 22-42 days				
Methionine sources*	DL-Methionine 0.18%	DL-HMTBA 0.2%	DL-Methioine 0.18%	DL-HMTBA 0.2%

*** Methionine product expressed as such**

Management and measurements:

The trial was performed at the China Agricultural University, Beijing, China. Broilers were randomly allocated in 4 treatments with 6 replicates per treatment. On day 8, half was challenged with 1mL of 2×10^{12} CFU of *Salmonella pullorum cvcc518.* This concentration was previously determined as optimal from a long term challenge trial. Birds from the other group (unchallenged) were administered with 1mL of saline water as negative control. Daily feed intake, daily weight gain and feed conversion rate were calculated during the two periods. Mortality was also recorded.

The effect of methionine sources has been compared using HMTBA to DLM performance ratio and results were expressed on a percentage basis of DLM performance (e.g. 100% means equal results between methionine sources on the considered parameter).

Statistical analysis:

Data were analyzed using the SAS 8.2 computer package. Firstly, effect of treatment on the growth parameters has been test using the ANOVA test. When significant effect was detected, means were separated by Tukey's pairwise at *P*=0.05.

Table 2. Composition and characteristics of the basal diets

Ingredients (%)	Starter (0-21 days)	Grower (22-42 days)	Calculated analyses	Starter (0-21 days)	Grower (22-42 days)
Corn	57.5	63.4	Met. Energy	3 100	3 100
Extruded soybeans	8.00	7.20	Crude protein (%)	19.99	17.97
Soybean meal	28.3	23.0	Calcium (%)	1.01	0.90
Limestone	1.30	1.30	Av. phosphorus	0.43	0.35
Dicalcium phosphate	1.70	1.30	Lysine (%)	1.09	0.99
Sodium chloride	0.30	0.30	Methionine (%)	0.59	0.47
Corn oil	1.90	2.50	Meth. + cystine	0.93	0.78
Vit. and min. premix	1.00	1.00			

3. Results and discussion

The growth performance results are summarized in table 3 for the 2 growth periods and the whole period. Globally, the challenged group showed significant lower growth rate ($p<0.05$) mainly due lower feed intake than unchallenged birds without clear impact on FCR. The effect of salmonella challenge on feed intake depression without impact on FCR has been reported by Marcq *et al*. (2011).

In this study, a significant effect of methionine sources was observed in unchallenged condition where HMTBA fed birds exhibited a higher daily gain during the finishing period and the global rearing period. This better daily gain appeared clearly related to a better feed intake for HMTBA fed birds compared to DLM fed birds.

In the challenged condition the relative daily feed intake appeared numerically better improved with HMTBA compared to DLM than in unchallenged condition (109.5% vs 107.9%). However, due to higher variability in the challenged condition the numerical improvement didn't reach the significant level of $p<0.05$. Interestingly, this numerical improvement of feed intake resulted also in a numerical improvement of daily gain with HMTBA to DLM and a performance ratio of 106.1%.

Table 3. Effect of methionine source on performance parameters with or without pathogen challenge

Conditions	Unchallenged		Challenged	
Treatments	T1	T2	T3	T4
0-21 days				
Average daily feed intake (g)	44,1	43,8	40,7	38,9
HMTBA to DLM perf. Ratio	99,3%		95,6%	
Average daily weight gain (g)	25,8	26,5	24	23,1
HMTBA to DLM perf. Ratio	102,7%		96,3%	
Feed conversion rate	1,71	1,65	1,7	1,68
HMTBA to DLM perf. Ratio	104,2%		100,0%	
22-42 days				
Average daily feed intake (g)	115,5	122,5	106,8	116,7
HMTBA to DLM perf. Ratio	106,1%		109,3%	
Average daily weight gain (g)	59.4 a	66.0 b	58,2	60,2
HMTBA to DLM perf. Ratio	111,1%		103,4%	
Feed conversion rate	1,94	1,86	1,84	1,94
HMTBA to DLM perf. Ratio	104,9%		94,8%	
0-42 days				
Average daily feed intake (g)	77,1	83,2	73,7	80,7
HMTBA to DLM perf. Ratio	107,9%		109,5%	
Average daily weight gain (g)	41.2 a	46.3 b	40,7	43,2
HMTBA to DLM perf. Ratio	112,4%		106,1%	
Feed conversion rate	1,87	1,8	1,81	1,87
HMTBA to DLM perf. Ratio	103,9%		96,8%	

Table 4 shows the effect of the different treatments and conditions on the content gut pH in the different parts, from duodenum to caecum. These results showed that in controlled condition HMTBA fed birds exhibited a significantly lower pH for duodenal content and numerically lower at jejunum level. Adversely, no effect of methionine sources on gut content pH was observed in the challenged condition. This interactive effect should be due to the higher microbial pressure in the challenged condition that led to acidic function consumption in upper part of Gastro Intestinal Tract (GIT).

Moreover Table 5 to 7 shows the effect of methionine and challenge conditions on the counting of Salmonella, E. coli and lactobacillus species at the different parts of the GIT. Surprisingly for Salmonella species the challenged group exhibited higher number of bacteria compared to unchallenged but without statistical significance. However, the number of *Salmonella* and *E. Coli* was lower, when HMTBA was supplied in the feed, as compared to DLM. This effect was significant for E. Coli counts at jejunal level whatever the tested condition. This effect of HMTBA in challenged condition could be related to its acidifying effect and the possibility to lower Salmonella pressure at gut level like organic acid, as reported by Skinner et al. (1991) or Moran, (2005).

Interestingly, the effect of HTMBA observed on *Salmonella* and *E. Coli* was not observed on the *Lactobacillus* species that appeared numerically throughout the GIT in HMTBA fed birds compared to DLM fed birds. This observation is consistent with those of Mercier et al. 2007 who reported HMTBA effect on some bacterial population at the ileal level.

Table 4. pH value in different deposition of intestine

Conditions	Treatement	Duodenum	Jejunum	Ileum	Cecum
Unchallenged	T1 DLM	6.38 ± 0.13b	6.43 ± 0.26	6.24 ± 0.28	6.86 ± 0.10
	T2 HMTBA	5.98 ± 0.10a	6.09 ± 0.03	6.29 ± 0.15	6.76 ± 0.06
Challenged	T3 DLM	6.20 ± 0.11ab	6.27 ± 0.04	6.48 ± 0.25	6.95 ± 0.13
	T4 HMTBA	6.41 ± 0.09b	6.47 ± 0.12	6.45 ± 0.21	6.75 ± 0.13

Table 5. *Salmonella* counting of in different deposition of intestine expressed in log of CFU

Conditions	Treatement	Duodenum	Jejunum	Ileum	Caecum
Unchallenged	T1 DLM	2.83 ± 0.36	3.27 ± 0.42	4.00 ± 0.44	6.58 ± 0.31
	T2 HMTBA	1.53 ± 0.78	2.63 ± 0.14	3.71 ± 0.50	5.69 ± 0.38
Challenged	T3 DLM	3.12 ± 0.24	3.56 ± 0.25	4.84 ± 0.05	6.97 ± 0.24
	T4 HMTBA	2.26 ± 0.14	3.29 ± 0.12	4.25 ± 0.19	7.01 ± 0.13

Table 6. *Escherichia coli* counting of in different deposition of intestine in log of CFU

Conditions	Treatement	Duodenum	Jejunum	Ileum	Cecum
Unchallenged	T1 DLM	3.01 ± 0.36	4.76 ± 0.08 b	4.65 ± 0.20	7.65 ± 0.06
	T2 HMTBA	1.99 ± 1.04	3.55 ± 0.05 a	4.19 ± 0.45	7.54 ± 0.07
Challenged	T3 DLM	3.35 ± 0.62	4.64 ± 0.15 b	4.28 ± 0.66	7.87 ± 0.18
	T4 HMTBA	2.68 ± 0.19	3.74 ± 0.11 a	4.15 ± 0.17	6.98 ± 0.44

Table 7: *Lactobacillus* counting of in different deposition of intestine in log of CFU

Conditions	Treatement	Duodenum	Jejunum	Ileum	Cecum
Unchallenged	T1 DLM	3.69 ± 0.28	4.29 ± 0.19	4.75 ± 0.69	7.65 ± 0.59
	T2 HMTBA	4.32 ± 0.31	4.62 ± 0.23	5.68 ± 0.46	8.24 ± 0.15
Challenged	T3 DLM	3.90 ± 0.41	4.24 ± 1.14	4.99 ± 0.45	7.89 ± 0.27
	T4 HMTBA	4.39 ± 0.22	4.69 ± 0.10	5.51 ± 0.09	7.60 ± 0.17

4. Conclusion

The results obtained in this study demonstrated that, when birds were challenged by *Salmonella pullorum* in the early stage of 8 days, the growth performances were affected during the overall rearing period. HMTBA used as methionine source in this condition tended to give some numerical improvement on feed intake and daily weight gain and also lower bacterial counting in the upper part of gut compared to DLM. These results seems indicated that HMTBA beyond it methionine source function could be considered as part of the solution to control challenging conditions.

5. Literature cited

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